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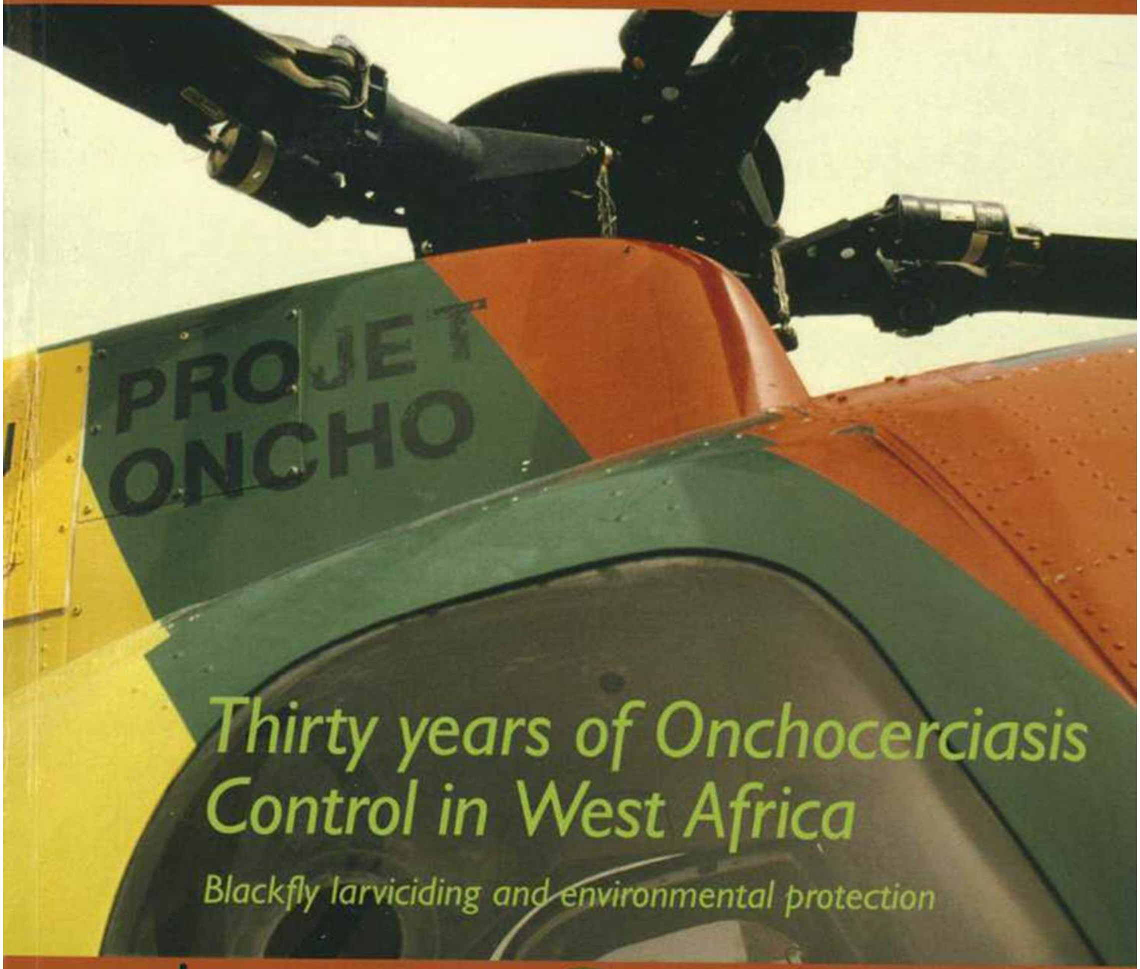
Trente ans de lutte contre l'onchocercose en Afrique de l'Ouest

Traitements larvicides et protection de l'environnement



Thirty years of Onchocerciasis Control in West Africa

Blackfly larviciding and environmental protection

Organisation mondiale de la Santé
World Health Organization

Programme de lutte contre l'Onchocercose en Afrique de l'Ouest (OCP)
Onchocerciasis Control Programme in West Africa

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Trente ans de lutte contre l’onchocercose en Afrique de l’Ouest. Traitements larvicides et protection de l’environnement

Thirty years de onchocerciasis control in West Africa. Blackfly larviciding and environmental protection

Laurent Yaméogo, Christian Levêque et Jean-Marc Hougard (dir.)

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La lutte contre l'onchocercose, ou cécité des rivières, une maladie parasitaire endémique, fut entreprise en Afrique de l'Ouest dans une perspective do développement durable. Tous les moyens technologiques disponibles ont de ce fait été mobilisés pour le contrôle du vecteur, une simulie, puis du parasite responsables de cette maladie, par le Programme de Lutte contre l'Onchocercose en Afrique de l'Ouest (OCP). La lutte antivectorielle consistant en épandages d'insecticides chimiques sur les sites de développement de la simulie dans les rivières, il est apparu indispensable d'assurer la sauvegarde de l'environnement aquatique qui fournit aux communautés riveraines eau et ressources biologiques. Les technologies les plus modernes ont été mises en œuvre dès leur mise au point, pour combattre la maladie, contribuant ainsi à la protection de ce milieu. Le programme de surveillance écologique des rivières traitées par des larvicides anti-simulies a été mis en place dès le lancement d'OCP. et assuré par des spécialistes de I' hydrobiologie des pays africains participants du Programme, sous la supervision d'un groupe international d'experts indépendants, le Groupe Écologique.
OCP est incontestablement un succès aussi bien pour ce qui est du contrôle de la maladie que de la protection de l'environnement. Il est l'exemple unique au monde d'un programme de santé publique de longue durée qui depuis son origine a mis en œuvre tout ce qui était possible pour harmoniser les enjeux de l'amélioration de la santé et ceux de la protection de l'environnement. Il s'est achevé avec la satisfaction de laisser aux générations montantes un environnement non dégradé et des vallées libérées de l'onchocercose, qui permettront d'accroître la productivité agricole des pays africains.

The control of onchocerciasis, or river blindness, an endemic parasitic disease, was implemented in West Africa in the perspective of sustainable development AH the available technological means to fight this disease, by way of the control of its blackfly vector, then its parasite, were therefore implemented by the Onchocerciasis Control Programme in West Africa (OCP). Vector control being achieved through applications of chemicals on its river breeding sites, it was necessary, at the same time, to fight for the preservation of the aquatic environment, which supplies the communities that live along the rivers with water and biological resources. This was the spirit in which the OCP was set up and implemented, and the most modem technologies were used as they become available to fight the disease, thus facilitating the preservation of the aquatic environment. This Programme has indisputably been a success as regards the control of the disease as also from the point of view of the preservation of the environment The aquatic monitoring programme of the rivers under treatment with anti-simulid larvicides was set up right from the very beginning, and performed by national experts of the Participating Countries of the Programme, under the aegis of a group of international independent experts, the Ecological Croup The Onchocerciasis Control Programme in West Africa is an unique example in the world of a long-term public health programme which has made every effort possible from its inception to adequately combine health and environment issues. It ended with the satisfaction of bequeathing to the coming generations a non degraded environment and valleys freed from onchocerciasis which would increase the agricultural productivity of the countries.

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Joint US-AID/OCCGE/WHO technical-meeting on the feasibility of onchocerciasis control

Avant-propos

Bernard Philippon

1 On connaît le rôle déterminant qu'ont joué les entomologistes médicaux de l'Orstom, devenu IRD en 1998, dans la conception, la préparation et le lancement d'OCR puis pendant près de 30 ans dans son exécution et son suivi. On sait moins que l'Orstom s'était investi d'emblée dans l'une des activités les plus originales d'OCP : la surveillance de l'environnement des cours d'eau soumis à la menace chimique des insecticides antisimulies. L'Orstom a maintenu pendant plus d'une décennie une forte équipe d'ichtyologues et d'invertébristes dans la zone d'OCP ; il a ouvert très tôt un poste d'hydrobiologiste au sein de l'Unité de lutte antivectorielle du Programme, et ses experts ont siégé durablement au Groupe écologique, y compris à sa tête.

2 Il était logique que dans la ligne de l'Orstom, accompagnateur de bout en bout d'OCR l'IRD fût aussi le vulgarisateur de l'impressionnant bilan de l'un des volets les plus audacieux de l'oeuvre de développement réussie d'OCR Ce bilan compte plusieurs réalisations majeures :

- la faune des poissons reste indemne et celle des invertébrés n'a subi aucun outrage irréversible grâce à la vigilance des hydrobiologistes, guides et gendarmes de la lutte antivectorielle ;
- la connaissance de la faune des rivières africaines et de ses réactions face aux transformations de toutes sortes de l'environnement a fait des progrès spectaculaires. Des méthodes de monitorage, mises au point *de nihilo,* ont été validées au stade opérationnel ;
- et surtout, des équipes africaines d'hydrobiologistes dûment formées ont pris en charge la surveillance de l'environnement de leurs propres vallées, une tâche qu'elles sont pleinement en mesure de poursuivre, pourvu que la communauté internationale les seconde dans cette activité encore pionnière dans nombre de pays du Sud.

3 The *outstanding role played by ORSTOM (IRD since 1998) medical entomologiste in the design, preparation and launching of OCP and then in the implementation and monitoring of the programme for almost 30 years is well known. ORSTOM's early contribution to the surveillance of the watercourses endangered by Chemical larvicides for control of blackfly, one of the most original undertakings in OCR is not as familar. ORSTOM maintained a strong team of ichthyologists and specialiste on invertebrates in the OCP area for more than a decade; one of its hydrobiologists was a*

full member of the OCP vector control unit and several of its experts have been members and even chairmen of the Ecological Group.

4 *In line with ORSTOM's policy of continued association with OCR IRD logically agreed to assemble the numerous resulte of this most challenging domain of OCP' s successful contribution to development. Several achievements can be emphasised :*

- *the hydrobiologists have been advisors and guides for vector control and their vigilance has mode it possible to prevent harm to fish and to avoid any irreversible damage to invertebrate aquatic fauna;*
- *tremendous progress has been made in knowledge of the aquatic fauna in African rivers and understanding of its reaction to all kinds of environmental changes. Monitoring methods were developed from scratch and validated under operational conditions.*
- *above all, African teams of hydrobiologists have received a high levei training and are responsible for the environmental monitoring of their own valleys. Their experience makes them fully capable of continuing this activity—while awareness of its usefulness is still only just emerging in most developing countries—provided that the international community of environmentalists continues to assist them in their tasks.*

Préface

Boakye A. Boatin

1 En moins d'une décennie, le développement durable est devenu le leitmotiv de toutes les actions de développement. Le développement durable ne signifie pas pour autant une protection aveugle et inconsidérée de l'environnement. Son but ultime est en effet de rechercher des compromis entre, d'une part, un développement dont l'objectif essentiel reste l'amélioration des conditions de vie des sociétés humaines et le bien-être de l'homme et, d'autre part, une bonne gestion de l'environnement et de ses ressources.

2 Pour y parvenir ; il est parfois nécessaire de modifier le cours des choses. Car à côté des nombreux biens et services qu'elle nous prodigue (ressources vivantes, eau, sols, etc.), la nature est également une importante source de nuisances pour l'homme. Ainsi, diverses maladies sont le produit de la co-évolution entre l'homme et ses parasites, et personne ne remettra en cause la nécessité de les contrô1er. Tous ceux qui ont vécu près des fleuves africains il y a quelques décennies se souviennent des nombreuses endémies qui sévissaient dans des milieux naturels encore peu modifiés par les activités humaines. Certaines de ces endémies perdurent encore, mais nous sommes en mesure aujourd'hui, dans la majorité des cas, d'en contrôler l'extension et la virulence.

3 Le contrôle d'une endémie parasitaire comme l'onchocercose s'est inscrit dans une perspective de développement durable. Il n'était pas acceptable que les populations vivant dans les vallées fertiles continuent à souffrir de cette maladie, ou soient contraintes par la cécité de se replier sur des terres pauvres et arides. À partir des années 1970, la sécheresse persistante, s'ajoutant à la croissance démographique, imposait aussi de rendre à l'agriculture les terres riveraines irrigables infestées pour accueillir les populations de paysans chassées des terres desséchées. Tous les moyens technologiques disponibles ont donc été mis en œuvre pour lutter l'onchocercose. Cette lutte a été dirigée contre son vecteur une simulie, puis son parasite. La stratégie première de lutte consistant en un contrôle chimique des larves de ce vecteur dans les gîtes larvaires des rivières, il a fallu parallèlement s'attacher à la préservation de l'environnement aquatique qui fournit aux sociétés riveraines l'eau et des ressources biologiques. C'est dans cet esprit qu'a été lancé le programme de lutte contre l'onchocercose en Afrique de l'Ouest (Onchocerciasis Control Programme in West Africa : OCP).

AUTEUR

BOAKYE A. BOATIN

Ancien Directeur de l'OCP

Foreword

Boakye A. Boatin

1 Within the decade, sustainable development has become the leitmotiv of all development activities. It is worth noting however that sustainable development does not in any way mean blind and mindless protection of the environment. The ultmate goal is indeed to find compromises in which the primary objective of development remains the improvement of living conditions in human societies and the welfare of human beings on the one hand, and a good management of the environment and its resources on the other hand.

2 To reach this goal, it is sometimes necessary to modify the natural trend of events. In fact, this rich environment which provides us with numerous goods and services (living resources, water soils, etc) can also be harmful to human beings. Many diseases are induced by the coexistence of man and his parasites, and nobody would question the urgency to bring them under control. All those who lived near the African rivers some decades ago remember numerous endemic diseases affecting natural environments which were only slightly modified by human activities. Some of these endemic diseases are still present, but there is the capacity today to control, in the majority of cases, their extension and virulence.

3 The control of an endemic parasitic disease as onchocerciasis falls within the framework of sustainable development. In fact, it was unacceptable that the populations living in these fertile valleys continued to suffer from this disease, or leave for less fertile and arid zones to prevent blindness. Moreover persisting draughts imposed to re-colonize riverside irrigable lands to host migrant farmers chased out from desertified areas. All the available technological means to control the disease were therefore implemented. Control was first directed against the blackfly vector then the parasite. The initial strategy being based on vector control through applications of Chemicals In its river breeding sites, it was necessary at the same time to fight for the preservation of the aquatic environment, which supplies the communities that lived along the rivers with good quality water and biological resources. The Onchocerciasis Control Programme in West Africa (OCP) was set up to meet this challenge.

4 Concerning the preservation of the environment, the OCP set up, right from the very beginning, an aquatic monitoring programme of the rivers treated with larvicides, conducted by national experts from the African participating countries, under the aegis of a group of national and international independent experts, the Ecological Group. One of the specific purposes was to preserve the fish populations, En matière de préservation de l'environnement, l'OCP a mis en place, dès l'origine, un programme de surveillance aquatique des rivières traitées aux insecticides anti-simulies, exécuté par des experts nationaux des pays africains participants du Programme, sous l'égide d'un groupe d'experts indépendants internationaux, le Groupe écologique. L'un des buts affichés était de préserver les populations de poissons qui constituent une source importante de protéines pour les populations riveraines.

5 Pour cela, il a fallu utiliser les technologies les plus modernes au fur et à mesure de leur développement, tant en matière de lutte contre la maladie qu'en matière de protection de l'environnement. Ainsi, un important travail de sélection des insecticides a permis de mettre en œuvre une stratégie de rotation des larvicides tenant compte non seulement de critères de coût et d'efficacité mais aussi d'impact sur la faune aquatique non cible. Il s'agissait donc de trouver des compromis entre ce qui était techniquement disponible, écologiquement acceptable, et économiquement viable. L'utilisation des satellites pour connaître les débits des fleuves au moment précis des épandages a permis des progrès importants dans l'utilisation des insecticides en évitant aussi bien les surdosages néfastes à la faune non cible que les sous-dosages préjudiciables au contrôle efficace des simulies. Dans le domaine de l'épidémiologie, la mise au point de sondes ADN a également permis de mieux faire la part des parasites d'origine humain et animale dans les simulies infectantes et d'ajuster ainsi les opérations de lutte.

6 Le programme de lutte contre l'onchocercose en Afrique de l'Ouest est indubitablement un succès en ce qui concerne le contrôle de la maladie et la protection de l'environnement. C'est un exemple unique au monde de programme de santé publique à long terme à s'être doté dès son lancement en 1974, de tous les moyens possibles pour préserver l'environnement aquatique. Il a été un précurseur avant la lettre, des principes du développement durable. Il s'est achevé avec la satisfaction de léguer aux générations futures un environnement non dégradé, des vallées libérées de l'onchocercose qui pourront accroître la production agricole des pays participant, ainsi que des connaissances nouvelles sur les fleuves africains et sur les moyens de contrôle des endémies parasitaires.

7 C'est le lieu d'adresser ici nos remerciements les plus sincères à toute la communauté scientifique (Comité consultatif d'experts et Groupe écologique de l'OCR hydrobiologistes, entomologistes et épidémiologistes des pays participants et des différents instituts de recherche de par le monde qui ont collaboré avec l'OCP à cette importante mission), à la communauté des donateurs qui a fait confiance à l'OCP pendant de si longues années malgré les multiples défis à relever et au personnel du Programme dont le dévouement, la persévérance et l'esprit de suite et de sacrifice ont été salutaires.

8 Notre souhait est que cette expérience unique de mobilisation de la communauté internationale en faveur d'un réel développement durable de zones « laissées pour compte » fasse école pour le plus grand bien des populations locales. which constitute a considerable source of protein for the riverside populations. Therefore, it was necessary to use the most modem technologies as they became available, to fight the disease as well as to preserve the environment. A programme of insecticides selection was launched and

allowed to use larvicides on a rotational basis taking into account not only their cost/efficacy ratio, but also their impact on the non target aquatic fauna. The question was to find compromises among what was technically available, ecologically acceptable, and economically viable. Using satellites to estímate the river discharges when spraying is was applied allowed to make considerable progress in the use of insecticides, thus avoiding both the fatal overdoses on the non target fauna and under-dosages which would be ineffective against the blackfly populations. In the field of epidemiology, the development of DNA probes also provided a better knowledge of human and animal parasites in the infective females of the vector and accordingly, an adjustment of the control strategy.

9 The Onchocerciasis Control Programme in West Africa is indisputably a success as regards the control of the disease and the preservation of the environment. It is a quite unique example of long-term public health programme which has made every effort possible to preserve the aquatic environment since its inception in 1974. It has been a precursor of sustainable development long before the term existed. It ended with the satisfaction of bequeathing to the coming generations a non degraded environment and valleys free from onchocerciasis which would increase the agricultural productivity of the countries, together with novel knowledge on African rivers and means of combating parasitic endemic diseases.

10 We are therefore pleased to extend our sincere thanks to the whole scientific community (the Expert Advisory Committee and the Ecological Group of the OCR hydrobiologists, entomologists and epidemiologists from the participating countries and from the various research institutes throughout the world which collaborated with the OCP), to the donor community which trusted the OCP during so many years despite the multiple challenges to be met, and to the staff of the Programme for their unvaluable dedication, perseverance, consistency and spirit of total sacrifice.

11 We hope that this quite unique involvement of the international community in a genuine sustainable development of "forgotten areas" becomes widespread for the benefit of the local communities.

AUTHOR

BOAKYE A. BOATIN

Ancien Directeur de l'OCP

Introduction

Qu’est-ce que l’onchocercose ?

Une maladie de santé publique d'importance socio-économique

1 L'onchocercose est une filariose cutanée provoquée par le développement dans le corps humain du ver filarien *Onchocerca volvulus* (DUKE, 1990). Ce parasite est transmis à l'homme par une simulie du genre *Simulium.* En Afrique de l’Ouest, il s’agit de simulies appartenant au complexe *Simulium damnosum* dont les stades larvaires se développent exclusivement dans les eaux courantes des rivières. C'est pourquoi la maladie se concentre dans des foyers situés le long des cours d’eaux, en particulier dans les zones de savane sèche, d’où son nom de « cécité des rivières ».

2 En dehors de l’Afrique tropicale (30 pays affectés), l’onchocercose est présente dans des petits foyers isolés (et transmise par des vecteurs locaux) de six pays d'Amérique centrale et du nord de l’Amérique du Sud, ainsi que du sud-est du Yémen. Il n’en reste pas moins que 99 % des 18 millions d’onchocerquiens estimés dans le monde sont africains.

3 Les principales manifestations cliniques de l’onchocercose se traduisent par des lésions cutanées (atrophie ou épaississement de la peau, dépigmentation) associées à d'insupportables démangeaisons. La principale manifestation reste toutefois les lésions oculaires qui vont des lésions réversibles du segment antérieur de l’œil jusqu’aux lésions irréversibles du segment postérieur ou antérieur.

4 L’onchocercose est une maladie cumulative, ce qui signifie que, pour une région bioclimatique donnée, la sévérité des lésions oculaires est liée à l’intensité de l’infection par *O. volvulus* au sein de la communauté humaine concernée, elle-même dépendante de la densité de piqûres infectantes du vecteur L’onchocercose est aussi une maladie invalidante et débilitante qui affecte plus spécifiquement les populations rurales. Dans les vallées de savane ouest-africaine, le taux de cécité onchocerquienne peut atteindre 10 % de la population totale, soit 25 % de la population active.

5 L'onchocercose, et par-dessus tout ses effets sur les yeux, mène à la détresse chez les populations affectées, poussant les jeunes à abandonner les terres riveraines les plus fertiles, ce qui conduit à la désintégration des rapports sociaux dans les villages. Bien que

l'onchocercose n'ait pas toujours été l'unique cause du dépeuplement des vallées des rivières en Afrique, elle est néanmoins le principal obstacle à leur développement et à l'installation de communautés dans ces zones (MARCHAL, 1978).

Un ver parasite transmis par un vecteur

O. volvulus chez l'homme et chez le vecteur

6 La femelle adulte d'*O. volvulus* (macrofilaire, environ 50 cm de long), se loge principalement dans les tissus sous-cutanés humains, libre ou le plus souvent enchevêtrée dans des nodules fibreux (SCHULZ-KEY, 1990). Pendant sa durée de vie féconde, elle produit des millions de très petits embryons (250-330 microns) appelés microfilaires, qui peuvent survivre plus de deux ans dans la peau humaine. Ces microfilaires sont les stades pathogènes du parasite qui envahissent les tissus dermiques, en particulier au niveau des tissus oculaires, pouvant causer des lésions sévères. Les microfilaires sont transmises d'une personne à une autre par une simulie femelle qui appartient en Afrique de l'Ouest au complexe S. *damnosum.* Seules les femelles piquent l'homme, et parfois les animaux, parce qu'elles ont besoin de sang pour la maturation de leurs œufs. Il n'y a pas à ce jour de réservoir animal du parasite connu autre que l'homme.

7 Une fois le sang dans l'estomac de la simulie, la plupart des microfilaires sont digérées, mais certaines d'entre elles traversent la paroi intestinale et atteignent la cavité abdominale et les muscles thoraciques où elles subissent une transformation (BAIN, 1971). Les microfilaires se transforment en larves infectantes d'environ 650 microns qui migrent vers la région buccale et peuvent ainsi être transmises à l'homme lors du repas de sang suivant. Le cycle de maturation des larves chez la simulie dure environ sept jours à 27-30 °C. Le nombre de larves infectantes présentes dans une simulie est généralement inférieur à 10 et, dans la plupart des cas, de une à trois, alors qu'une seule femelle de S. *damnosum* peut ingérer des centaines de microfilaires au cours d'un repas de sang. Lorsque des simulies infectées piquent un homme, elles déposent les larves infectantes sur la peau de celui-ci. Les larves pénètrent les couches superficielles de la peau, muent, s'accouplent et des nodules apparaissent sept à douze mois plus tard, parfois jusqu'à trois ans.

Le vecteur : *S. damnosum*

8 Les simulies adultes mâles se nourrissent uniquement de jus de plantes et ne jouent aucun rôle direct dans la transmission de l'onchocercose. Les femelles sont hématophages et peuvent absorber 1 mg de sang à chaque repas. Des études effectuées sous l'égide de l'OCP ont montré qu'il n'y pas de préférence particulière pour les gîtes de repos, les simulies adultes peuplant toute la forêt galerie, d'où les difficultés de traitements efficaces (BELLEC et HEBRARD, 1980). Les femelles adultes de *S. damnosum* ont une durée de vie pouvant aller jusqu'à quatre semaines. Elles vont à la recherche d'un repas sucré (jus des plantes, nectar) puis d'un repas de sang, ce qui peut conduire à l'ingestion de microfilaires si le repas de sang est prélevé chez une personne infectée par *O. volvulus.* Les femelles de *S. damnosum* pondent leurs œufs dans les parties rapides des cours d'eau où les larves éclosent et se développent jusqu'au stade adulte en l'espace de huit à douze jours. Les formes pré-imaglnales (œufs, larves et nymphes) sont toutes aquatiques et fortement réophiles. Après leur éclosion, les jeunes larves restent attachées aux substrats

présents dans l'eau mais elles peuvent également dériver avec le courant. Elles s'alimentent en se servant de leurs soles mandibulaires rigides pour attraper de façon aléatoire les particules suspendues dans l'eau courante, y compris les nutriments dont elles ont besoin. Si un insecticide est adsorbé sur les particules en suspension dans l'eau, ou est lui-même présenté sous forme de particule, il est ingéré par les larves comme nourriture.

Des manifestations cliniques graves

9 Les manifestations cliniques de l'onchocercose sont principalement dues aux microfilaires qui sont les stades pathogènes du parasite. Les microfilaires provoquent des démangeaisons et, en cas d'infection, une onchodermatite (MURDOCH *et al.,* 1993). Elles peuvent envahir l'œil, causant des troubles graves de la vision qui mènent à la cécité. Dans le cas d'une invasion massive et prolongée de l'œil par des microfilaires, des lésions permanentes apparaissent : la kératite qui opacifie la cornée de l'œil, l'iridocyclite qui cause le glaucome, et l'inflammation de la rétine et du nerf optique. Étant donné que l'onchocercose est une maladie cumulative, les complications oculaires apparaissent après une accumulation des infections pendant plusieurs années. Dans certaines régions hyper-endémiques, la cécité survient entre 30 et 40 ans mais peut se produire plus tôt chez quelques sujets (REMME *et al.,* 1989). Les nodules onchocerquiens sont une réaction d'enkystement du parasite *O. volvulus* par l'hôte humain. Ils sont localisés principalement là où les os sont superficiels, près des hanches, au niveau de la cage thoracique et très souvent sur la tête et les jambes. Ils sont généralement petits, 1-2 cm, mais peuvent parfois dépasser 5 cm. La fréquence et la gravité des symptômes sont souvent en étroite corrélation avec le nombre de microfilaires qui, à son tour, dépend du nombre de filaires adultes présentes chez l'homme. Ce nombre de vers adultes dépend du nombre de larves Infectantes reçues par le sujet, et donc du nombre de piqûres de simulies Infectées par rapport à la durée de temps passé dans une zone endémique (fig. 1).

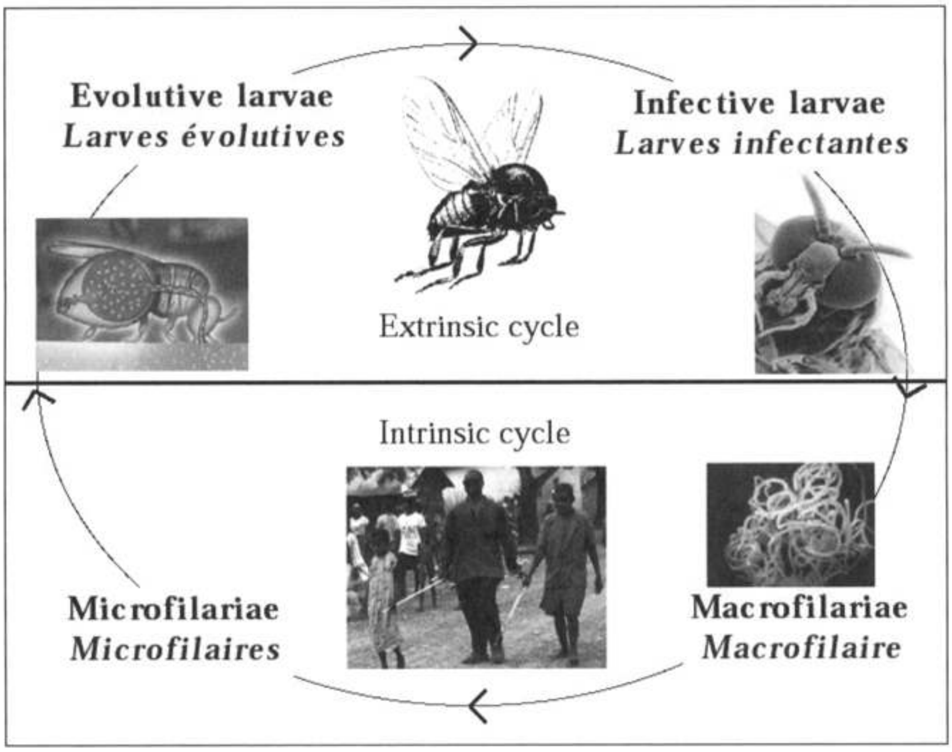


Fig. 1
Le cycle de vie de Onchocerca volvulus

Un contrôle de la maladie par des traitements larvicides et par chimiothérapie

10 Comme la plupart des maladies transmises par un vecteur ; l'onchocercose peut être combattue par des opérations appropriées de lutte antivectorielle. Selon le contexte géographique et la biologie du vecteur ; la stratégie de lutte peut avoir différents objectifs.

11 La stratégie d'élimination définitive du vecteur a été envisagée, particulièrement contre le complexe *S. neavei,* le vecteur est-africain. Dans le cas du complexe *S. damnosum,* en raison des capacités importantes de vol de ces moucherons, les tentatives d'élimination doivent être limitées à des situations exceptionnelles de foyers réduits, au travers d'actions limitées dans le temps et l'espace. Cette stratégie constitue une composante minime du Programme africain de lutte contre l'onchocercose (APOC - DADZIE, 1997). Elle est en cours d'essai dans quatre foyers limités d'Afrique centrale et de l'Est.

12 Une autre stratégie consiste à interrompre la transmission d'*O. volvulus* en éliminant les populations simulidiennes pendant un temps au moins équivalent à la durée de vie du ver adulte, actuellement estimée à quatorze ans. Cette stratégie a été appliquée et son efficacité a été démontrée dans certaines parties de l'aire initiale du Programme de lutte contre l'onchocercose en Afrique de l'Ouest (OCP) où la lutte antivectorielle, seule, a été appliquée pendant plus de quatorze années consécutives et où la maladie n'est plus un problème de santé publique (HOUGARD *et al.,* 2001 - fig. 2). Comme les simulies adultes sont difficiles à atteindre, les opérations de contrôle du vecteur consistent à traiter avec des insecticides appropriés les gîtes de reproduction situés le long des rivières où se

développent les stades larvaires réophiles. Chez *S. damnosum,* le développement du stade aquatique de l'œuf à la chrysalide est d'environ une semaine, d'où l'application hebdomadaire des insecticides.

13 L'onchocercose peut également être contrôlée par la chimiothérapie. Cependant, en dépit des efforts importants de recherche déployés, seul l'ivermectine (Mectizan ®) s'est avéré être efficace, bien toléré et accepté, et sans effet secondaire indésirable. Aujourd'hui, c'est la seule molécule utilisée pour le contrôle de la morbidité onchocerquienne (ABIOSE *et al.,* 2000). Contrairement à la diéthylcarbamazine (DEC), l'ivermectine est un microfilaricide qui est efficace en une seule dose sans causer de réactions secondaires (en particulier la réaction de Mazzotti) ou d'aggravation des troubles oculaires même lorsque la dose et la charge parasitaire sont élevées. Il réduit la charge microfilarienne de 90 % et cette réduction est maintenue pendant au moins six mois. L'objectif de l'utilisation de l'ivermectine à l'APOC et à l'OCP est de contrôler la morbidité onchocerquienne et donc d'empêcher les troubles oculaires et la cécité d'origine onchocerquienne. Pour tirer le meilleur profit du médicament, le traitement doit être régulièrement administré pendant une longue période, dont la durée n'a pas encore été déterminée et qui dépend du niveau d'endémicité de la maladie. Cela est dû à l'effet limité du médicament sur le ver adulte et sur l'Interruption de la transmission (WINNEN *et al.,* 2000). L'ivermectine est maintenant délivré à large échelle dans le cadre des Programmes OCP et APOC grâce à une stratégie institutionnalisée de traitement par l'ivermectine sous directives communautaires (TIDC). Le principe de cette stratégie repose sur la distribution de l'ivermectine par des agents de santé communautaire choisis par la population elle-même et spécialement formés dans les diverses activités liées au traitement (DARRA, 1998).

Qu'y a-t-il de nouveau au sujet du vecteur ouest-africain de l'onchocercose et du parasite ?

Un complexe d'espèces de simulies

14 Les simulies sont de petites mouches hématophages du genre *Simulium.* Il y a plus de 2 000 espèces dans le monde, dont seulement un petit nombre transmet l'onchocercose. *S. damnosum,* se rencontre dans la majeure partie de l'Afrique tropicale, non comme un seul groupe taxonomique homogène mais comme un complexe d'espèces jumelles ayant leurs caractéristiques écologiques propres (LE BERRE, 1966). On rencontre neuf espèces dans l'aire de l'OCP (BOAKYE, 1993). *S. damnosum s.s., S. sirbanum* et *S. dieguerense* se rencontrent en zone de savane jusqu'à la limite nord d'endémicité de l'onchocercose. *S. soubrense, S. sanctipauli, S. konkourense* et *S. leonense* vivent en zone de forêt dense ; elles peuvent aussi se rencontrer en zone de forêt clairsemée et, à certains endroits, atteignent même les zones de savane. La présence de *S. yahense* est limitée à de petits cours d'eau de forêt tandis que *S. squamosum* appartient probablement à plusieurs groupes différents et se rencontre à la fois dans les zones de forêt et de savane. La différentiation des espèces est basée sur l'examen des chromosomes polythènes des glandes salivaires des larves de simulies. Des critères morphologiques et morphométriques qui permettent de séparer la plupart des espèces ou groupes de femelles adultes ont également été identifiés. Depuis 1995, une technique basée sur les séquences de gènes mitochondriaux a été mise au point et permet d'identifier la plupart des espèces du complexe (TANG *et al,* 1995).

Plusieurs souches de parasites

15 Pendant de nombreuses années, l'OCP a été confronté au problème de l'identification des parasites parce que l'outil taxonomique disponible ne permettait pas une identification fiable des parasites trouvés dans les simulies femelles pendant les dissections. Au stade infectant, *O. ochengi,* la principale filaire du bétail qui peut être transmise par *S. damnosum s.l.,* ne peut être différenciée morphologiquement de *O. volvulus* qui est responsable de l'onchocercose humaine. En conséquence, la mesure de la transmission pouvait être surestimée puisqu'elle prenait en compte toutes les larves isolées lors des dissections (PHILIPPON, 1977). C'est pourquoi une méthode permettant de différencier d'une part, *O. volvulus* des parasites d'origine animale et, d'autre part, les souches de *O. volvulus* les plus pathogènes, fut mise au point. La découverte d'une famille de séquences répétitives composées de 150 paires de bases dans le génotype d'*Onchocerca* sp. a permis la mise au point de sondes d'ADN pour l'identification des parasites (ZIMMERMAN *et al,* 1993). Plusieurs sondes ont été isolées. La sonde OCH a permis de différencier *O. volvulus* de *O. ochengi.* Les sondes PFS-I et pSS-IBT ont permis de différencier les souches de savane et de forêt de *O. volvulus.* Elles ont été couramment utilisées par l'OCP à partir de 1992.

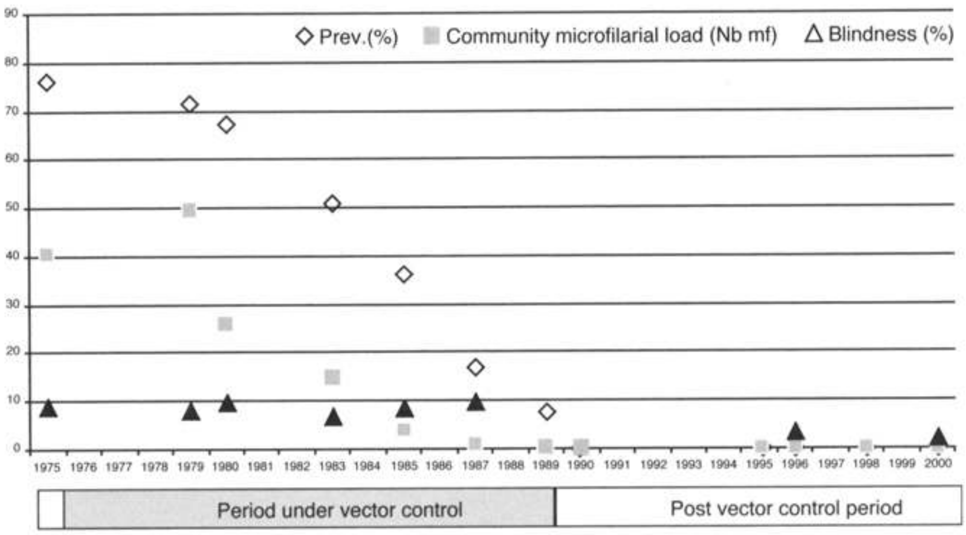


Fig. 2
*Tendances épidémiologiques lapèvalence de microfilaire, de la charge microfilarienne des communautés et de la cécité de 1975 à 2000 dans un village de Taire initiale de l'OCP (*HOUGARD et al, *2001)*

Comment le Programme de lutte contre l'onchocercose a-t-il été conçu ?

Une stratégie globale et intégrée

16 L'OCP a cessé ses activités le 31 décembre 2002, après 29 ans d'existence. Aucun autre programme de santé publique n'a jamais bénéficié pendant aussi longtemps de l'aide financière et logistique de la communauté Internationale. La raison de cet appui est que les résultats obtenus ont toujours convaincu les donateurs de l'efficacité des stratégies de lutte : lutte antivectorielle de 1975 à 1989 et/ou lutte thérapeutique jusqu'en 2002 (

MOLYNEUX, 1995). L'OCP a commencé ses activités précisément en janvier 1974. Son objectif était d'éliminer l'onchocercose en tant que maladie d'importance pour la santé publique et obstacle au développement socio-économique (WHO, 1969). La stratégie de base du Programme a consisté à interrompre la transmission de la souche cécitante de *O. volvulus* en détruisant *S. damnosum s.l* à son stade larvaire par des épandages aériens d'insecticides sélectifs sur les rivières infestées (HOUGARD *et al,* 1993). Les premiers traitements aériens ont commencé fin décembre 1974 dans les zones où l'incidence de la cécité était la plus élevée. Ils ont été plus tard progressivement étendus pour couvrir vers la fin de 1977 une aire de 654 000 km^2 répartie entre sept pays (le Burkina Faso, le sud-est du Mali, le sud-ouest du Niger ; les parties nord de la Côte d'Ivoire, du Bénin, du Ghana et du Togo). On se rendit cependant très vite compte que la frontière de cette aire était infestée par des simulies infectieuses provenant de zones situées en dehors de l'aire du Programme. Afin de protéger de manière permanente les régions objet de cette ré-invasion et de nettoyer également les bassins sources de ré-invasions, les zones hyper-endémiques incriminées furent identifiées et mises sous traitements larvicides. À l'ouest de l'aire initiale du Programme (Extension ouest), il s'agissait des bassins de l'ouest du Mali, du sud-est de la Guinée et du nord de la Sierra Leone. Au sud et à l'est de l'aire initiale du Programme (Extension sud-est), il s'agissait des bassins sud de la Côte d'Ivoire, du Bénin, du Ghana et du Togo (fig. 3).

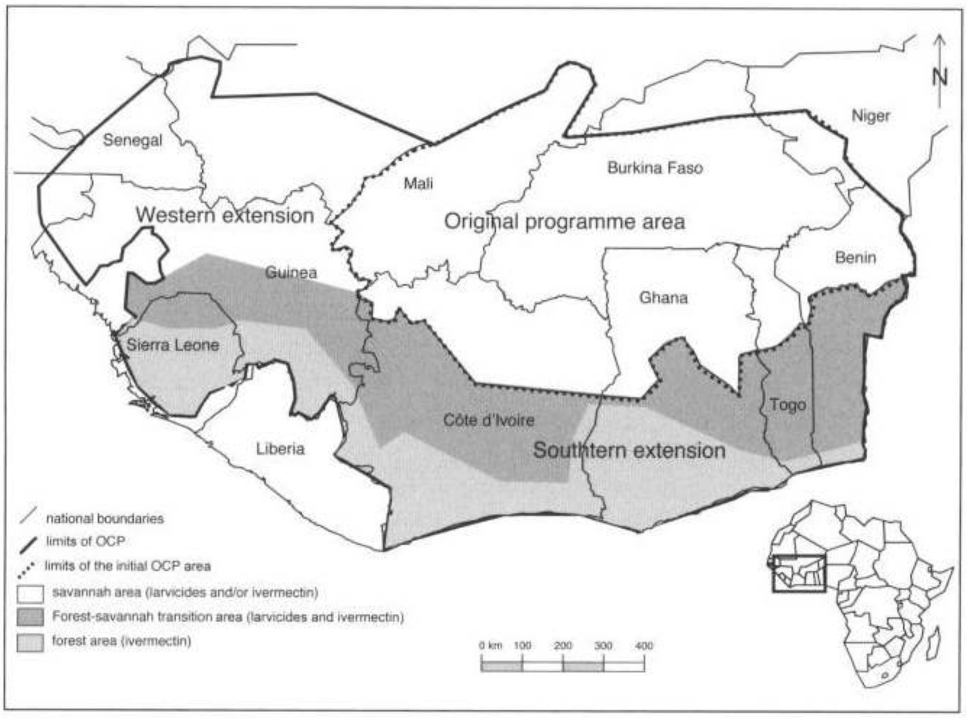


Fig. 3
L'aire du Programme de lutte contre l'onchocercose

17 Le lancement des opérations de lutte antivectorielle dans les zones d'extension s'est achevé vers la fin des années quatre-vingts, alors que tous les bassins de l'aire initiale étaient encore sous traitement. La couverture des traitements larvicides atteignait alors son apogée avec plus de 40 000 kilomètres de rivières traitées, correspondant à un million de kilomètres carrés répartis sur neuf pays du Programme. En raison du succès de cette stratégie, les opérations de lutte antivectorielle furent progressivement arrêtées à partir

de 1989 dans les bassins de l'aire initiale. Dans les zones d'extension, les traitements larvicides se poursuivirent de manière satisfaisante en combinaison avec le traitement à l'ivermectine. Aujourd'hui, l'onchocercose humaine ne constitue plus un problème d'importance pour la santé publique ni un obstacle au développement socio-économique dans toute l'aire traitée. La lutte contre cette filariose n'est cependant pas terminée puisque l'objectif de l'OCP n'a jamais été l'éradication, ni du parasite ni de son vecteur. Depuis 2003, les onze pays participants de l'OCP assument la responsabilité des activités résiduelles de surveillance et de lutte contre cette maladie. Cette tâche est d'une grande importance parce que toute recrudescence de la transmission mènerait à la réapparition des signes cliniques de l'onchocercose, voire de ses manifestations les plus graves.

Une structure opérationnelle prééminente

18 L'OCP a compris jusqu'à 11 pays participants : le Bénin, le Burkina Faso, la Côte d'Ivoire, le Ghana, la Guinée, la Guinée Bissau, le Mali, le Niger le Sénégal, la Sierra Leone et le Togo. Il a été parrainé par le Programme des Nations unies pour le développement (PNUD), l'Organisation des Nations unies pour l'alimentation et l'agriculture (FAO), la Banque mondiale et l'Organisation mondiale de la santé (OMS) et a bénéficié depuis son lancement du soutien financier de 28 donateurs. Un accord opérationnel signé en 1973 entre les pays participants et l'OMS a fixé le champ d'application, les objectifs, les structures de concertation et de gestion de l'OCP et les moyens par lesquels les opérations de lutte et les procédures d'évaluation devaient être effectuées (ANONYME, 1973). Le niveau le plus élevé de la structure actuelle de l'OCP était le Comité conjoint du Programme (CCP) qui était doté des pleins pouvoirs de décision en ce qui concerne la politique globale du Programme, la recherche des stratégies et les questions budgétaires. Ensuite venait le « niveau consultatif » représenté par le Comité consultatif d'experts (CCE). Le troisième niveau était celui de « l'appui et de la collaboration », comprenant le Comité des agences parrainantes, la Banque mondiale pour la mobilisation des fonds, le siège et le bureau régional de l'OMS pour l'Afrique pour l'administration des ressources financières et l'assistance administrative. Ce niveau comprenait également la collaboration avec les pays participants et les donateurs. Enfin, le dernier niveau consistait en la planification, la programmation et la mise en œuvre des opérations de terrain mises au point sur la base des recommandations du CCE approuvées par le CCP.

19 En 2002, l'OCP était composé de deux unités techniques, l'Unité de planification, évaluation et transfert (PET) et l'Unité de lutte antivectorielle (VCU). L'unité PET était chargée de l'évaluation et de la surveillance épidémiologique, de l'analyse bio-statistique et du soutien en matière de système d'information, de la formation et du transfert. L'unité VCU, de loin la plus importante en termes de personnel et de budget, avait quatre principales fonctions : (i) effectuer la surveillance entomologique en vue de guider les opérations aériennes selon la présence ou l'absence de larves de simulies dans les gîtes de reproduction et celle de mouches adultes infectieuses, (ii) effectuer les traitements larvicides aériens dans le but d'interrompre la transmission, (iii) surveiller l'impact des opérations de l'OCP sur l'environnement et, le cas échéant, ajuster les opérations pour éviter tout dommage à la faune non cible, et (iv) effectuer des recherches sur le vecteur (en vue d'accroître l'efficacité des mesures de lutte), et sur les insecticides, leurs formulations et leur stratégie d'utilisation (en vue d'améliorer la capacité du Programme

à faire face à un développement éventuel de la résistance aux insecticides et de maximiser l'innocuité et la rentabilité des traitements larvicides).

Un défi permanent : la recherche de nouveaux larvicides

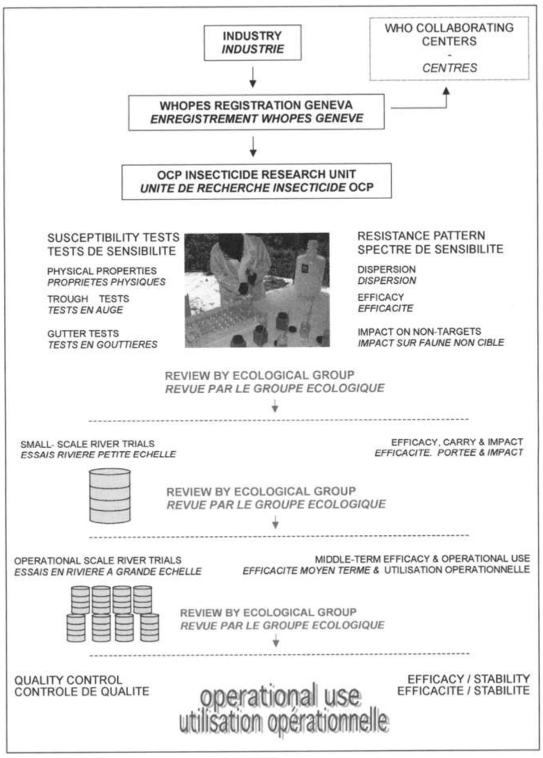


Fig. 4
Des essais en laboratoire à l'utilisation sur le terrain : les différentes étapes du processus de criblage des insecticides à l'OCP

De la nécessité de surmonter la résistance aux organophosphorés

20 La stratégie de lutte anti-larvaire pratiquée par l'OCP a été étroitement liée à l'apparition de la résistance des simulies au téméphos, le seul insecticide utilisé par le Programme de 1974 à 1979. La description en 1980 d'un foyer de résistance au téméphos sur le Bas Bandama en Côte d'Ivoire (GUILLET *et al.*, 1980), puis au chlorphoxime, une année seulement après son utilisation sur le même bassin (KURTAK *et al.*, 1982) conduisit le Programme à intensifier son appui à la recherche, dans un premier temps par l'intermédiaire d'instituts de recherche tels que l'Institut Pierre Richet (IPR) de l'OCCGE à Bouaké en Côte d'Ivoire, puis dans un deuxième temps par le développement de ses propres capacités de recherche, en créant en 1986 une structure de recherche sur les insecticides à Bouaké. Ces recherches avaient pour objectif de sélectionner des composés d'un bon rapport coût-efficacité, non toxiques pour les mammifères et la faune aquatique non-cible et n'offrant pas *a priori* de perspectives de résistance croisée avec le téméphos et le chlorphoxime. Ces recherches avaient également pour but d'optimiser leur utilisation en rivière afin de diminuer les risques d'apparition de résistance, tout en

préservant l'environnement aquatique avec des coûts raisonnables d'application (CALAMARI *et al.,* 1998).

Un criblage intensif de nouveaux larvicides

21 Pour être utilisable par le Programme, un insecticide antisimulidien devait répondre à un certain nombre de critères, dont les plus importants étaient l'efficacité, la sélectivité et l'innocuité (HOUGARD *et al.,* 1993). À des concentrations suffisamment faibles, l'insecticide devait garantir un contrôle total des larves de simulies le plus en aval possible des points d'épandage, de manière à réduire le nombre d'épandages aériens sur un même bief, économisant ainsi les heures de vol. À la concentration létale pour les larves de simulies, l'insecticide devait avoir un impact minimal sur la faune aquatique non-cible (insectes, poissons, mollusques et crustacés aquatiques), à la fois sur le court terme (aucune toxicité aiguë) et sur le long terme (absence de bio-accumulation).

22 Du point de vue de la sélectivité, certains larvicides opérationnels de l'OCP avaient une marge d'innocuité plus faible que d'autres, ce qui limitait leur utilisation à quelques applications par an, et uniquement à des débits élevés, de manière à garantir une précision maximale de concentration. Naturellement, tous les larvicides opérationnels devaient avoir une faible toxicité pour l'homme, afin de réduire au minimum les risques de leur manipulation par les opérateurs. En plus de ces trois critères, un larvicide antisimulidien devait être correctement formulé afin de s'assurer que l'insecticide avait une couverture uniforme des gîtes de reproduction larvaire, et un maximum de portée en aval du point d'épandage. Techniquement, la formulation choisie devait également garantir une fluidité suffisante pour permettre un pompage facile du réservoir de l'aéronef ainsi qu'une application uniforme par les buses de pulvérisation. Un larvicide antisimulidien devait enfin avoir une bonne stabilité dans les conditions habituelles d'entreposage dans un environnement tropical. Les stocks opérationnels d'insecticides devaient en effet être entreposés près des rivières, dans des dépôts à ciel ouvert, pendant des périodes qui pouvaient dépasser un an. Il était donc capital que les larvicides aient une stabilité suffisante pour conserver leur efficacité et leurs propriétés physiques, avant leur application par hélicoptère.

23 Il y a plusieurs étapes à franchir dans le choix d'un nouveau composé. Ce processus nécessite une collaboration étroite avec les fabricants de nouveaux composés et/ou formulations. La première étape du processus a consisté à choisir, parmi les composés commercialisés et approuvés par le Pesticide Evaluation Scheme de l'OMS (WHOPES), les produits qui avaient à la fois un impact significatif sur les larves de simulies et une faible toxicité pour les mammifères. Ces composés devaient appartenir ; si possible, à des familles différentes des insecticides opérationnels, ou du moins, à différents groupes de la même famille, ceci afin d'éviter tout risque de résistance croisée entre les insecticides. Dans la mesure où il est impossible de maintenir durablement les simulies en élevage au laboratoire, l'efficacité des nouveaux composés était évaluée par l'OCP sur le terrain, près des gîtes larvaires. Les insecticides étaient alors soumis à un criblage intensif sur simulies dans un système en circuit fermé, sans risque pour l'environnement (KURTAK *et al.,* 1987). À cette fin, un laboratoire fut installé *in situ* par le Programme dans la partie sud-ouest de la Côte d'Ivoire, près d'une rivière située en dehors de la zone sous traitements larvicides. Parallèlement à ces essais, des essais standardisés de sensibilité étaient effectués avec les matières actives des composés testés afin d'évaluer leur sensibilité de base. L'étape

suivante consistait à réaliser à échelle réduite quelques essais sur la faune non cible. Cette étape nécessitait la collaboration de l'équipe d'hydrobiologie de VCU, responsable de la surveillance de l'environnement aquatique sur toute l'aire du Programme. Des essais en gouttières sur la faune aquatique non-cible (insectes principalement) permirent d'évaluer la marge de sécurité entre la quantité opérationnelle estimée et la dose susceptible de causer un impact indésirable (YAMÉOGO *et al.*, 1991). Si cette marge était acceptable, comparée aux insecticides déjà opérationnels, un rapport technique était alors soumis au Groupe écologique, seule autorité habilitée à autoriser ou non la poursuite de l'évaluation à une plus grande échelle. Si le Groupe écologique donnait son accord, des essais à grande échelle pouvaient être effectués en rivière afin de vérifier l'efficacité du produit et sa portée, et mesurer dans les conditions naturelles son impact sur la faune non-cible. En cas de résultats prometteurs, des traitements à grande échelle étaient alors effectués.

Un choix de sept insecticides opérationnels

24 Plusieurs centaines de composés et/ou de formulations ont été évalués par l'OCP, dans le cadre d'un programme Intensif de criblage. Cette recherche aboutit, en 1997, au choix de sept insecticides opérationnels, dont six produits chimiques et un agent biologique (tabl. 1).

Tableau I. Principales caractéristiques des larvicides antisimulidiens utilisés par l'OCP

Famille		Organophosphorés			Pyréthrinoïdes		Carbamates	Bactéries
nom commun		téméphos	phoxime [1]	pyraclofos	perméthrine	etofenprox	carbosulfan	B.t H-14
formulation		EC [2]	EC	EC	EC	EC	EC	WD [3]
% de substance active		20	50	50	20	30	25	< 2
classe de toxicité [4]		III	II	II	II	III	II	III
toxicité contre la FNC [5]		faible	moyenne	moyenne	élevée	moyenne	élevée	faible
dose [6]		150 [7]	150	120	45	60	120	500
portée moyenne à (km)	10 m³/s	12	3	Non utilisé	Non utilisé	Non utilisé	Non utilisé	1,5
	100 m³/s	16	5	18	7	6	9	5
	300 m³/s	20	Non utilisé	23	8	8	Non utilisé	Non utilisé

[1] Chlorphoxime jusqu'en 1991 ; [2] concentré émulsifiable ; [3] dispersible dans l'eau ; [4] selon la classification de l'OMS des substances actives : II, assez dangereux ; III, légèrement dangereux ; [5] toxicité contre la faune non-cible selon les critères du Groupe écologique ; [6] en ml de formulation par m^3/sec ; [7] 300 ml dans l'eau claire.

25 LE TÉMÉPHOS, introduit dès le début de l'OCP, a été un larvicide exceptionnel pour le Programme. C'est un composé organophosphoré présentant une très faible toxicité pour les vertébrés. Il est doté d'une bonne sélectivité pour les simulies, et a donc un impact très faible sur les invertébrés non cibles. De plus, sa portée peut atteindre 50 kilomètres à des débits élevés. La quantité normalement appliquée est de 300 ml de concentrée émulsifiable (EC 20 %) par mètre cube/seconde de débit. Étant donné son excellente portée et sa plus grande efficacité en eau turbide, la quantité peut descendre à 150 ml par mètre cube/seconde en saison des pluies. Malheureusement, son utilisation a été limitée à partir de 1980 en raison de l'apparition d'une résistance dans les bassins du sud de la Côte d'Ivoire.

26 LE CHLORPHOXIME, le seul insecticide de remplacement disponible en 1980, a été introduit pour remplacer le téméphos dans les zones de résistance. Comme il s'agissait d'un

organophosphoré, une résistance croisée apparut bientôt chez les espèces forestières des bassins sud de l'OCP, mais cette résistance ne s'étendit pas. Le chlorphoxime, utilisé à 120 ml par mètre cube/seconde (EC 20 %), est moins sélectif que le téméphos et a une portée inférieure. Suite à l'abandon de sa production industrielle, il a été remplacé en 1991 par le phoxime, à 150 ml par mètre cube/seconde (EC 50 %). Son utilisation était aussi limitée que celle du chlorphoxime.

27 LE PYRACLOFOS a été introduit en 1990. Sa portée est comparable à celle du téméphos. Il était utilisé à 120 ml par mètre cube/seconde (EC 50 %). Potentiellement plus toxique pour les poissons que le téméphos ou le phoxime, son utilisation était limitée aux débits supérieurs à 15 mètres cubes/seconde. Bien que le pyraclofos n'ait pas de résistance croisée spontanée avec le téméphos ou le phoxime, il a été recommandé de ne pas l'utiliser plus de huit cycles de traitements hebdomadaires consécutifs. Grâce à la stratégie de rotation des insecticides, la sensibilité des larves au pyraclofos est demeurée sans changement dans toute l'aire de l'OCP, malgré son utilisation intensive sur plusieurs bassins.

28 LA PERMÉTHRINE, comme la plupart des pyréthrinoïdes, est un insecticide très efficace à faible dose contre les larves de simulies, (45 ml de CE 20 % par mètre cube/seconde). Sa portée est faible, comparée à celle du téméphos et du pyraclofos (inférieure à 10 km, même à fort débit). Bien que peu toxique pour les vertébrés à sang chaud, cet insecticide est moins sélectif que les organophosphorés pour la faune invertébrée non cible (insectes, mollusques et crustacés) et les poissons. C'est pourquoi, le Groupe écologique a recommandé qu'il ne soit pas utilisé pendant plus de six cycles consécutifs par an sur le même bief et jamais à un débit inférieur à 70 mètres cubes/seconde. Malgré ces contraintes, la perméthrine a joué un rôle significatif dans la stratégie de rotation des insecticides appliquée par l'OCP. Cet insecticide est en effet très efficace contre les simulies résistantes aux organophosphorés. De plus, son faible coût et son faible dosage a permis des traitements en rivière à des débits supérieurs à 500 mètres cubes/seconde. Aucune baisse de sensibilité de *S. damnosum s.l.* à la perméthrine n'a été signalée pendant toute la durée du Programme malgré les nombreux cas de résistance enregistrés çà et là chez certains ravageurs des cultures et autres insectes vecteurs de maladies, tels que les moustiques.

29 L'ETOFENPROX est un « pseudo-pyréthrinoïde » beaucoup moins toxique pour les poissons que la perméthrine. Introduit dans le Programme en 1994, il fut utilisé à 60 ml de CE 30 % par mètre cube/seconde et, pour des raisons de toxicité, à des débits supérieurs à 15 mètres cubes/seconde.

30 LE CARBOSULFAN est un insecticide de la classe des carbamates. Il a été introduit en 1985 afin de servir avec la perméthrine comme insecticides de substitution aux organophosphorés pour le traitement des grandes rivières. Il a été utilisé à la dose de 120 ml par mètre cube/seconde (EC 25 %). Sa sélectivité relativement faible et son risque d'impact sur les poissons ont restreint les limites de son utilisation, comme pour la perméthrine, à des débits supérieurs à 70 mètres cubes/seconde avec un maximum de six cycles consécutifs de traitements hebdomadaires par an et par bief. Sa faible portée et son coût relativement élevé ont limité son utilisation à une gamme étroite de débits. Aucune résistance de *S. damnosum* à cet insecticide n'a été détectée depuis son introduction jusqu'à la fin du Programme.

31 BACILLUS THURINGIENSIS H-14 (B.t. H-14) est un insecticide d'origine biologique. Découverte en 1977, cette bactérie produit des cristaux protéiques toxiques pour les larves de

simulies et plusieurs autres diptères. Des formulations commerciales ont été utilisées par l'OCP à grande échelle depuis 1982 (GUILLET *et al.,* 1982). La toxine du B.t. H-14 est en effet extrêmement sélective pour les larves de simulies, et il n'a donc pratiquement aucun effet sur la faune non cible. Son mode d'action est tout à fait unique et aucune résistance croisée avec les insecticides chimiques n'a jamais été signalée depuis son introduction. En effet, plusieurs rivières de l'aire de l'OCP ont été traitées avec le B.t. H-14 pendant plus de vingt ans sans aucune diminution de la sensibilité des simulies. Ces qualités ont fait du B.t. H-14 l'insecticide de choix pour parer à la résistance aux organophosphorés. Cependant, sa dose opérationnelle relativement élevée a limité son utilisation à des débits relativement faibles. À partir de 1985, l'amélioration des formulations commerciales a toutefois rendu possible le traitement des rivières à débit compris entre 75 et 100 mètres cubes/seconde.

Une stratégie de lutte adaptée

Gérer la résistance du vecteur aux insecticides

32 Parallèlement au programme de développement des larvicides antisimulidiens, l'OCP a mis au point une stratégie d'utilisation de ces composés qui a permis, d'une part, de contenir la résistance du vecteur aux organophosphorés (téméphos, chlorphoxime, phoxime, pyraclofos) et, d'autre part, d'éviter le développement de la résistance aux autres familles d'insecticides. Parmi les stratégies possibles de gestion de la résistance, l'une d'entre elles consiste à alterner dans le temps, des insecticides appartenant à différentes familles. Cette stratégie de rotation, retenue par OCP, a permis de réduire la pression insecticide des différentes familles insecticides et, ainsi, de diminuer les chances de développement de gènes de résistance au sein de cette population. Cette stratégie aurait été relativement simple à mettre en œuvre si tous les produits de remplacement avaient eu les mêmes caractéristiques que le téméphos. Le choix des insecticides aurait été alors seulement dicté par la gestion de la résistance, sans tenir compte des autres facteurs, tels que l'efficacité, le coût, les propriétés physiques et la toxicité. Ce ne fut malheureusement pas le cas, ce qui rendit la mise en œuvre de cette stratégie encore plus délicate (GUILLET *et al.,* 1991).

33 Afin de surveiller la sensibilité des simulies aux insecticides, le Programme a mis rapidement au point des méthodes simples et fiables d'évaluation de la sensibilité des larves de *S. damnosum* aux insecticides. Ces tests, dont les principes de base ont été décrits par MOUCHET *et al.* (1977) pour les insecticides chimiques et GUILLET *et al.* (1985) pour le B.t. H-14, sont facilement réalisables sur le terrain. Ils ont aidé à déterminer les doses diagnostiques de chaque insecticide, afin de détecter rapidement la moindre baisse de sensibilité des simulies à un insecticide donné. Après plusieurs années de mise en pratique de cette stratégie, une amélioration considérable de la situation de la résistance au téméphos a été notée, puisque la résistance a persisté seulement sur le Bas-Bandama et la Basse-Comoé en Côte d'Ivoire, à un niveau relativement faible. Le téméphos a donc pu être à nouveau utilisé sur 90 % de l'aire du Programme, en rotation avec d'autres insecticides opérationnels. Il en a été de même pour le phoxime, qui avait remplacé le chlorphoxime. En ce qui concerne le pyraclofos, seulement un cas de résistance a été signalé sur le Marahoué, après une succession de 16 cycles hebdomadaires consécutifs, effectués à titre expérimental. Cette résistance, heureusement, s'est rapidement révélée

réversible en l'absence d'une pression de sélection au pyraclofos. Aucune résistance n'a jamais été détectée avec les autres familles d'insecticides (fig. 5).

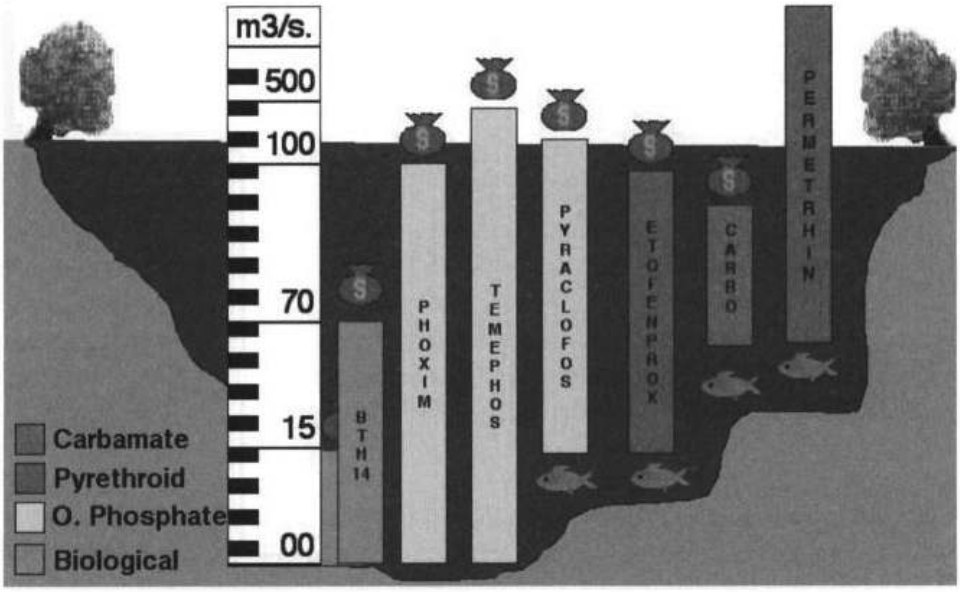


Fig 5
Fourchettes opérationnelles d'utilisation des larvicides utilisés par l'OCP

Exercer le moins de pression possible sur l'environnement aquatique

34 La périodicité hebdomadaire des traitements, et l'importance du réseau hydrologique à traiter ; ont constitué le premier facteur de complexité dans le processus de traitement. Une décision de traitement devait en effet être prise chaque semaine pour plusieurs milliers de kilomètres de rivières, c'est-à-dire, plusieurs centaines de points d'épandages (fig. 6). La première étape du processus de prise de décision était de déterminer pour un cours d'eau ou une partie d'un cours d'eau, si les traitements larvicides devaient avoir lieu ou pas. Cette décision dépendait d'un certain nombre de facteurs parmi lesquels les résultats de l'évaluation entomologique hebdomadaire ou le niveau d'endémicité dans la zone concernée. Une fois prise la décision de traitement, le choix de l'insecticide dépendait, non seulement de la dynamique de la résistance au niveau local, mais également des caractéristiques de chaque insecticide comme décrit plus haut.

35 En dessous de 1 mètre cube/seconde, seul le B.t. H-14 pouvait être utilisé. L'utilisation du téméphos n'était pas exclue, mais son utilisation en eau peu agitée augmentait le temps de contact avec l'insecticide, et entraînait des sous-dosages favorables au développement d'individus résistants. Entre 1 et 15 mètres cubes/seconde, trois des sept insecticides disponibles pouvaient être utilisés : le téméphos, le phoxime et le B.t. H-14. De 15 à 70 mètres cubes/seconde, on pouvait utiliser ; en plus des insecticides précédents, le pyraclofos et l'étofenprox, ce qui permettait de disposer de cinq insecticides. Entre 70 et 150 mètres cubes/seconde, l'utilisation du B.t. H-14 devenait onéreuse, mais cette fourchette de débit offrait cependant le plus grand choix et la plus grande diversité de composés avec trois organophosphorés (le téméphos, le phoxime et le pyraclofos), un pyréthrinoïde (la perméthrine), un pseudo-pyréthrinoïde (l'étofenprox) et un carbamate

(le carbosulfan). Cette diversité se réduisait entre 150 et 300 mètres cubes/seconde parce que le phoxime et le carbosulfan devenaient trop chers pour être utilisés. Entre 300 et 450 mètres cubes/seconde, seuls le téméphos et la perméthrine étaient rentables. Au-dessus de 450 mètres cubes/seconde, seule la perméthrine était utilisée opérationnellement. De 1999 à 2002, la réduction progressive de la couverture des traitements larvicides a permis de diminuer le nombre d'insecticides, en se limitant aux cinq les plus efficaces : le téméphos, le pyraclofos, la perméthrine, l'étofenprox et le B.t. H-14.

36 L'épandage d'un insecticide nécessite la connaissance précise en temps réel du débit des rivières à traiter Une mauvaise appréciation des quantités d'insecticides à pulvériser peut en effet avoir des conséquences sur le succès du traitement. Un surdosage peut avoir des conséquences financières et écologiques importantes, si le produit utilisé est cher ou relativement toxique. C'est pourquoi, un réseau hydrologique de surveillance a été mis en place dès le début du Programme sur toutes les rivières traitées. Il comprenait, à l'apogée des traitements larvicides, jusqu'à 185 échelles de crue, dont 103 équipées de balises hydrologiques, permettant une transmission par satellite des niveaux d'eau enregistrés (SERVAT et LAPETITE, 1990). Dans le même temps, la compagnie aérienne responsable des traitements, avec l'OCP, mit au point un dispositif de traitement permettant un dosage précis de l'insecticide (HOUGARD *et al.*, 1996).

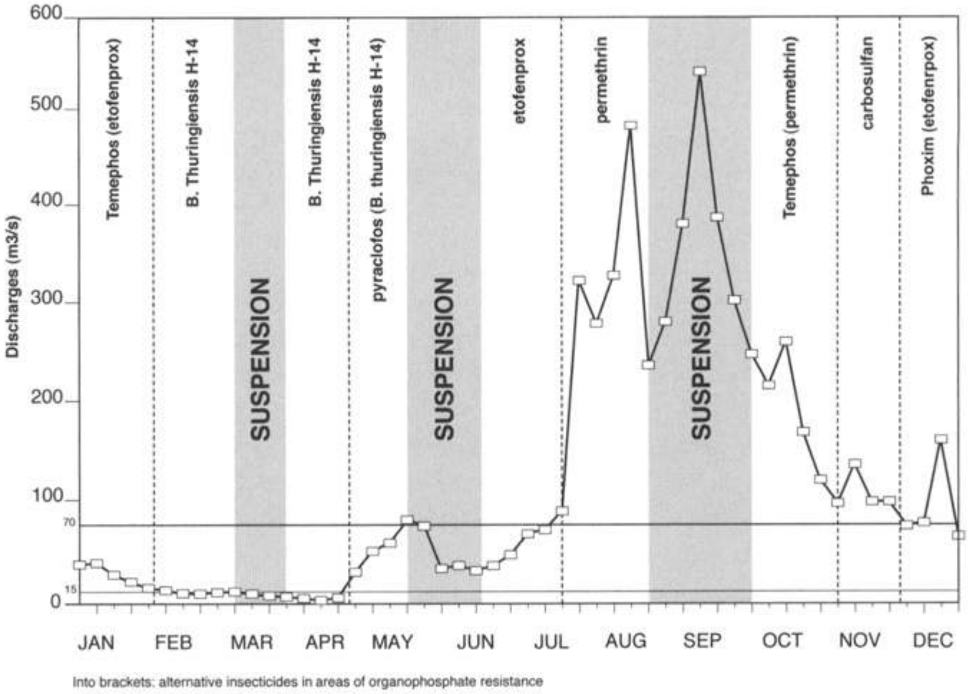


Fig. 6

UN EXEMPLE D'UTILISATION DES INSECTICIDES EN ROTATION DANS UNE RIVIÈRE STANDARD À PROXIMITÉ DE L'AIRE DE L'OCP

Un impératif : la protection de l'environnement

Un souci constant de surveillance de l'environnement

37 Le seul fait d'utiliser régulièrement des insecticides pendant de nombreuses années a soulevé des inquiétudes au sujet des conséquences potentielles que de telles opérations pouvaient avoir pour l'environnement aquatique. En effet, lorsque l'OCP a été lancé, plusieurs études avaient mis en évidence les conséquences néfastes du DDT sur les plans biologique et écologique. Échaudé par le « syndrome du DDT », la communauté internationale, les pays participants, ainsi que les donateurs qui soutenaient le Programme (28 pays et fondations), avaient des raisons de craindre que vingt ans d'applications répétées des insecticides sur les cours d'eau ne causent de graves perturbations des écosystèmes aquatiques.

38 En 1974, juste avant le début des activités sur le terrain, l'OCP mit en place un programme de surveillance aquatique des rivières qui devaient être régulièrement traitées avec des insecticides (LÉVÊQUE *et al.*, 1979). Ce programme fut mis en œuvre pour répondre à trois préoccupations principales :

- fournir sur le court terme un signal d'alarme précoce à la disposition de ceux qui effectuaient les traitements, au cas où des effets toxiques seraient notés, et s'assurer sur le long terme (c'est-à-dire pendant toute la durée de l'OCP) que les épandages d'insecticides ne perturbaient pas d'une manière excessive le bon fonctionnement des écosystèmes des rivières traitées ;
- éviter l'utilisation courante de produits chimiques qui pourraient avoir des effets nocifs sur les populations humaines vivant près des bassins hydrographiques et/ou qui pourraient s'accumuler dans la chaîne alimentaire comme ce fut le cas avec le DDT ;
- empêcher la perte irréversible de la biodiversité aquatique en Afrique de l'Ouest à la fois parce que les poissons d'eau douce sont une source importante de nourriture ainsi qu'une activité économique pour les populations ouest-africaines, et pour répondre à l'objectif de la convention sur la Biodiversité qui stipule que les pays sont responsables de la conservation de leur biodiversité.

39 Au début de l'OCP, la connaissance des systèmes écologiques des rivières et de la flore et faune associées était encore très faible. Dans une telle situation, les activités de surveillance aquatiques étaient conçues à la fois pour collecter les informations de base sur la structure et le fonctionnement des rivières, et pour étudier l'impact potentiel des larvicides sur la faune aquatique. En particulier il était nécessaire d'identifier (et parfois de décrire) les différentes espèces collectées, d'étudier la biologie et la dynamique saisonnière de la faune non cible, pour comprendre, sur le long terme, les tendances des débits des rivières et leur relation avec la dynamique des communautés aquatiques. Cette recherche fondamentale, essentielle pour l'interprétation des données de surveillance, fut progressivement réalisée par plusieurs équipes, en particulier l'équipe hydrobiologique de l'Orstom basée d'abord à Bouaké, puis à Bamako.

La mise en œuvre d'une organisation efficace

40 La surveillance de l'environnement aquatique a été possible grâce à la mise en œuvre d'une organisation spécifique reposant à la fois sur des études de laboratoire et de

terrain, ainsi qu'à l'analyse et l'interprétation périodiques des données collectées. Avant même la fin du programme, toute l'activité de surveillance était entièrement exécutée par les pays participants, grâce à un protocole standard, tout en recevant un soutien financier et technique du Programme. Des études spéciales et l'analyse indépendante des données étaient effectuées périodiquement en collaboration avec des consultants ou des institutions spécialisées afin de compléter ou de confirmer les études entreprises par le Programme et les équipes nationales d'hydrobiologie. Le Groupe écologique évaluait les différents résultats, ainsi que le niveau de toxicité des nouveaux produits chimiques proposés, et approuvait ou rejetait leur utilisation opérationnelle par le Programme (fig. 6).

Le Groupe écologique

41 Avant le lancement du Programme, les Agences parrainantes mirent en place un organe consultatif indépendant, le Panel écologique, qui plus tard devint le Groupe écologique (GE). Le Groupe a toujours été composé au maximum de cinq scientifiques indépendants qui rendaient compte au Comité consultatif d'experts de l'OCP. Son rôle était de s'assurer que la lutte antivectorielle menée par l'OCP ne mettait pas en danger l'environnement et de faire des recommandations au Programme pour la protection effective de l'environnement. Plus spécifiquement, les objectifs et le mandat du GE étaient :

- concevoir et évaluer un programme de surveillance à long terme de la faune aquatique ;
- évaluer le niveau de toxicité des nouveaux produits ou formulations et approuver ou rejeter leur utilisation opérationnelle par le Programme ;
- passer en revue la nature et l'importance des problèmes écologiques liés au Programme et aux projets associés de développement économique proposés dans les zones libérées de l'onchocercose, afin d'identifier les implications écologiques environnementales et humaines de tels développements.

Les équipes nationales de surveillance

42 Les activités de surveillance ont été menées par des équipes nationales d'hydrobiologie qui ont été créées et établies dans la plupart des pays participants où il y avait des traitements larvicides (Burkina Faso, Côte d'Ivoire, Ghana, Togo, Bénin, Mali, Guinée, Sierra Leone). La majorité des scientifiques nationaux de ces équipes ont bénéficié de bourses de l'OCP pour se former aux méthodologies utilisées par le Programme. Certains d'entre eux ont passé des thèses de doctorat. Ils ont également reçu l'appui de l'OCP pour mener leurs activités de surveillance et de recherche, et ils se réunissaient une fois par an pour discuter de leurs résultats avec le Groupe écologique. Des échanges entre équipes de surveillance ont été favorisés par le Programme.

L'unité de lutte antivectorielle

43 L'Unité de lutte antivectorielle (VCU) de l'OCP a été, au travers de plusieurs de ses activités, parmi les principaux contributeurs à la protection de l'environnement. À titre d'exemple : (i) elle a été responsable de l'utilisation judicieuse des insecticides dans l'aire de l'OCP ; (ii) elle a été responsable du criblage des nouveaux insecticides ; (iii) elle a rendu compte de ses activités lors des réunions du Groupe écologique.

La section hydrobiologie

44 La section hydrobiologie de l'OCP a été créée en 1981 au siège de l'OCP à Ouagadougou comme composante de VCU, et placée sous la responsabilité d'un scientifique confirmé. Ses principales fonctions étaient (i) d'effectuer les recherches éco-toxicologiques ; (ii) de coordonner les activités de surveillance et d'aider les équipes nationales dans leurs travaux sur le terrain dans l'aire de l'OCP ; (iii) de gérer les données de surveillance fournies par les équipes nationales (fig. 7).

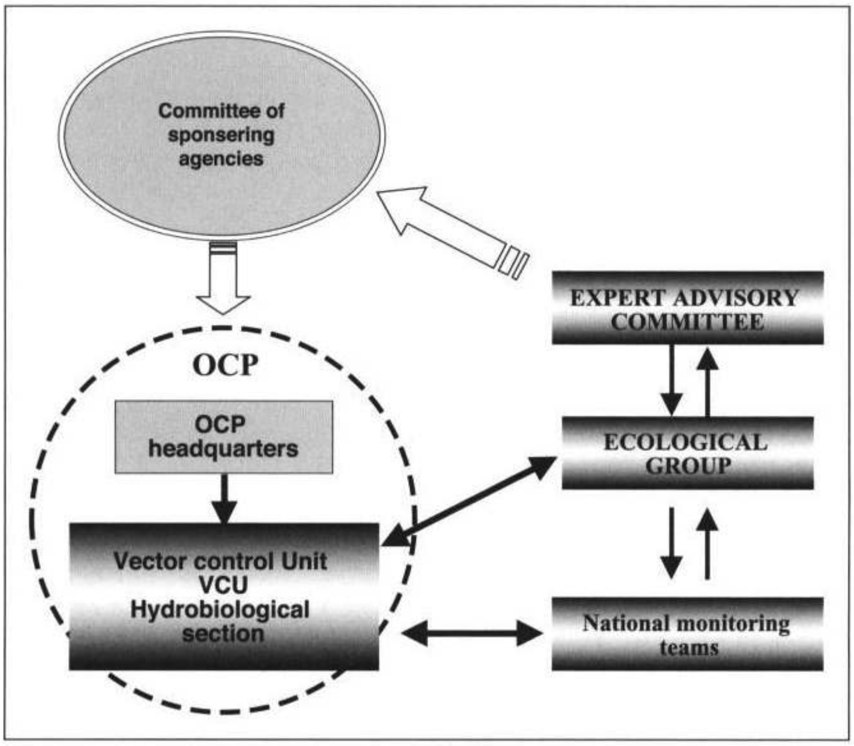


Fig. 7
Structure of OCP and position of the environmental monitoring activities

L'évaluation des risques à court terme des nouveaux larvicides

45 Une évaluation des risques était effectuée pour chaque nouveau larvicide susceptible d'être utilisé par l'OCP. Après les premiers tests destinés à évaluer l'efficacité du produit sur simulies, une revue de la littérature était réalisée ainsi que des essais en laboratoire sur les poissons et des essais en gouttière sur les invertébrés (CALAMARI *et al.*, 1998). Des tests de toxicologie aiguë ont été réalisés sur les différentes espèces africaines de poissons selon des protocoles standards (YAMÉOGO *et al.*, 1991) afin d'obtenir des données de base sur la toxicologie des poissons. L'impact à court terme sur les invertébrés non cibles a également été étudié en utilisant un système de gouttières artificielles et différentes concentrations d'insecticides (YAMÉOGO *et al.*, 1993). À partir de ces tests, les larvicides ont été classifiés selon leur toxicité générale et une typologie de la sensibilité des taxons les plus courants a été établie (YAMÉOGO *et al.*, 1991, 1993).

46 Une grande quantité d'informations a également été collectée à partir des tests insecticides effectués sur le terrain : B.t. H-14 (DEJOUX, 1983 ; DEJOUX *et al*, 1985) ;

deltaméthrine (DEJOUX, 1983), Gh 14 (TROUBAT et LARDEUX, 1982), téméphos (DEJOUX et ELOUARD, 1977 ; ELOUARD et JESTIN, 1982), pyraclofos (YAMÉOGO *et al.*, 1993), perméthrine (YAMÉOGO *et al*, 1993), étofenprox (YAMÉOGO *et al.*, 2001). En comparant les différents larvicides opérationnels, le B. t H-14 s'est avéré le moins nocif pour l'environnement, suivi du téméphos, du chlorphoxime, du pyraclofos, de l'étofenprox, de la perméthrine et du carbosulfan, en ordre croissant de toxicité. Parmi les taxons, les Baetidae (Ephémeroptères) étaient les plus sensibles aux larvicides chimiques tandis que les chironomidae (Diptères) étaient les moins sensibles à la plupart des insecticides.

47 Après avoir passé en revue ces résultats, le Groupe écologique recommandait, en cas de résultats toxicologiques acceptables, des études pilotes à petite échelle sur le terrain. Pour les insecticides les plus toxiques mais potentiellement les plus prometteurs (tels que leur faible coût, leurs grandes possibilités d'application par rapport aux débits des rivières et leur portée sur une plus grande distance), des études à grande échelle en dessous de la dose opérationnelle étaient recommandées. Cela permettait d'obtenir un aperçu complet de l'évaluation des risques avant que l'insecticide ne soit utilisé comme larvicide opérationnel. La perméthrine, par exemple, a été évalué de cette façon (YAMÉOGO *et al*, 1993 ; CALAMARI *et al*, 1998).

La surveillance à long terme de l'environnement aquatique

48 Les critères retenus par le Groupe écologique pour l'évaluation de l'impact à long terme des insecticides sur l'environnement aquatique étaient les suivants (LÉVÊQUE *et al*, 1988) :

- les activités de lutte antivectorielle ne devaient pas réduire le nombre des espèces d'invertébrés, ou causer un changement marqué de l'abondance relative des espèces ;
- les pesticides appliqués ne devaient avoir un impact direct ni sur les poissons, ni sur le cycle de vie des différentes espèces de poissons ;
- la bioaccumulation et la bioamplification à travers les réseaux alimentaires devaient être évitées ;
- Les Activités Humaines Dans La Zone De Lutte Ne Devaient Pas être gênées ;
- Les Variations Temporaires et Saisonnières des Populations d'invertébrés non cibles dues aux insecticides devaient être d'un niveau acceptable.

49 Le Programme De Surveillance était principalement concerné par deux grandes catégories d'organismes : (i) les invertébrés benthiques qui Abondent dans les cours d'eau et qui sont directement menacés par l'insecticide de la Même manière que les larves de *Simulium damnosum* ; (ii) les poissons en raison de leur intérêt économique pour les populations vivant le long des rivières, mais également pour des raisons psychologiques consistant à montrer aux villageois occupés par les activités de pêche que toutes les mesures étaient prises pour éviter les risques de pollution.

Méthodes et protocoles

50 Afin d'évaluer l'importance du risque environnemental, les hydrobiologistes ont utilisé des méthodes et des protocoles rigoureux pour surveiller les effets potentiels à long terme de l'utilisation répétée des larvicides sur les populations aquatiques (YAMÉOGO *et al.*,

2001 ; CROSA *et al.*, 1998 ; LÉVÊQUE *et al.*, 1979). en mettant en place le protocole de surveillance, il était nécessaire de garder à l'esprit plusieurs considérations importantes :

- la surveillance devait comporter un échantillonnage régulier et à long terme visant à étudier les effets écologiques des traitements sur toute la durée du programme, combiné à des programmes de recherche de plus courte durée sur certains problèmes spécifiques ;
- la périodicité des échantillonnages, les sites choisis pour la surveillance, et les méthodes de terrain utilisés devaient allier la fiabilité des techniques d'échantillonnage à la fiabilité de l'accès aux sites aussi bien en saison sèche qu'en saison des pluies, sur plusieurs kilomètres de routes ou de pistes non encore bitumées ;
- les techniques de surveillance devaient fonctionner aussi bien dans les rivières peu profondes, au cours lent en saison sèche, que dans les mêmes rivières, profondes et au cours rapide en saison des pluies ;
- afin de garantir une comparabilité raisonnable des résultats, toutes les équipes devaient utiliser les mêmes méthodes.

51 Un réseau de stations d'échantillonnage fut mis en place sur toute l'aire du Programme (fig. 8). Quarante sites d'échantillonnage furent utilisés au début du Programme, mais à mesure que le Programme évoluait ainsi que la stratégie de traitement, ce nombre fut réduit récemment à dix pour les invertébrés et à dix pour les poissons, après qu'une évaluation ait été faite de l'impact des traitements au cours des dix premières années.

52 Pour les invertébrés, trois principales méthodes d'échantillonnage ont été utilisées (YAMÉOGO *et al.*, 2001) :

- Échantillonnage au filet dérivant en utilisant de longs filets de 2 m, avec 20 x 20 cm d'ouverture, et 300 mm de maillage. les techniques de base de l'échantillonnage au filet dérivant utilisées par le programme ont été standardisées.
- Échantillonnage au Surber en utilisant un Surber d'échantillonnage de 15 x 15 cm. Cette méthode simple, qui permet de prélever les substrats rocheux, ne peut pas être utilisée dans des eaux profondes et son utilisation était donc limitée à la période d'étiage.
- Substrats artificiels : des instruments spéciaux ont été conçus et utilisés, depuis les blocs de ciment qu'on laisse immergés au fond de l'eau, jusqu'aux substrats flottants faits de touffes de fibres en plastique.

53 Pour les poissons, le programme de surveillance était principalement concerné (LÉVÊQUE *et al.*, 1988) par :

- l'étude des changements dans les prises (exprimés en poids ou en nombre d'individus) et dans la composition des espèces dans les pêches expérimentales effectuées à intervalles réguliers (habituellement 2 ou 3 mois) avec une gamme standardisée de filets maillants ;
- l'étude des paramètres biologiques, plus particulièrement le coefficient de condition qui est une mesure de la santé des poissons. Des recherches complémentaires étaient également effectuées par l'analyse du contenu de l'estomac de certaines espèces choisies, des périodes de fraie et de fécondité, ainsi que de l'impact des composés organophosphorés sur l'activité de l'acétylcholinestérase du cerveau.

54 Certaines méthodes et protocoles des programmes de surveillance et d'évaluation des risques ont évolué depuis 1974, du fait de la dynamique du Programme face aux situations continuellement changeantes (par exemple, les ré-invasions d'insectes, la résistance aux pesticides, la variabilité de l'hydrologie, etc.). Cependant, les concepts de base et les problèmes sont restés les mêmes.

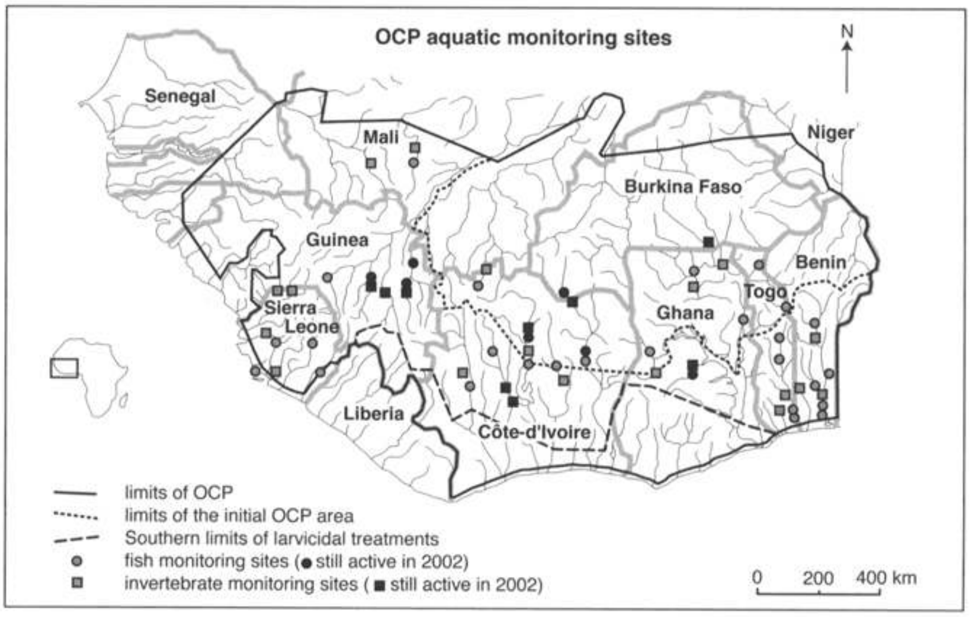


Fig. 8
Emplacement des sites de surveillance dans l'aire de l'OCP

Vingt-cinq ans de surveillance aquatique

55 Le principal souci de l'OCP concernant l'impact des insecticides sur les environnements aquatiques était d'éviter des changements à long terme ou durables de la biodiversité aquatique. Les principaux groupes d'organismes qui ont été surveillés ces dernières vingt années pour des indications de changements anormaux ont été les poissons et les invertébrés non cibles (fig. 9).

Principaux résultats de la surveillance des poissons

56 Les impacts potentiels des traitements larvicides sur les poissons ont été évalués par la mesure des changements dans la richesse spécifique des prises, des prises par unité d'effort (PUE) des pêches et du coefficient de condition des différentes espèces de poissons (LÉVÊQUE *et al.*, 1988 ; PAUGY *et al.*, 1999).

57 Par rapport à la richesse spécifique des prises expérimentales (qui désigne le nombre d'espèces attrapées par un ensemble standard de filets maillants expérimentaux pendant deux nuits de pêche), les tendances à long terme observées dans trois zones principales du Programme (c'est-à-dire les rivières de Côte d'Ivoire, les rivières du bassin de la Volta au Ghana et les rivières du bassin du Niger en Guinée) étaient différentes. Cependant, après une période de baisse, particulièrement dans les rivières de Côte d'Ivoire et du bassin de la Volta, un rétablissement et une amélioration de la richesse spécifique des prises ont été observés dans toutes les rivières depuis respectivement 1994 et 1996. Ainsi, après plusieurs années de traitements larvicides avec plusieurs insecticides, jusqu'à vingt ans dans l'aire initiale du Programme, il n'y a eu aucun signe indiquant une diminution de la diversité des espèces de poissons dans les rivières traitées. Une observation semblable a été faite après les dix premières années de surveillance durant lesquelles seulement trois insecticides avaient été utilisés. La comparaison des changements dans la richesse spécifique avec les tendances hydrologiques suggère que les tendances observées dans la

richesse spécifique pourraient être attribuées à des facteurs climatiques et à la longue période de sécheresse qui est survenue pendant plusieurs années en Afrique de l'Ouest (fig. 10 et 11).

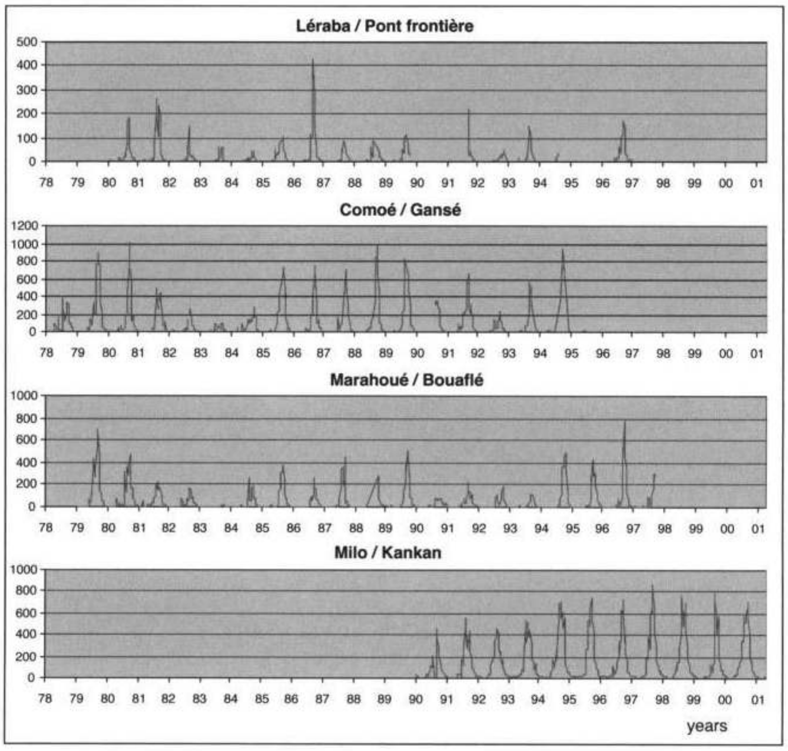


Fig. 9
Changements à long terme des débits annuels pour certaines des rivières surveillées. Les faibles débits sont caractéristiques des années 1980 et début 1990 dans beaucoup de rivières de l'aire de l'OCP

58 Les tendances globales des prises par unité d'effort (PUE) en relation avec les traitements larvicides durant vingt ans indiquent également divers scénarios dans les différents bassins principaux du Programme et parfois entre rivières d'un même bassin, et ce malgré un régime de traitements larvicides généralement similaire dans toute faire du Programme. Par exemple, les prises ont diminué dans les rivières de Côte d'Ivoire jusqu'en 1989 et 1993 selon les rivières tandis que des augmentations ont été observées dans toutes les rivières depuis 1995 alors que les traitements larvicides avaient été arrêtés à des moments différents selon les rivières. Dans le bassin du Niger, on n'a observé aucune diminution des prises depuis le début de la surveillance, alors que depuis 1994 on a observé des augmentations par rapport à la situation initiale dans les trois rivières surveillées du bassin. Dans le bassin de la Volta, on a observé différentes tendances dans les prises au fil des années. Cependant, une tendance saisonnière commune des prises, élevée en période d'étiage (décembre-janvier jusqu'en avril-mai) et faible en période de crue (juillet-août jusqu'à octobre-novembre), a été observée pour tous les bassins et rivières. L'influence des changements hydrologiques sur les prises a été suspectée plus haut.

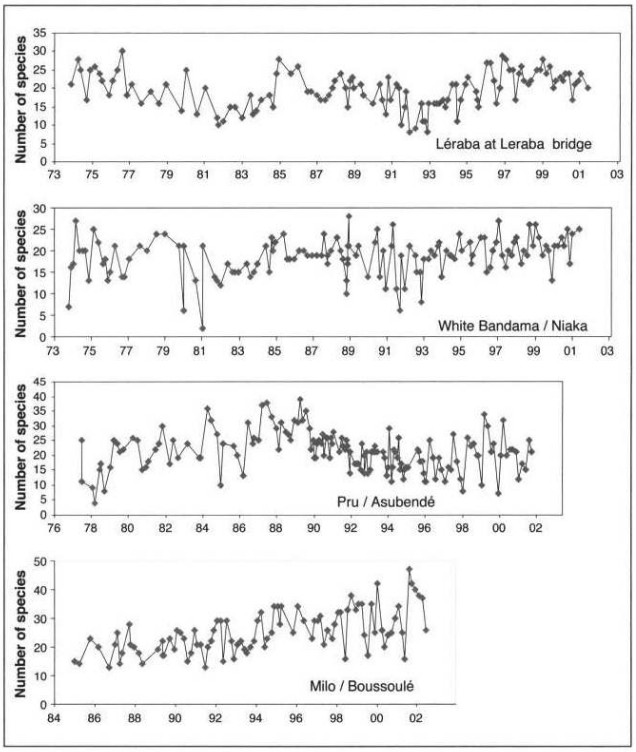


Fig. 10
Changements à long terme de la richesse spécifique des poissons par échantillon dans quelques rivières surveillées

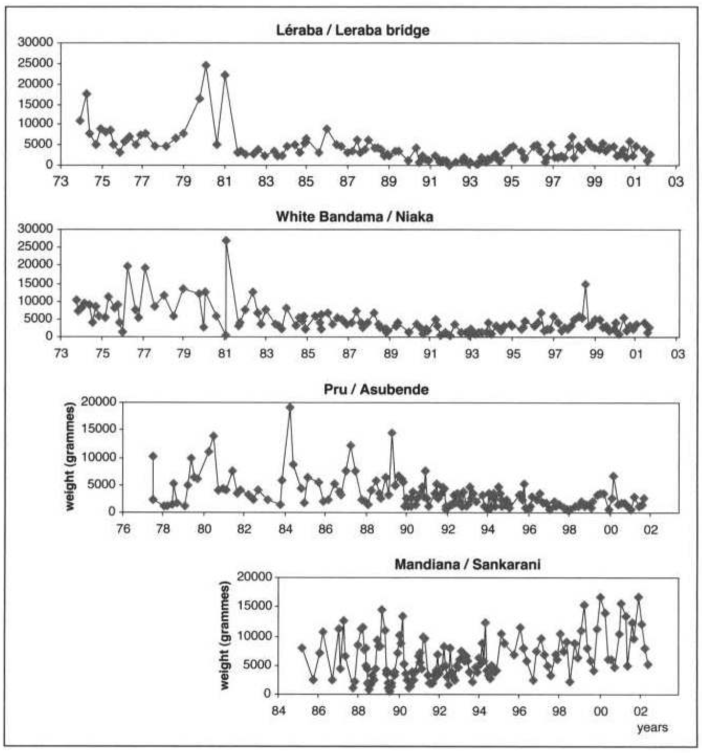


Fig. 11
Changements à long terme des prises par unité d'effort (PUE) et par échantillon en utilisant un ensemble standard de filets maillants expérimentaux dans quelques rivières surveillées

59 Les valeurs du coefficient de condition (le ratio poids - longueur) qui expriment le « bien-être » des poissons ont été également surveillées afin d'évaluer l'effet direct des larvicides sur les poissons (toxicité aiguë) et/ou les effets indirects à travers l'impact des larvicides sur leurs sources de nourriture (qui sont dans beaucoup de cas les invertébrés aquatiques). Au cours des années, diverses évaluations des tendances de la « condition » de plusieurs espèces de poissons dans faire du Programme ont indiqué uniquement des fluctuations autour des moyennes attendues mais pas de changements significatifs des valeurs. Les traitements larvicides n'ont donc pas directement affecté les poissons, ce qui suggère que là où certains éléments de la nourriture des poissons ont été affectés, d'autres éléments ont été trouvés et raisonnablement bien utilisés à leur place.

60 La bioaccumulation des pesticides dans les poissons était un souci majeur avec l'utilisation du DDT En effet, l'étude en laboratoire des effets des organophosphorés a montré que les poissons pouvaient accumuler le téméphos (MATTHIESSEN et JOHNSON, 1978). Mais cette accumulation semble avoir été limitée et n'a pas augmenté jusqu'au point observé avec le DDT Les données de terrain récoltées dans faire de l'OCP ont confirmé que le téméphos ne s'accumulait pas dans les poissons (QUÉLENNEC *et al.*, 1977). Par ailleurs, dans les conditions de terrain, l'activité de l'acétylcholinestérase dans le cerveau des poissons n'a pas semblé être significativement différente dans les rivières traitées avec le téméphos ou celles non traitées (ANTWI, 1985 ; SCHERINGA *et al.*, 1981).

Principaux résultats de la surveillance pour les invertébrés

61 Le souci spécifique de l'OCP par rapport aux invertébrés non cibles en relation avec les traitements larvicides des rivières était d'empêcher la perte de la diversité faunique et de préserver la qualité de la biomasse disponible pour les niveaux supérieurs du réseau alimentaire de l'écosystème aquatique. L'évaluation de l'impact des larvicides a été basée sur deux types de données. Ce sont les données d'échantillons du Surber et les données de la dérive (de jour et de nuit).

62 En analysant les données sur les Invertébrées collectées au moyen de diverses stratégies d'échantillonnage entre 1977 et 1996, YAMÉOGO *et al.*, (2001) ont évalué les changements à long terme des populations d'invertébrés par rapport à leur composition taxonomique ainsi que leurs structures trophiques. Les échantillons de Surber fournissaient une évaluation qualitative et quantitative de la communauté des invertébrés vivant à l'endroit de l'échantillonnage. Ils permettaient un examen clair des changements de la communauté à la fois du point de vue taxonomique net du point de vue structure fonctionnelle. D'une manière générale, les résultats ont indiqué que les différents larvicides avaient différents impacts sur les rivières et montraient un certain nombre d'effets sur les différents groupes d'invertébrés. La plus grande diminution de la diversité et de l'abondance des groupes d'invertébrés a été détectée pendant les traitements au phoxime, à la perméthrine, au carbosulfan et au pyraclofos (YAMÉOGO *et al.*, 1992). Le téméphos et le B.t H-14 se sont avérés être les larvicides les moins agressifs. Les groupes taxonomiques qui montraient les plus grands changements en termes d'abondance relative étaient les Tricorythidae, les Leptoceridae, les Chironomidae, et les Baetidae.

63 Du point de vue trophique, toutes les communautés étaient dominées par les collecteurs rassembleurs et, à un moindre degré, par les collecteurs filtreurs. L'abondance de ces groupes d'aliments a été une preuve directe de la disponibilité des matières organiques particulaires fines qui caractérisent les ressources en nourriture dans les rivières

étudiées. Cette structure dominée a tendu à l'augmentation avec l'application de tous les insecticides à l'exception du B.t H-14 (YAMÉOGO *et al.*, 2001). Dans l'ensemble, les résultats ont suggéré que ni les structures taxonomiques ni les structures trophiques n'étaient considérablement changées dans la gamme des variations biologiques, liées à l'écoulement, qui se produisent normalement dans les rivières étudiées (YAMÉOGO *et al.*, 2001, CROSA *et al.*, 2001) (fig. 12).

64 Cela a permis de conclure que l'effet des insecticides sur la faune aquatique était habituellement faible mais provoquait chez les invertébrés des changements dans la composition des espèces et dans la structure de la communauté. Cependant, ces impacts n'ont pas affecté le fonctionnement général du système aquatique et étaient donc écologiquement acceptables. Afin de s'assurer qu'il n'y avait aucune perte irréversible d'espèce, la question du rétablissement de la faune aquatique a été soulevée par le Groupe écologique. La question fondamentale était celle-ci : la communauté aquatique retourne-t-elle à une structure et à une composition spécifique plus ou moins semblables à celles d'avant les traitements à la fin de la période de traitement ? En effet, les données de terrain fournissaient des indications qu'une recolonisation par les taxons (par exemple, *Neoperla* sp. et *Caridina* sp.) qui avaient été affectés pendant la période de traitement était observée dans la plupart des stations après l'arrêt des traitements larvicides. La capacité de rétablissement de la faune aquatique dans les rivières traitées a donc été démontrée, même si elle s'est faite à un rythme plutôt lent.

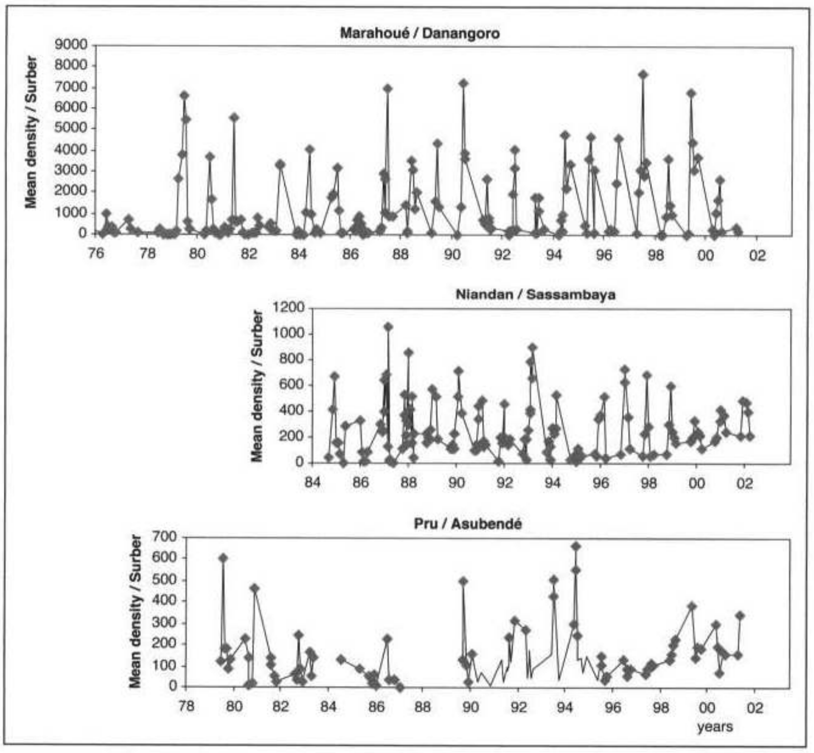


Fig. 12

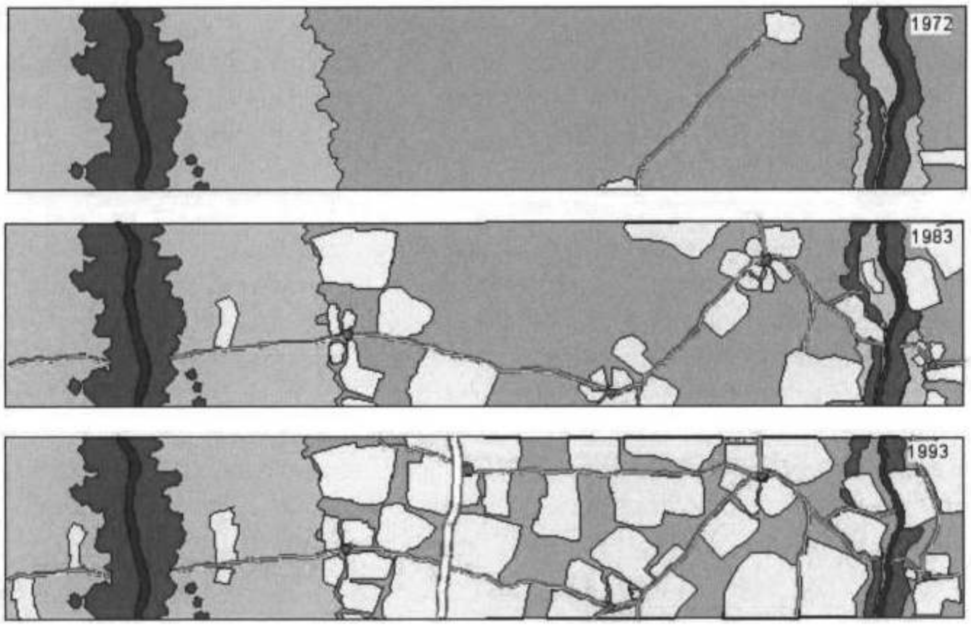
Changements à long terme dans la densité des invertébrés sur les dalles rocheuses, selon les estimations faites à partir des échantillons de Surber.

L’objectif est atteint, mais qu’en est-il de l’avenir ?

65 L'OCP en Afrique de l’Ouest a été clôturé en décembre 2002, après vingt-neuf ans d'activités. Aucun programme de santé publique n’a bénéficié pendant aussi longtemps de l'aide financière de la communauté Internationale. Une des raisons de cet appui est que l'OCP a toujours convaincu les donateurs de l'efficacité des stratégies de lutte utilisées. L'autre raison a été le souci permanent de l'OCP pour la préservation de l'environnement aquatique avec la participation des équipes nationales et de l'expertise internationale. La mise en œuvre à long terme d'un programme de surveillance visant à évaluer les effets potentiels des traitements larvicides, et le criblage à grande échelle des larvicides pour choisir les plus efficaces contre *S. damnosum* tout en étant les moins agressifs pour la faune non cible, sont des traits uniques dans les grands programmes de gestion de la santé. Ces efforts ont eu un coût économique en termes de consommation des insecticides et des stratégies opérationnelles utilisées, mais ils ont permis de préserver la qualité de l'eau utilisée par les populations riveraines ainsi que les ressources en poissons qui constituent une partie importante des produits alimentaires de ces populations.

66 L’objectif a été atteint : l'onchocercose a presque entièrement été éliminée de l’aire de l’OCP en tant que maladie d'importance pour la santé publique et obstacle au développement socio-économique. En éliminant la menace de la cécité, l’OCP a permis le repeuplement de ces vallées de rivières qui avaient été autrefois abandonnées par crainte de la maladie. Pratiquement, aucune nouvelle infection n'a jusqu'ici été détectée dans cette zone et un des résultats les plus significatifs est que 18 millions d'enfants nés dans l’aire de l’OCP ne sont plus infectés par l'onchocercose. Cependant, la lutte contre cette filariose n'est pas terminée. En effet, l'OCP n'a jamais visé à éradiquer ni le parasite ni son vecteur et l’onchocercose existera toujours. La capacité à contrôler le « risque » de l’onchocercose constituera donc un nouveau défi dans l'avenir.

67 Du point de vue environnemental, le succès de l’OCP peut être compromis par une utilisation déraisonnable de terres libérées de l'onchocercose. Par exemple, une étude pilote menée dans la région de la Léraba a montré que 75 % de la savane boisée originelle avait été défrichée pour le développement agricole et l'installation de nouveaux villages (BALDRY *et al.*, 1995). Les forêts riveraines de nombreuses petites rivières ont été détruites et sur certaines des berges, l'érosion du sol continue. Par contre, les forêts et les plaines facilement inondées en bordure des rivières plus grandes n'ont subi aucune perturbation de cette envergure. Il est donc nécessaire de prendre à la fois des mesures et de sensibiliser les populations riveraines sur la nécessité de protéger l'environnement et de bien gérer la biodiversité, parallèlement au développement des activités agricoles. En d'autres termes, il s'agit ici d'appliquer sur le terrain les principes du développement durable tels qu'élaborés à la conférence sur la Planète Terre tenue à Rio en 1992, et à Johannesburg en 2002 (fig. 13).



LE CÔTE BURKINABE DU FLEUVE LERABA
Fig. 13
Changements dans l'utilisation des terres près de la station de surveillance de la Léraba

Nuisance simulidienne

68 Dans certaines zones, l'importante diminution des piqûres de simulies dès le début du Programme a été un grand soulagement pour les populations riveraines dont les conditions de vie et/ou de travail ont ainsi été améliorées. Ce résultat était l'avantage le plus immédiat perçu par les populations sur les activités de l'OCP.

69 Les traitements larvicides ont progressivement cessé depuis 1990 dans les zones où la maladie était sous contrôle et les taux de piqûres de simulies sont parfois remontés à des niveaux très élevés. Bien que les simulies ne transmettaient plus l'onchocercose, leur réapparition a été perçue par les populations, qui associaient ces insectes à la transmission de la maladie, comme un retour de l'onchocercose. D'autre part, les piqûres des simulies constituent également une véritable nuisance qui pourrait gêner le développement socio-économique en cours dans les vallées des rivières (HOUGARD *et al*, 1998).

70 Afin de répondre à la préoccupation des populations, l'OCP a encouragé certaines actions individuelles contre les simulies, grâce à des techniques peu coûteuses de traitement au sol des gîtes larvaires. Le transfert de ces techniques aux villages et aux unités de développement a été réalisé dans certaines zones. Cependant, ces applications de larvicides exécutées par des non-spécialistes pendant une période indéterminée pourraient comporter un risque énorme de pollution environnementale. L'utilisation de deux insecticides présentant de faibles dangers pour l'environnement (le B.t. H-14 et le téméphos) a donc été recommandée par l'OCP, Cependant, depuis la fermeture de l'OCP, il y a un risque que ces communautés utilisent à cet effet les insecticides disponibles localement, en particulier ceux à usage agricole, avec le danger que cela représente en termes de résistance et de pollution de l'environnement.

Accroissement des connaissances et de l'expertise

71 Indépendamment du succès de l'OCP dans la lutte contre la maladie, il y a également plusieurs résultats positifs qui méritent d'être soulignés.

Amélioration de l'expertise nationale dans le domaine de l'écologie riveraine et de sa gestion

72 Une contribution significative de l'OCP est la formation des scientifiques nationaux dans le domaine de la surveillance de l'environnement aquatique et des programmes de recherche associés. Dans le cadre de cette formation, plusieurs scientifiques nationaux ont reçu des bourses pour être formés en Afrique et dans les pays du Nord. Le résultat est une amélioration générale de l'expertise dans le domaine de la biologie aquatique et des sciences environnementales.

Une meilleure connaissance de l'écologie des rivières de l'Afrique de l'Ouest

73 Avant l'OCP, la connaissance de l'écologie des rivières ouest-africaines était très pauvre. La mise en œuvre du programme de surveillance a conduit à :

- Une meilleure connaissance de la faune et de l'écologie des rivières ouest-africaines, en particulier en ce qui concerne les insectes et les poissons (DEJOUX *et al.*, 1981 ; de MÉRONA, 1981 ; ILTIS, 1983 ; ILTIS et LÉVÊQUE, 1982 ; GIBON et STATZNER, 1985 ; LÉVÊQUE *et al.*, 1990, 1992) ;
- Une meilleure connaissance de la dynamique à long terme des populations aquatiques en relation avec les changements et les influences climatiques et humaines ;
- L'acquisition d'une masse d'informations sur la réaction de la faune aquatique ouest-africaine à divers produits chimiques ;

74 Toutes ces connaissances seront utiles pour d'autres activités de développement et pour la conservation de l'environnement ouest-africain.

Constitution d'une base de données de la surveillance aquatique : une ressource exceptionnelle

75 Tous les résultats enregistrés durant la surveillance environnementale du Programme ont été compilés dans une base de données gérée et disponible au siège du Programme. Cette base de données, mise à jour et validée sur l'écologie des rivières ouest-africaines, générée pendant une période de plus de vingt-cinq ans, est un patrimoine unique.

Introduction

What is Onchocerciasis ?

A public health disease of socio-economic importance

1 Onchocerciasis is a dermal filariasis caused by the development in human of the filarial worm *Onchocerca volvulus* (DUKE, 1990).The main clinical manifestations of the disease are skin lesions and visual impairment which may result in blindness. *O. volvulus* is necessarily transmitted to man by the bites of a blackfly vector of the genus *Simulium* in West Africa. This fly breeds exclusively in fast-flowing stretches of rivers. Thus the disease is essentially concentrated in foci alongside watercourses, especially in dry savanna areas, hence its name “river blindness”.

2 Onchocerciasis is prevalent in 30 African tropical countries from Senegal to Ethiopia, between 12-15° North and 12° South.The disease is also prevalent in small isolated foci in six countries of Central and Northern South America, (transmitted by local blackfly vector species) and South-Eastern Yemen. More than 99 % of the estimated 18 million onchocerciasis patients live in Africa.

3 Onchocerciasis main clinical manifestations include skin lesions (skin atrophies or thickenings, depigmentations :“leopard skin, caiman skin...”), which are associated with intolerable itching and scratching ; however the main ones are eye lesions, which may range from mild reversible anterior lesions to severe, irreversible and ultimately blinding anterior or posterior lesions.

4 Onchocerciasis is a cumulative disease : in a given bioclimatic area the severity of eye lesions are related to the mean intensity of infection of the human communities by *O. volvulus,* itself dependent on the density of infective biting vector Onchocerciasis is a disabling and debilitating disease which affect more severely small rural communities. In savannah African valleys, onchocerciasis blinding rates may affect up to 10 % of the total population, and 25 % of the adult active populations.

5 Onchocerciasis, and above all its effects on the eyes, leads to distress in the affected populations, prompting young people to abandon the most fertile riverside lands which lead to the breakdown of the social relationships in the villages. While onchocerciasis has

not always been the unique cause of depopulation of the valleys in Africa, it is none the less the main obstacle to their development and to the settlement of communities in those regions (MARCHAL, 1978).

A vector-borne parasitic disease

O. volvulus in man and vector

6 The adult *O. volvulus* female (macrofilaria, about half a meter long) lives in the human subcutaneous tissues, free or more often tangled in fibrous nodules (SCHULZ-KEY, 1990). It is sexually active for 9 to 14 years. During its fertile life span it produces millions of very small embryos (250-330 microns) called microfilariae which can survive more than two years in the human skin. The microfilariae are the pathogenic stages which invade dermal tissues and cause severe disorder, in ocular tissues inclusively. They have to be carried from one person to the other by a female blackfly belonging to the *Simulium damnosum* complex in West Africa. Only females of S. *damnosum* bite man, and sometimes animals, as they need blood for the maturation of each batch of eggs laid. There is no known animal reservoir.

7 Once the blood arrives in the blackfly stomach most of the microfilariae are digested, but a few of them pass through the intestinal wall and reach the abdominal cavity, and the thoracic muscles where they transform (BAIN, 1971): the microfilariae give rise to infective larvae, measuring 650 micros, which find their way in the mouth parts and may thus be transmitted to man during subsequent blood meal. The maturation cycle of the larvae in the blackfly takes about seven days at 27-30 °C. The number of infective larvae in blackfly is generally less than 10 and in most cases from one to three, while one female S. *damnosum* may ingest hundreds of microfilariae during one blood meal. When infective blackflies bite man they deposit the infective larvae in the skin. The larvae penetrate the skin superficial layers, moult, mate and the nodules appear between seven to twelve months, sometimes up to three years.

The vector : *S. damnosum*

8 As in all the Simuliids, only *S. damnosum* females are hematophagous. Male adult blackflies feed solely on plant juices and play no direct role in onchocerciasis transmission. The females are largely anthropophilic and may absorb I mg of blood at each meal. Studies carried out on behalf of OCP showed that there are no preferred locations for resting sites and that the resting blackflies occupy the entire gallery forest, hence the difficulties for an efficient adulticidal coverage (BELLEC and HEBRARD, 1980). The female *S. damnosum* lives for up to four weeks. This female seeks a sugar meal (plant juices or nectars), then a bloodmeal and may thus ingest microfilariae if the meal is taken from a person infested with *O. volvulus.* S. *damnosum* females lay their eggs in fast flowing parts of rivers from which larvae hatch and develop to pupae then to the adult stage in eight to twelve days. The pre-imaginal forms (eggs, larvae and pupae - ELSEN, 1979) are all aquatic and strongly rheophilic. After hatching, young larvae stay attached to the substrates but may also drift with the current. They feed by using their unfolded mandibular fans indiscriminately to catch particles suspended in the flowing water ; including the nutrients as they pass through the digestive tube. If an insecticide has been adsorbed on

the water suspended particles, or is itself particulate, it is ingested by the larvae in the same way as food.

A serious clinical disease

9 The clinical manifestations of onchocerciasis are primarily due to the microfilariae, which are the pathogenic stages of the parasite. They provoke itching and, in case of infections, onchodermatitis (MURDOCH *et al.,* 1993). The microfilariae may invade the eye, causing severe eye disorders culminating in blindness. In the case of intense and prolonged invasion of the eye by microfilariae, permanent lesions appear : keratisis which opacifies the cornea, iridocyclisis causing glaucoma, and inflammation of the retina and the optic nerve. Since onchocerciasis is an accumulative disease, the ocular complications appear after an accumulation of infections over several years. In certain hyperendemic areas, blindness occurs between the ages of 30-40 but may occur earlier in some subjects (REMME *et al.,* 1989). Onchocercal nodules are an encysting reaction to the parasite *O. volvulus* by the human host. They are located mainly where bones are superficial, near the hips, the rib cage and quite often on the head and the legs. They are generally small, 1-2 cm, but may sometimes exceeded 5 cm. The frequency and severity of the symptoms are often closely correlated with the number of microfilariae which, in turn, depends on the number of adult filariae. This number of adult worms is governed by the number of infective larvae received by the subject, and therefore by the number of bites received from infected blackflies in relation to the length of time spent in an endemic area.

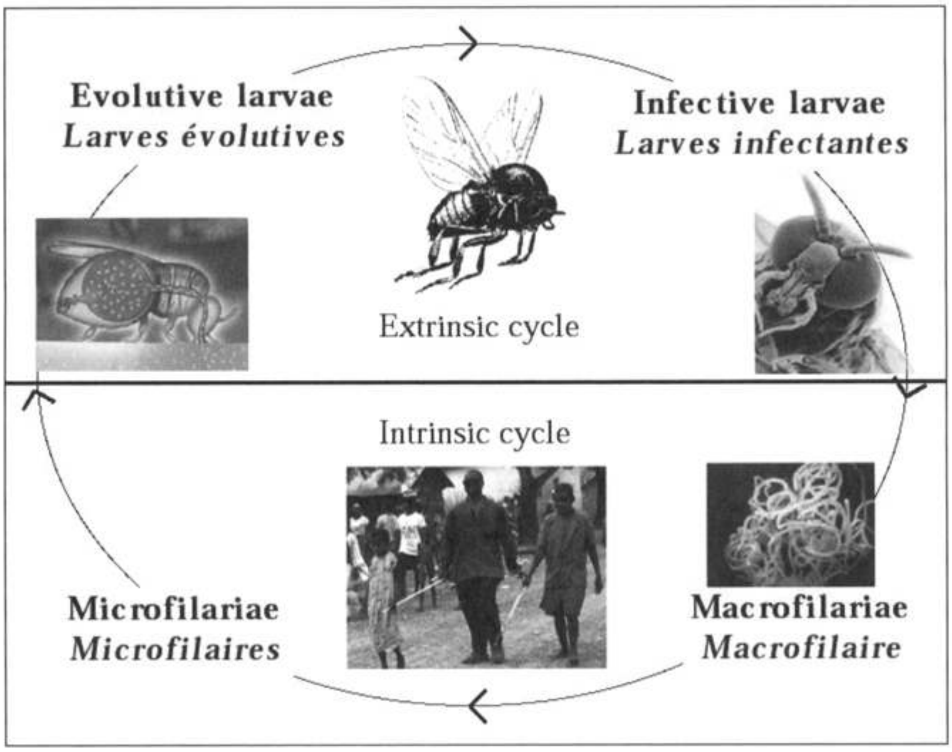


Fig. 1
THE LIFE CYCLE OF *ONCHOCERCA VOLVULUS*

A disease controlled through larviciding and chemotherapy

10 As most of vector-borne diseases, onchocerciasis can be combated by appropriate vector control operations. According to the geographical context and vector biology, the vector control strategy can have different objectives.

11 The strategy of definite elimination of the vector has been envisaged, especially against the *S. neavei* complex, the East African vector In the case of the *S. damnosum* complex, because of the important flight potential of those flies, attempts must be restricted to exceptional situations of small isolated foci, through actions limited in time and space. This strategy, which is a little component of the African Programme for Onchocerciasis Control (APOC) (DADZIE, 1997), is being implemented by this programme in four limited foci in Central and East Africa.

12 Another strategy consists of bringing the transmission of the parasite to an end. The objective is to arrest transmission of the *O. volvulus* by eliminating vector population for the duration of the lifespan of the adult worm from the human reservoir which at present is calculated to be around 14 years. This has been applied and demonstrated in parts of the original area of the Onchocerciasis Control Programme in West Africa (OCP) where vector control alone has been carried out for more than 14 consecutive years and where the disease is no longer a public health problem (HOUGARD *et al.,* 2001 – see figure 2). As blackfly adults are difficult to target, the vector control operations consist of treating with appropriate insecticides the breeding sites of rivers where the reophilic larval stages develop. As far as *S. damnosum* is concerned, the development of the aquatic stage from egg to pupae is around one week, hence the insecticide application is undertaken weekly.

13 Onchocerciasis can also be controlled by chemotherapy. However despite the importance of the research effort, only ivermectin has proven to be effective, well tolerated and accepted, and without side effects. Today, it is the only molecule used to control onchocerdasis morbidity (ABIOSE *et al.,* 2000). In contrast to diethylcarbamazine (DEC), ivermectin is a microfilaricide which is effective at a single dose without causing any side effects (particularly Mazzotti reaction) or serious aggravation of ocular troubles even when the dose and the parasitic load are high. It reduces the microfilarial load by 90 % and this reduction is maintained for six months at least. The objective of the use of ivermectin in APOC and OCP is to control onchocercal morbidity and therefore to prevent onchocercal ocular disease and blindness. To get maximum benefit from the drug, the treatment has to be regularly administrated over a long period of time, the duration of which has not yet been determined and depends on the level of endemicity. This is due to the limited effect of the drug on the adult worm and on the interruption of the transmission (WINNEN *et al., 2002*). Ivermectin is now extensively delivered within OCP and APOC through an institutionalized strategy of Community Directed Treatment with Ivermectin (CDTI). Its principle rests on the distribution of ivermectin by community workers selected by the population itself and specially trained in the various activities related to the treatment (DIARRA, 1998).

What’s new about the West African onchocerciasis vector and parasite ?

A complex of blackfly species

14 Blackflies are small hematophagous flies of the genus *Simulium.* There are over 2000 species around the world, only a few of which transmit onchocerciasis. *S. damnosum* is distributed throughout most of tropical Africa, not as a single taxonomic entity but as a complex of sibling species with own ecological characteristics (LE BERRE, 1966). Nine species are present in the OCP area (BOAKYE, 1993). *S. damnosum* s.s., *S. sirbanum* and *S. dieguerense* are found in the savannah zone until the northern limit of the endemic area for onchocerciasis. *S. soubrense, S. sanctipauli, S. konkourense* and *S. leonense* are great dense forest-dwelling ; they may also be found in the zone of light west forests and, in places, even reaches savannah zones. *S. yahense* is limited to small forest watercourses and S. *squamosum* probably covers several different entities and is widespread in both forest and savannah zones. Species differentiation is based on the examination of the polytene chromosomes of the salivary glands of the larvae. The morphological and morphometric criteria that enable us to separate most species or groups of females adults have also been identified. Since 1995, a technique based upon mitochondrial encoded gene sequences has been developed and makes it possible to identify most species of the complex (TANG *et al.,* 1995).

Several parasite strains

15 Considering the geographical differences in the clinical pattern and focalization of onchocerciasis, the severity of ocular lesions and prevalence of blindness, the existence of several *O. volvulus* strains differing in their pathogenicity to man and their adaptation to various species of the *S. damnosum* complex, had been suspected since the years 60's (DUKE *et al.,* 1966). For many years OCP has been faced to the problem of parasite identification because the available taxonomic tools could not allow for a reliable identification of the parasite found in the blackfly females during dissections. At the infective stage, *O. ochengi,* the main cattle filaria which can be transmitted by *S. damnosum s.l.*, cannot be morphologically differentiated from *O. volvulus* which is responsible for human onchocerciasis. As a result, the measure of the transmission could be overestimated since it took into account all the larvae found during dissections (PHILIPPON, 1977). That was the reason to develop a method for differentiating, on one hand, *O. volvulus* from the parasites of animal origin and, on the other hand, the *O. volvulus* strains that are the most pathogenic. The discovery of a family of repetitive sequences composed of 150 basic pairs in the genotype of *Onchocerca* sp. made possible the development of DNA probes for parasite identification (ZIMMERMAN *et al.,* 1993). Several probes were isolated. OCH probe is able to differentiate *O. volvulus* from *O. ochengi.* PFSI and pSS-IBT probes made it possible to differentiate the savannah and forest strains of 0. *volvulus.* They have been commonly used by OCP from 1992.

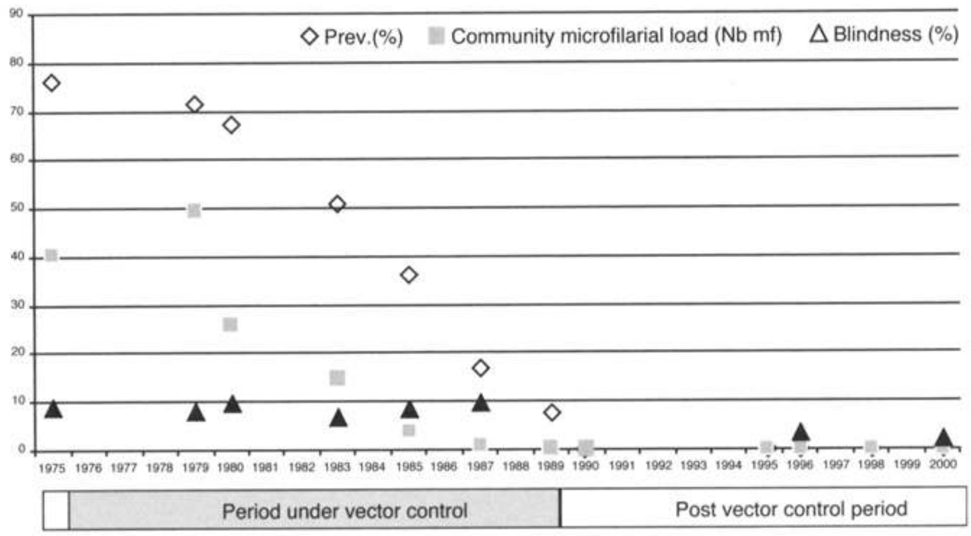


Fig. 2

EPIDEMIOLOGICAL TRENDS FROM 1975 TO 2000 IN A VILLAGE OF THE CORE AREA OF OCP (FROM HOUGARD *ET AL.*, 2001)

How was devised the Onchocerciasis Control Programme ?

An overall integrated strategy

16 OCP ceased rts activites on December 31, 2002, after 29 years of life. No other public health programme ever benefited for so long from the logistical and financial support of the international community, the reason for this support being that the results obtained have always convinced the donors of the effectiveness of the control strategies used: vector control from 1975 to 1989, then vector control and/or therapeutic control until 2002 (MOLYNEUX, 1995). OCP precisely began its activities in January 1974. Its objective was to eliminate onchocerciasis as a disease of public health importance and an obstacle to socio-economic development (WHO, 1969). The basic strategy of the Programme consisted in interrupting the transmission of the blinding strain of *O. volvulus* by destroying *S. damnosum s.l.* at its larval stage through aerial application of selective insecticides on the rivers infested (HOUGARD *et al.*, 1993). The first aerial treatments began at the very end of 1974 in areas where the incidence of blindness was highest. They were later gradually extended to cover by the end of 1977 an area of 654 000 km^2 spread over seven countries (Burkina Faso, south-eastern Mali, south-western Niger ; the northern parts of Côte d'Ivoire, Benin, Ghana and Togo). However ; it was very soon clearly established that the border of this area was affected by infective blackflies originating from regions outside the Programme area. In order to protect permanently the reinvaded area and also clean the basins which were a source of reinvasions, the incriminated hyperendemic regions were identified and then put under larvicidal treatment. To the west of the original area (western extension), these were the basins of western Mali, south-eastern Guinea and northern Sierra Leone. To the south and east of the original area (south-eastern extension), these were the Southern basins of Côte d'Ivoire, Bénin, Ghana and Togo (Fig. 3).

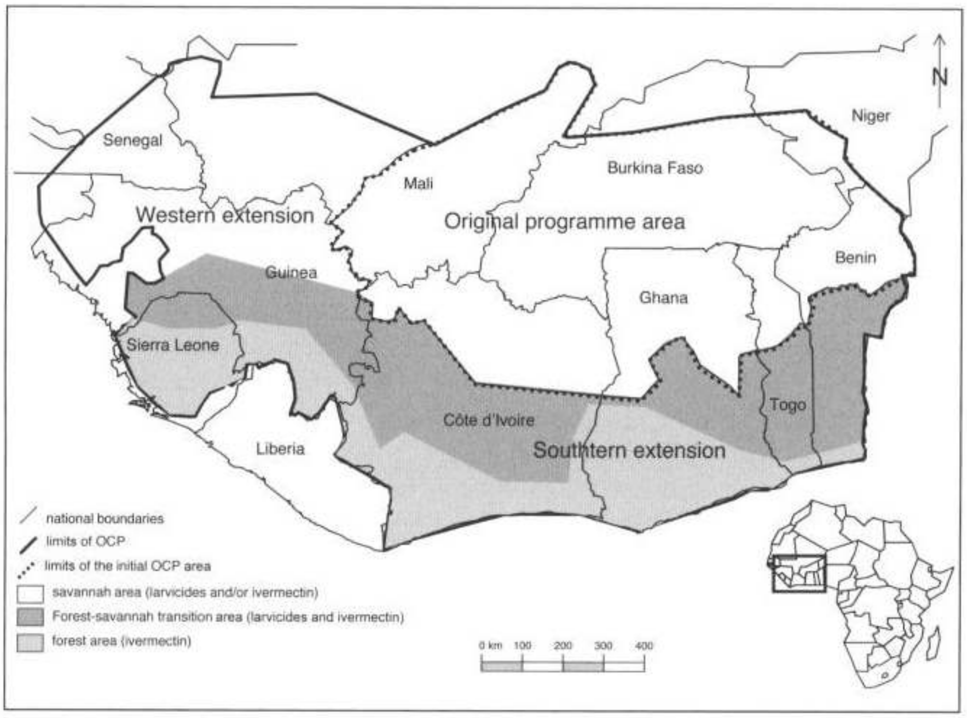


Fig. 3
THE ONCHOCERCIASIS CONTROL PROGRAMME AREA

17 The set up of vector control operations in the extension area was completed towards the end of the 80's, while all the basins of the original area were still under treatment. The larviciding coverage then reached its peak with more than 40,000 km of river stretches treated, corresponding to a million square kilometres, spread over nine countries of the Programme. Because of the success of the larval control strategy, larviciding operations progressively stopped from 1989 in the basins of the original area. In the extension areas, larviciding was going on satisfactorily in combination with ivermectin. To date, human onchocerciasis is no longer a problem of public health importance nor an obstacle to socio-economic development in all of the treated area. The control of this filariasis is however not over since OCP never aimed at eradication, neither of the parasite nor of its vector In 2003, the eleven participating countries of OCP have taken over the responsibility of carrying out the residual activities of monitoring and control of this disease. This task is of great importance because any recrudescence of the transmission would lead in the long run to the reappearance of the clinical signs of onchocerciasis, if not its most serious manifestations.

A pre-eminent operational structure

18 OCP included up to 11 participating countries : Bénin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Guinea Bissau, Mali, Niger Senegal, Sierra Leone and Togo. It was sponsored by the United Development Programme (UNDP), the Food and Agricultural Organizaron (FAO), the World Bank and the World Health Organizaron (WHO) and has received since its inception a financial support from 28 donors. An operational agreement signed in 1973 between the Participating Countries and WHO determined the scope, objectives, consultation and management structures of OCP and the means by which control

operations and the evaluation procedures were to be conducted (ANONYMOUS, 1973).The uppermost level of the current OCP structure was the Joint Programme Committee (JPC) which has exercised full directional powers as regards overall programme policy, strategy development and budgetary matters. Next came the "advisory level" represented by the Expert Advisory Committee (EAC). The third level was that of "support and collaboration", consisting of the Committee of Sponsoring Agencies, the World Bank for the mobillzation of funds and the WHO headquarters and regional office for Africa for the administration of financial resources and the administrative support. This level also included collaboration with the participating countries and donors. Finally, the last level consisted in planning, programming and implementing the field operations developed on the basis of EAC recommendations approved by JPC.

19 In 2002, OCP was composed of two technical units, the Planning, Evaluation and Transfer unit (PET) and the Vector Control Unit (VCU). PET was involved in the epidemiological evaluation and surveillance, biostatistical analysis and information Systems support, training and transfer. VCU, by far the most important unit in terms of staff and budget, had four main functions: (i) to carry out entomological surveillance with a view to guiding aerial operations according to the observed presence or absence of blackfly larvae at breeding sites and of infective adults, (ii) to conduct aerial larviciding for the purpose of interrupting transmission, (iii) to monitor the environmental effect, if any, of OCP operations and, if required, to adjust operations to avoid damage to the non-target fauna, and (iv) to conduct research on the vector aimed at increasing the effectiveness of control measures, and on insecticide compounds, their formulations and their strategies of use, with a view improving the Programme's ability to deal with development of resistance to insecticide and of maximizing safety and cost-effectiveness of larviciding.

A permanent challenge: a search for new larvicides

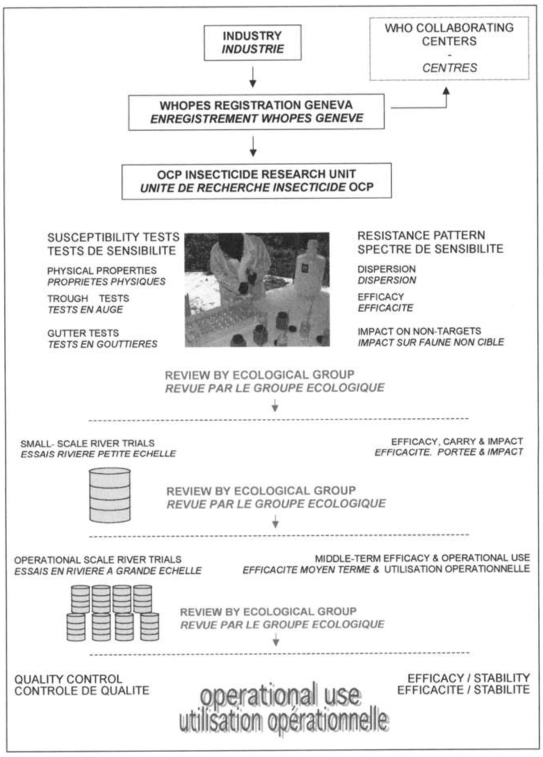


Fig. 4

FROM LABORATORY* TESTS TO *OPERATIONAL USES: THE DIFFERENT STEPS OF THE INSECTICIDE SCREENING PROCESS IN OCP

The need to overcome the organophosphate resistance

20 The strategy of larval control, which was currently practised by OCP, was closely related to the appearance of blackfly resistance to temephos, the only insecticide used by the Programme from l974 to 1979. The identification in 1980 of a focus of resistance on Lower Bandama in Côte d'Ivoire (GUILLET *et al.*, 1980) urged the OCP to abandon the "all-temephos" strategy to the benefit of an alternate use of several insecticides that was intended to contain this resistance. The choice of new anti-blackfly larvicides became a major concern, especially after the appearance of resistance to chlorphoxim, approximately a year after its use had started on the same stretch of river (KURTAK *et al.*, 1982): From 1980 to 1997, the Programme invested heavily in operational research on insecticides. First of all it was necessary to select compounds, which were cost-effective, and not toxic for mammals and non-target aquatic fauna. It was then essential to optimize their use so as to manage the eventual emergence of resistance, while preserving the aquatic environment and maintaining reasonable application costs (CALAMARI *et al.*, 1998).

21 The programme initially intensified its support for research involving laboratories such as the OCCGE "Institut Pierre Richet" (IPR) at Bouaké in Côte d'Ivoire, and then developed by 1986 its own research capacities, by creating an insecticide research structure in Bouaké (Fig. 4).

An intensive screening of new larvicides

22 In order to make it usable by the Programme, an anti-blackfly larvicide had to meet a number of criteria, the most important of which was efficacy, selectivity and harmlessness (HOUGARD *et al.*, 1993). At sufficiently low concentrations, the larvicide compound might guarantee the total control of blackflies larvae as far as possible downstream the spraying point, in order to reduce the number of aerial applications, on a given stretch of river thus saving flight hours. At the concentrations lethal for blackfly larvae, the insecticide might have a minimum impact on non-target aquatic fauna (aquatic insects, fish, shellfish), both in the short term (no acute toxicity) and in the long term (absence of bio-accumulation).

23 From the point of view of selectivity, certain operational larvicides in OCP had a low safety margin, which restricted their use a few applications per year only In the watercourses where the high flow rate guaranteed a high degree of accuracy of dosage. Of course, all the operational larvicides might have a low toxicity for mammals, in order to minimize the risks of handling by operators and affecting the health of men and animal using rivers as sources of drinkable water Apart from these three criteria, an anti-blackfly larvicide had to be correctly formulated so as to ensure that the insecticide had a uniform coverage of the larval breeding sites, a maximum carry downstream from the spraying point. Technically, the selected formulation might also guarantee sufficient fluidity to allow an easy pumping in the tank of the aircraft as well as a uniform application by the spraying tubes. A blackfly larvicide might finally have a good stability under the usual conditions of warehousing in a tropical environment. Operational insecticide stocks might indeed be stored close to the rivers, in open air deposits, for periods that could exceed one year It was thus critical that the larvicides had a sufficient stability to preserve their efficacy and their physical properties, prior to application by helicopter.

24 There are several steps to meet in the selection of a new compound. This process relates on a close cooperation with the manufacturer In charge of the development of new compounds and formulations. The first stage consisted in selecting, from among the compounds provided by the manufacturer and approved by the WHO Pesticide Evaluation Scheme (WHOPES), the products that had, at the same time, a significant impact on mosquitoes larvae and a low toxicity for mammals. These compounds might belong, if possible, to families different from that of operational Insecticides, or at least to different groups within the same family, In order to minimize the risk of crossed resistance among insecticides. They were then proposed for evaluation by OCP for screening on blackflies in a closed System, without any risk to the environment (KURTAK *et al.*, 1987). Insofar as it was impossible to maintain permanent colonies of blackflies in the laboratory, the efficacy of the new compounds was evaluated in the field, near larval breeding sites. For this purpose, a laboratory was established in situ by the Programme in the south-western part of Côte d'Ivoire, close to a river located outside the rivers subject to larviciding. Alongside these tests, standard susceptibillty tests were carried out with the active ingredient of the tested compounds in order to assess their basic susceptibility. The following stage consisted in realizing at a small scale some tests on non-target fauna. This stage implied the collaboration of the hydrobiology team of VCU, in charge of monitoring the aquatic environment for the entire Programme. Tests in gutters on non-target aquatic fauna (insects mainly) made it possible to evaluate the safety margin between the

estimated operational amount and the dose likely to cause an undesirable impact (YAMÉOGO *et al.,* 1991). When this margin was acceptable, compared to the already operational larvicldes, a technical report was submitted to the Ecological Group, the authority allowed to authorize the follow-up of the evaluation at a larger scale. If the Ecological Group agreed, large-scale tests might be carried out in river to check the efficacy of the product and its carry, and to measure its impact on non-target fauna under field conditions. Should results-promising, large-scale treatments were carried out.

A selection of seven operational compounds

25 Several hundreds of compounds and/or formulations have been evaluated by OCP, under an intensive screening programme. This research led to the selection of seven operational insecticides, six Chemicals and one biological control agent (table 1).

Table I
Main characteristics of the blackfly larvicides used by OCP

Family		Organophosphorous			Pyrethrioïds		Carbamates	Bacteria
common name		temephos	phoxim [1]	pyraclofos	permethrin	etofenprox	carbosulfan	B.t H-14
formulation		EC [2]	EC	EC	EC	EC	EC	WD [3]
% active ingredient		20	50	50	20	30	25	< 2
class of toxicity [4]		III	II	II	II	III	II	III
toxicity against FNC [5]		slight	medium	medium	high	medium	high	slight
dose [6]		150 [7]	150	120	45	60	120	500
mean	10 m³ / sec	12	3	Not used	Not used	Not used	Not used	1.5
carry	100 m³ / sec	16	5	18	7	6	9	5
(km)	300 m³ / sec	20	Not used	23	8	8	Not used	Not used

[1] ***CHLORPHOXIM UP TO 1991;*** [2] ***EMULSIfIATE CONCENTRATE;*** [3] ***WATER DISPERSIBLE;*** [4] ***ACCORDINg TO THE WHO CLASSIfICATION OF ACTIVE INgREDIENT: II, QUITE HAZARDOUS; III, SLIgHTLY HAZARDOUS;*** [5] ***TOXICITY AgAINST NON AQUATIC FAUNA ACCORDINg TO THE CRITERIA OF THE ECOLOgICAL GROUPE;*** [6] ***IN ML OF FORMULATION PER CUMECS;*** [7] ***300 ML IN CLEAR WATER.***

26 TEMEPHOS, introduced from the very beginning of OCP, was an exceptional larvicide. It is an organophosphorous compound, with very low toxicity for vertebrates. It has a good selectivity for blackflies, resulting in a very low impact on nontarget invertebrates. Moreover, the carry can reach 50 kilometres in favourable conditions. The amount normally applied is 300 ml of an Emulsifiable Concentrate formulation (EC 20%) per cumecs of flow in the river Given its excellent carry and its greater efficacy in high turbid water the amount can drop to 150 ml per cumecs in the rainy season. Unfortunately its use was limited since 1980 as a result of the appearance of resistance among *Simulium* populations.

27 CHLORPHOXIM, the only alternative compound operationally available in 1980, was introduced to replace temephos in the areas of resistance. As it is also an organophosphate, a cross resistance soon appeared in some species in the Southern basins of OCP, but this resistance did not extend. Chlorphoxim, used at 120 ml per cumecs (EC 20%), is less selective than temephos and has a lower carry. Because its industrial production was abandoned it was replaced in 1991 by phoxim, at 150 ml per cumecs (EC 50%). Its use was as limited as chlorphoxim.

28 PYRACLOFOS was introduced in 1990. Its carry is comparable to temephos. It was used at 120 ml per cumecs (EC 50%). It is potentially more toxic for fish than temephos or phoxim, and it was thus restricted to discharge higher than 15 cumecs. Although pyraclofos does not have spontaneous cross resistance with temephos or phoxim, it had been recommended not to use pyraclofos beyond eight consecutive weekly cycles. Thanks to the strategy of rotation, the susceptibility to pyraclofos remained unchanged in the OCP area, in spite of an intensive use on several basins.

29 PERMETHRIN, like most of pyrethroids, is a very effective insecticide against blackflies, used at no more than 45 ml per cumecs (EC 20%). While not being very toxic for hot-blooded vertebrates, it is less selective than organophosphates for the non-target invertebrates fauna (insects, shellfish) and fish. Its carry is low, compared to that of temephos and pyraclofos (lower than 10 km even in favourable conditions). The Ecological Group recommended no more than six consecutive cycles per year on the same river stretch and never below 70 cumecs. In spite of these constraints, permethrin played a significant role in the strategy of rotation of insecticides applied to the OCP. It proved indeed very active against blackflies resistant to organophosphates. Moreover its low cost and low operational dosage allowed river treatments over 500 cumecs discharge. No decrease of *Simulium* susceptibility to permethrin had been detected in spite of the many cases of resistance recorded here and there in some crop rodents and other insect vectors, such as mosquitoes.

30 ETOFENPROX is a “pseudo-pyrethroid” which is much less toxic for fish than permethrin. It was used operationally since 1994 at 60 ml per cumecs (EC 30%) for discharge above 15 cumecs.

31 CARBOSULFAN is a carbamate insecticide. It was introduced in 1985 in order to serve with permethrin as an alternative to organophosphates for the treatment of large rivers. It has been used at 120 ml per cumecs (EC 25%). Its relatively low selectivity and its risk of impact on fish raised the limits of use, as for permethrin, to discharge higher than 70 cumecs with a maximum of six consecutive weekly treatments per year and per river stretch. Its low carry and relatively high cost limited its use to a low range of river discharges. No *Simulium* resistance was detected since its introduction.

32 BACILLUS THURINGIENSIS H-14 (B.t. H-14) is a biological control agent. Discovered in 1977, this bacterium produces protein crystals toxic for *Simulium* larvae and several other diptera. Commercial formulations were used by OCP on a large scale, since 1982 (GUILLET *et al.,* 1982).The toxin of B.t. H-14 is indeed extremely selective for blackfly larvae, and operational spraying does not practically have any effect on non-target fauna. Its mode of action is completely unique and no crossed resistance with Chemical insecticides has ever been recorded. In fact, several rivers in the OCP area have been treated by B.t. H-14 for nearly 20 years without any decrease of *Simulium* susceptibility. This made B.t. H-14 the insecticide of choice to counter resistance to organophosphates. However its operational dose was relatively high, limiting its use at relatively low discharges. By 1985, the improvement in the commercial formulations made it possible to treat rivers at a discharge of 75 to 100 cumecs.

An adapted operational control strategy

Managing vector resistance to insecticides

33 Alongside the programme of development of *Simulium* larvicides, OCP developed a strategy of use of these compounds which allowed, on the one hand, to contain resistance to temephos, chlorphoxim and phoxim and, on the other hand, to avoid the development of blackfly population resistance to the other insecticide families. Among the possible strategies of management of resistance, one consisted ¡n alternating, in time, the insecticides belonging to different families. This rotation made it possible to reduce the insecticide pressure on a given *Simulium* population and, thus, to decrease the chances of development of genes of resistance in this population. This strategy would have been relatively simple to implement if all the products of replacement had shown the same characteristics as temephos. The choice of insecticides would then have been limited only to the considerations of management of resistance, other than any other factors, such as efficacy, cost, physical properties and toxicity. Unfortunately, it was not the case, which made the implementation of this strategy even more complex (GUILLET *et al.*, 1991).

34 In order to monitor the susceptibility of *Simulium* to Insecticides, the Programme quickly developed simple and reliable methods of evaluation of the susceptibility of *S. damnosum* larvae to insecticides. These tests, the guiding principles of which were described by MOUCHET *et al.* (1977) for chemical insecticides and GUILLET *et al.* (1985) for B.t. H-14, are easily achievable in the field. They helped to determine, for each insecticide, the diagnostic doses, so as to quickly detect the least fall in susceptibility of the blackflies to a given insecticide. A considerable improvement in the situation of resistance to temephos has, however been noted these last years, since the resistance now persisted only on the lower-Bandama and the lower-Comoé in Côte d'Ivoire, on a relatively low level. Temephos could, thus, be used again in 90% of the Programme area, in rotation with other operational Insecticides. The same goes for phoxim, which had replaced chlorphoxim. With regard to pyraclofos, only one case of resistance was reported on the Marahoué (Côte d'Ivoire), following a succession of 16 consecutive weekly cycles, carried out on an experimental basis. This resistance, fortunately, quickly proved reversible in the absence of pressure of selection. No resistance has, never; been detected with the other insecticide families (Fig. 5).

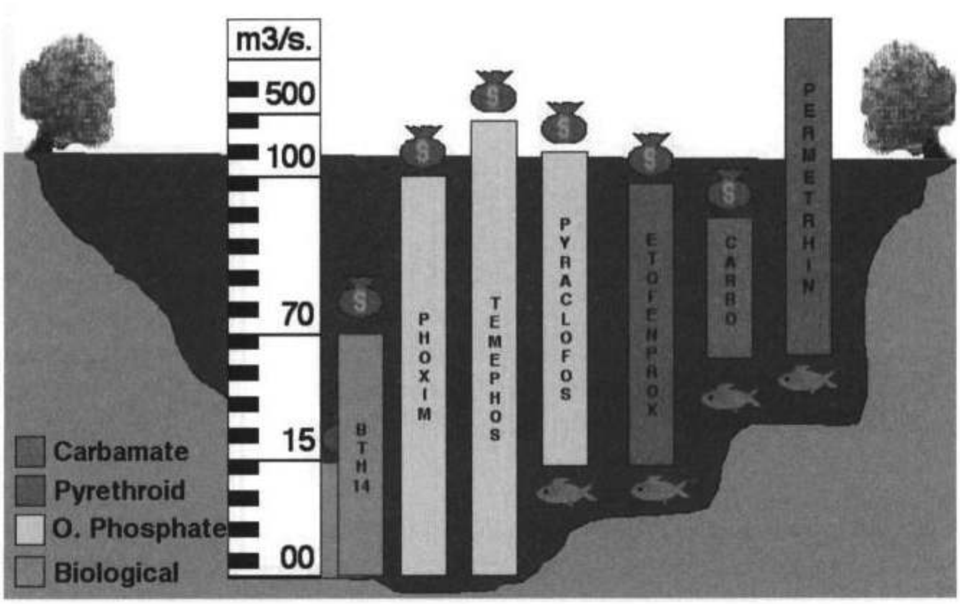


Fig 5
OPERATIONAL RANGE OF LARVICIDES USED BY OCP

Less pressuring the aquatic environment

35 The weekly periodicity of treatments, and the importance of the hydrological network to be treated, constituted the first factor of complexity. A decision to treat should indeed be taken each week on several thousands of kilometres of rivers, that is to say, several hundreds of spraying points (fig. 6). The first step among the decision-making process was to determine, for a watercourse or a portion of it, if larviciding must take place. This decision depended on a number of factors among which were the results of the weekly entomological evaluation or the level of endemicity of the zone under study. Once the decision for treatment was taken, the choice of insecticide depended, not only on the dynamics of resistance at the local level, but also on the characteristics of each insecticide as described previously.

36 Below I cumecs, only B.t. H-14 might be used. The use of temephos was not excluded, but its use in little agitated water courses increases the time of contact of the insecticide with substrates, and the risks of under-dosages, favourable to the survival of the individual heterozygotes, in the case of recessive or semi-recessive resistance gene. Between 1 and 15 cumecs, three of seven insecticides available could be used: temephos, phoxim and B.t. H-14. From 15 to 70 cumecs, two compounds could be added, pyraclofos and etofenprox, bringing the number of available larvicides up to five. Between 70 and 150 cumecs, the use of B.t. H-14 became expensive, but this range of flow allowed the greatest choice and the greatest diversity of compounds with three organophosphates (temephos, phoxim and pyraclofos), a pyrethroid (permethrin), a pseudo-pyrethroid (etofenprox) and a carbamate (carbosulfan).This diversity was reduced between 150 and 300 cumecs because phoxim and carbosulfan become too expensive for use. Between 300 and 450 cumecs, only temephos and permethrin were profitable. Above 450 cumecs, only permethrin was used operationally. By 1999, the gradual reduction in larvicide coverage has made it possible to decrease the number of insecticides, by limiting to the five most effective ones : temephos, pyraclofos, permethrin, etofenprox and B.t H-14.

37 The spraying of an insecticide requires precise knowledge in real time of the discharge of rivers to be treated. A mis appreciation of the quantities of insecticides to be sprayed can indeed have several effects on the success of treatment. An overdosage can have significant financial and ecological consequences, if the product used is expensive or relatively toxic. This is why an hydrological surveillance network was set up from the very start of the programme on the entire treated rivers. It included, at the height of larviciding, up to 185 river gauges, 103 of which were equipped with hydrological beacons, allowing a transmission by satellites of recorded water levels (SERVAT and LAPETITE, 1990). In the same time, the aircraft company in charge of treatments, along with OCP, developed a treatment device allowing precise dosage (HOUGARD *et al.*, 1996).

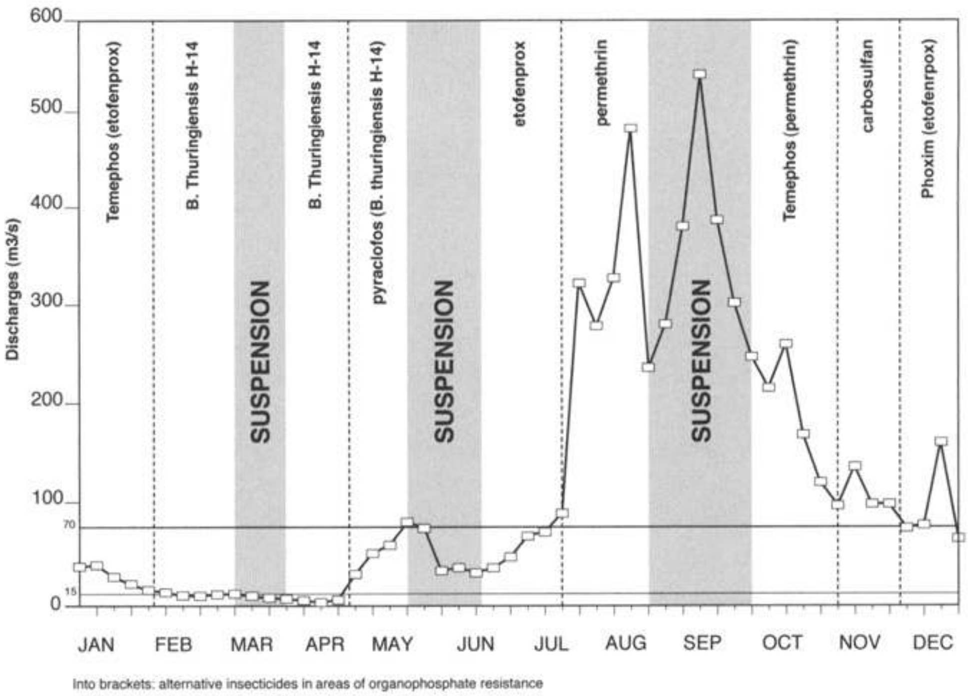


Fig. 6

AN EXAMPLE OF ROTATIONAL USE OF LARVICIDES IN A STANDARD RIVER CLOSE TO OCP AREA

Environmental protection: a need to deal with

A strong concern about the fate of the environment

38 The mere fact of regularly using insecticides for many years raised the concern of the potential risk such operations could have for the aquatic environment. Indeed, at the time OCP was launched, there was much evidence on biological and ecological consequences of DDT With the awareness of the "DDT syndrom" of the international community, the Participating countries, as well as the Donors that support the Programme (28 countries and foundations), had reasons to fear that 20 years' repeated

applications of insecticides in the watercourses would cause serious disturbances of the freshwater ecosystems.

39 In 1974, just before the beginning of operational activities, OCP set up an aquatic monitoring programme of rivers planned to be regularly treated with insecticides (LÉVÊQUE *et al.,* 1979). It was implemented to satisfy three major concerns:

- to provide early warning to those carrying out treatments, should toxic effects be noted on the short term and to ensure that the insecticide release did not excessively disturb the functioning of the treated ecosystems on a long term basis (the expected duration of OCP);
- to avoid the widespread use of Chemicals which may have adverse effects on human populations near the river Systems and/or might accumulate in the food chain as DDT has been known to do;
- to prevent the irreversible loss of aquatic biodiversity in West Africa both because freshwater fish are a major source of food as well as an economic activity for West African populations, and to meet the objective of the Convention on Biodiversity that stipulates that countries are responsible for the conservation of their biodiversity.

40 At the beginning of OCP, the knowledge of riverine Systems and their associated flora and fauna was still very poor In such a situation, the aquatic monitoring activities were devised both to collect basic knowledge on the structure and functioning of the rivers, and to investigate the potential impact of larvicides on the aquatic fauna. In particular ; it was necessary to identify (and sometimes describe) the different species collected, to investigate the biology and seasonal dynamics of the non-target fauna, to understand the long-term trends in river water discharges and their relations to the dynamics of aquatic communities. This basic research, highly critical for the interpretation of the monitoring data, was progressively achieved by several teams, and particularly by the Orstom hydrobiological team based first in Bouaké, and then in Bamako.

The implementation of an efficient organization

41 The monitoring of the aquatic environment was made possible through the implementation of a specific organization devoted to laboratory and field studies, as well as to the periodical analysis and interpretation of collected data. Currently, the entire monitoring activity is catered for by the participating Countries using a standard protocol, but is backed by financial and technical support to the Programme. Special studies and independent analysis of data are carried out periodically in collaboration with consultants or specialized institutions to supplement or support studies conducted by the Programme and National hydrobiology teams. The Ecological Group evaluates the different results, assesses the level of toxicity of proposed new Chemicals, and approves or rejects their operational use under the Programme (see figure 6).

The Ecological Group

42 Before the Programme was launched, the Sponsoring Agencies set up an independent advisory body, the Ecological Panel, which later became the Ecological Group (E.G.).The Group always consists at maximum of five independent scientists and reports to the Expert Advisory Committee of OCP. Its role is to ensure that vector control carried out by OCP does not endanger the environment and to make recommendations to the

Programme for effective protection of the environment. More specifically, the objectives and mandate of the EG are to:

- organize and evaluate a long-term monitoring programme of the aquatic fauna;
- assess the level of toxicity of new products or formulations and approve or reject their operational use under the Programme;
- review the nature and magnitude of ecological problems connected with the programme and with associated economic development projects, proposed in areas freed from onchocerciasis, in order to identify the environmental and human ecological implications of such developments.

National Monitoring Teams

43 The monitoring activities have been conducted by National Hydrobiological Teams which were developed and established in most of the Participating Countries where larvicide treatments occurred (Burkina Faso, Côte d'Ivoire, Ghana, Togo, Bénin, Mali, Guinée, Sierra Leone). Most national scientists received OCP grants to be trained in the methodologies used by OCP. Some of them received PhDs. They also received support from OCP to conduct their monitoring and research activities and they meet annually to discuss their results with the Ecological Group. Exchanges between monitoring teams were promoted during the programme.

The Vector Control Unit

44 The Vector Control Unit (VCU) in OCP has been one of the major contributors to environmental protection through many of its activities. For example: (i) it has been responsible for the judicious operational use of insecticides in the OCP area; (ii) it has been in charge of the screening of new insecticides; (iii) it reports its activities at the Ecological Group meetings.

The OCP Hydrobiological Section

45 The OCP Hydrobiological Section was created in 1981 at the headquarters of OCP in Ouagadougou, as a component of VCU, and under the leadership of a senior scientist. Its main functions were (i) to carry out the ecotoxicological research; (ii) to coordinate monitoring activities, and to assist the national teams in their field work in the OCP area; (iii) to manage the monitoring data provided by the national teams (Fig. 7).

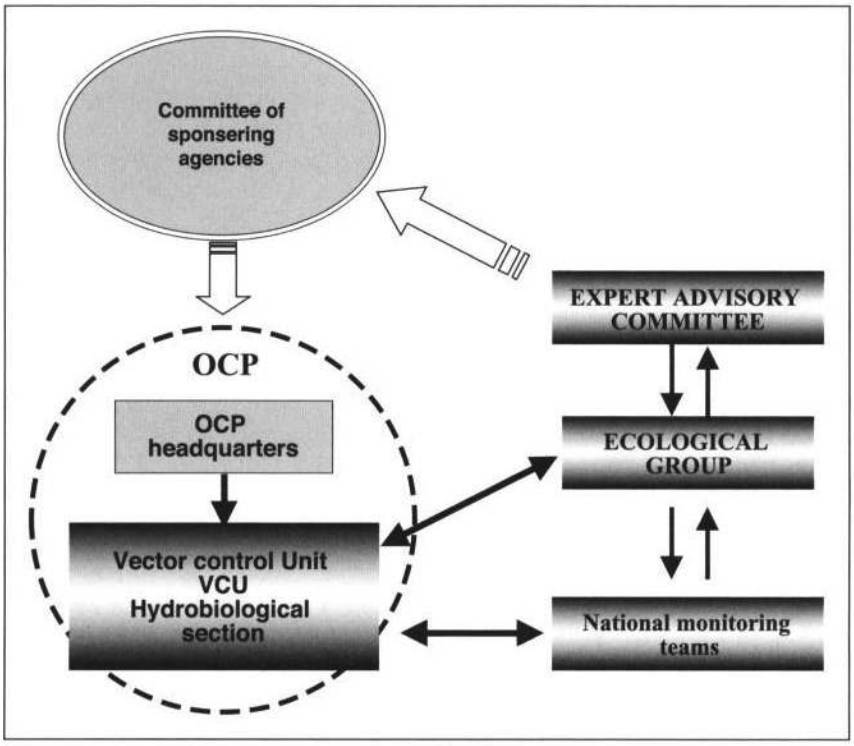


***Fig.* 7**
STRUCTURE DE L'OCP ET POSITION DES ACTIVITÉS DE SURVEILLANCE DE L'ENVIRONNEMENT

The short-term risk assessment of new larvicides

46 A risk assessment was performed for every new larvicide to be used in OCP. After the first trials devised to evaluate the efficacy on blackflies, a review of literature was performed together with laboratory tests on fish and gutter tests on invertebrates (CALAMARI *et al.,* 1998). Acute toxicological tests have been performed on African fish species according to standard protocols (YAMÉOGO *et al.,* 1991) to obtain original data on fish toxicology. Short-term impact on non-target invertebrates was also studied using a System of artificial gutters and different concentrations of insecticides (YAMÉOGO *et al.,* 1993). From these tests, larvicides were classified according to their general toxicity and a typology of the susceptibility of the most common taxa was established (YAMÉOGO *et al.,* 1991, 1993).

47 Quite a lot of information has been collected also from field trials of insecticides : B. t. H-14 (DEJOUX, 1983 ; DEJOUX *et al.,* 1985); deltamethrin (DEJOUX, 1983), Gh 14 (TROUBAT et LARDEUX, 1982), temephos (DEJOUX et ELOUARD, 1977; ELOUARD et JESTIN, 1982) pyrachlofos (YAMÉOGO *et al.,* 1993) permethrine (YAMÉOGO *et al.,* 1993) etofenprox (YAMÉOGO *et al.,* 2001)

48 Comparing the different operational larvicides, B. t. H-14 proved to be the least environmental damaging, followed by temephos, chlorphoxym, pyraclofos, etofenprox, permethrin and carbosulfan, in increasing order of toxicity. Among the taxa, the Baetidae (Ephemeroptera) were the most susceptible to the Chemical larvicides while the chironomidae (Diptera) were the least susceptible to most of the insecticides.

49 The Ecological Group reviewing the results recommended, in cases of acceptable toxicological results, small-scale pilot studies on the field. For the most toxic Insecticides that have many advantages to their possible use (such as low cost, wide range of

application in relation to discharges and long carry distance), large-scale studies below the operational dose were recommended. This allows a complete risk assessment scheme to be obtained before the insecticide is used as an operational larvicide. Permethrine for example was tested this way (YAMÉOGO *et al.*, 1993; CALAMARI *et al.* 1998).

The long-term monitoring of the aquatic environment

50 The criteria retained by the Ecological Group for the evaluation of the long-term impact of insecticides on aquatic environment have been the following (LÉVÊQUE *et al.*, 1988):

- the vector control activities should not reduce the number of invertebrate species, or cause a marked shift in the relative abundance of species;
- the pesticides applied should have a direct impact neither on fish, nor on the life cycle of fish species;
- bioaccumulation and biomagnification through food webs should be avoided ;
- human activities in the control area should not be impaired;
- temporary and seasonal variations in non-target invertebrate populations due to insecticides should be acceptable.

51 The monitoring programme was primarily concerned with two major categories of organisms: (i) the benthic invertebrates that abound in the watercourses and that are directly threatened by the insecticide in the same way as *Simulium damnosum* larvae; (ii) the fishes, by virtue of their economic interest for the people living along the rivers, but also for the psychological reasons to show the villagers occupied in fishing that care was taken to avoid the risks of pollution.

Methods and protocols

52 In order to evaluate the magnitude of the environmental risk, hydrobiologists have used consistent methods and protocols to monitor potential long-term effects of continuous use of larvicides on aquatic populations (YAMÉOGO *et al.*, 2001; CROSA *et al.*, 1998; LÉVÊQUE *et al.* l979). When setting up the monitoring protocol, several important considerations had to be kept in mind:

- the monitoring had to deal with a long-term regular sampling aimed at investigating the ecological effects of treatment over the duration of the programme, combined with shorter duration research programmes looking at specific short-term problems;
- the periodicity of sampling, the sites selected for monitoring, and the field methods used had to combine reliability of sampling techniques with reliability of access in both wet and dry seasons, over many kilometres of road or tracks which are not yet hard surfaced;
- the monitoring techniques had to work equally well in shallow, slow-flowing rivers in the dry season, and in the same rivers, deep and flowing fast in the wet season;
- in order to ensure reasonable comparability of results, all teams had to use the same methods.

53 A network of sampling stations throughout the Programme area was established (Fig. 8). Forty sampling sites were used at the start of the Programme, but as the programme evolved together with the treatment strategy, the number has been reduced recently to

ten for invertebrates and ten for fish, after an evaluation was made on the impact of the first ten years.

54 For invertebrates, three main sampling methods were used (YAMÉOGO *et al.,* 2001):

- Drift net sampling using 2 m long nets, 20 x 20 cm aperture, 300 mm mesh size. The basic techniques of drift sampling used in the Programme were standardized.
- Surber samples using 15 x 15 cm Surber sampler. This simple method, which allows rocky substrates to be sampled, cannot be used in deep waters and was therefore limited to the low-water period.
- Artificial substrates: special apparatus were designed and used, from concrete blocks which were left immersed on the bottom, to floating substrate made of a bunch of plastic fibres.

55 For fishes, the monitoring programme mainly concerned (LÉVÊQUE *et al,* 1988):

56 The study of changes in the catch (expressed in weight or number of individuals) and species composition of experimental fishing carried out at regular intervals (usually 2 or 3 months) with a standardized set of gill nets.

- The study of biological parameters, more especially the coefficient of condition which is a measure of the health of fishes. Complementary research was also conducted on the analysis of stomach contents of selected species, spawning periods and fecundity, as well as the impact of organophosphorous compounds on brain acetylcholinesterase activity.

57 Some methods and protocols for the monitoring and risk assessment programmes have evolved since 1974, the Programme being dynamic in the face of continually changing situations (e.g. insect reinvasion, resistance to pesticides, variability in hydrology, etc.). However the basic concepts and issues have remained.

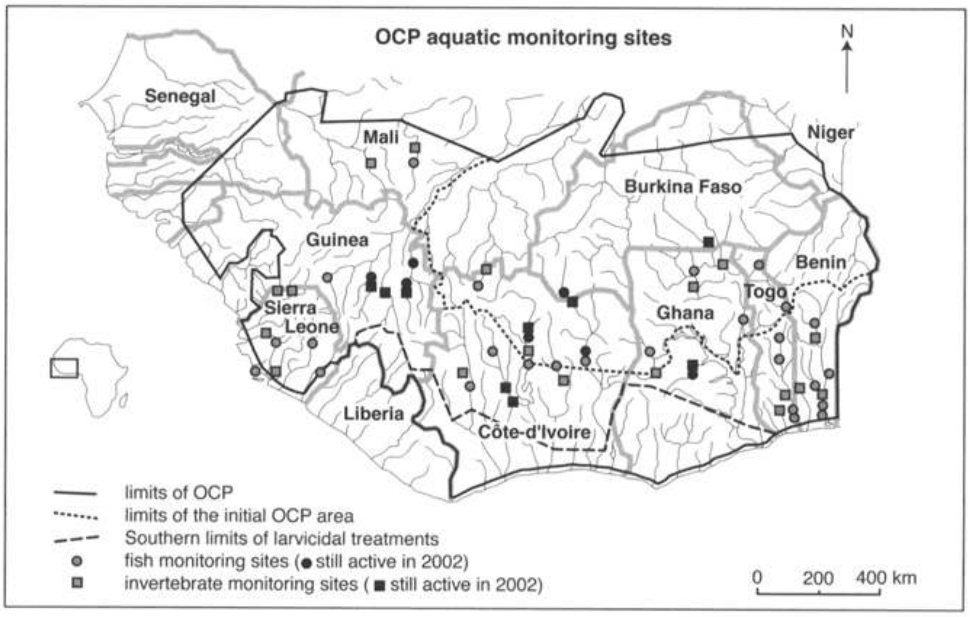


Fig. 8
MONITORING SITES LOCATION IN OCP AREA

Twenty five years aquatic monitoring

58 The major concern of OCP regarding the aquatic environments exposed to insecticides has been to avoid long-term or lasting changes in aquatic biodiversity. Major groups of

organisms which have been monitored during over twenty years of OCP operation for any indications of undue changes are fishes and non-target invertebrates (Fig. 9).

Main fish monitoring results

59 Potential impacts of larviciding on fish have been evaluated by assessment of changes in species richness of catch, catch per unit effort (CPUE) of fishing and coefficient of condition of fish species (LÉVÊQUE *et al.*, 1988 ; PAUGY *et al.*, 1999). In relation to species richness of experimental catch (which is the number of species caught in a standard set of experimental gill nets during two nights'n fishing), long-term trends observed in three major areas of the Programme (ie. rivers in Côte d'Ivoire, Volta basin rivers in Ghana and Niger basin rivers in Guinea) have been different. However after a period of declines especially in Côte d'Ivoire and Volta basin rivers, recovery and improvements in species richness of catch in all rivers have been observed since 1994 and l996. Thus, after several years of larviciding with several insecticides, up to twenty years in the original Programme area, there is no evidence of reduction in fish species diversity in treated rivers. A similar observation was made after the initial ten years of monitoring during which only three larvicides had been used. Comparison of species richness changes with hydrological trends suggests that the observed trends in species richness might be attributed to climatic factors and the long period of drought that occurred for many years in West Africa (Fig. 10 and 11).

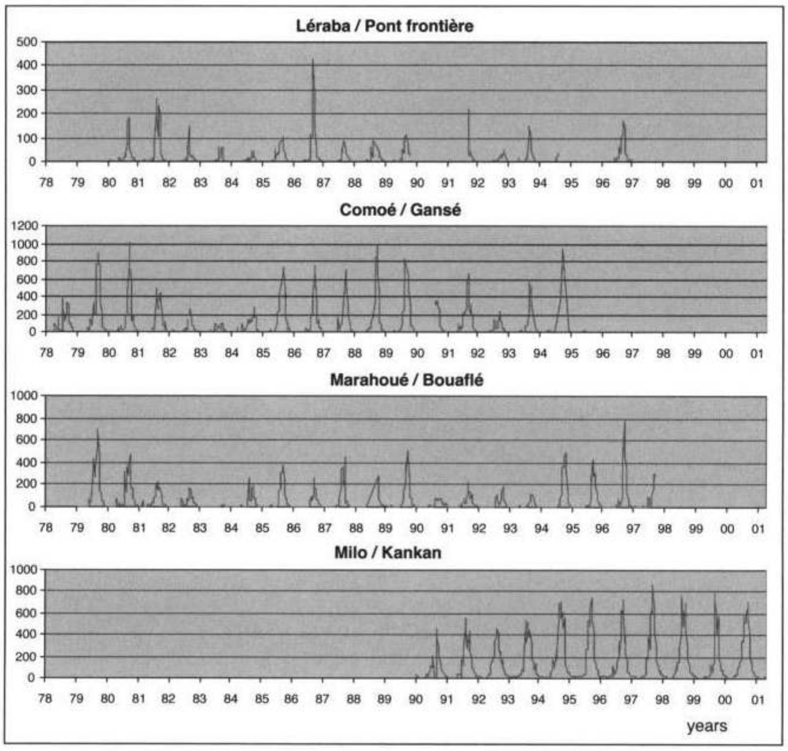


Fig. 9
LONG-TERM CHANGES IN THE ANNUAL DISCHARGE FOR SOME OF THE MONITORED RIVERS. LOW DISCHARGES ARE CHARACTERISTICS OF THE EARLY 1980S AND THE EARLY 1990S IN MANY RIVERS OF THE OCP AREA

60 Overall trends of catch per unit effort (CPUE) in relation to larviciding during 20 years also indicate various scenarios in the different major Programme areas/basins and

sometimes among rivers of the same basin in spite of a generally similar larviciding regime in the Programme area. For example, catches reduced in Côte d'Ivoire rivers up till 1989 and 1993 and increases were observed in all rivers since 1995 although larviciding was stopped on different rivers at different times. In the Niger basin, no decrease in catch has been observed since monitoring began, while increases over the original status have been observed in the three rivers monitored in the basin since 1994. In the Volta basin, different trends in catch are being observed over the years. However, a common seasonal pattern of catch, high at low water periods (Dec./Jan. till Apr/May) and low at high water periods (Jly/Aug. till Oct./Nov.) is observed for all basins and rivers. The influence of hydrological changes on fish catch has been previously suspected.

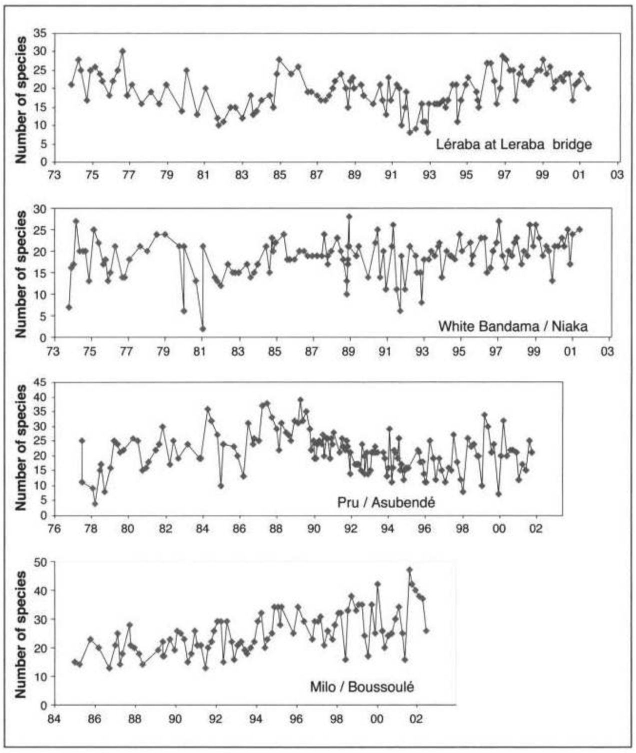


Fig. 10
Long-term changes in fish species richness per sample in some monitored rivers

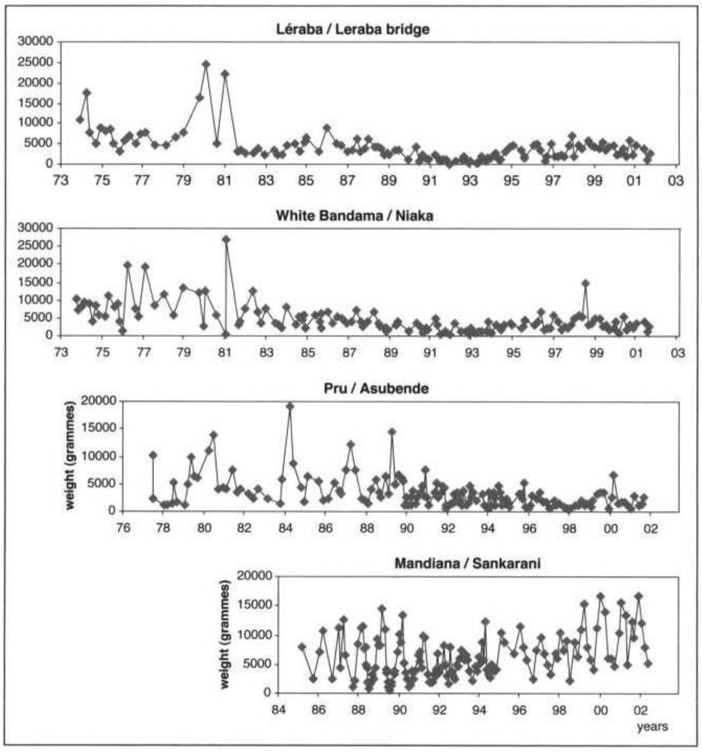


Fig. 11
LONG-TERM CHANGES IN FISH CATCH PER UNIT EFFORT (CPUE) PER SAMPLE USING A STANDARD SET OF GILL NETS SAMPLE IN SOME MONITORED RIVERS

61 Coefficient of condition values (a ratio between weight and length) which express the “well being” of fish have also been monitored to assess direct effect of larvicides on fishes (acute toxicity) and/or indirect effects through larvicide impacts on their food sources which are in many cases aquatic invertebrates. Over the years, various assessments of trends of ‘condition’ of several fish species in the Programme area indicate only fluctuations around expected means but no significant changes in values. The situation indicates that larviciding has not directly affected fish and suggests that where fish food items have been affected, others have been found and used reasonably well in their place.

62 Bioaccumulation of pesticides in fish was a major concern with DDT. Actually, the effects of organophospates in laboratory experiments showed that fish were able to accumulate temephos (MATTHIESSEN and JOHNSON, 1978). But this accumulation seems to be limited and does not increase to a point observed with DDT Field data in OCP area confirmed that temephos did not accumulate in fish (QUÉLENNEC *et al.,* 1977). Moreover, in field conditions, the acetylcholinesterase activity in the fish brain does not seem to be significantly different in the rivers treated with temephos or untreated (ANTWI, 1985; SCHERINGA *et al.,* 1981).

Main monitoring results for invertebrates

63 The specific concern of OCP with regard to non-target invertebrates and larviciding of rivers has been to prevent loss of faunal diversity and to maintain the quality of biomass available for higher levels of the aquatic ecosystem food web. Impact assessment of larvicides has been based on evaluation of two types of data. These are Surber samples data and drift (day and night) data.

64 By analysing the invertebrate data which were collected using various sampling strategies between 1977 and 1996, Yaméogo *et al.* (2001) evaluate the long-term changes of the invertebrate populations with respect of their taxonomic composition as well as their trophic structures. Surber samples provide a qualitative and quantitative assessment of the invertebrate community at the sampling location. They allow a clear examination of the community changes both as taxonomic as well as functional structure. Generally, results indicate that individual larvicides have different impacts on rivers and exhibit a range of effects on various groups of invertebrates. The greatest reduction in the diversity and abundance of the invertebrate assemblages has been detected during phoxim, permethrin, carbosulfan and pyraclofos treatments (Yaméogo *et al.,* 1992). Temephos and B.t H-14 are the less stressing larvicides. The taxonomic units that present the wider changes in relative abundance are Tricorythidae, Leptoceridae, Chironomidae, and Baetidae. From a trophic point of view, all the communities are dominated by the gathering collectors and, to a less extent, by the filtering collectors. The abundance of these feeding groups is a direct evidence of the availability of the fine particulate organic matter that characterizes the food resources within the studied rivers. This dominated structure tends to increase with the application of all insecticides but the B. t. H-14 (Yaméogo *et al.,* 2001). As a whole, results suggest that neither the taxonomic nor the trophic structures are greatly altered from the range of biological, flow-related variation that normally occurs in the studied rivers (Yaméogo *et al.,* 2001, Crosa *et al.,* 2001).

65 This allows concluding that the effect of insecticides on the aquatic fauna is usually low but results for invertebrates in changes of species composition and community structure. However this impact does not affect the general functioning of the aquatic system and is therefore ecologically acceptable. To be sure that there were not irreversible losts of species the question of recovery of the aquatic fauna has been raised by the Ecological Group. The basic question was: does the aquatic community return to a structure and species composition more or less similar to the pre-treatment one at the end of the treatment period? Actually, field data provide indications that recolonization by taxa (for example, *Neoperla* sp. and *Caridina* sp.) which had been affected during treatment period was observed at a majority of stations after larviciding stopped. The ability of the aquatic fauna to recover in treated rivers has therefore been demonstrated, even if it is at a slow rate.

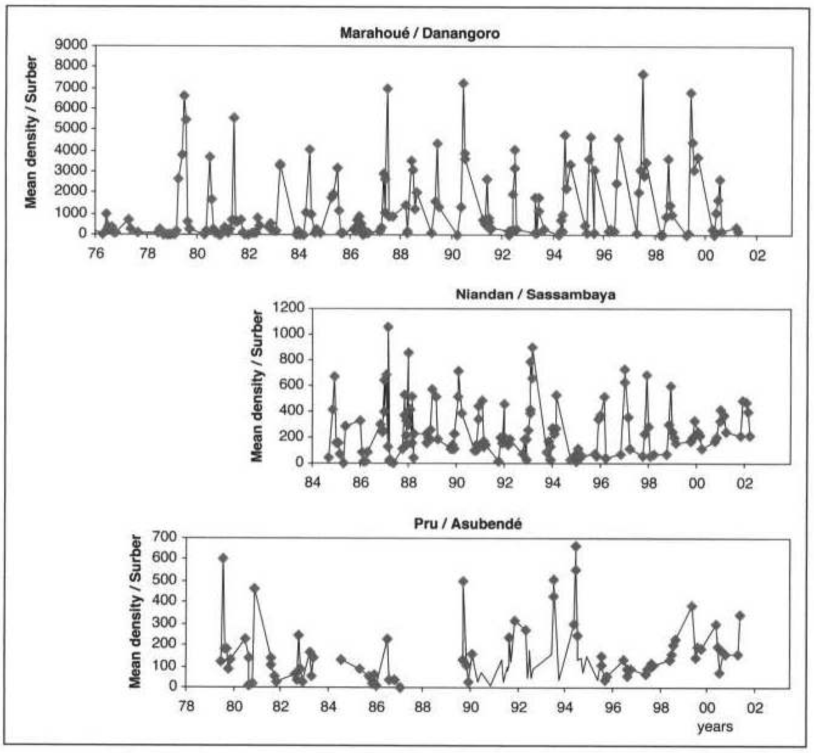


Fig. 12
LONG-TERM CHANGES IN THE DENSITY OF INVERTEBRATES ON THE ROCKS, AS ESTIMATED USING SURBER SAMPLERS.

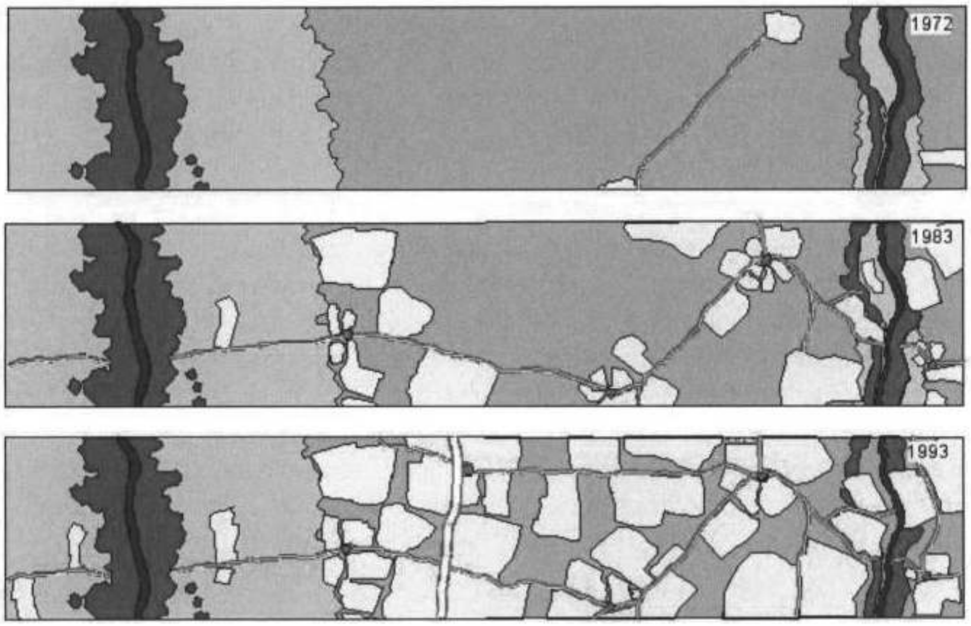
The goal is achieved, but what about the future?

66 The OCP in West Africa closed in December 2002, after 29 years activities. There is no equivalent of a public health programme benefiting for so long a financial support of the international community. One of the reasons for this support is that OCP always convinced the donors of the effectiveness of the control strategies used. The other reason has been the permanent concern of OCP to take care of the aquatic environment with the involvement of national teams and international expertise. The implementation of a long-term monitoring programme to assess the potential effects of larviciding, and the large-scale screening of larvicides to select the most efficient for *S. damnosum* while the less drastic for the non-target fauna, are unique features in large health control programmes. These efforts had an economic cost in terms of insecticide consumption and operational strategies but they made it possible to preserve the quality of the water used by the riverine populations as well as the fishing resources which constitute a significant part of the foodstuff of these populations.

67 The goal is achieved: onchocerciasis has been virtually eliminated from the OCP area as a disease of public health importance, and as an obstacle to socio-economic development. By eliminating the threat of blindness, OCP has made possible the repopulation of those river valleys which had formerly been deserted by fear of the disease. Practically, no new infection is currently recorded in this zone and one of the most significant results is that 18 million children born in the OCP area have no longer been infected by onchocerciasis. However the control of filariasis is not over. Indeed, OCP never aimed at the eradication, neither of the parasite nor of its vector and onchocerciasis will still be present. The

capacity to manage the “risk” of onchocerciasis will constitute a new challenge in the future.

68 From the environmental point of view, the success of OCP may be jeopardized by an unsustainable use of the freed land. For example, a pilot study conducted in the Léraba area thus showed that 75 % of the original wooded savannah was cleared for agricultural development and the settlement of villages (BALDRY *et al.,* 1995).The riverine forests of many small rivers were destroyed and on some of the banks, soil erosion is going on. On the other hand, the bordering forests and the easily flooded plains of the larger rivers did not undergo any disturbance of this scale. It is therefore necessary both to take measures and sensitize the riverine populations on the need for environmental protection and biodiversity management along with the development of agricultural activities. In other words, that is the field application of the principles of sustainable development as developed at the conference on the planet Earth in Rio in 1992, and in Johanesburg in 2002.



BURKINA FASO 'S SIDE OF THE LERABA RIVER
Fig. 13
CHANGES IN LAND USE IN THE VICINITY OF THE LERABA MONITORING STATION

Blackfly nuisance

69 In certain areas, the strong decrease of blackfly bites has been a relief for riverine populations whose life and/or working conditions have been improved. That was the most immediate benefit perceived by populations from the activities of OCP at the beginning of the Programme.

70 Larviciding has, however progressively ceased from 1990 in the regions where the disease was under control and the blackfly bite rates again reached high levels in some areas. Even though blackflies no longer transmit onchocerciasis their reappearance was perceived by populations who associated these insects with transmission of the disease, as the comeback of onchocerciasis. On the other hand, blackfly bites are also a real

nuisance that could hinder the ongoing socio-economic development of the river valleys (HOUGARD *et al.,* 1998).

71 To take care of the public concerns OCP encouraged individual actions against blackflies, through low-cost control techniques on the ground for dealing with breeding sites. Transfer to villages and development units has been achieved in different areas. However; larvicide applications performed by non specialists for an indeterminate period could carry an enormous risk of environmental pollution. The use of two insecticides which present low environmental hazards (B.t. H-14 and temephos) has been recommended by OCP. However, once OCP closed, there is a risk that these communities will use the insecticides that are available locally for agriculture purposes for instance, with the attendant danger of causing resistance and contaminating the environment.

Increasing knowledge and expertise

72 Apart from the success of OCP in controlling the disease, there are also several positive consequences which may be stressed.

Improvement of national expertise in the field of river ecology and management

73 A significant contribution of OCP is the training of national scientists in relation with the monitoring of aquatic environment and related research programmes. As part of the training, several national scientists received grants to be trained in Africa and in northern countries. The result is in an overall improvement of the expertise in aquatic biology and in environmental sciences.

A better knowledge of the ecology of West African rivers

74 Before OCP, the knowledge of the ecology of African rivers was very poor. The implementation of the monitoring programme leads to:

- A better knowledge of the fauna and ecology of West African rivers, particularly for insects and fish (DEJOUX *et al.,* 1981; de MÉRONA, 1981; ILTIS, 1983; ILTIS and LÉVÊQUE 1982; GIBON and STATZNER, 1985; LÉVÊQUE *et al.,* 1990, 1992);
- A better knowledge of the-long term dynamics of aquatic populations in relation with climatic changes and human influences ;
- The acquisition of a bulk of information on reaction of African aquatic fauna to diverse Chemical products ;

75 All this knowledge will be useful for other developmental activities and for conservation of the west African environment.

Establishment of an aquatic monitoring data base : an exceptional property

76 All results recorded during the environmental monitoring of the Programme have been constituted into a data base managed and available at the OMS Head-quarters in Ouagadougou. This data base, updated and validated on the ecology of West African rivers generated during a period of more than 25 years, is a unique heritage.

Sélection d’articles du programme OCP / Selected OCP papers

Sélection d'articles du programme OCP

Selected OCP papers

Nous remercions les éditeurs qui ont accepté de nous céder les droits de reproduction sur les articles publiés dans leur revue par les chercheurs du programme de lutte contre l'onchocercose en Afrique de l'Ouest. Ils nous ont ainsi permis de rassembler en un volume unique l'ensemble des travaux réalisés dans le cadre de ce programme scientifique.

Our thanks to all the publishers who let us have the reproduction rights to articles published in their journals by researchers in the Onchocerciasis Control Programme in West Africa. This has allowed us to put together in one volume all the research conducted under this programme.

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Environmental Assessment of Larvicide Use in the Onchocerciasis Control Programme

D. Calamari, L. Yaméogo, J-M. Hougard and C. Lévêque

1 *The objective of the Onchocerciasis Control Programme (OCP) is to eliminate onchocerciasis as a disease of public importance and as an obstacle to socio-economic development. The OCP was initially based solely on the control of the blackfly vector,* Simulium damnosum *sensu lato, by insecticide spraying of the breeding sites on river Systems, where larval stages develop. Results of monitoring the environmental effects and the process of risk assessment for new insecticides are reviewed. The achievements of this strategy are outlined here by Davide Calamari, Laurent Yameogo, Jean-Marc Hougard and Christian Leveque.*

2 Onchocerciasis (river blindness) is caused by a filarial worm *Onchocerca volvulus;* the microfilariae live in the skin and cause debilitating skin lesions and, ultimately, blindness. West Africa used to be the most affected area in terms of the distribution and severity of clinical manifestations of the disease. Since 1974, successful control has been undertaken through the Onchocerciasis Control Programme (OCP)[1-3].

3 The strategy of larviciding is designed to interrupt the transmission of the parasite for longer than the longevity of the adult worms in a human host (estimated to be ~14 years) by destroying larval stages of the vector through aerial application of insecticides at breeding sites. The development of the aquatic stage from egg to pupae is around one week, hence insecticide application is undertaken weekly. At the peak of larviciding activities, during the years 1986-1991, ~50 000 km of river were treated in an area of over 1 000 000 km^2.

4 Recently, the introduction of a microfilaricide (ivermectin) for mass chemotherapy suggests that the combined use of ivermectin and larviciding should reduce the required duration of vector control to 12 years[4]. Despite the introduction of ivermectin, and in the absence of a macrofilaricide, vector control remains the method of choice to achieve the goals of the OCP[5]

5 Because prolonged and regular use of insecticides presents a potential risk to the aquatic environment, an ecological group was set up, prior to launching the OCP, in order to: (1) organize a long-term monitoring programme of the aquatic fauna; (2) identify criteria for the selection of pesticides for operational use, and determine the conditions by which insecticide use can be optimized in relation to season and environmental factors in different areas of the OCP; and (3) review the nature of the agricultural development process being undertaken and proposed in the areas liberated from onchocerciasis, and identify the environmental and human ecological implications of such development.

6 Although methods, procedures and protocols for the monitoring and risk assessment programmes have evolved since 1974, with the OCP responding to continually changing situations (insect re-invasion, resistance, variability in hydrology, etc.), basic concepts remain. The monitoring activities conducted by National Hydrobiological Teams and the ecotoxicological research carried on by the Hydrobiological Unit of the OCP, which also coordinates and assists the field work in the OCP area, are reviewed annually.

7 The criteria for the evaluation of insecticide impact on aquatic environment include: (1) the vector control activities should not reduce the number of invertebrate species, or cause a marked shift in the relative abundance of species; (2) the pesticides applied should have neither a direct impact on fish, nor an effect on the life cycle of fish species; (3) bioaccumulation and biomagnification through food webs should be avoided; (4) human activities in the control area should not be impaired; and (5) temporary and seasonal variations in non-target invertebrate populations are acceptable.

8 A network of sampling stations exists throughout the OCP area where selection criteria have been met[6]. Initially, there were 40 sampling sites, but as the programme evolved together with the treatment strategy, the number has been reduced to ten for invertebrates and ten for fish (Fig. 1).

9 Details of the methods and sampling techniques for OCP monitoring programmes have been described[7-9]. It was realized, however, that information on the biology of the aquatic fauna in West Africa was generally limited, and so specific studies were undertaken to provide ecological baseline information.

Vector control

10 Vector control has faced three major obstacles[5]. (1) It was established that the border of the initial area (654 000 km^2 spread over seven countries) was invaded by migrating infective blackflies originating from outside the designated area. To protect the core area, vector control was extended (between 1986 and 1990) in the west and southeast, with two additional countries joining the OCP. (2) Five years after the beginning of the OCP, resistance of *Simulium damnosum* to temephos was detected[10]. This organophosphorous compound, the only insecticide used at the beginning of the programme, was selected because of its efficacy, its carry (distance over which it remains effective), its limited impact on non-target fauna and also its acceptable cost. After the development of insect resistance to another organophosphate (chlorphoxim) was detected, the OCP adopted a strategy of rotational use of different classes of insecticides, with different modes of action, to prevent development of further resistance. (3) Seven insecticides are currently used[5]: six of them formulated as emulsifiable concentrates (temephos, phoxim, pyraclofos, permethrin, etofenprox and carbosulfan), while the seventh is a liquid

concentrate of a biological insecticide, *Bacillus thuringiensis* H-14 (Bt-14). The rotational use of insecticides has been particularly effective, with only limited resistance to the organophosphates currently in use, while the susceptibility of the *Simulium* populations to other classes of compounds remains unchanged.

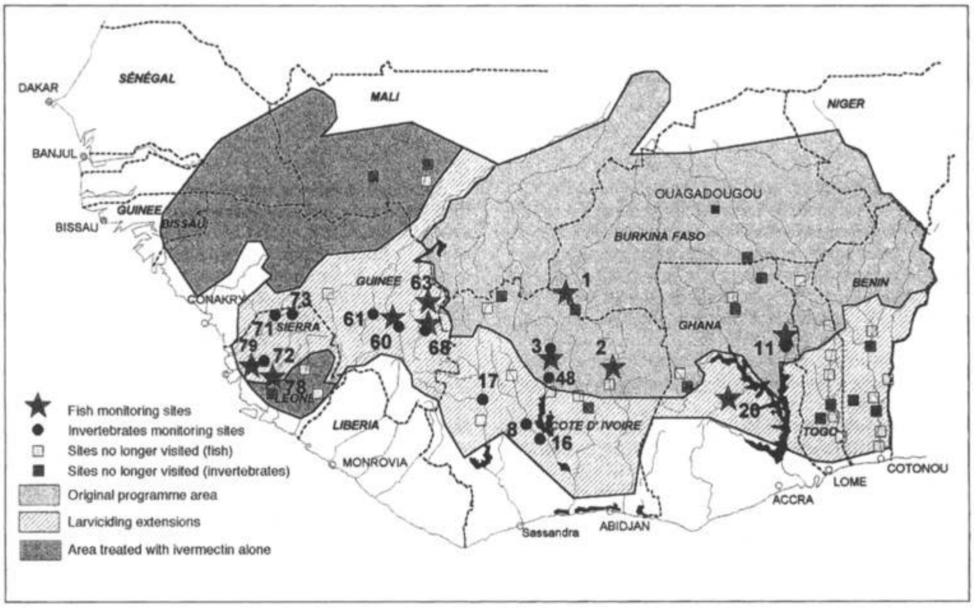

Fig. I. Onchocerciasis Control Programme (OCP) area and location of invertebrate and fish monitoring stations.

11 The consideration of environmental concerns has resulted in a number of limitations and restrictions in the use of the available insecticides for the rotational vector control.

Invertebrate and fish monitoring results

12 For the monitoring process, invertebrate data have been collected in each river and analysed independently, distinguishing, for each river, the three types of sampling technique adopted. Invertebrates collected directly from the river-bed by means of a Surber net (named invertebrate community, living exactly at the sampling station), day and night drift, where the nets capture animals transported by water (named invertebrate assemblages, which include organisms from upper river locations).

13 Numerical analysis methods to assess the variations in invertebrate community structure during the periods of treatment and during the periods when larviciding is suspended permit the evaluation of two principal properties of the biological communities: (1) their resistance (capacity of contrasting stress factors); and (2) their resilience (capacity to recover after stress).

14 The taxonomic levels as well as the functional feeding group (trophic role) of the invertebrates sampled have been determined; the analysis of the biological variation has been focused on these structural and functional attributes. The OCP concern is to prevent the loss of faunal biodiversity, and to maintain the quality of the biomass available for the higher trophic levels in the food webs, while conserving the stability of the energetic flows (quantity). As the invertebrate communities represent lower levels of the aquatic

food webs, any change can be a signal of potential detrimental effects on the ecological characteristics of the whole river System.

15 On the basis of preliminary results, the following analyses were undertaken: (1) invertebrate taxonomic diversity; (2) relative abundance of functional groups; (3) rank abundance models; and (4) multivariate analysis[11].

16 The taxonomie diversity indices calculated during pretreatment periods showed a normal structure of invertebrate communities. The result was obtained from relative abundance of functional groups and diversity indices and from rank abundance models.

17 During separate insecticide treatments, some rivers showed only limited changes in invertebrate community structure; in others, great changes occurred.

18 Individual larvicides have different impacts on river fauna, depending on the river System to which they are applied. Moreover, each insecticide shows a characteristic distribution of effects on various invertebrate taxa, thereby providing a 'fingerprint', because of selective action on particular groups of insects, according to the mode of action of different types of larvicides[12].

19 Rotational use of the larvicides has led to difficulties in interpreting some biological data because of the additional effects related to previous treatment history. This is most frequently seen during a period of suspension, when the invertebrate recovery is related to the type and the severity of the stresses that occurred during past treatments.

20 A first survey of the impact of the larviciding campaign was made after the initial phase of the programme, when only temephos, chlorphoxim and Bt-14 were applied. This demonstrated an initial deleterious impact on non-target fauna during the first year (limited reduction in biomass and number of taxa). However, not-withstanding the continuation of the treatments, there was a partial recovery within a year. Long-term studies (several years) showed such types of insecticides had little impact[6,7].

21 A recent evaluation of 20 years of OCP monitoring data[11,13], for a period when different groups of Chemical substances (eg. carbosulfan and permethrin) were used, showed greater effects of the insecticides on the invertebrate fauna, particularly the rarefaction of a few taxa and, for example, in the case of permethrin, a biomass reduction. However, the ability of the aquatic biota to recover was demonstrated, even if, at a slow rate. Therefore, the taxonomic and functional variability in non-target invertebrate fauna seems to be compatible with the range of biological variation that would normally occur in these river Systems[11,13].

22 In the normal situation, invertebrate communities are rarely constant in taxonomic and trophic composition because of natural stresses (eg. unusual hydrological conditions, such as drought and spate events), which occur frequently. When these factors are taken into consideration, the 'biological variations previously discussed are ecologically acceptable': a conclusion confirmed by analysis on fish monitoring[14,15].

23 Fish populations at most monitoring sites show no evidence of a reduction in species richness after almost 20 years of larviciding in the original area of the programme, and seven years of larviciding in Guinea[15]. In an earlier evaluation[6,16], the same conclusions were reached after ten years of larviciding with temephos, chlorphoxim and Bt H-14. Although the total number of species could mask changes in species composition, results from all rivers showed no loss of species after 20 years of larviciding.

24 Fish catches per unit of effort (ie. a standard time during which nets remain in the river) with a standardized set of gill nets has demonstrated a seasonal pattern, with high catches at the low water period (January – April) and lower catches in the wet season (August – November). Long-term, yearly fluctuations are well correlated to the hydrological pattern, as has been observed for African rivers[17].

25 The coefficient of condition, a ratio between weight and length (corpulence), of the principal species of fish fluctuates around the expected means, which have not changed significantly during the programme. This suggests that larviciding has no effects on food availability, and that the feeding habits of fish have not altered[18].

26 There has, therefore, been no evidence of fish mortality due to larviciding at the operational doses in the OCP area. However, fluctuations have been observed in the abundance of biomass and richness of fish species. These variations are correlated to hydrological patterns, and can also be attributed to the pressure exerted by local fisherman, in certain areas.

27 As a result of the first ten years monitoring the use of organophosphate insecticides and Bt-14, the Ecological Group decided to reduce the number of monitoring stations, and to change monitoring strategies by abandoning drift-sampling[19].

28 Moreover, during the annual evaluation of the research on new insecticides (see below), and as derived from monitoring data, several means of reducing the impacts of new Chemicals have been suggested. The following proposals have been made: (1) temephos and Bt-14 are considered as harmless for the environment and can be used without restrictions; (2) pyraclofos might show some toxicity (for example, in an accidental overdose) against the non-target fauna, particularly fish, and it is recommended that it be used at discharges above $15m^3\ s^{-1}$ but without any restriction on the number of cycles; (3) permethrin and carbosulfan should only be used above $70m^3\ s^{-1}$ and (if possible) for not more than six weeks per year on the same stretch of river; and (4) although the toxicity of etofenprox for fish and crustaceans is less than permethrin, its utilization should remain limited to discharges above $15\ m^3\ s^{-1}$ but without any restriction on the number of annual cycles.

29 For these reasons, the OCP has established a satellite transmission network for recording water discharge, allowing for in-time management of hydrological data in the treatment of the rivers[20].

Risk assessment for new larvicides

30 In addition to the above monitoring activities, the OCP has carried out a number of ecotoxicological studies on candidate larvicides.

31 Acute toxicological tests have been performed on African fish species according to standard protocols[21] to obtain original data on fish toxicology. Short-term impact on non-target invertebrates was also studied using a System of artificial gutters (semifield equipment). Qualitative and quantitative drift in such Systems have been evaluated at different concentrations, including the operational dose, and the larvicides classified according to their general toxicity and the typology of the susceptibility of the principal taxa established[22-23].

32 Bt-14 has proved to be the most selective and the least environmentally damaging larvicide of the seven. The other insecticides can be classified as follows, in increasing order of toxicity: temephos, chlorphoxim, pyraclofos, etophenprox, permethrin and carbosulfan.

33 Among the taxa studied, the Baetidae were the most susceptible to the Chemical larvicides. The Hydropsychidae were fairly susceptible, while the Chironomidae were the organisms least susceptible to most of the insecticides. Results suggest that the more toxic the insecticides were in gutter tests, the more the population abundance differed from the reference ones in the field.

34 A risk assessment in different steps was performed for every new larvicide to be used in the OCP. After the first trials, where efficacy on blackflies was evaluated (as well as physical properties such as dispersion and viscosity), a review of the literature was performed, together with some laboratory tests on fish, and gutter tests on invertebrates. The Ecological Group reviewing the results recommended, in cases of acceptable toxicological results, small-scale pilot studies in the field, with spraying to be carried out a few meters upstream of the evaluation zone.

35 For the most toxic insecticides that have many advantages to their possible use (such as low cost, wide range of applications in relation to discharges and long carry distance), large-scale studies below the operational dose were recommended. This allows a complete risk assessment scheme to be obtained[24] before the insecticide is used as an operational larvicide. Permethrin, for example, was tested in this way[25]. After 15 weekly applications at its operational dose, benthic fauna density and diversity were severely affected. However, both drift and benthic fauna recovered almost to pretreatment levels a month after the treatment was terminated, but one taxa remains absent. Fish showed some evidence of stress, but remained in the treatment zone, allowing catches to be compared with those of the control station. No fish mortality was observed, nor any variation in the condition factors of the fish.

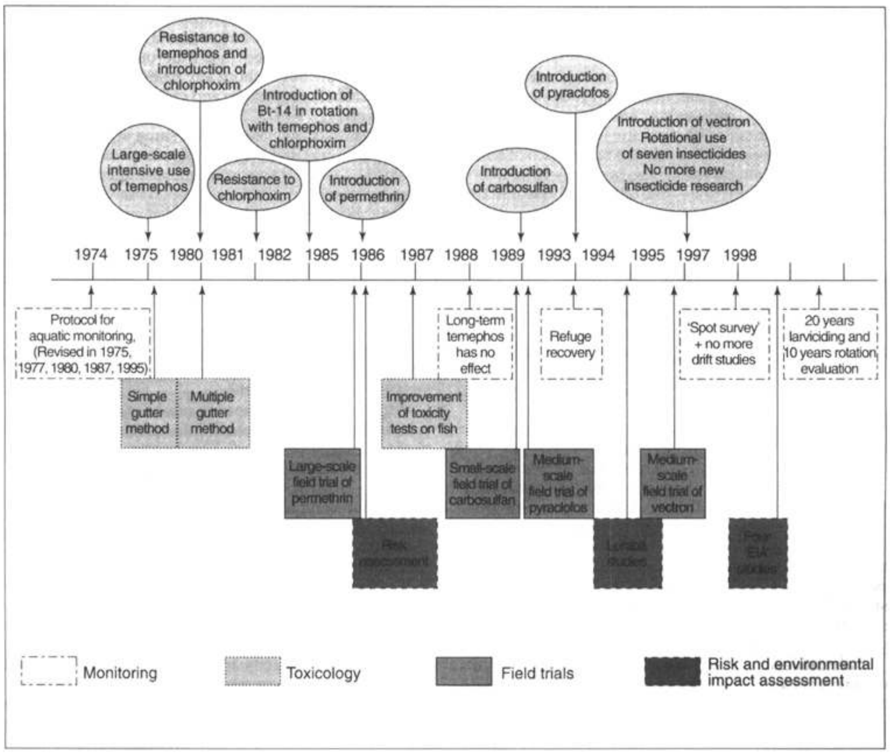

Fig. 2. Synopsis of the Ecological Group activities. Introduction of the operational insecticides and the different toxicity tests in the programme. Bt-14, *Bacillus thuringiensis* H-14 ; EIA, environmental impact assessment.

36 All these studies have allowed the OCP and the Ecological Group to agree on the optimal use of larvicides that limits the environmental impact. A synopsis of the activities of the Ecological Group is shown in Fig. 2.

Environmental impact and development

37 The success of the OCP in vector control in West Africa has allowed extensive areas of previously abandoned valleys to be resettled and developed.

38 Thus, despite the long-term use of larvicides in the rivers of the OCP area, the quality of the aquatic environment has been preserved, largely because of the precautions taken, and because of the absence of human population pressure in the areas most severely affected by the illness. However, the situation is now changing rapidly, and there is an increasing concern for the aquatic and terrestrial environments, because of population movements and land recolonization.

39 It was recognized, therefore, that there was a need for the formulation of an appropriate methodology for environmental impact assessment. A pilot project in the Upper Leraba Basin was launched in 1993, the primary objectives being: (1) to assess the present environmental situation in the basin, with a view to determining potential sources of impact on the aquatic environment; (2) to quantify Chemical loads and assess the modification to the physical environment; and (3) to identify simple study methods applicable to other areas, and investigate ways to minimize impacts.

40 An evaluation of the changes in the physical environment was made for the period between 1972 and 1993. These studies showed that about 75% of the original savanna

woodland had been cleared in the past few years for settlement and agricultural development. There had been no observed significant disturbance of the riverine forest and associated floodplain grasslands of the main rivers System, and organic loads and nutrients were only of relevance along limited stretches of the main Leraba River, where point sources of contamination can be identified (eg. human and cattle wastes).

41 The presence in river water of pesticides used for cotton protection was calculated from the quantity of Chemicals used locally by means of simulation models, which permitted the concentrations of pesticides in the river to be estimated. Although the predicted concentrations of pesticides in river water were not at levels that would cause damage or give cause for alarm, they could nevertheless be considered as early warning signais[26]

Conclusions

42 The OCP has repeatedly been able to demonstrate that, despite the weekly application of larviciding over a long period, there has been no significant deterioration of the aquatic environment.

43 The resuit of the OCP activity should not only be regarded as a success in curbing and controlling river blindness, but also as an important example of Sound environmental management in a programme that, from the beginning, had included ecological considerations in planning vector control activities.

Acknowledgements

44 The authors are grateful to the OCP Director Yankum K. Dadzie for support and encouragement, and to David Molyneux, Chairman of the Expert Advisory Committee, for his help and suggestions about the manuscript.

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Long term quantitative ecological assessment of insecticides treatments in four African rivers: a methodological approach

G. Crosa, L. Yamaego, D. Calamari and J.M. Hougard

1. Introduction

1 *Onchocerca volvulus,* a parasitic worm giving rise to skin reactions and eventually severe ocular lesions followed by blindness [1], had been a major public health problem in many fertile valleys of West African countries. The parasite is transmitted by the female blackfly of the *Simulium damnosum* complex [2] and, being difficult to control the adults, it was decided to destroy with insecticides the vector at its larval stages whose distributions is limited to rapids.

2 The initial phase of the project (Onchocerciasis Control Programme - OCP) covered a vast area of 764 000 km^2 in which up to 18 000 km of rivers had been partly weekly sprayed with larvicides. To prevent flies reinvasion, from 1989 the original programme was expanded to 1 235 000 km^2, controlling about 50 000 km of rivers.

3 Obviously such an extensive and prolonged use of larvicides could have important environmental risks, therefore an aquatic monitoring programme has been settle up ffom the initial phase of the program to evaluate the possible long-term effects of the larvicides on the non target fauna.

4 From 1975 to 1985 temephos, chlorphoxim (two organophosphorous compounds) and a biological insecticide, *Bacillus thuringiensis* var. *israelensis* [B.t. H-14] had been the larvicides used.

5 From 1980, the appearing of certain forest cytotypes of the vector resistant to temephos [3] and to chlorphoxim by 1982 [4] forced the search of new compounds and the implementation of a renewed treatment strategy based on the rotational use of the

insecticides with suspension periods. The new compounds were: permethrin (pyrethroid), carbosulfan (carbamate), pyraclofos (organo-phosphorus) and vectron (pseudo-pyrethroid). After the papers of Lévêque et al. and Yaméogo et al. [5,6] that provided a comprehensive evaluation of the first ten years monitoring of fish and non target insects populations, the biological data collected within a wide area till 1998 allow to face new questions arising from the implementation of the OCP.

6 It is now possible a better evaluation of: i) the long-term changes of the invertebrate and fish populations with respect to their taxonomic composition as well as to their trophic structures, ii) the severity of each specific larvicide used during the programme, iii) the resistance of the communities to the induced stresses and iv) their recovery capacity.

7 Approaching the analysis of the invertebrate data collected in four countries during a period ranging from 1977 to 1996 for addressing the above mentioned questions, this paper presents and discusses the data collections and the applied quantitative and qualitative numerical methods of analysis used in ecology, with the major objective of commenting their different contribution and the complementary informations they provide to the evaluation and comprehension of the data variation.

8 Examples of the application of the discussed methods are presented, while the detailed results of the analyses will be published in a forthcoming paper which inelude fish data.

RIVER	STATION	COUNTRY	MAXIMUM SAMPLING PERIOD
Entomokro	Danangoro	Ivory Coast	Dec. ‘77-Feb.’ 96
Pru	Asubende	Ghana	Gen. ‘80-Apr. 95
Niandan	Sansambaya	Guinea	Dec. ‘84 - Apr. ‘94
Kaba	Outamba	Sierra Leone	Mar. ‘89 - Apr. ‘94

TABLE 1: LOCATION OF THE SAMPLING STATIONS AND MAXIMUM SAMPLING PERIODS.

2. Available data and sampling methods

9 The study addresses the invertebrates collected during the dry season in four rivers located in West Africa during the time extents and within the sampling stations indicated in table 1. The rivers are savanna type with a water regime characterised by high discharges from July to November and a low water period from January to June, details on hydrological and physicochemical charactristics of the rivers are reported in Ilitis and Lévêque [7] and in Moniod *et al* [8].

10 The organisms were sampled, by means of Surber net and as day and night drift, during the application of the larvicides as well as during suspension and pre-treatment periods. Details of monitoring and sampling methods can be found in Lévêque et al. [9] and Dejoux et al. [10].

11 The three sampling strategies, Surber samples, day and night drift, had been employed in order to collect biological data showing different informations.

12 Night drift, which is supposed to be mainly voluntary, reflects an active period while the number of the day drift organisms is related with their health condition.

13 As regards the use of the Surber net, this technique allows to sample, in a quantitative way, the organisms living in specific river areas and for this reason the collections reflect the structure of the benthic communities, where this term indicate an assemblage of interacting individuals sharing at the same time the same space. Obviously this concept of community is less applicable to the drift samples which represent collections of organism turning up from a wider area located upstream the sampling site and thus mainly controlled by factors externai than those related to the sample location.

14 Because of this possible source of bias the presented examples of the numerical methods have been applied to the Surber samples.

15 All the sampled individuals were classified according to their taxonomic level as well as their trophic role, this second classification method being based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories: gathering and filtering collectors, predators, shredders and scrapers [11]. To avoid the presence of rare taxa only the principal systematic units belonging to the Ephemeroptera, Trichoptera and Chironomidae were used for the analyses.

3. Aims and Protocols

16 The data analysis strategy has proceeded along two relatively distinct paths, the first considered the evaluation of the changes in the invertebrate taxonomic and functional structures, the second has been oriented towards the examination of the main fractions of the taxonomic variation by means of multivariate techniques of analysis.

17 On the basis of the results of a preliminary data inspection, the following analysis techniques were selected for the study.

18 - *Invertebrate taxonomic diversity* Three aspects of the taxonomic diversity have been estimated: taxa richness, the distribution of the individuals among those taxa (equitability or evenness) and the heterogeneity, a measure that encompasses the first two diversity measures. The nonparametric indexes utilised were, respectively, the Margalef richness index: (S-1)/ln N, the Pielou evenness index: H'/H' max and the Shannon heterogeneity index: $H' = -\Sigma p_i \ln(p_i)$.

19 For the representation of the invertebrate taxonomic structure, occurring during the pretreatment, treatment and suspension periods, the median values of the indexes was preferred to the average values, for that the median values are less outliner sensitive.

20 - *Relative abundance of the functional groups.* The relative abundance of the invertebrates classified as functional groups were estimates for each treatment period. On the fact that the trophic structure is a property of the living organisms emerging at community level, only the Surber samples have been used for this analysis.

21 - *Rank abundance models.* This graph-approach to the analysis of the biological structures consists in a conventional form of presenting the importance of each taxon as abundance (*y*-axis) with the different taxa concerned arranged in rank order along the *x*-axis from the commonest to the rarest. The pattern of the line connecting the taxa of each sample allows a visual examination of the invertebrates structures: S-shaped curves are related

to high heterogeneity values, on the contrary high slopes indicate more dominated structures The comparison of the curves permits to detect the changes that can take place in the invertebrates structures during the different sampling periods.

22 In the example graphs the species abundance are represented by means of the median values occurring during the pre-treatment, treatment and suspension periods.

23 - *Multivariate analysis.* Because of the linear response of the invertebrates abundance Principal Components Analysis (PCA) was preferred to the unimodal multivariate analysis approaches (i.e. Correspondence Analysis sensu ter Braak [12]). The PCA was performed to the log-transformed abundance of the taxa collected by means of Surber net.

24 The analysis has been applied separately to each river communities; an all rivers data matrix has not been analysed because a preliminary ordination of this matrix showed a wide variation in the invertebrate communities among the rivers.

4. Discussion and Conclusions

25 For the selection of the appropriate analysis methodology, the following problems, other than the different sources of bias detailed by Lévêque [5] were considered:

i. insufficiencies of pre-treatments samples and replicates that constrained the use of non parametric analyses;
ii. the incremental biological variation because of the rotational use of the larvicides;
iii. the presence of outliner values and
iv. the rivers peculiarities that outlines the importance of considering the different antropic pressure that have been taking place, with different time, in the treated rivers.

26 It has to be firstly noted that the use of different analysis methods on different biological informations (taxonomical and functional) allows itself to face the possible source of bias because of the problems previously listed. Considering that no standard analysis procedures are available for answering the questions addressed by the monitoring programme or, more in general, in the long-term impact assessment studies, the use of different analytic techniques applied on different biological aspects are necessary to corroborate a comprehensive evaluation of the biological data.

27 Among the questions posed in the introduction are the measure of the biological variation because of the larviciding and the evaluation of the severity of each specific larvicide. For addressing these questions the commonly used analysis is PCA (or equivalent ones depending of the data property i.e. Correspondence Analysis for biological variation following unimodal pattern). In Fig. 1 an example of its application to Niandan samples is shown. PCA detects two biological gradients oriented 45° with respect the first two principal components. The first gradient explains the biological variation occurring because of the treatments irrespectively of the applied larvicide (bold arrow in the figure), the second one is related to the biological variation specifically related to each larvicide (dotted arrow in the figure), roughly opposing the data sampled during B.t. treatment and the ones sampled during the application of permethrin.

28 While these gradients demonstrate both a general effect of the larviciding and a different response of the communities to the applied larvicides, it is however difficult from the ordination diagram to define the severity of each specific larviciding (i.e. the taxonomic structures most affected are the ones related to the use of B.t. or to permethrin?).

29 Fig 2a answer to this question, clearly showing the previous severity gradient with the additional graphic information of the levels of the taxonomic structural changes related to each larvicide. Normally structured communities, at equilibrium, generally are composed by taxa having similar abundance and when these are plotted according to their decreasing abundance, the resulting curve is characterised by a clear "plateau". This S-shaped pattern, that in ecological literature is named "broken stick" [13], is clearly evident for the communities sampled during the pre-treatment periods (Fig 2a) and can be used as reference to compare the communities structure models of the treatment periods.

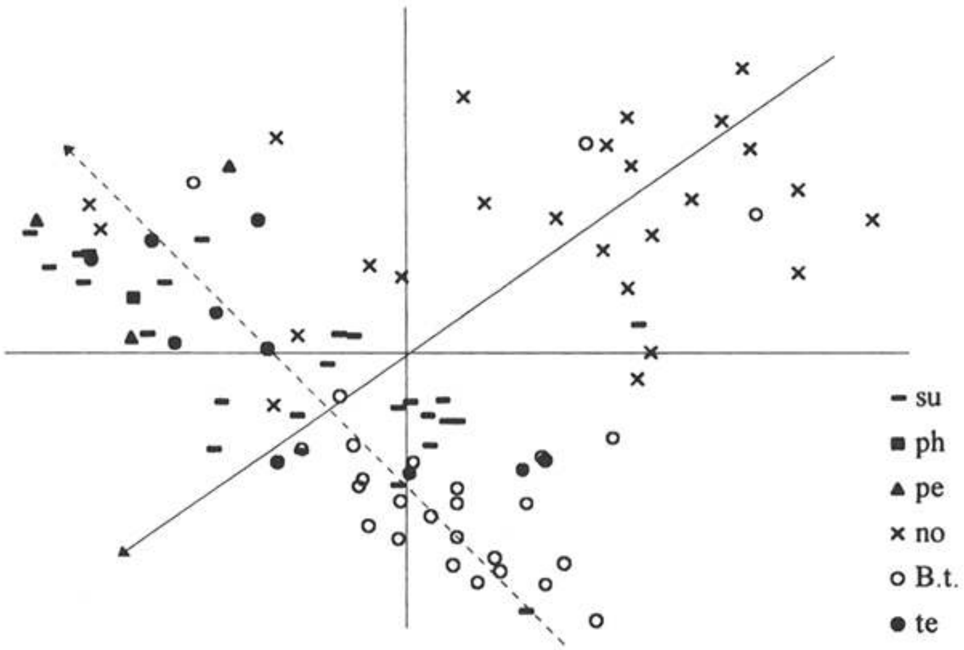

Fig 1. Scatterplot of the samples position according the first two components of the PCA explaining, respectively 43 and 22% of the total data variation, The bold and the dotted arrows show, respectively, the biological variation because of the treatments and the biological variation related to each specific larvicide. Su = suspension, ph = phoxim, pe = permethrin, no = pre-treatment, B.t. = *Bacillus thuringiensis,* te = temephos.

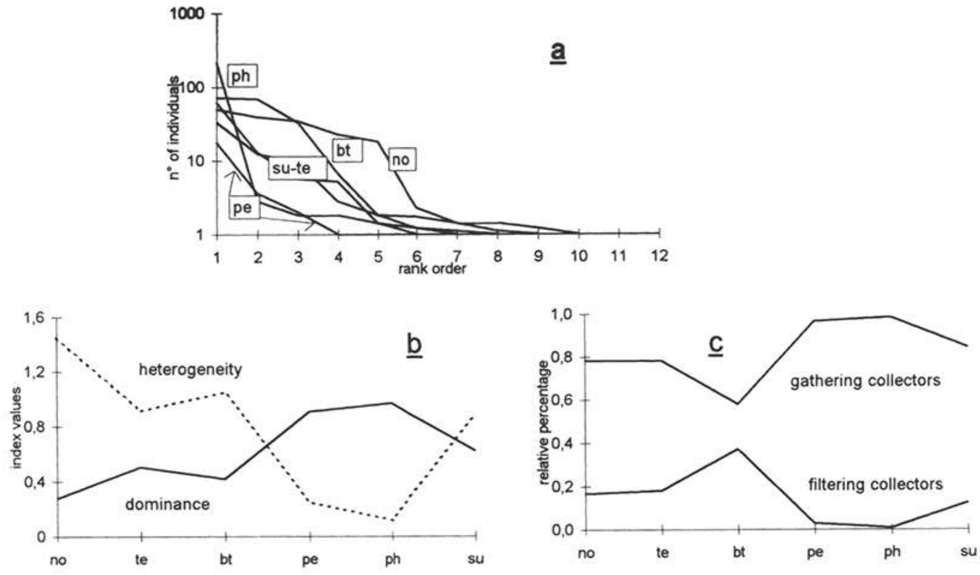

Fig 2. a) rank abundance models showing the taxonomic structure of the invertebrates communities sampled during the different treatments; b) diversity indexes variation according the treatments and c) relative percentage variation of the two most abundant functional guilds. Labels as in Fig. 1.

30 From the visual examination of the rank abundance models it is possible to suggest that the biological communities sampled during the application of permethrin present the highest taxonomical changes, both in terms of abundance (y-axis) and taxa richness (x-axis). On the contrary the model of the invertebrates communities sampled during the use of B.t. shows a pattern similar to the one of the pre-treatment samples. This information it can be now of help in answering to the question about the direction of the severity gradient outlined by the PCA (dotted line).

31 Similar communities response is corroborated by the diversity indexes (Fig. 2b) while Fig. 2c, showing the variation of the two dominant trophic guilds, adds further informations. From this figure a different biological response occurring during B.t. treatment is evident, compared to permethrin and phoxim that it is not clearly outlined by the previous analyses. With reference to the pre-treatment observations, during B.t. treatment, the gathering collectors show a reduction in their relative abundance, on the contrary during the use of permethrin and phoxim the filtering collectors result more affected. This different response of the invertebrate trophic structures can be partially justified by the larvicide properties and the feeding habits of the invertebrates; for example the gathering collectors feed on surface deposit and thus can be more affected by B.t. because to its rapid adsorption to soil particles [14].

32 Considering the third question, the resistance of the communities to the larvicides is measurable, with reference to the pre-treatment data, by the bold arrow in the PCA ordination diagram and by the slope of the rank abundance models.

33 Finally the invertebrates' resilience, can be addressed by inspecting the communities structure variation occurring during the suspension periods. For this, all the analyses applied show that during these suspension periods the communities still present changes in their taxonomic structures, it has to be noted however that these changes do not affect the relative abundance of the trophic guilds.

34 In choosing the analysis strategy, we adopted the concept of "significant ecological change" used by OCP in assessing the ecological impact of larviciding [9]. Whitin the

mandate of the Ecological Group two criteria were indicated "the vector control activities should not reduce the number of invertebrate species, nor causing a marked shift in the relative abundance of species" and "temporary and seasonal variations in invertebrate populations other then Simulium could be accepted."

35 For this we think that, other than considering the social benefit of having oncho free valley in the operational area, the range of biological variation that would normally occur in these river Systems should be taken in account. In fact in the natural situation these river invertebrate communities would rarely be in equilibrium (constant in taxonomic and trophic composition) because of the natural stresses, like drought and spate events, which would occur with great frequency and regularity. When these factors are considered, it can be suggested that the biological variation outlined in the discussed examples can be positively considered and it can be concluded that the OCP has produced limited damages and never caused irreversible canges on the non-target invertebrate populations.

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ABSTRACTS

In West Africa different insecticides had been applied in selected river areas for the reduction of the blackfly populations vectors of *Onchocerca volvulus,* a parasite causing blindness. To evaluate the possible long term effects of the larvicides on the non target fauna an aquatic monitoring programme has been up from the initial phase of the project. Addressing the attention to the invertebrates data collected in four countries during a maximum period ranging from 1977 to 1996, this paper shows and discusses the data analysis strategy for the measure and interpretation of the biological variation. In particular the application of quantitative ecological analysis methods: Principal Component Analysis, rank abundance models and the community diversity indexes, is critically discussed and comments are given to the ecological interpretation of the results.

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Mise en évidence d'une résistance au téméphos dans le complexe *Simulium damnosum* [*S. sanctipauli* et *S. soubrense*] en Côte d'ivoire

Zone du programme de lutte contre l'onchocercose dans la région du Bassin de la Volta

Demonstration of resistance to temephos in simulium damnosum complex (s. sanctipauli and s. soubrense) in ivory coast

Pierre Guillet, Henri Escaffre, Moussa Ouedraogo et Daniel Quillévéré

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1 Le programme de lutte contre l'Onchocercose (OCP) entrepris par l'Organisation Mondiale de la Santé en Afrique de l'Ouest repose sur l'utilisation de larvicides chimiques déversés dans les rivières afin d'éliminer les larves des espèces du complexe *S. damnosum* vectrices de cette endémie. Le téméphos, en concentré émulsionnable (abate [R]) est utilisé par le programme depuis sa création en 1974. Cet insecticide, de par sa forte toxicité pour les larves du complexe *S. damnosum* et sa relative innocuité pour la faune non cible, convient parfaitement à cette utilisation et a toujours donné d'excellents résultats. Cependant les captures de simulies effectuées par le programme sur le bas Bandama en République de Côte d'Ivoire ont montré la présence d'une population de femelles piqueuses anormalement élevée en dépit des traitements. Simultanément, des prospections dans les gîtes ont permis de mettre en évidence la présence de populations larvaires importantes 24 heures ou 48 heures après les traitements.

2 Une étude des facteurs pouvant expliquer ces échecs a été entreprise. Elle porte sur la sensibilité des larves au téméphos, l'efficacité des traitements, la détermination des espèces concernées et enfin la composition physicochimique de l'eau.

1. PRÉSENTATION DE LA ZONE ÉTUDIÉE

3 L'échec des traitements ne s'observe que sur le bas Bandama, en aval du barrage hydroélectrique de Taabo, en zone de forêt tropicale. Les derniers gîtes larvaires se situent au niveau de Tiassalé à 65 km du barrage. Ce bief présente un nombre important de gîtes de grande taille notamment au niveau du lieu dit « Les chutes Gauthier » où s'est déroulée toute cette étude (5° 57' N, 4° 50' O).

4 Le début du traitement de ce bief (mars 1979) est postérieur à la mise en eau du barrage. Le débit est relativement régularisé, variant entre 150 et 250m^3/s . On enregistre cependant des fluctuations journalières dues aux rythmes de fonctionnement des turbines du barrage. L'étendue des gîtes et l'abondance des supports pour les larves sont très favorables au développement d'une population composée d'environ 75 % de *S. sanctipauli* et de 25 % de *S. soubrense* (Vajime et Dunbar, 1975 ; Quillévéré et Pendriez, 1975 ; Vajime et Quillévéré, 1978). Les déplacements des femelles semblent très limités (Le Berre, 1966 ; Quillévéré, 1979). Les captures de femelles effectuées par OCP indiquent que depuis le début des traitements, ceux-ci n'ont pas toujours été efficaces à 100 %. Cela peut s'expliquer en partie par la complexité des gîtes et les fluctuations journalières de débit. Le délai écoulé entre la lecture de l'échelle de crues et le traitement a pu conduire souvent à des erreurs de dosage. Les concordances observées dans les débits indiqués simultanément par les échelles de crues relativement voisines ont permis d'éliminer l'hypothèse d'une éventuelle modification du tarrage de l'une ou l'autre d'entre elles.

2. MATÉRIEL ET MÉTHODES

5 2.1. La sensibilité des larves a été mesurée suivant la méthode préconisée par Mouchet *et al.* (1977). Les tests sont effectués dans des bols en verre où sont placées 25 larves de stades IV et V dans 250 ml d'eau distillée. Le contact dure 3 heures à l'issue desquelles est effectuée la lecture de mortalité. La température de l'eau est maintenue entre 20 et 25° C. Le téméphos utilisé est une solution éthanolique de téméphos technique. Le critère adopté pour différencier les larves moribondes des vivantes est la réaction immédiate de repli de ces dernières au contact de la pince. Au cours de la dernière série de tests, les larves moribondes aux concentrations de 0,125 mg/l et plus ont été mises en observation. Dès la fin du test, ces larves sont prélevées, rincées puis transférées dans 250 ml d'eau distillée aérée par un diffuseur relié à un compresseur d'air. La durée de l'observation est de 6 heures.

6 2.2. L'efficacité des traitements au téméphos a été évaluée en utilisant un dispositif de minigouttières. Celles-ci sont alimentées par gravité avec de l'eau de la rivière préalablement filtrée à 120 µ. Les larves sont installées dans les gouttières au minimum 2 heures avant le début des traitements. En l'absence de traitement, la dérive spontanée des larves dans ce type de gouttières est très faible (1 à 3 % en 24 heures).

7 2.3. Les larves de *S. damnosum* s. 1. survivant dans les tests de sensibilité aux concentrations supérieures ou égales à 0,25 mg/1 ainsi que dans les gouttières après les traitements sont fixées dans le liquide de Carnoy pour l'identification spécifique à partir des chromosomes.

8 2.4. Des échantillons d'eau ont été prélevés en trois points : en amont du barrage, immédiatement en aval et aux chutes Gauthier (à 55 km en aval). Ces échantillons ont été analysés par le laboratoire d'analyses de l'O.R.S.T.O.M. à Abidjan. Des mesures extemporanées de *p*H ont été effectuées *in situ* à l'aide d'un *p*H-mètre électrique au moment de la collecte des échantillons. Des prélèvements de phytoplancton ont également été effectués et identifiés au Centre de recherches océanographiques de l'O.R.S.T.O.M. à Abidjan.

3. RÉSULTATS

3.1. Sensiblité des larves au téméphos

3.1.1. Sensibilité avant les traitements

9 Deux séries de trois tests ont été effectuées aux chutes Gauthier en janvier et juin 1977. Les deux droites obtenues à partir des récapitulatifs de chaque série sont très voisines et parallèles (fig. 1). Les résultats obtenus sont très homogènes et dans tous les cas, la limite supérieure de la CL100 a été de 0,125 mg/1 (tabl. I). Il faut noter cependant que la population des chutes Gauthier avait fait l'objet de 7 séries de traitement à l'abate en 1976.

3.1.2. Sensibilité actuelle des larves

10 Les résultats obtenus au cours de deux premières séries de tests sont tout à fait semblables. La CL95 est d'environ 0,2 mg/1 et la limite supérieure de la CL100 de 0,5 et 0,625 mg/l (tabl. II et III, fig. 1).

11 Au cours de la troisième série de tests, les résultats obtenus sont plus hétérogènes. Un premier test, réalisé à partir d'un seul support abondamment peuplé de jeunes larves (majorité de stades III et IV) a indiqué une sensibilité presque normale avec une limite supérieure de la CL100 de 0,25 mg/1 (tabl. IV). Trois autres tests, réalisés à partir de larves provenant d'un grand nombre de supports ont donné des résultats très différents avec 14,7 % de survivants à 0,312 mg/1 et 3,7 % à 0,625 mg/l[1]. (La pente de la droite de régression est encore plus faible que celles obtenues lors des deux premières séries (tabl. V, fig. 1). Le rapport CL99,9 actuelle/CL99,9 avant traitement atteint des valeurs allant jusqu'à 45.

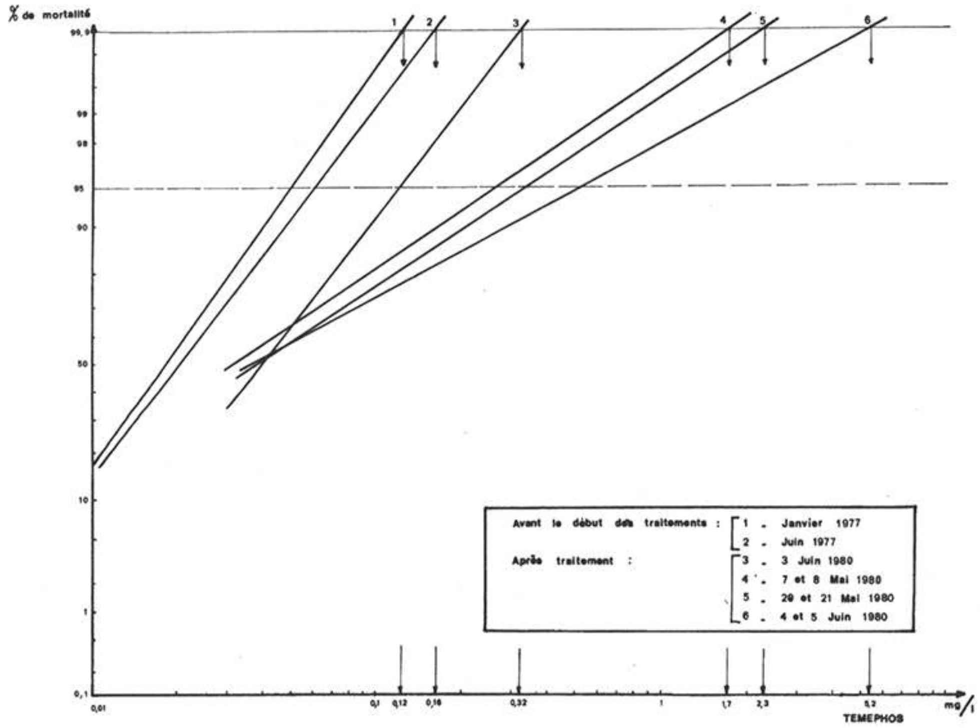

FIG. 1. — Sensibilité des larves de *S. sanctipauli* et *S. soubrense* au téméphos sur le bas Bandama.

12 *Sensibilité des larves du complexe* S. damnosum (75 % S. sanctipauli, 25 % S. soubrense) *au téméphos sur le bas Bandama* (Chutes Gauthier)

TABLEAU I. Avant le début des traitements (concentrations exprimées en mg/l)

	Janvier 1977				Juin 1977			
	CL50	CL95	Lim. sup. CL100	nb. larves testées	CL50	CL95	lim. sup. CL100	nb. larves testées
Test nº 1	0,020	0,055	0,125	421	0,021	0,062	0,125	301
Test nº 2	0,019	0,050	0,125	415	0,017	0,049	0,125	337
Test nº 3	0,016	0,047	0,125	346	0,023	0,074	0,125	489

TABLEAU II. Première série de tests (7 et 8/05/80)

Concentration en mg/l	Nombre de larves			% mortalité
	mortes	moribondes	vivantes	
0,625 (4) *	97	11	0	100
0,312 (8)	150	19	7	96
0,156 (5)	88	18	12	89,8
0,078 (4)	73	8	28	74,3
0,039 (4)	47	9	25	69,1
0 (4)	0	1	90	1,1

TABLEAU III. Deuxième série de tests (20 et 21/05/80)

Concentration en mg/l	Nombre de larves			% mortalité	% corrigé
	mortes	moribondes	vivantes		
0,5000 (1) *	13	0	0	100	100
0,2500 (6)	121	12	10	93	92,4
0,1250 (3)	38	14	10	83,8	82,5
0,0625 (3)	31	8	19	67,2	64,6
0	5	0	68	7,3	

* entre parenthèses : le nombre de répliques par concentration.

13 *Sensibilité des larves du complexe* S. damnosum (65 % S. sanctipauli, 35 % S. soubrense) *au téméphos sur le bas Bandama* (Chutes Gauthier)

TABLEAU IV. Troisième série de tests (3/06/80)

Concentration en mg/l	Nombre de larves			% mortalité	% corrigé
	mortes	moribondes	vivantes		
0,2500 (4) *	160	1	0	100	100
0,1250 (4)	100	11	4	96,5	96,2
0,0625 (3)	41	17	21	73,4	71,5
0,0312 (3)	9	17	28	48	38,8
0	2	4	86	6,5	—

TABLEAU V. Troisième série de tests (4 et 5/06/80)

Concentration en mg/l	Nombre de larves			% mortalité
	mortes	moribondes	vivantes	
0,625 (10) *	76	29	4	96,3
0,312 (11)	191	35	39	85,3
0,156 (10)	159	35	45	81,1
0,078 (4)	55	11	29	69,5

* entre parenthèses : le nombre de répliques par concentration.

14 Au cours de la troisième série de tests, des larves de *S. damnosum* s. l. provenant de la basse Comoé (zone non traitée) ont été testées à titre comparatif à la concentration de 0,156 mg/l. La mortalité a été de 100 % sur les 184 larves testées, ce qui indique une sensibilité normale pour cette population.

3.2. Efficacité des traitements au téméphos

15 Deux séries d'épandages ont été effectuées, l'une avec l'Abate Procida 20 % CE, l'autre avec 1'Abate Cyanamid 20 % CE. Lors de ces deux séries, les conditions opérationnelles ont été les mêmes : débit de $180 m^3/s$ épandage en trois points (une dose à l'entrée du gîte et deux demi-doses 500 à 600 m en aval, au même niveau sur les deux bras principaux du gîte). Les gouttières étaient situées environ 1 000 m en aval du deuxième point d'épandage.

16 Le premier traitement à l'Abate Procida à 0,1 mg/l pendant 10 mn (le 22-05-80) a provoqué 3,5 % de décrochement des larves et le deuxième à 0,2 mg/l pendant 10 mn le lendemain, 17,9 % de décrochement des larves restant dans les gouttières. Le décrochement global pour ces deux traitement est de 19,1 % (tabl. VI). Dans tous les cas, les larves s'étant nymphosées après passage de la vague d'insecticide ont été comptées comme vivantes.

17 Le premier traitement à l'Abate Cyanamid à 0,1 mg/l pendant 10 mn (le 5-06-80) a provoqué 25,3 % de décrochement et le deuxième, à 0,4 mg/l pendant 10 mn le lendemain, 41,8 % de décrochement des larves restant dans les gouttières. L'effet global pour les deux traitements est de 52 % (tabl. VII). Lors de ces deux traitements, le décrochement des larves a commencé 3 heures après l'épandage et s'est étalé sur 4 à 5 heures. Passé ce délai, il devient pratiquement nul.

18 *Efficacité * des traitements au téméphos évaluée à l'aide des Gouttières*

TABLEAU VI. Abate Procida 20 % CE

	% de décrochement des larves			
	stades 2-3	stades 4-5	stades 6-7	total
Premier traitement 0,1 mg/l pendant 10 mn, 22-05-80	0	6,6	3,4	3,5
Deuxième traitement 0,2 mg/l pendant 10 mn, 23-05-80	40	25	13,6	17,9
1er et 2e traitements	40 (20) **	30 (30)	14,8 (176)	19,1 (226)

TABLEAU VII. Abate Cyanaraid 20 % CE

	% de décrochement des larves			
	stades 2-3	stades 4-5	stades 6-7	total
Premier traitement 0,1 mg/l pendant 10 mm 5-06-80	33,6	38,5	23,8	25,3
Deuxième traitement 0,4 mg/l pendant 10 mn, 6-06-80	76,1	80,6	22,7	41,8
1er et 2e traitements	84,1 (101) **	88,1 (109)	41,2 (1613)	52 (1823)

* L'efficacité du deuxième traitement est calculée sur l'effectif de larves restant dans les gouttières. Les larves s'étant nymphosées après le passage de la vague d'insecticide sont considérées comme vivantes.
** Entre parenthèses : le nombre de larves mises en place dans les gouttières.

19 Tous les contrôles larvaires effectués dans les gîtes après ces deux séries de traitements ont montré dans tous les cas une importante population larvaire résiduelle, même à moins de 200 m en aval des points d'épandage. Cette population, comme dans les gouttières, est composée à la fois de larves jeunes et âgées.

3.3. Déterminations spécifique des larves du gîte et des larves survivantes aux tests

20 La population larvaire du gîte Gauthier au moment de l'expérimentation se composait approximativement de 65 % de larves de *S. sanctipauli* et de 35 % de *S. soubrense.* On note chez les larves survivantes aux tests à 0,312 et 0,625 mg/l, ainsi que dans le gîte après deux traitements à l'Abate Cyanamid une forte majorité de *S. sanctipauli* (environ 80 %). Il est également remarquable de noter sur les larves survivantes de *S. sanctipauli* la fréquence d'une inversion hétérozygote sur le III L (située entre les bandes 90 à 95). Il est difficile de numéroter cette inversion ne connaissant pas à l'heure actuelle la totalité des inversions du complexe. La fréquence de cette inversion chez *S. sanctipauli* était de 2 pour 26 dans le gîte contre 15 pour 32 chez les larves survivantes aux tests à 0,625 et 0,312 mg/l.

21 Étant donné le peu de larves déterminées (40 larves dans le gîte et 82 larves survivantes), il est difficile d'établir des conclusions définitives. Toutefois, il semble bien que la résistance se manifeste plus chez *S. sanctipauli* que chez *S. soubrense.* La relation apparente entre l'inversion hétérozygote sur le chromosome III L et cette résistance mérite d'être signalée.

3.4. Analyse des eaux du Bandama

22 Les différences minimes observées dans la composition physico-chimique de l'eau des trois points de prélèvement ne peuvent certainement pas expliquer l'échec des

traitements (tabl. V). Aux *p*H rencontrés, la stabilité du téméphos n'est pas altérée. On enregistre peu de différences au niveau de la composition ionique et de la résistivité. On note cependant une différence importante dans les matières en suspension. Elles sont cinq fois plus abondantes au niveau des chutes Gauthier qu'en amont. On sait actuellement que l'abondance de particules en suspension accroît sensiblement l'efficacité du téméphos (Guillet et Escaffre, 1979).

23 On constate en aval du barrage la présence d'un peuplement de phytoplancton nettement moins diversifié qu'en amont, avec prédominance très nette de pyrrhophytes (*Peridinium* sp.) et présence de quelques diatomées (*Melosia* sp.) et cyanophycées.

24 Aucune des quelques caractéristiques physicochimiques des eaux étudiées ne peut expliquer l'échec des traitements à l'abate sur le bas Bandama.

TABLEAU VIII. Composition physico-chimique de l'eau du Bandama

Lieu de prélèvement	*p*H *	Résistivité Ω/cm à 20° C	Total cations en méq/l	Total anions en méq/l	Matières en suspension en mg/l (séchées à 105° C)	Carbone en % dans les matières sèches	O^2 dissous en mg/l	O^2 consommé par la matière organique
Amont du barrage de Taabo	7,6	10 000	0,98	1	3,85	13,5	7,58	3,6
Aval immédiat du barrage	6,4	9 708	1,02	1,03	3,99	64,5	7,53	4,2
Chutes Gauthier	7,1	9 900	1,03	1,03	20,31	56,3	7,74	4,4

* Mesure extemporanée.

4. DISCUSSION - CONCLUSION

25 La méthode préconisée par Mouchet *et al.* (*loc.. cit.*) a permis de mettre très clairement en évidence la résistance au téméphos. Cette méthode, qui donne des résultats fiables, est tout à fait adaptée à la détermination de la sensibilité des larves de *S. damnosum* s. l. aux insecticides. Elle vient d'être approuvée par le dernier comité O.M.S. d'experts sur la résistance des vecteurs aux pesticides.

26 L'analyse des résultats des tests de sensibilité effectués avant le début des traitements et 16 mois après indique que les larves de *S. sanctipauli* et de *S. soubrense* du bas Bandama ont rapidement développé une résistance au téméphos. Le coefficient de résistance enregistré (jusqu'à 45) a une incidence opérationnelle immédiate et évidente. Une dose 4 à 5 fois supérieure à la dose opérationnelle efficace élimine à peine la moitié des larves présentes dans les gîtes, et ce indépendamment de la formulation de téméphos employée. Il faudrait probablement des concentrations beaucoup plus élevées pour obtenir une efficacité totale.

27 Deux éléments confirment cette résistance mise en évidence par les tests. D'une part, le pourcentage de décrochement lors du deuxième traitement à l'Abate Cyanamid n'a pas augmenté proportionnellement à la dose. En augmentant celle-ci de 4 fois, la mortalité des larves n'augmente que de 1,6 fois, ce qui indique que la plupart des larves sensibles ont décroché lors du premier traitement. L'effet du deuxième traitement est donc minimisé du fait qu'il s'applique à des larves génétiquement moins sensibles. Il faut noter cependant que cette moindre sensibilité a été en partie masquée par le premier

traitement qui a probablement sensibilisé les larves au téméphos. Ce phénomène s'observe couramment chez les larves de moustiques lors d'expositions répétées à des doses sublétales d'insecticides. D'autre part, en dépit de concentrations de téméphos très élevées, les jeunes larves (stades II à IV) n'ont décroché qu'à 70-80 %. Ces larves, plus sensibles que les larves âgées, décrochent en général toutes lors de sous-dosages accidentels sur des populations sensibles.

28 L'échec des traitements à l'abate ne vient pas d'une moindre efficacité des lots utilisés. Simultanément, ces lots ont donné ailleurs d'excellents résultats. Il n'est pas imputable non plus aux conditions hydrologiques puisqu'un traitement au chlorphoxim (O.M.S. 1197 20 % CE) à 0,05 mg/l pendant 10 mn dans les mêmes conditions opérationnelles a été efficace à 100 %.

29 Si la résistance aux insecticides chez les simulies n'est pas un phénomène couramment observé, c'est probablement que celles-ci n'ont pas fait souvent l'objet de campagnes de lutte systématique. Des baisses de sensibilité au D.D.T. ont été enregistrées aux États-Unis (Jamnback et West, 1970), au Japon (Susuki *et al.,* 1963 ; Asahina *et al.,* 1966) ainsi qu'en Afrique chez *S. damnosum* s. 1. (Walsh, 1970 ; Kuzoe et Noamesi, 1973) et *S. hargreavesi* (Quélennec et Vervent, 1970). Récemment un niveau élevé de résistance au D.D.T. a été mis en évidence chez *S. damnosum* s. l. dans la zone du programme (Guillet *et al.,* 1977).

30 Le développement d'une résistance au téméphos sur le bas Bandama, 16 mois seulement après le début des traitements, est assez surprenant. Toutefois, un certain nombre de facteurs ont été favorables à son apparition : l'importance de la population et son isolement, le manque d'une efficacité totale des traitements opérationnels et enfin, facteurs communs à toutes les populations de *S. damnosum* s. l., la prolificité des femelles et la brièveté du cycle de développement. L'apparition de cette résistance souligne la nécessité impérative pour les traitements d'être systématiquement efficaces à 100 %. La surveillance entomologique du programme repose essentiellement sur la capture de femelles piqueuses. Il serait souhaitable, chaque fois qu'il est possible d'une part de renforcer les contrôles larvaires dans les gîtes après les traitements, d'autre part d'utiliser les doses diagnostiques proposées par Mouchet *et al.* (loc. cit.) afin de suivre régulièrement la sensibilité des larves surtout dans les zones où l'on constate une moindre efficacité des traitements.

31 La population résistante du bas Bandama est actuellement contrôlée à l'aide d'un alternatif du téméphos : le chlorphoxim qui s'est révélé efficace à 100 % à la dose de 0,05 mg/l pendant 10 mn. Il est difficile de statuer sur l'évolution de la résistance au téméphos après un certain nombre de cycles de traitement au chlorphoxim. Toutefois, cela souligne la nécessité d'intensifier les recherches dans le domaine de l'utilisation d'autres types de larvicides et notamment les formulations à base de bactéries entomopathogènes telles que le *Bacillus thuringiensis* sérotype H 14.

REMERCIEMENTS

32 Nous tenons à remercier les responsables du programme de lutte contre l'onchocercose dans la région du bassin de la Volta qui ont mis à notre disposition tous les moyens matériels nécessaires à la réalisation de cette étude.

33 *Manuscrit reçu au Service des Publications de l'O.R.S.T.O.M. le 17 septembre 1980.*

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NOTES

1. Les larves survivantes de ces tests n'ont pas été mises en survie ; en revanche, la totalité des larves moribondes mises en observation sont mortes dans les trois heures qui ont suivi le test.

RÉSUMÉS

Des espèces du complexe S. damnosum *ont pu, en zone de forêt et dans certaines conditions d'isolement, développer rapidement une sérieuse résistance au téméphos. Cette résistance semble se manifester davantage chez* S. sanctipauli *que chez* S. soubrense.

In Ivory Coast, on the lower Bandama River, in forest area, larvae of the Simulium damnosum *complex have rapidly developed a serions resistance to temephos* (*abate* [(R)]). *The resistance ratio to the LC 99,9 level can reach 45. This resistance seems to be more apparent in* S. sanctipauli. *We have noted for this species a high frequency of a heterozygote inversion on the chromosome III L in resistant larvae. Treatments fait completely at dosage 4 to 8 times the normal operational dosage (0,05 mg/l during 10 mn). The resistance occurs on a very limited portion of the river, 65 km long and limited upstream by a dam and downstream by the absence of breeding sites in the final 90 km of the river. The resistant population is currently being controlled with chlorphoxim, an alternative insecticide.*

INDEX

Mots-clés : S. damnosum, résistance, téméphos
Keywords : S. damnosum, resistance, Temephos

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Criteria for the selection of larvicides by the Onchocerciasis Control Programme in West Africa

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1 One of the weekly decisions the Onchocerciasis Control Programme has to make, in its operations in 11 west African countries, is the selection of one insecticide out of the six used that is most appropriate to the river stretches to be treated. This decision depends on several criteria, linked not only to the compounds themselves but also the hydrological conditions and blackfly populations involved. Given the great number of breeding sites (gites) to be treated, in 23 000 km of rivers at the height of the rainy season, this paper identifies the parameters needed to facilítate the choice of insecticide and to optimize the larviciding in terms of cost effectiveness, management of resistance and minimizing the environmental impact.

2 The Onchocerciasis Control Programme in West Africa (OCP), which was launched in 1974, is executed by the World Health Organization. There are 11 participating countries in the Programme area, which covers more than a million km^2. The Programme's objective is to control blinding onchocerciasis caused by savanna strains of *Onchocerca volvulus* (Zimmerman *et al.,* 1992) transmitted by the *Simulium damnosum* complex, particularly the savanna vectors *S. sirbanum* and *S. damnosum* s.s. Despite the introduction of ivermectin, a microfilaricide which Controls the ocular morbidity (Dadzie *et al.,* 1990) and in the absence of a macrofilaricide, vector control remains OCP's method of choice to bring the transmission of the parasite to an end. The objective of the vector control strategy is to arrest transmission of *O. volvulus* by eliminating vector populations for the duration of the life span of the adult worm in the human host (WHO, 1968), at present calculated to be about 14 years (Plaisier *et al.,* 1991). Onchocerciasis is thus no longer a public health problem in the original Programme area, where control, that has only involved the vectors, has been maintained for more than 14 years (WHO, 1992).

3 The vector control operations consist of treating breeding sites (gites), where the larval stages of *S. damnosum* s.l. develop, with insecticides. As development of the aquatic stage

takes about a week front egg to pupa, the insecticide is applied weekly. As there are a large number of breeding sites (over 23 000 km of river are treated each rainy season), some of which are inaccessible on the ground, the larvicides have always been applied as aerial sprays. Temephos, an organophosphorous compound and the only insecticide used for the first 5 years of the Programme, was selected because of its efficiency, its carry (the distance over which it remains effective), its lack of impact on non-target fauna, and its acceptable cost. After resistance of *S. damnosum* to temephos and phoxim (another organophosphate) was detected (Guillet *et al.,* 1980), the Programme adopted a strategy of rotational use of insecticides (which, if possible, came from different Chemical groups and had different modes of action) to slow down and suppress the appearance of new cases of resistance (Guillet *et al.,* 1990; Kurtack, 1990). This rotational use of insecticides has, to date, been so effective that there is now very little resistance left to the organophosphates in use and the susceptibility of the *Simulium* population to the other compounds remains unchanged.

4 The vector control operations staff must decide each week which insecticide to use on which stretch of river. The decision whether or not to include a river, tributary or stretch in the aerial larviciding depends on: (1) the results of a weekly entomological evaluation of the efficiency of earlier treatment on the aquatic stages (involving a search for larvae and pupae in rivers) and adults (involving capture and dissection); (2) factors such as fly migrations; (3) the vectorial capacity of the species involved; (4) the levei of onchocerciasis endemicity in the area; and (5) the treatment coverage with ivermectin. When larviciding operations are foreseen, the second step consists of selecting, for each river, the appropriate compound to be used. Although many parameters used in the overall decision-making process are difficult to codify and subject to mathematical analysis, the criteria for selection of an insecticide are simpler and therefore easier to quantify for rational computer analysis. These criteria are discussed and reviewed here to facilítate the work of those in charge of the vector control operations.

CRITERIA FOR THE SELECTION OF INSECTICIDES

Efficiency and Potential Resistance

5 Six insecticides are presently used by OCP. Five of them are Chemical insecticides formulated as emulsifiable concentrates and the sixth is a liquid concentrate of a biological insecticide. Of these, two organophosphates, temephos and pyraclofos, are considered to be the most effective larvicides; their required operational doses are low and their carry may reach over 50 km when the river discharge is high (around 300m^3 Vs). Temephos, however, should be used with caution in areas where some species of the *S. damnosum* complex have retained low susceptibility to it since 1980, bearing in mind the risk of cross-resistance with other organophosphates (Kurtak *et al.,* 1987). Pyraclofos is therefore preferentially used at the beginning of the rainy season, when discharges are increasing and the breeding sites are being recolonized. Although permethrin, a synthetic pyrethroid, has a low carry compared with temephos and pyraclofos, its operational dose is very low and no case of resistance to it has been detected by the OCP. There seems negligible risk of such resistance developing; it took more than 3 years of consecutive weekly treatment with permethrin on a 30-km river stretch in Cameroon before a decrease in the sensitivity of the flies to this product could be detected and this

phenomenon was found to be reversible (Hougard *et al.*, 1992). Carbosulfan is a carbamate with almost the same carry as permethrin but with a higher operational dose. There are no restrictions on its use because of problems with resistance; it has been recently introduced into the Programme and the current policy of insecticide rotation should prevent any serious resistance developing. Use of the spore-forming bacterium *Bacillus thuringiensis* serotype H-14 (Bt), as a biological insecticide, has shown low cost-efficiency, both in terms of dose and carry. However, its use is justified because it has no effect on non-target organisms and, again, because there is Utile chance of resistance to it developing (Hougard and Back, 1992).

Environmental Toxicity

6 The insecticides used in the Programme are continually evaluated by an independent ecological group (Paugy, 1991), given the fact that a larvicide for *Simulium* control, no matter how effective it is, would not be accepted for use by OCP if it had short-, intermedíate-or longterm deleterious effects on the environment, particularly on fish. Temephos and Bt are considered harmless to the environment and are used freely. Pyraclofos may, in cases of accidental overdose, show some toxicity against non-target fauna, particularly to fish, and it is recommended that the use of pyraclofos be restricted to discharges above 15m^3/s. Permethrin and carbosulfan should also be used with caution, only when the discharge stays above 70 m^3/s and no more than six times a year on any stretch of river. In general, all Chemical insecticides should be used in a manner which avoids the possibility of an overdose likely to harm the environment. The Programme has established a satellite transmission network to record water discharges and permit rapid management of hydrological data for the safe treatment of rivers (Servat and Lapetite, 1990).

Cost of Application

7 Cost effectiveness is a key factor in the selection of a compound. To make a correct estímate of this, OCP requires that not only those variables related to the insecticide itself be considered (operational dose, carry, cost of formulation) but also those related to its transport and application (fuel and maintenance for vehicles, staff, maintenance and protection of depots, kerosene, flight hours). The discharge of the river must also be taken into account when assessing the treatment cost, since it influences the amount of insecticide used, its carry, its operational dose (in the case of temephos), the frequency at which depots need replenishing and the number of flight hours.

8 Each parameter can be evaluated accurately enough to allow the cost effectiveness for each kilometre of river treated to be assessed as an index for each range of discharges and each insecticide (see Table). This allows the comparative costs of applications of different insecticides to one river to be calculated and is also an important criterion for establishing priority among the six insecticides in use. Experience has shown that U.S. $45-50/km of river treated may be considered the maximum cost of treatment within the budgetary constraints of the Programme.

The Spraying System

9 Treatment helicopters are equipped with a spraying System that allows for dosing of insecticides with an accuracy of 10 cm^3. However, below a given quantity (which depends on the formulation of the product), it is difficult for the pilot to apply the correct amount of the insecticide to a breeding site, especially when it is impossible to fly at low altitude because of the vegetation. In particular, accurate treatment of discharges below 1 m^3/s with those compounds that have a low operational dose (temephos and phoxim) becomes hazardous. This constraint does not apply to Bt, which, due to its low solubility, is never applied in values less than 1 litre, even to very low discharges.

Hydrological Characteristics of the Rivers

10 In the majority of cases, the hydrological characteristics of the rivers do not pose any special problems if the selection criteria listed above are adhered to. However, the choice of insecticides for use on rivers that have a particularly high discharge over a long period of time may be limited by cost effectiveness, in particular when the ecological constraints on permethrin or carbosulfan use (i.e. not more than six cycles/year on any river stretch) are observed. Consequently, the hydrological characteristics of large rivers, such as the Niger at Bamako (where the discharge varies from 70 to 3000 m^3/s) and some rivers in the

forest zone of Sierra Leone (e.g. the Rockel) with discharges greater than 300 m Vs for many months each year, have to be carefully considered.

TABLE

*The cost-efficiency index (in U.S.$) for each kilometre of treated river**

Larvicide	Discharge (m^3/s) 0–5	5–10	10–15	15–50	50–100	100–150	150–200	200–250	250–300
Temephos	7	8	8	11	16	22	27	31	36
Pyraclofos	N.D.	N.D.	9	12	19	26	33	38	43
Phoxim	13	17	22	34	54	84	97	122	147
Permethrin	N.D.	N.D.	N.D.	12	14	16	20	21	24
Carbosulfan	N.D.	13	14	22	32	43	57	65	77
Bt	27	36	43	71	114	N.D.	N.D.	N.D.	N.D.

Larvicide	Discharge (m^3/s) 300–350	350–400	400–450	450–500	500–600	600–700	700–800	800–900	900–1000
Temephos	39	45	48	49	54	N.D.	N.D.	N.D.	N.D.
Pyraclofos	50	50	62	66	N.D.	N.D.	N.D.	N.D.	N.D.
Phoxim	172	198	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Permethrin	27	27	30	32	36	41	47	52	52
Carbosulfan	90	94	105	116	N.D.	N.D.	N.D.	N.D.	N.D.
Bt	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

*The index is calculated using the equation {[100/P] x *D* x *Q* x (*F+X*)]+ *Ty*}/ 100, where *P* is the carry, (*100/P*) is the number of applications or 'drops' needed to treat 100 km of river, *D* is the mid-point of the discharge range m^3/s) *Q* is the quantity of formulation to be dropped perm^3 /s discharge (litres), *F* is the cost of the larvicide (U.S.$/litre), *X* is the cost of transporting a litre of the larvicide close to the river (about U.S.$0-06), *T* is the time it takes to treat 100 km of river—including refuelling at the depot (min), and *y* is the total cost of the helicopter flight—including maintenance, kerosene and pilot salary (U.S.$13 57/min). Emboldened values exceed the budgetary constraints of the Programme.
N.D., Not determined; Bt, *Bacillus thuringiensis* H-14.

OPERATIONAL USE OF INSECTICIDES

11 After considering all the various constraints, it has been possible to prepare a scheme for the use of insecticides according to the discharge of the river to be treated (Fig. 1). Below 1 m^3/s only Bt should be used, and above 450 m^3/s only permethrin is appropriate. Between 1 and 15 m^3/s temephos, phoxim or Bt can be used. At 15-70 m^3/s the three organophosphorous compounds should be used; Bt becomes too expensive for use under these conditions. Between 70 and 150 m^3/s there is more choice; temephos, pyraclofos, permethrin and carbosulfan are all usable. Carbosulfan becomes too expensive to use at 150-300 m^3/s and between 300 and 450 m^3/s the cost-efficiency index reduces the choice to only temephos or permethrin. This scheme can be applied to most of the rivers in West Africa under larviciding once the characteristics of each river (hydrological conditions, configuration of breeding sites, and potential for insecticide resistance) are known. It does, however, need to be adapted at certain sites which have unusual features. Two such sites are outlined below.

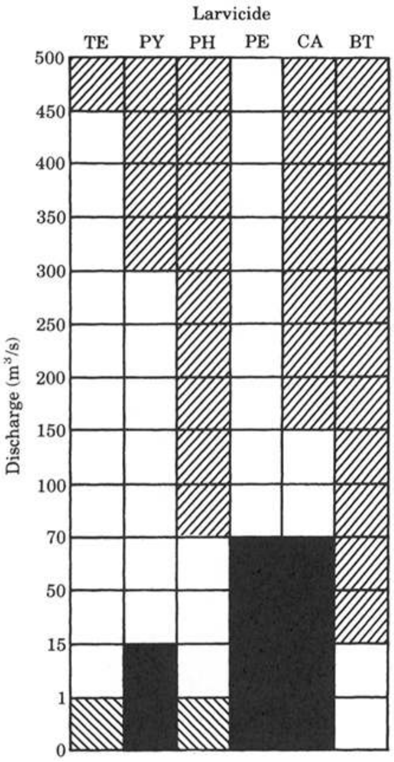

Fig. 1. The generally preferred insecticide(s) (□) for different discharges. Low cost efficiency (▨), potential environmental damage **(■),** or lack of accuracy in application (▧) make each insecticide unusable under certain conditions. TE, Temephos; PY, pyraclofos; PH, phoxim; PE, permethrin; CA, carbosulfan; BT, *Bacillus thuringiensis* H-14.

The Marahoué River in Côte d'ivoire

12 The Marahoué river begins in the north of Côte d'ivoire, in the area of Boundiali, and ends in the white Bandama near which, in 1980, the first cases of temephos resistance in species of the *S. damnosum* complex were detected. Although the level of susceptibility to this compound is now acceptable and even though the risk of cross-resistance remains, the two other organophosphates are used instead of temephos (Fig. 2). This ban on temephos use could be a handicap at discharges greater than 300 m^3/s because permethrin would be the only usable compound. In reality, the problem does not arise, since the discharge on the Marahoué seldom exceeds 300 m^3/s except during the temporary flood and this never lasts more than 1 week. In any case, since the Marahoué river has recently been selected for an evaluation of the impact of carbosulfan on non-target organisms, no permethrin will be used on it until this assessment is complete.

The Niger River near Bamako

13 The Niger river begins in Guinea, passes through Mali and Niger, and ends on the Nigerian coast. Around Bamako there is a stretch of tens of kilometres in which *Simulium* breed, although there are no breeding sites further up-or down-stream. Neither phoxim nor Bt can be used on this stretch as the discharge is seldom below 70 m^3/s (Fig. 2). Temephos is not used because resistance to it was detected in the area in 1988. Carbosulfan is seldom applied because the discharge is only suitable for its use (70-150 m^3

/s) for about 10 weeks each year. Pyraclofos is more frequently used, in preference to other products that could have been chosen for the discharges involved. As the discharge is about 300 m^3/s for about two thirds of the year, permethrin is widely used. As resistance to organophosphorous compounds decreases, susceptibility to synthetic pyrethroids increases (Kurtak *et al.,* 1987). However, to avoid using all six permethrin cycles permitted each year, permethrin is generally used only at high discharges, after which all treatment can be suspended for long periods of time because the breeding sites are not productive. A pressing need to interrupt transmission occasionally leads to use of more than six permethrin cycles and costs above the permitted threshold, provided these options do not affect non-target organisais or affect them only in the short-term (these organisms can often recolonize treated areas from non-treated areas).

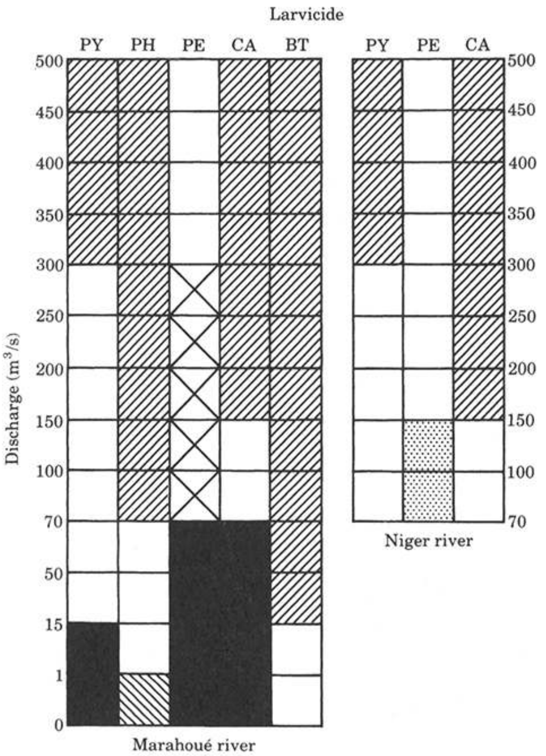

FIG. 2. The preferred insecticide(s) (□) for different discharges in the Marahoué and Niger rivers. Low cost-efficiency ( ), potential environmental damage (■), lack of accuracy in application ( ), hydrological conditions ( ), or a particular experimental need ( ) make each insecticide unusuable under certain conditions. TE, Temephos; PY, pyraclofos; PH, phoxim; PE, permethrin; CA, carbosulfan; BT, *Bacillus thuringiensis* H-14.

DISCUSSION

14 We are unaware of any other vector control programme for public health in which the selection of several insecticides can be made before each treatment, based on wellestablished criteria. Among such criteria, the efficiency of the formulation, its toxicity to non-target fauna, the spraying System and the hydrological conditions of the river are relatively easy to assess, provided the baseline data are available. On the other hand, the likelihood of resistance to the insecticides developing and the cost-effectiveness of each larvicide are more difficult to determine because of the numerous parameters involved. The genetic and biochemical mechanisms of resistance are not yet

well known and studies on the transfer of the genes involved between populations of insects are yet to be conducted (Poirié and Pasteur, 1991). Nevertheless, the use of the criteria proposed in this paper should facilítate the decisionmaking process.

15 The diagram outlining the choice of larvicide (Fig. 1) highlights two sets of conditions that are problematic in terms of treatment:

- Very Jow discharges (up to 1 m^3/s), where il is impossible to use any other product than Bt Despile the low risk of resistance to this biocide developing (because of the mode of action of bacterial toxins), research should continue to identify other potencial biological insecticides, such as *Clostridium bifermentans* Malaysia, which gave promising results in initial trials (De Barjac *et al.,* 1990), as a back up to Bt.
    - Discharges between 15 and 70 m^3/s, at which only use of organophosphates is permitted, with all the risks of cross-resistance. It is hoped that a pseudo-pyrethrinoid compound, etofenprox (Udagawa, 1988), can also be used within this range of discharges; preliminary results indícate it would be a cost-effective alternative.

CONCLUSIONS

16 It is now generally accepted that the rotational use of six insecticides, although based on empirical criteria and field experience, has proved successful in the OCP area (Guillet *et al.,* 1990; Kurtack, 1990), although it may be less useful in other circumstances (Curtís *et al.,* 1993). Given the consequences of treatment failure, the strategy of rotational use of insecticides will continue. Once the selection criteria for larvicides had been defined, the decision makers couid make objective selections based on cost efficiency and environmental impact. This approach has been applied successfully for several months in Mali, Côte d'ivoire, Guinea and Sierra Leone. The next step will be the development of a computer model.

17 ACKNOWLEDGEMENTS. We thank Dr. E. M. Samba, Director of the OCP, for his continuous support of this work. We also express our sincere gratitude to Professor D. H. Molyneux and Drs. O. Christensen, B. Philippon and A. Sékétéli for their interest in this study and their assistance in the final conecrions to the munuscript.

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Twenty-two Years of Blackfly Control in the Onchocerciasis Control Programme in West Africa

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1 *Twenty-two years after the launch of the Onchocerciasis Control Programme in West Africa (OCP), Jean-Marc Hougard and colleagues critically review the vector-control strategy adopted. They go on to identify the few hydrological basins where transmission of the infection remains difficult to control, to analyse the causes and to propose appropriate corrective measures on a case-by-case basis. Most of these measures, which are mainly based on ivermectin chemotherapy, will continue to be applied after the end of the OCP in 2002, under the control of the countries concerned.*

2 Onchocerciasis, a dermal filariasis caused by *Onchocerca volvulus,* is a disease transmitted to humans by a blood-sucking dipterous insect, the blackfly *(Simulium).* The most serious manifestations are blindness and debilitating skin lesions. This disease is found in 37 countries, 30 in Africa, six in America and one in the Arabian península[1]. Africa is by far the most affected continent in terms of the severity of the clinicai manifestations of the disease and the extent of its distribution. Hence, as early as 1968, representatives of the Organisation de Coopération et de Coordination pour la Lutte contre les Grandes Endémies, WHO, Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) and United States Agency for International Development, encouraged by the results of the pilot campaigns for the control of blackflies in the savanna zone of West Africa[2], laid down the foundations of an ambitious regional onchocerciasis control project at a meeting in Tunis (1-8 July 1968).

3 The Onchocerciasis Control Programme in West Africa (OCP)[3] began its activities in January 1974. Its objective was to eliminate onchocerciasis as a disease of public health importance and as an obstacle to socio-economic development. The basic strategy was to interrupt the transmission of the blinding strain of *O. volvulus* for a period longer than the longevity of the adult worm in its human host (about 14 years)[4]. The interruption of transmission is achieved by destroying the larval stages of the vector, *Simulium damnosum* s.l., through the aerial application of selective insecticides to infested rivers (Fig. 1). Each week, the OCP selects the most appropriate insecticide (out of seven, which are used in

rotation) for application in each river stretch to be treated (Fig. 1). This decision allows the optimization of larviciding in terms of cost-effectiveness, management of resistance and environmental protection[5-6]; several criteria need to be considered, not only relating to the insecticides, but also to the hydrological conditions and blackfly populations involved. One of the seven insecticides is a liquid concentrate of a biological control agent, *Bacillus thuringiensis* H-14 (Ref. 7). Of the six Chemicals, formulated as emulsified concentrates, three are organophosphates (temephos, phoxim and pyraclofos), two are synthetic pyrethroids (permethrin and etofenprox) and one is a carbarnate (carbosulfan).

4 The first aerial insecticide treatments began in February 1975, in areas where the incidence of blinding onchocerciasis was highest. Later, the area covered was gradually extended to 654000 km^2, spread over seven countries (Burkina Faso, south-eastern Mali, south-western Niger, the northern parts of Côte d'Ivoire, Benin, Ghana and Togo) by the end of 1977. However, it soon became apparent that the border of this area was infiltrated by infective blackflies originating from regions outside the OCP area[8]. To give permanent protection in the original OCP area and to control the vector in the basins where the re-invading *Simulium* originated, the hyperendemic regions were identified and larvicidal treatment was given[9]. This resulted in two extensions to the original OCP area, one to the west, comprising the basins of south-eastern Guinea and northern Sierra Leone (western extension), and a second to the south and east, comprising the southern basins of Côte d'Ivoire, Benin, Ghana and Togo (south-eastern extension) (Fig. 1).

5 The installation of vector-control operations in the extension areas was completed towards the end of the 1980s, while all the basins of the original area were still being treated. The larviciding coverage then reached its peak with>40000 km of river being treated over an area of 106 km^2, over nine countries of the OCP The satisfactory results achieved mean that larviciding operations have now stopped in almost all the basins of the original area. In the extension areas, larviciding is proceeding, and is combined with the distribution of the microfilaricide ivermectin, the only drug available so far which is suitable for the mass treatment of onchocerciasis[10]. By pursuing this combined drug and vector-control strategy, the whole of the basins treated should be free from onchocerciasis[10]. By 2002 at the latest, which is the end point of the OCP operations[11]. A few residual foci of infection will remain, however, after the conclusion of the OCP, both in the controlled areas and in the extension areas.

Residual foci in the original area

6 *The control strategy.* The original OCP area is a savanna zone and is relatively homogeneous in its blackfly species and parasite strains. Before the OCP was launched, the area was plagued mainly with the most severe form of onchocerciasis, the savanna type, which is characterized by high blindness rates, and is transmitted by the group of savanna species of the *S. damnosum* complex (Fig.1)[12-14]. In principie, the vector-control strategy was simple to implement, as it consisted of arresting transmission of the parasite regardless of its pathogenicity, through the indiscriminate elimination of vector species[15]. Although the blackflies could carry animal filariae, some of which could not be differentiated from O. *volvulus,* evaluation of the treatments posed no particular problem. Indeed, the savanna blackflies were largely anthropophilic, and the annual transmission potential (ATP; Fig. 1) reflected the intensity of the blinding form of human onchocerciasis[16].

7 The implementation of this control strategy made it possible to interrupt transmission of the blinding strain of the parasite. From 1990, the first decisions to stop larviciding were made for the basins in which the situation was deemed satisfactory from both the epidemiological and entomological points of view[17, 18]. Subsequently, vector-control operations ceased in other basins that had received at least 14 years of larviciding and where the trends of the epidemiological data indicated depletion of the parasite reservoir. However, because of contamination by infective blackflies, some rivers at the edge of the original area continued to be treated beyond the theoretical period of 14 years, and up to the time the extension areas were set up. Onchocerciasis is no longer a public health problem in the whole of the original area, that is, clinical signs of the disease have totally disappeared. WHO forecast in 1991 (Ref. 19) that the whole of the original area would be free from onchocerciasis by 1997; however, a few basins remain slightly endemic[20]

8 *The residual foci.* The persistence of some residual foci is a cause for concern. While the risk of contamination of the adjacent basins remains, there is no guarantee that an alternative control strategy would succeed fully where the current strategy has only been a partial success. The OCP and the participating countries are therefore paying particular attention to these foci in an attempt to identify the factors that have hindered success. To our knowledge, six residual foci of onchocerciasis remain in the original area; a succinct analysis of these foci follows.

9 (1) In the north-eastern part of Benin, a few villages located near two tributaries on the east side of the river Niger are still hypoendemic despite 18 years of uninterrupted vector control. The persistence of this focus is due to a seasonal contamination by infective blackflies carried by harmattan winds from untreated hyperendemic areas of Nigeria.

10 (2) The problem of the Lower Black Volta focus in Ghana is partly caused by demographic factors. A recent sociological study[21] has shown that this area attracted large numbers of migrants in search of new farmlands; the migrants mainly originated from areas to the east of Lake Volta, which were still endemic.

11 (3) The control programme in the White Volta focus in Ghana was hampered for many years by serious logistical problems related to the inaccessibility of two of its tributaries during the rainy season. Some larval breeding sites thus escaped larviciding, allowing an unsuspected transmission to continue as a result of the inappropriate location of one blackfly-catching point.

12 (4) In the Dienkoa focus in Burkina Faso, onchocerciasis remains mainly because of the premature decision to stop vector-control operations in the 1980s. This was partly a consequence of continuing undetected transmission as a result of an inappropriate reduction in the evaluation network.

13 (5) The decision to stop larviciding in the basin of the Bougouriba, Burkina Faso in 1989 might also have been premature. Indeed, although transmission had been successfully controlled for more than 14 consecutive years, focal entomological, epidemiological or demographic signs suggested that larviciding should have been continued or, at least, some backup measures (entomological and / or epidemiological monitoring or ivermectin distribution) instituted.

14 (6) Lastly, the causes of the persistence of high transmission levels and poor epidemiological results on three of the tributaries within the Oti focus are difficult to determine, but could result from interactions between climatic, demographic and

logistical factors. Aerial larviciding, in combination with ivermectin treatment, is being continued pending further investigations.

15 *The corrective measures.* The identification of the characteristic features of these foci has made it possible to take corrective actions, in particular, treatment with ivermectin and, to a lesser extent, larviciding. In the White Volta focus, considering the delay in controlling transmission and the fact that transmission is localized, the large-scale ivermectin treatment that was instituted in 1995, soon after larviciding was stopped, might accomplish more than a simple control of morbidity through more focal treatment. The relatively isolated nature of this focus and its low endemicity level, combined with the treatment regime employed (150 $\mu g kg^{-1}$ twice a year), permits hope that a significant impact on transmission might be achieved, as occurred in the savanna zone of northern Cameroon[22]. In the Dienkoa focus, satisfactory larvicidal treatments, combined with ivermectin distribution, were achieved (ATPs <100 infective larvae per person per year, and no new infections in humans) only from 1990. The ground larviciding by health service technicians in Burkina Faso should end in 2001, after a period of 12 years of successful control, which is long enough to reduce the risk of recrudescence to <1%. In the Oti focus, where vector control is planned only until 2002, and in the other remaining foci, the long-term control of morbidity through community-directed treatment with ivermectin is probably the only remaining solution in the absence of a macrofilaricide suitable for mass treatment. Community-directed ivermectin treatment, which is used by the African Programme for Onchocerciasis Control, directly involves the exposed communities[23], which is probably the best way to ensure optimal and sustainable protection of the populations after the conclusion of OCP, in the current socioeconomic context.

Potential residual foci in the extension areas

16 *History of onchocerciasis control.* Larviciding of the river basins that were the initial sources of invasion by infective savanna blackflies commenced as early as 1979 in Southern Côte d'ivoire[9]. Year-round larviciding was started in 1987 in the Southern parts of Ghana, Togo and Benin, which were the sources of invasion to the Southern borders of the original area[24, 25]. In 1989, insecticide treatment of the basins of eastern Guinea and then, in 1990, those of northern Sierra Leone was started[26, 27]. With the registration of ivermectin in 1987, these basins also benefited from large-scale ivermectin treatment from 1989. This strategy led to a rapid reduction in ocular morbidity[28] from the effects of ivermectin, as well as a longerterm reduction in transmission from the combined effects of ivermectin treatment and larviciding[29]. At the same time, the foci of Guinea Bissau, western Mali, Senegal and Southern Sierra Leone were treated with ivermectin only, because they were isolated, had low-level endemicity and were not a source of invasion into the original area. Thus, at the beginning of the 1990s, vector control and/or ivermectin treatment had brought blinding onchocerciasis under control over the entire area of almost 1 300 000 km^2 of the 11 countries of the OCP (Fig. 1).

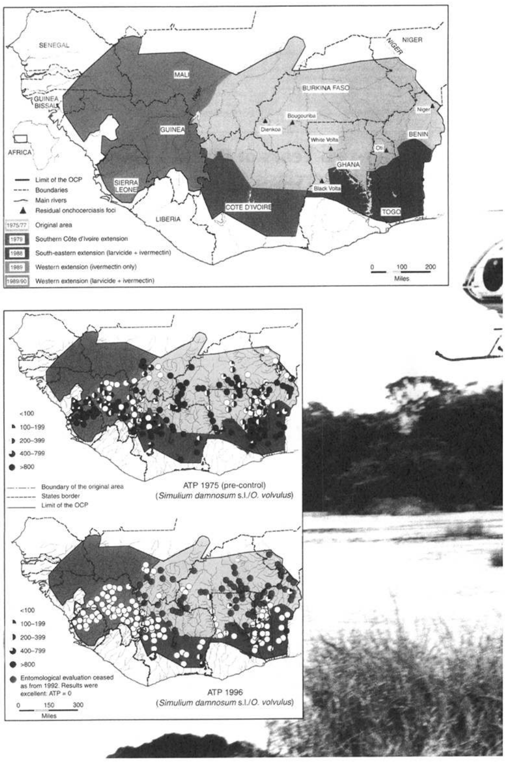
17 *The control strategy.* In the extension zones undergoing larviciding, the nature of the soil, the altitude, latitude, rainfall or human activities determine the different types of vegetation, which range from the savanna in Sudan and Guinea to the degraded forests. This diversity is reflected in the habitats of different cytospecies of the S. *damnosum* complex, from the savanna to the forest vectors[30]. When larvicidal treatments were

initiated, a selective strategy exclusively targeted at the savanna blackflies was considered. Flowever, the transmission dynamics of different parasite strains was unknown, and it was difficult to identify the infected adult females accurately. This option was therefore discarded in favour of a less targeted strategy, which took into account the seasonal abundance of the savanna blackflies in their larval habitat based on the cytotaxonomic tools available[31]. Species identification by cytotaxonomy is based on the analysis of the banding sequence on the polytene chromosomes from the larval salivary glands[12]. Among the nine sibling species of the S. *damnosum* complex found in the area covered by the OCP[13], six are the primary vectors for O. *volvulus* in West Africa. *Simulium sirbanum* and S. *damnosum* s.s. are the primary vectors in the savanna, while S. *squamosum* and S. *yahense* are the primary vectors in the rain forest. The remaining two vector species, S. *leonense* and S. *sanctipauli,* have a restricted distribution, which is mostly limited to the large Coastal rivers of Sierra Leone and Côte d'ivoire, respectively[30].

18 Morphological identification of adult blackflies has now improved, allowing the differentiation of the savanna species from the others at the same blackfly-catching point [30]. DNA probes can differentiate between the savanna strain and the forest strain of the parasite[32], and a technique involving mitochondrial DNA sequences is being developed, which allows the identification of the adult flies of the six main vector species[33]. These methods are providing insights into the mechanisms of transmission under natural conditions and, hence, into vector-control strategies. Toé and colleagues[34] have shown that the relation between savanna vectors and the blinding strain of the parasite is not as clear as was suggested by earlier experimental xenodiagnostic studies. The consequent overemphasis on the role of savanna species in transmission, when planning control activities, might favour the transmission of blinding onchocerciasis by the forest blackflies. This hypothesis is supported in that the vectorial capacity of forest species is higher than that of savanna species[35]. However, other observations made in several basins of the extension areas suggest that the current strategy can be maintained without jeopardizing the achievements of vector control. For example, at the Benin village of Kaboua in the degraded forest zone (Fig. 1), transmission of the savanna parasite strain is relatively high (an ATP of 421 infective larvae per person per year in 1996), although the transmission is mainly due to forest blackflies originating from the untreated basins of Nigeria (S. *soubrense* Beffa type)[36]. This level of transmission, in the savanna zone, would have been assodated with clinicai signs.

19 These different observations underline the uncertainties regarding the threat posed by the blinding strain of the parasite in the extension areas. Research is now under way to improve molecular tools (heteroduplex, DNA microsatellites) to facilitate the identification of the vector and parasite populations[33,37]. The heteroduplex technique permits the identification of individuais on the basis of hybridization with mitochondrial DNA fragments. Microsatellite DNA provides useful polymorphic markers for populations and even for individuals. The application of immunodiagnostic techniques to determine whether different levels of pathogenicity exist in the parasite or whether individuals can be predisposed to develop blinding onchocercal lesions is also being planned. Pending the results of these studies, with five years to go to the end of larvicide treatments, the current vector-control strategy – a realistic compromise between a selective option and a more global option – should be pursued without major changes until the conclusion of the OCP.

20 *The potential residual foci.* Vector control, combined with ivermectin distribution, is being continued in almost all the basins of the extension areas. Larviciding is planned to stop at the end of 1998 in the Côte d'Ivoire basins, and between 2001 and 2002 in the western and south-eastern extensions[38,39]. However, in a few river basins where the blinding strain of the parasite predominates, transmission sometimes remains difficult to control despite continued larviciding and the distribution of ivermectin (eg. the Lower Tinkisso, Guinea, in the western extension, and the Pru, Ghana, in the south-eastem extension). Therefore, as observed in the original area, it is possible that a few residual foci of savanna onchocerciasis will persist after the end of the OCP. After 2002, when vectorcontrol measures will have ceased, in the absence of a macrofilaricide suitable for mass treatment, ivermectin will be the only means of control for these residual foci. The research mentioned previously, if successful, will help to assess the threat represented by these foci, allowing the appropriate adjustment in ivermectin-treatment strategies on a case-by-case basis. Meanwhile, work on modelling onchocerciasis transmission and its control might help to determine precisely how long ivermectin distribution needs to be continued to make the recrudescence of blinding onchocerciasis unlikely[40]



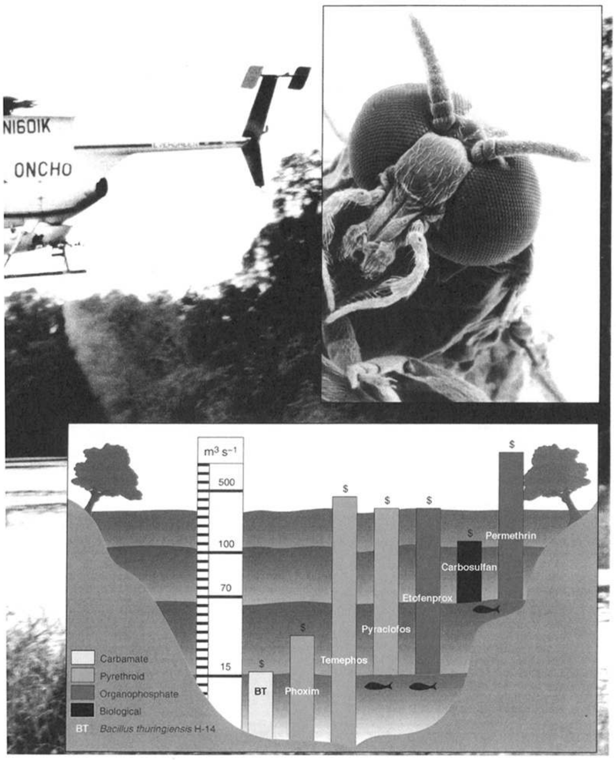


Fig. 1. An OverView of the vector-control activities in the Onchocerciasis Control Programme in West Africa (OCP). The map (top left) shows the different operational phases of the OCP and identifies the residual foci in the original OCP area. The photograph below (kindly supplied by the Institut Français de Recherche Scientifique pour le Développement en Coopération) shows an adult of *Simulium damnosum* s.l. complex species, which is responsible for the transmission of onchocerciasis. The background shows a helicopter (photograph kindly supplied by OCP) spraying one of seven larvicides selected according to their operational range (bottom right). In this figure, the position of the fish represents the minimum level of water discharge with respect to the water gauge at which the product is authorized to be used because of the risk of impact on the environment The dollar sign ($) represents the level above which the cost of use of the product is no longer acceptable. The entomological evaluation is also shown (bottom left). The entomological index most frequently used to quantify transmission is the annual transmission potential (ATP): the theoretical number of infective onchocerca larvae that a person at a catching point would receive if highly exposed to blackfly biting 12 hours a day for 12 months a year. In the savanna area, transmission of onchocerciasis at a level <100 infective parasite larvae per person per year is considered to give no risk of developing severe ocular lesions.

Transfer of residual activities

21 By 2002, the responsibility for maintaining the residual activities of the OCP will be transferred to the participating countries. A few residual foci will remain in both the original and the extension areas; however, their total size will be fairly small compared with the 25 million hectares of fertile land that will be disease free by the end of the OCP; furthermore, the disease will no longer be a public health problem. Although the studies conducted so far in the disease-freed zones have given encouraging results, it is possible that new onchocerciasis foci will emerge after 2002 in the areas currently considered to have been freed. In the basins of the White Volta in the southeast of Burkina Faso, for example, onchocerciasis has been reduced from hyperendemic to almost absent after 14 years of vector control alone. The latest parasitological surveys still show excellent results six years after larviciding was stopped.

22 The health services of the countries involved must be given the means to detect an onchocerciasis focus early enough that control is feasible. The necessary tools and know-how for entomological surveillance will be made available by the OCP to the national health services to enable them to detect a resurgence of transmission. A simple and cheap method for the detection of infective larvae of the parasite in blackflies is in the process of being tested for use in the field[41]. This method will provide an early warning of potential recrudescence, enabling the health services of the affected area to apply the various techniques currently available or in development[42] before the infection becomes difficult to control. In the Southern areas of the OCP, especially south-eastem Côte d'Ivoire, south-eastem Guinea and south-western Ghana, the national health services will also have to monitor closely the consequences of deforestation on vectorial dynamics and, hence, on the epidemiology of onchocerciasis. For the time being, to avoid any deterioration of the situation, OCP will provide the countries with logistical and technical support for the distribution of ivermectin to control onchocercal morbidity, regardless of pathogenicity.

23 All vector-control activities will probably have ceased by the end of 2002, as sustained vector-control action to arrest transmission in each residual focus would be beyond the budgets of the participating countries. However, the use of ground larviciding with non-toxic and non-persistent insecticides to control blackfly nuisance might increase, at least in socio-economically important sites, where the efficient and sustainable management of such treatments is possible[43]

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Evaluation of larvicides for the control of *simulium damnosum* s.l. (diptera: simuliidae) in west africa

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INTRODUCTION

1 Temephos resistance in larvae of some cytospecies of the *Simulium damnosum* s.l. complex was first reported in the Onchocerciasis Control Program (OCP) area in West Africa in 1980 (Guillet et al.) and chlorphoxim resistance was first reported in 1982 (Kurtak et al.). These findings led to large-scale use of *Bacillus thuringiensis* H-14 (Kurtak 1986) and to an acceleration of a screening program in a search for possible alternative larvicides.

2 Considerations in selecting a candidate larvicide for testing include the following:

1. It must be active against all larval instars of *S. damnosum* s.l. Ideally, the concentration required to kill all of the larvae with a 10-min. exposure should not exceed 0.1 mg/liter (equivalent to temephos). Concentrations up to 0.3 mg/liter might be accepted in some circumstances but higher application rates cause serious logistical problems because of the limited capacities of the helicopters and fixed-wing aircraft used for spraying.
2. The distance that the larvicide remains nearly 100 % effective downstream (“carry”) should approach 20 km in large rivers.
3. Since the larvicide is added to rivers which serve as village water supplies, formulations with a rat oral toxicity greater than 250 mg/kg (LD50) are ruled out. In practice, this implies that dilute formulations of moderately toxic materials can be tested. Danger to the Program and aerial contractor staff handling the product must also be limited, especially since pilots work alone in remote locations.
4. The formulation, ideally, should have no acute or long-term toxic effects on fish even at considerable overdoses. Toxicity to non-target invertebrates should be minimal, but it is recognized that some reduction will occur. In principle, no important group of invertebrates should be eliminated, but shifts in proportions are considered to be tolerable.

5. The preferred formulation is an emulsifiable concentrate which has a lower specific gravity than water and forms a stable emulsion without agitation. Formulation has an important influence on performance, but the factors influencing effectiveness are not well understood. Wettable powders and flowable formulations, especially those which must be diluted with water, are more difficult and dangerous to mix, load and apply, and are generally avoided. The formulation should not degrade rapidly when stored as a concentrate in unprotected containers in a hot climate where the air temperature often reaches 40°C.
6. The applied larvicide should not be so stable as to lead to its accumulation in the food web.
7. Formulations with solvents that corrode the spraying equipment and the aircraft itself should be avoided.

3 Of the insecticides currently used or proposed for use in the program, only temephos comes close to meeting all of these criteria. Other than temephos, chlorphoxim (another organophosphate) and *B.t.* H-14 have already been used extensively. A pyrethroid (permethrin) and a carbamate (carbosulfan) have recently undergone large-scale operational trials in OCP and are entering into routine use.

4 This paper will briefly describe the screening methods and criteria for acceptance and then compare the new larvicides with those already available and comment on possible modalities for their use.

SCREENING METHODS AND CRITERIA FOR SELECTION[1]

5 To be introduced into the OCP screening program, a compound must first have demonstrated that it kills mosquito larvae at low concentrations. Mosquito trials are carried out by the WHO Pesticide Evaluation Scheme (WHOPES) which is described in World Health Organization (1982). The compounds must also meet the toxicological criteria mentioned in the introduction, as well as can be determined from the manufacturers data.

6 *Susceptibility tests:* As a first step in screening, the compound is subjected to a series of susceptibility tests to determine its cross-resistance status vis à vis larvae resistant to organophosphates. These tests are carried out according to the method of Mouchet et al. (1977). Mature larvae are exposed for 3 hours to technical grade compound mixed in oxygenated distilled water. The compound is added to the water as an alcoholic solution. There is no agitation. Temperature is held constant between 20 and 25° C and the mortality is read immediately at the end of the exposure. If there is significantly less mortality with organophosphate-resistant versus susceptible larvae, it is concluded that cross-resistance exists and the compound is not tested further. To date such tests have demonstrated cross-resistance with carbamates, and a negative correlation with pyrethroids (Kurtak et al. 1987a).

7 *Trough test:* If there is no evidence of cross-resistance, the formulated product is next tested in a field laboratory in a closed circuit trough System similar to that described in an unpublished OCP report.[2] In this System, a small electric pump circulates river water between a 60 liter plastic garbage can and a small trough. The trough discharges back to the garbage can over a narrow, shallow lip where the water velocity reaches that required by *S. damnosum* (about 1 m/sec). In the bottom of the lip is a removable metal plate. Field-collected larvae, when placed in the trough, migrate to the lip and attach to the metal

plate. This plate is then transferred to the lip of another trough-bucket System where insecticide is circulating with the water. After a 10-minute exposure (length chosen to simulate a field application), the plate is transferred to a third untreated "holding" system where mortality is scored after 24 hours by counting the surviving larvae and comparing this number with the total exposed. All detached larvae are considered to have died. In the control plates subject to the same manipulations, detachment is less than 1%. In trough testing, the goal is to determine the minimum concentration which gives 100% or nearly 100% mortality. Insecticides that require more than 0.5 mg/liter/10 min to obtain this level of control are not tested further. The more active compounds are next tested in rivers.

8 *River tests:* If activity is sufficient, individual river applications are carried out to confirm the operational dose and observe the carry. These tests consist of applying the formulated product by spraying or pouring above a series of breeding sites (rapids) where trailing grass or other larval attachment sites have been checked and the densities of young (instar 1 to 5) and mature (instars 6 and 7) larvae have been estimated. Twenty-four hours after treatment the sites are examined again and the percentage reduction in larval numbers estimated. The initial dose used is the minimum which gave 100% effect in the troughs. If this result is not achieved in the field, river tests are continued until the minimum field dosing rate giving 100% is found. For a product to be considered for OCP operations this dosing rate should be no more than about 700 ml/cubic meter (m 3) of discharge (for a 25% EC formulation equivalent to 0.3 mg/1-10 min of AI or 0.5 mg/1-10 min for a 50% EC formulation). Compounds with dosing rates of 0.1 mg/1-10 min or less are much more attractive. If the product shows some promise, but the dose is high, the manufacturer will be asked to furnish more formulations.

9 The results of the carry observation are more difficult to interpret, since with any product carry will vary with the transport characteristics of the river. Among the characteristics influencing transport, discharge is the most important. An application of temephos at 0,1 mg/1-10 min in a river flowing at 2 m^3/sec in the dry season may only cover one rapids (a few hundred meters) while the same compound at 0.05 mg/1-10 min will clear 30 km or more of the same river in the rainy season at 200 m^3/second. In general, carry is considered "adequate" when results obtained are at least equivalent to the operational compounds, i.e., 2-5 km at 10 m^3/sec and 10-15 km at 100 m^3/second.

10 If activity and carry are adequate, several more river tests may be done to cover the range of turbidity and discharge to be encountered during operations. Later applications will be carried out with helicopter or fixed-wing aircraft equipped with standard OCP release equipment. Six or more river tests are often done. Several formulations may be tested in parallel to ensure that an optimum formulation has been found.

11 *Non-target fauna:* As soon as field effectiveness against *S. damnosum* s.l. is confirmed, evaluation of effects on the non-target invertebrates begins. Even before this, during the first river tests, a close watch is kept for drastic mortality of fish or Crustacea. Any evidence of this will cause the product to be dropped. Catastrophic effects almost never occur because of prescreening based on manufacturer'» data and literatura. Lacking catastrophic effects, a detailed evaluation is carried out. The first step is a test against non-target insects in troughs parallel with standard insecticides [see Troubat (1981) for details of method). Acute toxicity to fish and Crustacea is determined in aquaria. The effect of individual river applications is followed by surber sampling (before and after),

drift net samples before, during and after the application, and observation of detachment of non-target insects in gutters (Lévêque et al. 1977).

12 *Operational trial:* If the short-term effects of individual treatments on the non-target fauna are acceptable, and all other criteria (compatibility with application equipment, etc.) are met, an operational trial is carried out. An entire river basin is treated weekly by aircraft. These treatments continue for several months, so that the effectiveness of the formulation both in controlling larvae and in reducing the number of biting *S. damnosum* s.l. females over a wide area can be evaluated. At the same time changes in the non-target fauna are monitored by a combination of the methods described above plus fish net catches before, during and after treatments. The monitoring continues for several months after the applications stop, to follow recolonization. Sediment samples are recovered along the river at the end of the trial and at various times after the treatments stop. Insecticide residues are extracted and analysed by appropriate methods (usually GLC).

13 All of the results from the aforementioned procedures are considered in making a decision as to if, and under what conditions, the product will be used operationally.

RESULTS AND DISCUSSION

14 As of December 1986, 18 organophosphate compounds, 2 organochlorine compounds, 10 carbamates, 17 pyrethroids and 6 miscellaneous categories have been screened. This includes a total of 115 formulations. The results are summarized in Appendix 1. Where several formulations have been tested, only the results with the best one are given. In general, it can be seen that most of the organophosphates have been rejected because of cross-resistance with strains resistant to temephos and/or chlorphoxim. Only one pyrethroid, permethrin, and one carbamate, carbosulfan, have been subjected to operational trials. Several other pyrethroids with similar characteristics may be suitable for operational trial, but have not been given high priority because of the probability of cross-resistance between pyrethroids, if resistance to permethrin were once selected.

Table 1. Performance of Simulium larvicides operational in the Onchocerciasis Control Program.

Compound	Class[1]	Formulation	Operational dose (mg AI/ liter for 10 min)	Volume of formulation per m³/sec (liters)	Influence of[2]		Carry[3] (km)		Effect on non-target invertebrates
					Algae	Turbidity	Low discharge	High discharge	
temephos	O-P	emulsifiable concentrate	0.05–0.1	0.15–0.30	0	+	1–3	50	acceptable for continuous use in all seasons
chlorophoxim	O-P	emulsifiable concentrate	0.05	0.15	0	0	1–3	15	acceptable for long-term use in wet season
carbosulfan	Carb	emulsifiable concentrate	0.05	0.12	0	0	3–6	10–15	acceptable for short-term use in wet season
permethrin	Pyr	emulsifiable concentrate	0.015	0.045	–	0	1–3	15	acceptable for short-term use in wet season
B.t. H-14 (Sandoz H-PD or Abbott 12AS)	bacterial toxin	suspension	1.2	0.72	–	–	1–2	10	acceptable for continuous use in all seasons

[1] For abbreviationa see Appendix 1.
[2] 0 = neutral,+ = incressed efficacy, – = reduced efficacy.
[3] Distance below application point with 100% effect.
[4] 10 m^3/sec or less.
[5] 100 m^3/and above

Table 2. Cost effectiveness and risk of selecting resistance of *Simulium* larvicides operational in the Onchocerciasis Control Program.

Compound	Relative cost per km of river treated[1]		Risk of selecting resistance
	Dry season (10 m^3/sec)	Wet season (500 m^3/sec)	
temephos	1.0	1.0	high (forest species) moderate (savannah species)
chlorophoxim	0.7	1.3	high (forest species) moderate (savannah species)
carbosulfan	0.9	2.3	moderat
permethrin	0.6	0.7	high
Bacillus thuringiensis H-14 (Sandoz HP-D or Abbott 12AS)	1.4	4.3	low

[1] temephos = 1.0

15 In Tables 1 and 2, the newly-selected compounds are compared with the existing operational compounds (temephos, chlorphoxim and *Bacillus thuringiensis* H-14 (Sandox HP-D formulation)). These tables will be commented on in some detail since they represent the knowledge gained by considerable practical experience.[3] A general review of control methods is given by Kurtak et al. (1987b).

16 Temephos has the best combination of low dosage rate, long carry (up to 50 km), mineral effect on non-target fauna and cost effectiveness. It has a very low mammalian toxicity and the formulation used does not damage application equipment or aircraft. The emulsifiable concentrate retains its efficacy after 3 or more years of storage under tropical conditions. Turbidity and high discharge rates in the rivers enhance its effectiveness to the extent that the dosage can be cut in half during the wet season. Unfortunately, resistance to it has developed in both forest and savannah species of the *S. damnosum* complex (Kurtak 1986, Kurtak et al. 1987b).

17 Chlorphoxim is as effective as temephos at the application point, but has a much inferior carry. It also has a more severe effect on non-target organisms and so is not normally used over long periods or when rivera are low. The emulsifiable concentrate formulation used is corrosive to spray equipment and aircraft and its storage life is not more than 2 years. In temephos-resistant populations, resistance to chlorphoxim develops in less than one year of weekly treatments. This resistance is not always stable in the absence of treatment and the product can be used for brief periods each year in some areas.

18 *Bacillus thuringiensis* H-14 is effective against both susceptible and resistant populations of S. *damnosum* s.l. It is not injurious to most non-target organisms. Although formulations have improved over the last 6 years, a high dosage rate is required (0.72 liter/m^3/sec of river discharge compared with temephos which requires 0.15 to 0.3 liter/m^3/second). Given the type of aircraft used in OCP, the use of *B.t.* H-14 is normally limited to rivers with a discharge rate below 100 m^3/second. In exceptional cases, it has been used, with considerable difficulty and added expense, in larger rivers to eliminate temephos-resistant larvae before other alternatives were available. The product is formulated as a more or less viscous suspension. Some formulations require dilution before application. Even those which do not must be applied as a coarse spray or dumped at high airspeeds to ensure good dispersel. Placement must be precise for effective control and carry is much inferior to that of temephos.[4] As a result, in large complex rapids, as much as 20 times more product is needed as compared to temephos and the cost is at least 4 times greater (Table 2). In the dry season, the increased cost of *B.t* H-14 treatments is less, but still

substantial, given the scale of OCP. Another disadvantage of *Bacillus thuringiensis* H-14 is that at the beginning of treatments, more weekly cycles are needed to achieve 100% control than with other products. There are con8istently more small failures along treated rivers. Algae growing in stagnant pools between rapids in the dry season interfere with *B.t.* H-14 and make it necessary to increase the dosage. High turbidity during the wet season may also reduce effectiveness.

19 The potential for development of resistance to *B.t.* H-14 is thought to be low. Work with mosquitoes (World Health Organization 1986) showed only a low level of resistance after 60 generations. However, resistance to another strain of *Bacillus thuringiensis* has been detected in stored grain pests (McGaughey 1985). There remains considerable latitude for improving the effectiveness of the product through augmenting active toxin content and improving formulation.

20 Permethrin is effective against *S. damnosum* s.l. larvae at dosage rates 1/6 to 1/3 that of temephos. Permethrin is available as an emulsifiable concentrate that posts no problema so far as its stability in storage, its dispersal in river waters, or its corrosive effect on equipment are concerned. Unfortunately, the carry is less than for temephos, not exceeding 15 km under ideal conditions for any of the many formulations that have been tested (unpublished OCP data). Nonetheless, due to its effectiveness at low concentrations, the cost/km of river treated is only 60 to 70% that of temephos. As is the case with *B.t.* H-14, high concentrations of algae, such as may be found in nearly stagnant rivers during the dry season, can reduce the effectiveness of permethrin to almost zero.

21 Permethrin is somewhat more effective against a organophosphate-resistant population of *S. damnosum* than against a susceptible population. There is also some evidence that permethrin accelerates the reversion of chlorphoxim resistance. The operational usefulness of this phenomenon is being explorad (Kurtak et al. 1987a).

22 Permethrin causes the most severe reduction in non-target invertebrates of any of the larvicides in operational use. This reduction persists as long as the treatments are continued. However, after treatment stops, recovery to pretreatment levels occurs within a few months. Permethrin is only used for short periods at high river discharge to eliminate organophosphate resistant populations.

23 Many insects readily develop resistance to pyrathroids with widespraad cross-resistance to other pyrathroids (World Health Organization 1976). As pyrathroids are used to control cotton pests along the banks of some rivers in the OCP area, there is the possibility that some pre-selection has already occurred due to runoff. Even in areas where no *Simulium* larviciding has been carried out, seasonal changes in pyrethroid susceptibility have been recorded (OCP unpublished data). These may be related to agricultural use.

24 Carbosulfan, in its present formulation, requires the same dosage rate as chlorphoxim, and has the same limited carry of about 15 km below the treatment point under optimal conditions. The insecticide formulated as 250 g AI/liter EC is not corrosive to equipment. Its storage life under tropical conditions is at least 18 months.

25 Carbosulfan is less injurious to non-target organisme than permethrin, but more toxic than temephos or chlorphoxim. Its toxicity becomes more pronounced at river discharge rates below $75m^3$/sec. Below $25m^3$/sec there is a danger that fish will be traumatized (OCP unpublished data). Therefore, this product can only be used at high river discharges and for limited periods of time.

26 There is also a risk of resistance developing to carbamates, including carbosulfan, which could lead to cross-resistance to organophosphates but not to pyrathroids according to Hemingway and Lines (1985).

27 The pattern of use of these and other larvicides in future OCP operations will depend on the extent and character of resistance that develops in *S. damnosum* s.l. populations and on a further analysis of their effects on non-target fauna. The present strategy is to use *B.t.* H-14 during the dry season when the rivers are low because it is nearly as effective and much less injurious to non-target fauna than the other operational insecticides and also because it is less likely to provoke the development of resistance. These advantages compensate the moderate increase in cost. As river discharges increase, organophosphates, carbamates and pyrathroids will be used in a sequence determined by the susceptibility of the populations involved. Temephos will remain the product of choice during the rainy season when rivera are high and where there is no resistance. It is hoped that dry-season alternation with *B.t.* H-14 will retard the development of resistance to temephos.

28 Where resistance to temephos occurs, chlorphoxim will be used when there is no cross-resistance. An alternation between chlorphoxim and permethrin might be envisaged if the "negative correlation" proves to be operationally useful. If not, carbosulfan might be chosen over permethrin in spite of its high cost because it is not as injurious to non-target fauna and less likely to provoke the development of resistance as compared to permethrin.

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APPENDIXES

APPENDIX 1. Compounds testad as *Simulium damnotum* larvicides in the Oñchocerciasis Program since the beginning of accelerated screening in 1982.

			Results					
Compound[1]	Class[2]	Formulation[3]	Cross-resistance	Trough test[4]	River trial[5]	Effect on non-target fauna	Operational trial	Remarks
azamethiphos (Ciba-Geigy)	O-P	EC 10%	no	acceptable (0.2 to 0.3)	insufficient activity (0.3/3–6/10–20)	acceptable	none	poor storage life, 10% max. conc.
bromophos (Celamerck)	O-P	tech AI	yes	none	none	unknown	none	–
OMS 3036 CME 16002 (Celamerck)	O-P	EC 48%	no	acceptable (0.3)	in progress	in progress	none	–
chlorfenvinphos (Shell)	O-P	tech AI	yes	none	none	unknown	none	–
chlorphoxim (Bayer)	O-P	EC 20%	no[6]	acceptable (0.05)	acceptable (0.05/2–3/2) (0.05/2–8/35)	acceptable	1977	"improved" formulations
chlorpyrifosmethyl (Dow)	O-P	tech AI	yes	–	none	unknown	none	–
dichlorvos (Ciba-Geigy)	O-P	EC 50%	no	insufficient activity (0.53 = 98%)	none	unknown	none	–
etrimphos (Sandoz)	O-P	tech AI	yes	–	none	unknown	none	–
fenchlorvos (Dow)	O-P	tech AI	yes	–	none	unknown	none	–
fenthion (Bayer)	O-P	tech AI	yes	–	none	unknown	none	–
iodenphos (Ciba-Geigy)	O-P	tech AI	yes		none	unknown	none	–
pirimiphosmethyl (ICI)	O-P	tech AI	yes	–	none	unknown	none	–
propetamphos (Sandoz)	O-P	tech AI	yes	–	none	unknown	none	–
temephos (Phytagri)	O-P	EC 20%	N.A.	acceptable (0.2)	acceptable (0.1/6.5/7)	acceptable	successful	alternative supplier
temephos sulfone (Cyanamid)	O-P	tech	yes	none	none	unknown	none	–
temephos sulfoxide (Cyanamid)	O-P	tech AI	yes	none	none	unknown	none	–
tetrachlorvinphos (Shell)	O-P	tech AI	yes	none	none	unknown	none	–
trichlorphon (Bayer)	O-P	tech AI	yes	none	none	unknown	none	–
cartap (Takeda)	Carb	WSP 50%	no	insufficient activity (1.0 = 50%)	none	unknown	none	–
carbosulfan (FMC)	Carb	EC 25%	no	acceptable (0.05)	acceptable (0.05/10–15690) (0.05/8–9/17)	acceptable for short periods	successful	formulation under development
ethiofencarb (Bayer)	Carb	EC 50%	no	insufficient activity (2.0 = 93%)	none	unknown	none	–
macbal (Hodogaya)	Carb	EC 20%	no	insufficient activity (3.0 = 98%)	none	unknown	none	–
methiocarb (Bayer)	Carb	F 50%	no	acceptable (0.5)	acceptable (0.35/6/4)	unknown	none	formulation unsuitable for operational use
methiocarb (Bayer)	Carb	EC 10%	no	acceptable (0.5)	acceptable (0.3/3.5/2) but formulation too dilute	unknown	none, dosage almost 2 liters/m³	impossible to increase AI content
metolcarb (Sumitomo)	Carb	EC 30%	no	insufficient activity (2.0 = 75%)	none	unknown	none	–
pirimicarb (ICI)	Carb	EC 5%	no	insufficient activity (0.6 = 0%)	none	unknown	none	–
propoxur (Bayer)	Carb	EC 2%	no	insufficient activity (1.3 = 83%)	none	unknown	none	–
thiodicarb (Union Carbide)	Carb	F 37.5%	no	insufficient activity (1.1 = 83%)	none	unknown	none	–
xylylcarb (Sumitomo)	Carb	EC 30%	no	insufficient activity (0.7 = 93%)		unknown	–	–
DDT (Ligtermoet Chemical B.V)	O-C	EC 25%	no	acceptable (0.3)	acceptable (0.2/3/1.5)	residues pose serious problems	used sucessfully for many years before OCP	emergency use only authorized
methoxychlor (Nordisk)	O-C	EC 30%	no	acceptable (0.5)	insufficient activity (0.5 = 90%/0.5/2)	unknown	–	–
alphamethrin (Shell, FMC, ICI)	Pyr	EC 10%	no	acceptable (0.01)	acceptable (0.010/3/4)	more toxic than permethrin	none	–
cyfluthrin (Bayer)	Pyr	EC 5%	no	acceptable (0.015)	none	similar to permethrin	none	no advantage over permethrin
cyhalothrin (ICI)	Pyr	EC 10%	no	acceptable (0.025)	acceptable (0.025/7.8/4)	less than permethrin but kills Crustacea	none	too toxic to Crustacea
cypermethrin (Shell)	Pyr	EC 40%	no	acceptable (0.006)	acceptable (about 0.01)	very severe, especially Crustacea	none	more toxic to non-target fauna than to *Simulium*
cyphenothrin (Sumitomo)	Pyr	EC 5%	no	acceptable (0.005)	acceptable (0.01/1/11)	similar to permethrin	none	no advantage over permethrin
cypothrin (Cyanamid)	Pyr	EC 20%	no	acceptable (0.10)	insufficient activity	unknown	none	–
deltamethrin (Roussel-Uclaf)	Pyr	EC 1%	no	acceptable (0.0005)	acceptable (0.002/4/12)	severe mortality to Crustacea	none	effective, but too toxic for non-target fauna
esfenvalerate (Sumitomo)	Pyr	EC 2.5% F 5%	no	insufficient activity (1.0 to 2.0)	none	unknown	none	–
ethoproxyfen (Zoecon/Mitsui Tostsu)	Pyr	EC 60%	no	acceptable (0.1)	acceptable (0.2/3/1)	similar to permethrin	none	high dosage rate required
fenfluthrin (Bayer)	Pyr	EC 20%	no	acceptable (0.01)	acceptable (0.015/2.5/1.8)	similar to permethrin	none	no advantage over permethrin
flucythrinate (Cyanamid)	Pyr	EC 20%	no	acceptable (over 0.13)	none	probably severe	none	high mammalian toxicity
fluvalinate (FMC)	Pyr	EC 25% F 24%	no	acceptable (0.1 to 0.5)	insufficient activity (0.48 = 99% at 150m)	moderate at 0.25	none	dosage rate too high
OMS 3021 (ICI PP 321)	Pyr	EC 5%	no	acceptable (0.02)	acceptable (0.01 = 99%)	similar to permethrin	none	no advantage over permethrin
permethrin (Shell, Wellcome ICI, FMC)	Pyr	EC 20%	no	acceptable (0.010 to 0.015)	acceptable (0.015/15/86)	roughly 70% reduction, 80–90% of Emphemeroptera	1984 in operational use	–
Pynaminforte (Sumitomo)	Pyr	EC 5%	no	acceptable (0.02 to 0.1)	in progress	moderate at 0.02	none	–
neo-Pynaminforte (Sumitomo)	Pyr	EC 5%	no	acceptable (0.05 to 0.1)	in progress	unknown	none	–
Talstar® FMC 54800 (OMS 3024)	Pyr	EC 10	no	acceptable (0.01)	in progress	similar to permethrin	none	–

			Results					
Compound[1]	Class[2]	Formulation[3]	Cross-resistance	Trough test[4]	River trial[5]	Effect on non-target fauna	Operational trial	Remarks
avermectin (Merck, Sharpe and Dohme)	lactone	EC 1	no	acceptable (0.03)	none	unknown	none	mammalian toxicity too high
Bensultap (Takeda)	*Nereis* toxin	WP 50% EC 25%	no	insufficient activity (0.5 to 3.0+)	none	unknown	none	–
evisect (Sandoz)	*Nereis* toxin	WSP 25%	no	insufficient activity (over 6.0)	none	unknown	none	–
octyl propargyl sulfite (Uni-Royal)	sulfite	EC 90%	no	insufficient activity (0.5 = 50%)	none	unknown	none	–
BTS 49178, pure Zisomer OMS 3027 (FBC)	hydrazone	F	no	insufficient activity (1.2)	none	unknown	none	–
BTS 49178, Z + E isomers OMS 3028 (FBC)	hydrazone	F	no	insufficient activity (1.2)	none	unknown	none	–

[1] Common name, WHO code, or manufacturer's code. Manufacturer un parentheses.
[2] O-P = organophosphate, Carb = carbamate, Pyr = pyrethroid, O-C = organochlorine.
[3] EC = emulsifiable concentrate, F = flowable, WDP = water dispersable powder, WSP = water soluble powder.
[4] In parentheses = LC 100 in mg/liter/10 min followed by carry in km followed by river discharge in m^3 /sec.
[5] In parentheses = operational dose in mg/liter/10 min followed by carry in km followed by river discharge in m^3/sec.
[6] Multiple resistance develops rapidly.

NOTES

1. This section deals with the screening of Chemical compounds other than bacterial toxina and insect growth regulators which will be the subject of other reports.
2. Jamnback, H. 1982. Insecticide screening tests against *Simulium damnosum* larvae, Lama Kara, Togo. Unpublished OCP report.
3. In the height of the wet season, OCP aircraft apply about 8.5 tons of insecticide at up to 4,500 applications points on 15,000 km of river every week.
4. Emulsifiable concentrates, especially of temephos, can be applied quite successfully through a simple pipe or dump System. Their placement is less critical, since there is a spreading effect and the emulsions form spontaneously.

ABSTRACTS

The Onchocerciasis Control Program of the World Health Organisation is carrying out an extensive screening program in a search for new larvicides to be used for control of *Simulium damnosum* s.l. Emphasis has been given to finding a pyrethroid and a carbamate to supplement the organophosphates currently in use. These Chemicals with differing modes of action, together with *Bacillus thuringiensis* H-14, are being used in an attempt to cope with the development and spread of resistance to the organophosphates temephos and chlorphoxim.

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Onchocerciasis control programme in West Africa: ten years monitoring of fish populations

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INTRODUCTION

1 Human Onchocerciasis is a dermal filariasis particularly serious in Guinean and Sudanian African savannas where it causes irreversible blindness among exposed populations. The filaria Onchocerca volvulus is transmitted to man by female blackfly of the Simulium damnosum complex (Philippon, 1977). The larvae of these flies are aquatic and occur only in fast-flowing parts of rivers. Thus the disease is most prevalent near water courses.

2 In the absence of any effective cure suitable for large-scale use, vector control was the most effective way to prevent the spread of this disease. Adult control being difficult, Chemical treatment of larval stages in the rivers was considered the only feasible method (Anon., 1985).

3 The Onchocerciasis Control Programme (OCP) commenced in December 1974 under the auspices of the World Health Organisation (WHO) and was planned for a twenty year intensive implementation (Davies et al., 1978). The initial control area of 764,000 km’included Burkina Paso and parts of Ivory Coast, Ghana, Togo, Benin, Niger and Mali (Figure 1). The first routine insecticide treatments were in February 1975 in the central part of the OCP area, and have been progressively extended. Up to 18,000 km of rivers have been monitored, and treated when necessary in the weekly spraying programme.

4 Prolonged and extensive use of insecticides could have important environmental risks, and therefore it was necessary to evaluate the possible short-, medium-and long-term effects of insecticides on the non-target fauna (Leveque et al., 1979).

5 The aquatic monitoring programme is performed by national teams of scientists in the countries in the OCP area, aided by outside specialists. This support was essential because little information on the aquatic fauna was available. The surveillance has been primarily

concerned with two major categories of organisms: the fish, by virtue of their economic importance, and the benthic invertebrates, which may more quickly respond to insecticides. An important consideration for OCP is the demonstration to the local human population that care is being taken in reducing the risks of pollution.

6 An independent Ecological Group, consisting of experts of international repute, meets every year and is in charge of the evaluation of the collected data which has been analysed by outside specialists (Cummins, 1985). The group advises OCP on safe insecticide use and new monitoring procedures.

AIMS AND PROTOCOL

7 All insecticides used by OCP have to follow an intensive screening procedure in order to prove their high toxicity against the S. damnosum larvae, and their low toxicity for non-target fauna (Leveque, 1987). The criteria are that the pesticides should have neither any direct impact on fish nor any effect on their life cycles. Among hundreds of insecticide formulations tested by OCP, few are selected after operational field trials.

8 The fish monitoring programme was based on two fundamental ideas: -

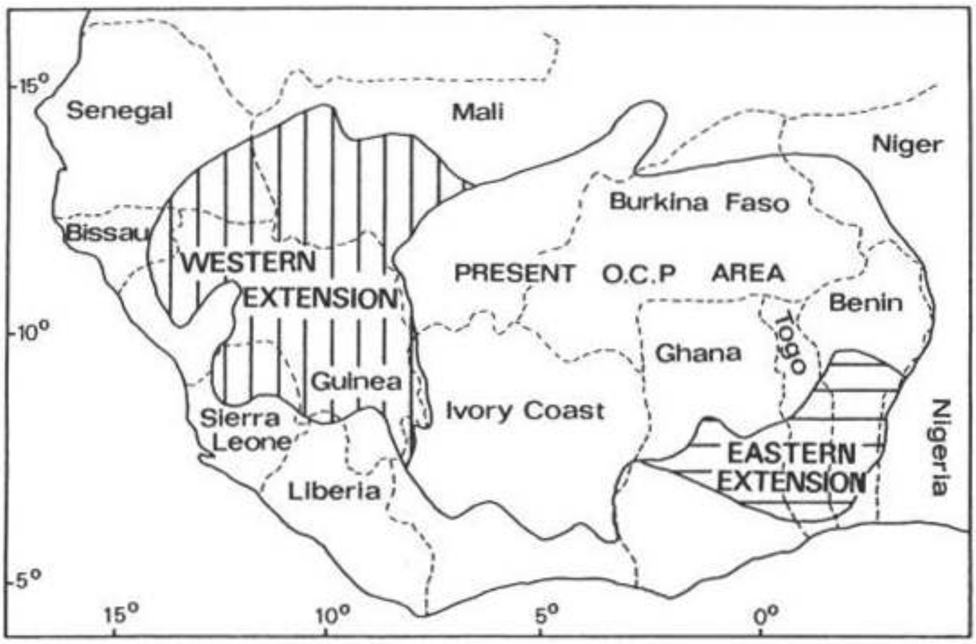


FIGURE 1 - Map of OCP area.

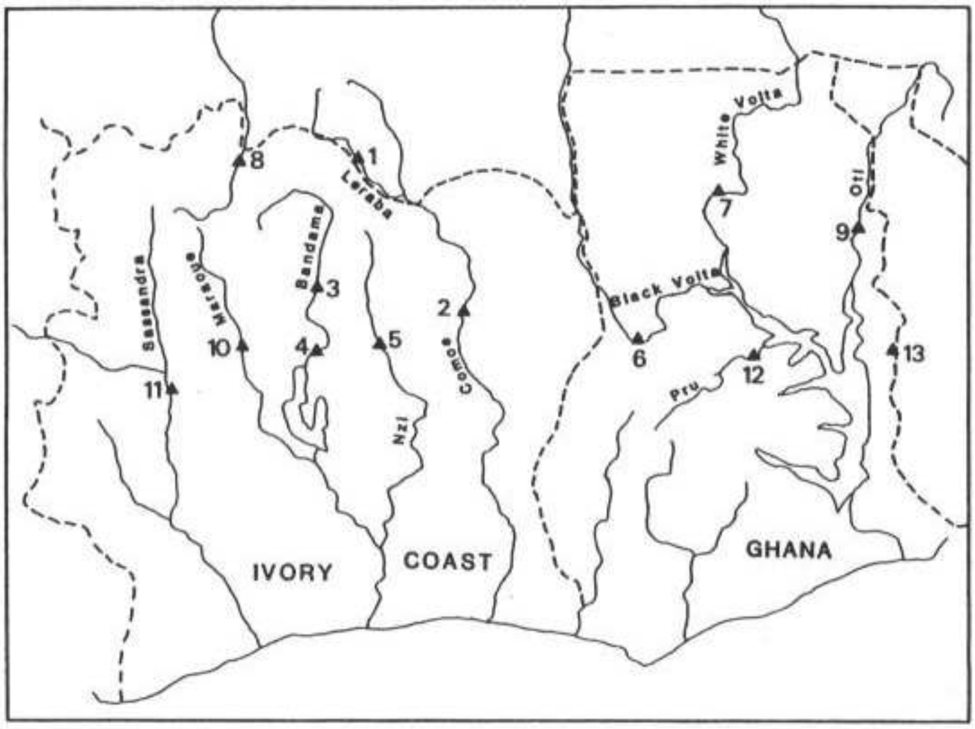


FIGURE 2 - Location of Major Fish Monitoring Stations in Ivory Coast and Ghana.
1. Leraba - Pont Frontiere
3. Bandama - Niakaramandougou
5. Nzi - bridge of Dabakala Road
7. White Volta - Daboya
9. Oti - Sabari
11. Sassandra - Semien
13. Wawa - Dodo Papasse
2. Comoe - Ganse
4. Bandama - Marabadiassa
6. Black Volta - Bamboi
8. Bagoe - Kouto
10. Maraoue - Mankono
12. Pru - Asubende

9 a) repeated long-term treatments could change the reproductive cycle of fishes, either by affecting the physiology or by direct effect on eggs or juveniles. If so, there could be changes in fish recruitment and, on a long-term basis, a decrease in fish abundance. This would apply to the fish community as a whole, or to particular species which could be more sensitive to insecticides.

10 b) Insecticides could affect the food chain leading to a serious reduction in diet.

11 It should be noted that such investigations could hardly be conducted in the laboratory because of the number and diversity of fish species involved, and the difficulties of maintaining most of the species in rearing conditions to complete their life cycle.

12 The monitoring stations (Figure 2) were chosen on the basis of accessibility at all seasons, suitability for sampling, availability of hydrological data and abundance of fish stocks.

13 In establishing the monitoring programme, the terms of reference included the introduction of simple standardized sampling techniques for use by different teams and under various environmental conditions (Leveque et al., 1979). Experimental fishing is therefore carried out using sets of gill nets 25 metres long and two metres deep with various mesh sizes (15, 20, 25, 30 and 40 mm).

14 Usually each collection is the result of two sets of gill nets fishing on two consecutive nights, but some protocol variations have taken place, particularly in the early years of the programme. Data sheets for each sample record the number and total weight of individual species caught in the different mesh sizes. for comparison and standardization, resulta are expressed as catch per unit effort (CRUE) which is the number or weight of fish caught in 100 m^2 of net per night. Most of the monitoring stations were investigated every three months, but again there were some protocol variations during the ten years, mainly due to the accessibility of stations and availability of teams.

15 From the results obtained in sampling the different stations, it was therefore possible to follow long-term changes in:

a. total catch for the set of gill nets with different mesh sizes or combinations of mesh sizes.
b. the number of species caught.
c. the quantity of each species caught.
d. the structure of the fish catch i.e. relative abundance of species in each mesh size.

16 Coefficient of condition, is a standard expression of the health of fishes which provides an assessment of feeding and ecological conditions. fish were individually measured and weighed, to estimate the coefficient of condition (K) derived from the formula: - $K = W \times 10^5 / L^3$. where W is the weight in grammes and L is the standard length in mm.

17 Before the monitoring programme there were very few detailed studies on the biology and ecology of West African fishes. It was soon apparent that additional research was essential if the results of the monitoring programme were to be correctly interpreted. Various studies have since provided a better knowledge of the biology of the main species: Alestes baremoze, Brycinus nurse, B. imberi, B. macrolepidotus, B. lonqipinnis (Paugy, 1978, 1980a, 1980b, 1982a, 1982b), Petrocephalus bovei (Merona, 1980), Schilbe mystus and Eutropius mentalis (Leveque & Herbinet, 1980, 1982).

18 A study of the Bandama basin (Merona, 1981) provided information on the ecology of the fish species and confirmed the representative nature of monitoring stations. Electro-fishing has also been carried out in the rapids of some rivers to give a better understanding of fish populations of these habitats which cannot be sampled by gill nets, and of their changes over time. In order to help the different teams in identification of species, a catalogue of fishes was produced (Leveque & Paugy, 1984). All this information will be developed further in other publications.

INSECTICIDE TREATMENTS

19 Temephos ("Abate") is an organophosphûrus larvicide which was used exclusively from 1975 to 1980. A 20 % emulsion concentrate was applied at a dosage of 0.05 mgl^{-1} per 10 mn during the wet season, and at 0.1 mgl^{-1} per 10 mn in dry seasons.

20 In December 1979 temephos resistance developed in larvae of some cytospecies of the S. damnosum complex (Guillet et al., 1980; Kurtak, 1986) and spread rapidly to the Southern forest zone and part of the humid savanna zone. This situation led to a large-scale application of Bacillus thurinqiensis H14 ("Teknar") during the dry season (dose rate 1.2 mgl^{-1} per 10 mn) in these areas of resistance, together with "Chlorphoxim", another organophosphate, during the wet season (0.025 mgl^{-1} per 10 mn). However, a resistance to Chlorphoxim was discovered in July 1981 in the forest species already resistant to

temephos (Kurtak et al., 1982). This necessitated an acceleration of screening of other alternative insecticides, and "Permethrin" and "Carbosulfan" appeared to be promising. These had only been used in the field during the rainy season (0.015 mgl^{-1} per 10 mn) where resistance to organophosphates was observed. No direct effect on fishes was apparent.

21 By the end of 1985 the treatment situation could be summarised as follows. Temephos was still used in those regions of, the OCP area where no resistance had developed among Simulium populations; in the south-west, where strains resistant to this larvicide had appeared, Teknar was used where river discharge was below $75m^3s^{-1}$. Above this level, the strategy was to alternate other larvicides such as temephos, Chlorphoxim and, when necessary, Permethrin (WHO, 1986). As a result, insecticide treatment varied between rivers. Figure 3 illustrates the treatment regime for the main fish monitoring stations since the start of observations. More details of insecticides and treatment strategies will be found in Anon (1985).

22 It must be remembered that other insecticides may also affect the rivers in Ghana and Ivory Coast. Large amounts of agricultural pesticides, which are difficult to evaluate, may reach the water courses. According to Calamari (1985), 300 t of DDT, 600 t of Lindane, 100 t of methylparathion and 30 t of other compounds were used in 1976 in Ivory Coast. Since 1979 DDT has been replaced by organophosphates, carbamates and pyrethroids. Similar values are given for Ivory Coast by Balk and Koeman (1984) and are expected to increase two to three fold by the end of the century.

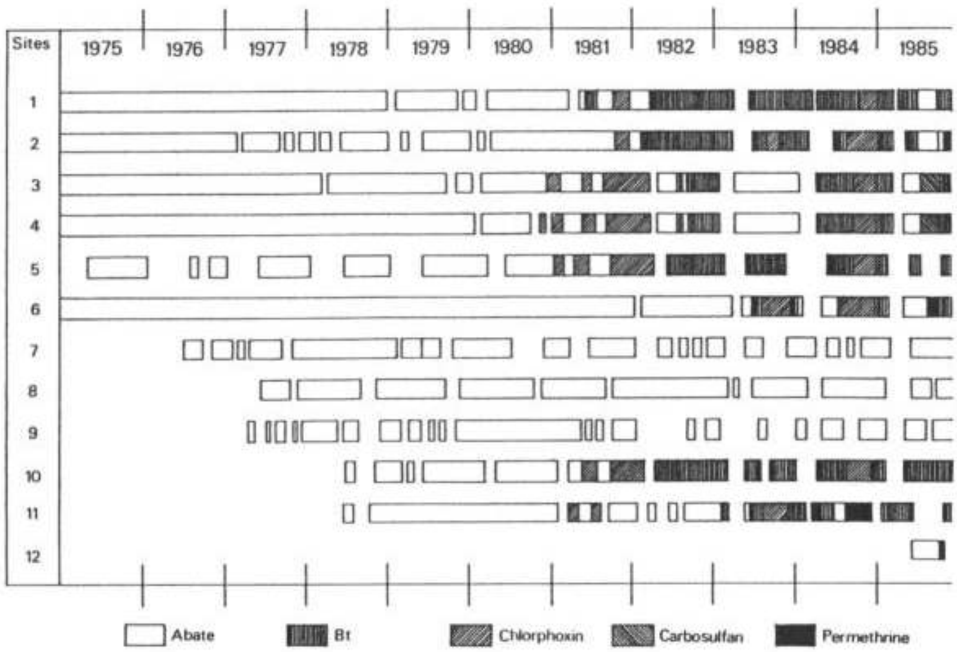


FIGURE 3 - Insecticide Treatment at the Major Aquatic Monitoring Stations

23 The areas surrounding some West African rivers are also treated with insecticides to control tsetse flies. The side effects of helicopter applications of dieldrin, endosulfan, permethrin and azamethiphos were monitored (Everts et al., 1983a, 1983b: Takken et al., 1978). No acute fish mortality was observed, except in Nigeria where a mass mortality of fish occurred following endosulfan spraying (Koeman et al., 1978) as well as in Ivory Coast (Everts et al., 1983b).

24 Another source of pollution is the sugar and fruit factories often situated along the rivers. The residues from this organic pollution could cause fish mortalities, such as those reported from the Sassandra upstream of the Semien monitoring station in 1985-1986.

RIVER HYDROLOGY AND BIOLOGICAL SIGNIFICANCE

25 The OCP area covers major river Systems in West Africa, such as the Volta basin, part of the Niger basin, the northern parts of the Sassandra, Bandama, Comoe, Mono and Oueme basins. Most of these rivers are savanna type, with a water regime characterised by a flood period from July to November, with a peak in September and a lengthy low water period from January to June. for Ivory Coast, hydrological and physicochemical characteristics for the main water courses are summarised in Iltis and Leveque (1982). For the Volta basin, Moniod et al (1977) gave a synthesis of hydrological data.

26 Many of the rivers in the central part of the OCP area are intermittent and may dry up completely. For permanent rivers discharge is very low during the dry season, and the upper course is sometimes reduced to a series of pools. There are therefore several seasonal changes in flow which result in major ecological changes for the fish species. However, the importance of the flood period is also directly related to the abundance of seasonal rains and as a result of climatic fluctuations, the water discharge of rivers exhibits large changes from year to year (Figure 4). There may be differences between basins, but it is clear that a poor hydrological situation prevailed in the whole OCP area from 1982 to 1984.

27 In tropical rivers fish biologists recognise that hydrology plays a major role in fish behaviour. Fish reproduction tends to be highly seasonal and correlated primarily with flow (Welcomme, 1985). This is the case for many West African species which spawn during the earlier part of the flood: Alestes baremoze, Brycinus nurse, Petrocephalus bovei, Marcusenius furcidens, M. ussheri, Labeo senegalensis, L. coubie, Schilbe mystus, Eutropius mandibularis etc. (Albaret, 1982; Leveque & Herbinet, 1980, 1982; Paugy, 1978, 1980). However, some species are known to breed throughout the year: B. imberi, B. macrolepidotus, Hydrocynus forskalii, Tilapia zillii, Hemichromis fasciatus (Albaret, 1982; Paugy, 1980, 1982).

28 It is also assumed that breeding success and survival of fry of many species could be related to the duration and water level of the flood period (for review see Welcomme, 1979, 1985). In years when there is insufficient water, the young fish have fewer refuges, are more vulnerable to predators and have fewer sources of food. Dansoko et al (1976) have shown that the reduction in commercial catches of Hydrocynus brevis and H. Forskalii in 1972 and 1973 was a consequence of inadequate levels during the flood periods, and resulted in a poor condition factor, limited growth and weak recruitment to the fish stocks.

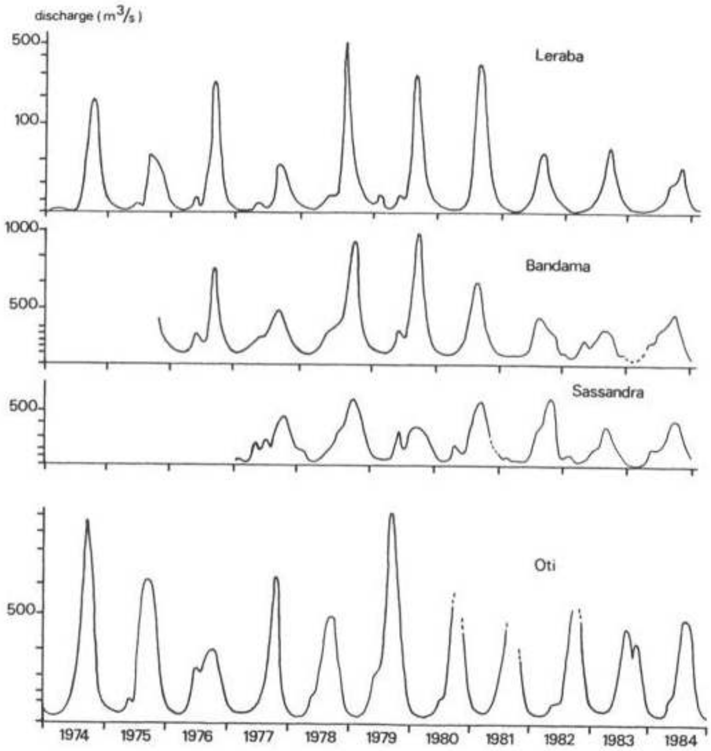


FIGURE 4 - Discharge ($m^3 s^{-1}$) per month for Some Monitoring Stations in the OCP area.

29 In a detailed study of fish populations from the Logone flood plain, Benech and Quensiere (1983) were also able to demonstrate over ten years the existence of a positive correlation between fish production and flood volume, as well as changes in species composition and community structure related to drought periods. Many fish species migrate long distances upstream at the beginning of the flood in order to spawn and find good conditions favouring the development of fry. This is the case in the rivers considered here for A. baremose, B. leuciscus, Mormyrus rume, Mormyrops deliciosus, Distichodus rostratus, Eutropius niloticus, E. mandibularis, tabeo senegalensis, etc. These migration patterns could be modified as a result of water management schemes such as dams and impoundments, whose numbers and surface area are expected to increase greatly by the end of the century (Clay, 1984). These dams could act as barriers interrupting upstream migrations, but could also favour species which develop in the lakes where they find good ecological conditions and migrate upstream during flood (See Bernaczek, 1984, for review). Such examples are the Volta and Kainji lakes (Kapetsky & Petr, 1984) as well as the Kossou lake on the Bandama. Since the beginning of the monitoring programme other dams have been built, such as the Taabo on the Bandama, Buyo on the Sassandra and numerous smaller irrigation reservoirs on the upper reaches of the rivers. Other projects are planned for the future in Ivory Coast and Ghana. As a result of this management, changes in fish community structures are expected in many rivers both upstream and downstream of the dams (Bernaczek, 1984).

30 In conclusion, the composition of experimental fish catches could be subject to: a) seasonal changes as a result of migrations; b) year to year changes as a result of climatic fluctuations; c) long-term changes following effects of impoundments; d) possible larvicide impacts.

ECOTOXICOLOGICAL STUDIES

31 The effects of organophosphates (temephos and Chlorphoxim) in laboratory experiments showed that fish were able to accumulate temephos (Miles et al., 1976; Matthiessen & Johnson, 1978), but this accumulation seems to be limited and does not increase indefinitely, as was observed with DDT for instance. As an example, Sarotherodon mossambicus exposed weekly to operational doses (0.05 mg 1^{-1} for 10 minutes) accumulated 3-4 mg kg^{-1} by direct absorption. They could also accumulate residues by eating contaminated food. An affinity of temephos for fatty tissues has been observed, but in contrast to organochlorides there is no accumulation in the liver.

32 According to results obtained in field conditions (Quelennec et al, 1977) fish captured during the dry season just below the spraying point exhibited traces of temephos (between 1.3 and 14.3 mg 1^{-1} according to species) one day after treatment. Six days later contamination was lower (between 1 and 7 mg 1^{-1}). At a distance of 1 km below the spraying point, fish were weakly contaminated (between 0 and 0.25 mg 1^{-1}). In the rainy season, accumulation of temephos is much lower; 0-0.4 mgl^{-1} five hours after spraying, just below the spraying point, and 0-0.03 mg 1^{-1} five days later. It should be mentioned that DDT residues were also found (0.01-0.35 mg 1^{-1}) in the fish studied, probably as a result of the use of this pesticide in agriculture.

33 The inhibition of acetylcholinesterase activity due to organophosphates was aiso studied in laboratory and field conditions. In the laboratory (Gras et al., 1982; Pelissier et al., 1982, 1983), where operational doses were tested (0.05 mg 1^{-1} for 10 minutes) inhibition by temephos was about 25" for Tilapia quineensis, but no fish intoxication was noted after repeated weekly exposures. When fish were exposed to the operational dose for 24 hours, the inhibitory effect is much higher; 38" after one exposure ad 69 % after three weekly exposures. In the latter case the fish did not survive. The inhibition of acetylcholinesterase activity appears more important with Chlorphoxim.

34 In field conditions, the acetycholinesterase activity in the fish brain does not seem to be significantly different in rivers treated with temephos or untreated (Antwi, 1983, 1984; Scheringa et al, 1981). When Chlorphoxim is used, fish captured below spraying points exhibited a 20" reduction in enzymatic activity, but this inhibition was shown to be reversible (Antwi, 1983, 1985).

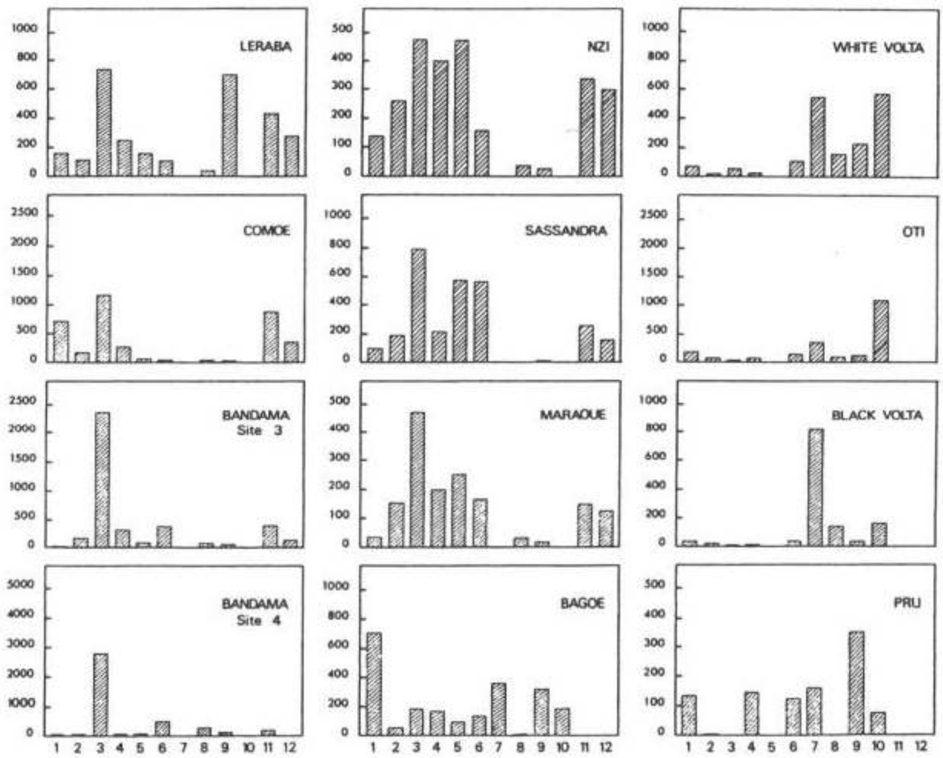


FIGURE 5 - Total Numbers of the Most Common Fish Caught in Gill Nets per Unit Effort at the Major Aquatic Monitoring Stations.
1. Petrocephalus bovei
3. Alestes baremoze
5. Brvcinus imberi
7. Brycinus leuciscus
9. Schilbe mystus
11. Eutropius mandibularis
2. Hydrocynus forskalii
4. Brycinus macrolepidotus
6. Brycinus nurse
8. Labeo senegalensis
10. Eutropius niloticus
12. Chrysichthys velifer

RESULTS OF ECOLOGICAL MONITORING

1. Species Composition

35 The monitoring stations are situated in different river basins and at different levels of the water course. It is therefore not surprising to observe differences between stations in the relative abundance between species (Figure 5). Alestes baremoze is the dominant species in the south-flowing Ivory Coast rivers except in the Leraba-Comoe basin where Schilbe mystus is co-dominant. The Sassandra demonstrates a spectrum similar to the Maraoue. The Bagoe (Nigerbasin) and Volta exhibit a somewhat different fish fauna: Alestes leuciscus, Eutropius niloticus and Chrysichthys auratus instead of Brycinus imberi, Eutropius mandibularis and Chrysichthys velifer respectively.

2. Number of Fish Species

36 The number of fish species caught at selected stations (Figure 6) exhibits a seasonal change with a maximum at low water. This represents the greatest efficiency of the gill nets. There are different long-term trends in each river, but overall there is no evidence of a reduction in species richness over the ten year period. for Ivory Coast, there is a decrease in the number of fish species between 1981 and 1984 which could be related to the poor river discharges during that period (Figure 4). The recovery observed in 1985 follows a season of particularly heavy rain.

37 The total number of species could mask changes in species composition, some species being replaced. However, the results did not show evidence of disappearance of fish species in the experimental catches.

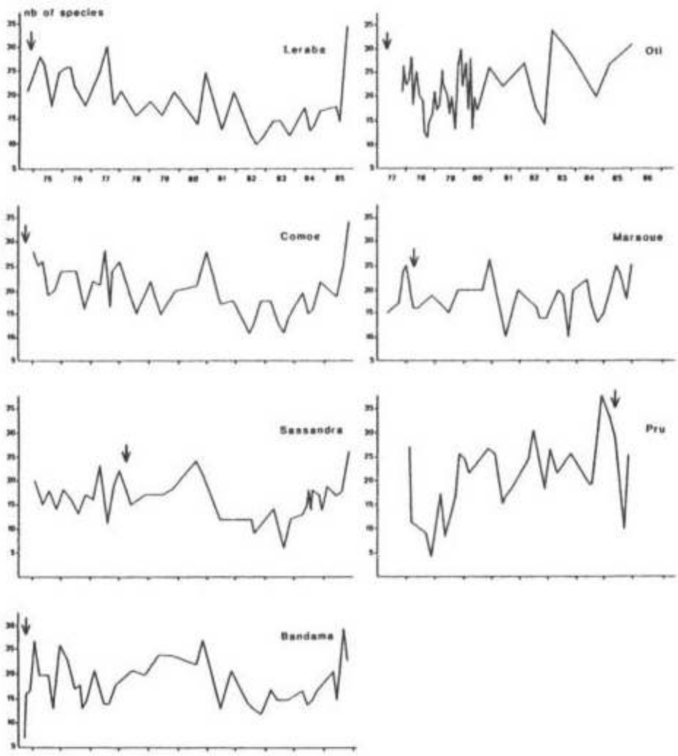


FIGURE 6 - Changes in Number of Fish Species per Sample at Various Stations Based on expectation of 2 sets of Gills nets on 2 consecutive nights.

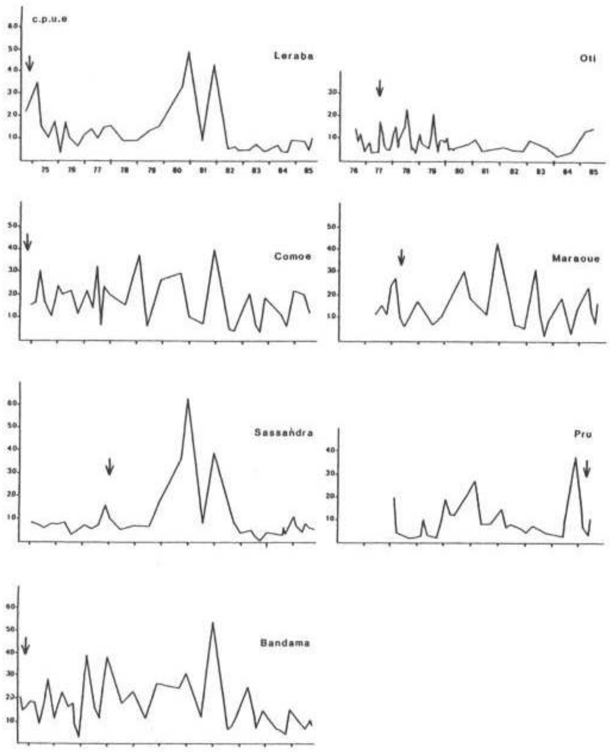


FIGURE 7 - Changes in Mean C.P.U.E. (Catch per 100 m^2 per night) For the Whole Set of Gill Nets in Various Stations

3. Changes in Total Experimental Catches

38 When considering changes in the fish catches, expressed as the mean CPUE For the standardized set of gill nets (Figure 7) » a seasonal pattern is generally observed, with higher catches at the end of the high water period (November to January) and lower catches during the flood (August to September). Again this pattern is partly the result of the Fishing gear being more eFFicient in low water conditions. The high CPUE values observed in the Sassandra in 1900 appear to be due to an increase in Alestes/Brycinus species (Alestes baremoze, Brycinus nurse, B. imberi). A similar phenomenon was observed in 1980-1901 in the Leraba due to increased catches of Alestes baremoze, Eutropius mandibularis, Schilbe mystus and Lates niloticus. Over the ten year period no long-term reductions in mean catch can be discerned.

39 When considering different mesh sizes (Figure 8), and particularly the smaller ones eFFicient For juveniles, there is a more obvious correlation with year-to-year changes in hydrology. At Niaka on the Bandama, For instance, catches in the 15 mm mesh size are lower in 1976-1977 and 1982-1984 which correspond to a poor Flood (Figure 4). The catch was better with more Favourable hydrology between 1979 and 1981. A similar situation occurred on the Leraba (Figure 8b).

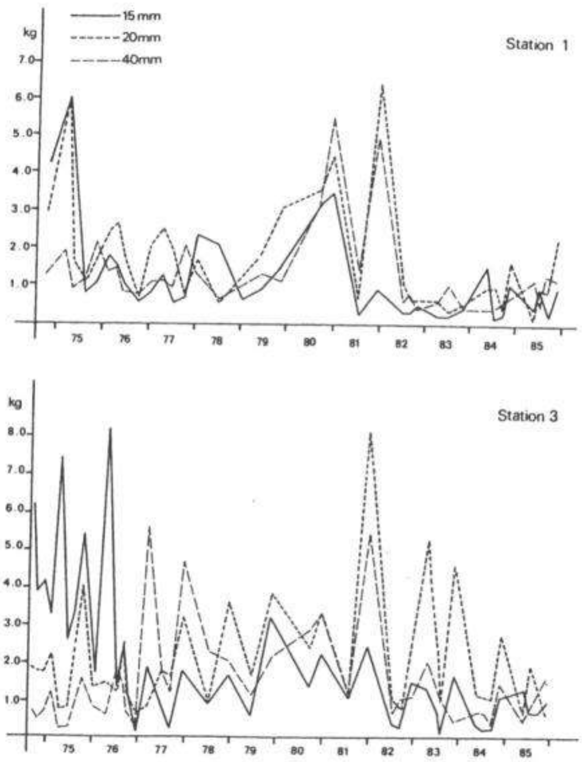


FIGURE 8 - Changes in Catch (C.P.U.E.) for Different Mesh Sizes at Two Monitoring Stations (Leraba and Bandama)

4. Changes in the Structure of the Fish catches

40 It is difficult to compare many graphs of species abundance against time and overlay these with abiotic factors. Therefore multivariate analyses are appropriate and the method used here is the factorial analysis of correspondance, the salient points of which are amply described elsewhere (Benzecri, 1973: Lebard **&** Fenelon, 1973: Hill, 1974). This method is particularly informative as it permits simultaneous graphical representation of species and samples.

41 Two examples are given here, the first being the river Oti at Sabari with only Abate treatment being applied. Although treatments have been less frequent in recent years, the river Mo has received heavy insecticide applications. This tributary joins the Oti before the Volta Lake so all migratory fish would be exposed to Abate at some time.

42 In the ordination presented in Figure 9a. the first axis separates the samples into low and high water periods. Dry period collections are characterised by Alestes, Labeo and Chrysichthys species, as well as Synodontis filamentosus and Schilbe mystus. The second axis has a group of high water samples in the middle, with rising and falling water periods being more diffuse. Comparison with Figure 10 gives some reasons for this pattern, as the Petrocephalus spp. are not common after 1979, while Eutropius niloticus continues to be abundant throughout the sampling period. Apparent reductions in abundance can also be correlated with a reduction in frequency of monitoring from monthly to quarterly. Therefore species such as E. niloticus may be under-represented as the main migration period is more likely to be missed.

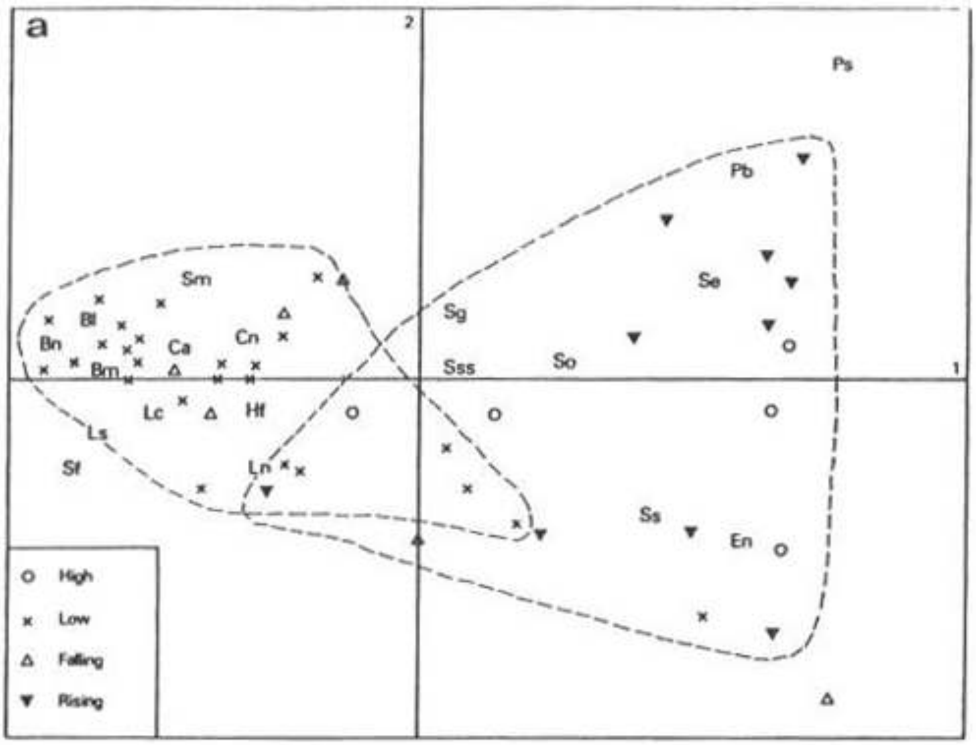
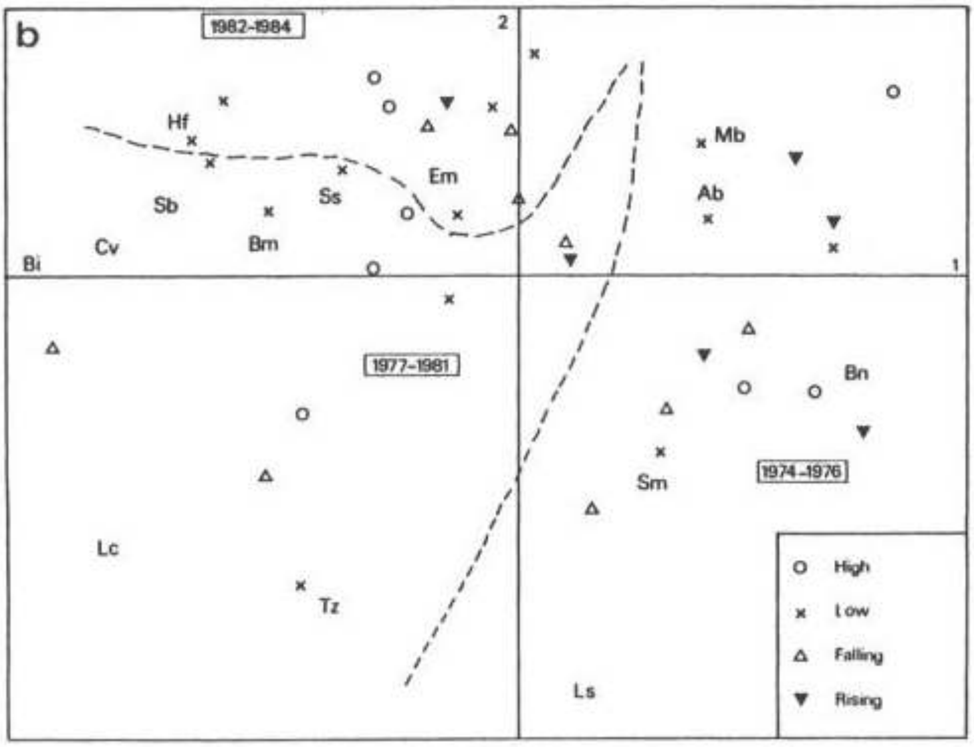


FIGURE 9 - Correspondance Analysis of experimental catches (in fish numbers) at:
a) Oti at Sabari
b) Bandama at Niakaramandougou
Pb Petrocephalus bovei
Ps Petrocephalus simus
Se Synodontis eupterus
So Synodontis ocellifer
Sss Synodontis sorex
Sg Synodontis gambiensis
Ss Synodontis schall
SF Synodontis filamentosus
Sb Synodontis bastiani
Em Eutropius mandibularis
En Eutropius niloticus
HF Hydrocynus forskalii
Bm Brycinus macrolepidotus
Bn Brycinus nurse
B1 Brycinus leuciscus
Bi Brycinus imberi
Ab Alestes baremoze
Cv Chrysichthys velifer
Ls Labeo senegalensis
Lc Labeo coubie
Sm Schilbe mystus
Ln Lates niloticus
Tg Tilapia galilea
Mb Marcusenius bruyerei



43 In conclusion, the major patterns of community change reflect seasonal influences rather than year-to-year differences. Changes in abundance of individual species over time have a partial explanation in reduction of sampling frequency, but species such as Petrocephalus bovei and P. simus appear to become rarer, a trend which is more difficult to explain in terms of hydrology.

44 The second example is the river Bandama at Niakaramandougou. The river feeds into the Kossou Dam, and early treatments were with Abate, other insecticides being employed from the end of 1980. The ordination (Figure 9b) appears to separate Abate treatments from the rest, but doser examination reveals a distinct community change at the end of 1976, with A. nurse becoming more rare and species such as A. imberi, C. velifer and Hydrocynus forskalii increasing in abundance. The second axis is dominated by Labeo senegalensis, Schilbe mystus and Tilapia galilaea, indicative of another change at the beginning of 1982 when these species became a smaller proportion of the catch (Figure 16). In the centre of the ordination lies Eutropius mandibularis which has maintained a relatively constant proportion of the catch throughout the sampling period. The reason for the changes could lie in a combination of a lag effect of the filling of the Kossou Lake and the wet years of 1979 and 1980. Therefore, effects of treatment are again not obvious, and this conclusion is reached for other sampling stations.

5. Coefficient of Condition

45 Long-term changes in the mean coefficient of condition (K) were studied for the most abundant species at the different monitoring sites. Selected examples are given in Figure 10 for species whose food is primarily based on aquatic invertebrates. The values of the coefficient of condition are relatively random, fluctuating around a mean which does not seem appreciably altered over the ten year period of treatment. There is no evidence of a long-term decrease or irreversible modification in K. Nevertheless, when examined in

detail, significant short-term decreases were observed for a few species. This is the case for Alestes baremoze and to a lesser extent for Eutropius mandibularis in the Bandama river in 1976-1977. A similar phenomenon occurred for Brycinus nurse in the Sassandra and Leraba rivers between 1981 and 1983. Such decreases, which apparently do not affect all species in the same river, or the same species at every site, seem difficult to explain. However, the drop in K for A. baremoze in 1976-1977 was restricted to the course of the Bandama between the Kossou and Ferkessedougou dams (Paugy, 1978) and coincided with a period of poor floods (Figure 4). The fall in value of K for A. nurse between 1981-1983 coincided with severe drought in the Sassandra and Leraba. For some species, therefore, there should be a relationship between K and changes in hydrology. Minor fluctuations in K relate to the life cycle and the seasons (Paugy, 1978, 1980; Leveque & Herbinet, 1980). It was also observed that for the same species there could be differences in K for the different river basins.

46 The relative stability of coefficient of condition indicates that fish are able to feed normally in treated rivers. In fact, stomach content analysis carried out in 1975, just after the start of treatment (Vidy, 1978), and in 1976-1977, did not reveal significant changes in the diet of fish species feeding entirely (Petrocephalus bovei) or partially (A. baremoze, B. imberi, B. nurse, E. mentalis) on the aquatic invertebrate fauna. It has been observed that temephos treatment resulted in a temporary reduction of aquatic insects by only 30-40 % (Dejoux, 1983), and it does not seem that treatment could lower food stocks to a critical threshold. Moreover, the adaptability of many freshwater fishes to various types of food has been demonstrated a number of times (Lauzanne, 1976; Welcomme, 1985). Nevertheless, Corbet (1956) observed a change in the diet of some fish, particularly Mormyridae. Some species with a specialized diet, such as Mastacembelus victoriae apparently suffered from malnutrition or migrated.

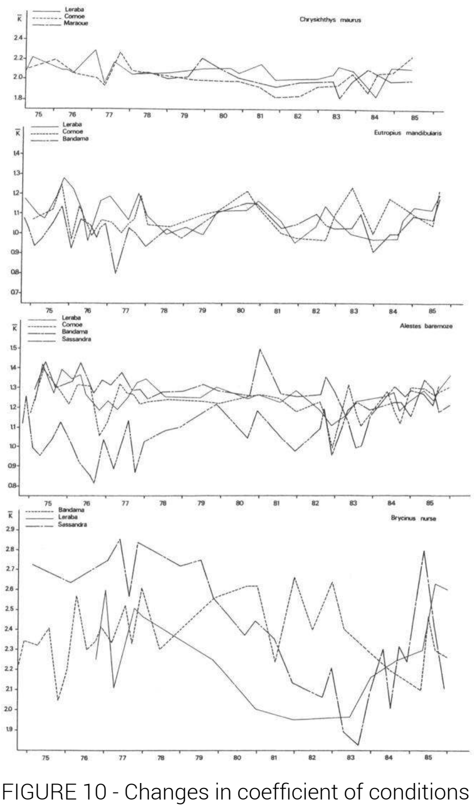


FIGURE 10 - Changes in coefficient of conditions for selected species

CONCLUSIONS

47 Experimental fish sampling has been carried out for ten years in West African rivers treated weekly with insecticides by OCP using the standard protocol. The monitoring stationsinvestigated differed in many ways, with:-

a. their location on different river basins
b. the relative abundance of fish species, despite many species being common to different basins
c. the insecticides used, some stations always being treated with temephos, others with temephos up to 1980 and later with an alternation of insecticides (Figure 3).

48 For the interpretation of data collected, we were faced with different sources of bias:-

a. the human factor, in that data were collected by different teams and there were changes in the personnel responsible for fish monitoring in Ivory Coast and Ghana. This could lead to some differences which on occasion could be recognised by detailed statistical analysis. This source of bias did not appear to change the overall picture.
b. lack of knowledge concerning the changes in fish community structure as the result of changes in natural environmental factors. This is the case for instance for river discharge, which could vary greatly between years, and is known to affect the reproductive success of fishes.
c. lack of knowledge concerning the uses and abuses of insecticides other than those applied by OCP. It is known (Calamari, 1985; Balk & Koeman, 1984) that large amounts of agricultural pesticides are used in the OCP area, but the questions are: what reaches the river beds, and what is the impact on the aquatic fauna? A few direct, observations also showed that insecticides are sometimes used as poison when fishing in rivers. However, the extent of this influence is not known.

ACKNOWLEDGEMENTS

49 The independent Ecological Group of O.C.P. is recognised as the general instigator of this presentation. The authors would like to acknowledge the work of many people in the collection, identification and data analysis phases. Particular mention should be made of J.J. Albaret, L.A.K. Antwi, R. Bigorne, H.R. Dankwa and B.de Merona. OCP in general, and especially L. Yameogo, J. Henderickx and P. Pangalet of the Vector Control Unit, have given considerable assistance.

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ABSTRACTS

Commencing in 1975, under the auspices of W.H.O., weekly applications of insecticides have been employed to control the blackfly which transmits human river blindness in West Africa. The results provided in this paper do not show, as a whole, any clear impact of OCP-applied pesticides on fish populations. The total catch, the number of species caught in each sample and coefficient of condition, appeared to fluctuate around a mean value, and no long-term drop was observed over the period investigated. The seasonal pattern is generally clear. In some cases longer term declines occur, generally being followed by a rise correlating with changing hydrological conditions.

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The Onchocerciasis Control Programme and the monitoring of its effect on the riverine biology of the Volta River Basin

C. Lévêque, M. Odei and M. Pugh Thomas

1 This paper outlines the problems both logistic and scientific, which arose when designing and setting up the World Health Organization's monitoring programme in connection with the control of river blindness in the Volta River Basin. At present Simulium damnosum Theo. is being controlled by the application of the organo-phosphorous pesticide 'abate' to the larval breeding sites. The monitoring programme, using simple but effective techniques, has now been running for more than 18 months and the techniques of monitoring are described and early results are discussed.

INTRODUCTION

2 Onchocerciasis is caused by a thread like filarial worm which amongst other symptoms produces lesions in the eye which may eventually lead to total blindness.

3 The disease occurs in Africa, the Yemen and in parts of Central and South America, affecting in all some 20 million people. The largest endemic areas occur in Africa and one of the most serious of these is located in the savanna region of the Volta River Basin. In an area of approximately 700,000 km^2 about one million people have the disease, and of these some 70,000 are either totally or partially blind. The location of the programme area is shown in Fig. 1.

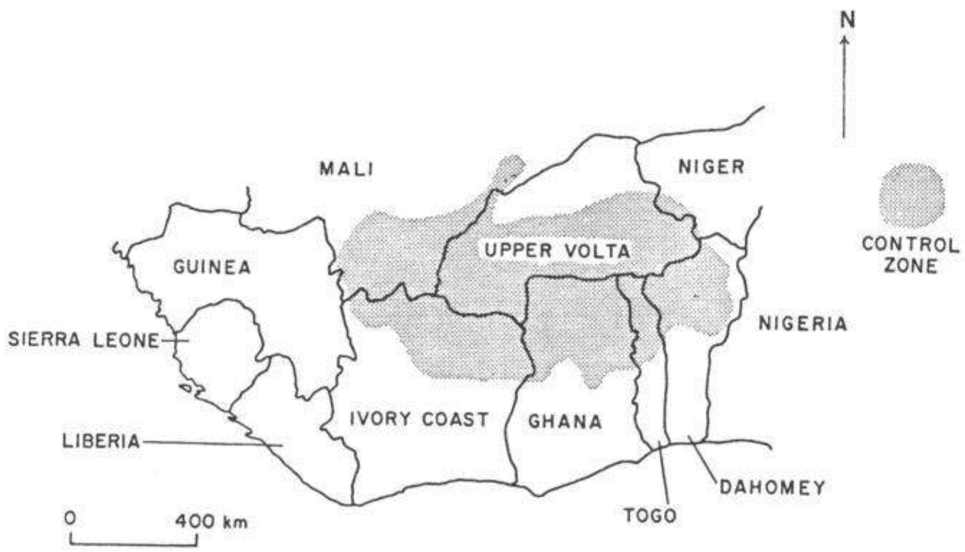

Figure 1. The location of the World Health Organisation, Onchocerciasis Control Programme in the Volta River Basin of West Africa.

4 The filarial worm is carried from person to person by the female adult of the black fly *Simulium damnosum* Theo. The larvae of these Aies occur only in fast flowing water, requiring a minimum flow of about 50 cm/second for survival. Because of this association with fast flowing waters, the disease has the common name of river blindness.

5 In addition to causing blindness, the disease produces other symptoms including hanging groin and intense irritation of the skin. Because of these symptoms and of the threat of blindness, people have tended to leave the river valleys and move to the uplands. Here, they have increased the pressure on already over-taxed soils and this led to soil erosion and loss of fertility. In 1970, the United Nations Development Programme funded the preparation of a strategy for the control of onchocerciasis on behalf of the governments of Benin, Ghana, Ivory Coast, Mali, Niger, Togo and Upper Volta (P.A.G., 1973). The preparation of this report was completed in 1973 and control operations commenced in 1974. The programme is run by the World Health Organisation and is to continue for some twenty years.

6 Because of the large area of West Africa to be covered, the poor roads, the general difficulties of the terrain and the restricted and often localised distribution of the fly breeding sites, the disease is being attacked by controlling the numbers of *Simulium* larvae by aerial spraying. At present the pesticide used is 'abate', (a proprietory formulation of'temephos', $C_{16}H_{20}O_6P_2S_3$, 000'0-tetramethyl 00'-thiodip-phenylene diphosphorothiote, an insecticide with a low toxicity to mammals, birds and aquatic organisais) but other pesticides are being considered. Because of the ecological implications of the heavy use of pesticides over so large a part of West Africa, an Ecological Panel was appointed to check on the ecological effects of the work. The Monitoring Programme was set up at the suggestion of the Panel and reports to them. The Panel contains observers from the World Health but no staff of the Organisation sit on it as members.

THE MONITORING PROGRAMME

7 The ecological monitoring seeks to evaluate the effect of abate on the non-target organisms. The effect of the pesticide on *Simulium damnosum* is watched over by the Vector Control staff of the Onchocerciasis Control Programme itself. When setting up the monitoring programme several important considerations had to be kept in mind.

8 (i) Would the monitoring aim principally at investigating the long term effects of the abate on the river ecology or would it look only at the short term effects. The techniques for each approach being different. In effect, the monitoring work is divided into two parts. A long term regular sampling programme aimed at investigating the ecological effects of treatment over the duration of the programme; combined with shorter duration research programmes looking at specific short term problems.

9 (ii) The best possible use has to be made of the available manpower and of local facilities. The monitoring is based on three teams; one in each of the following countries: Ivory Coast, Ghana and Upper Volta. In order to ensure reasonable comparability of results, all three teams had to use the same methods.

10 (iii) The periodicity of sampling, the sites selected for monitoring, and the field methods used had to combine reliability of sampling technique with reliability of access in both wet and dry seasons over many kilometres of roads or tracks which are not yet hard surfaced.

11 (iv) The monitoring techniques had to work equally well in shallow slow flowing rivers in the dry season and in the same rivers flowing fast and deep in the wet season.

12 (v) The sequence of sampling had to be sufficiently frequent to make it possible to at least attempt to differentiate between naturally and artificially induced changes in the populations. In most cases a monthly sequence was established.

13 In summary a monitoring programme had to be devised which would not only give the desired levei of scientific information, but which would be reliable and consistent when applied to the conditions pertaining in the Programme Area. It is, therefore, worth expanding some of the points listed above. There is no long term monitoring of the effect of pesticide on the phytoplankton and other river algae. The bulk of the monitoring is being done by the Institute of Aquatic Biology in Ghana and the Laboratoire d'Hydrobiologie at Bouaké in Ivory Coast. Neither team had the full facilities, or more importantly, the fully trained algologists needed to monitor the algae in the rivers in the savanna region of the Volta River Basin. In addition, this effectively would have required an extensive preliminary research programme on the phyto-plankton of African rivers. The absence of a vast fund of knowledge on African river biology, similar to that available for temperate rivers, is one of the major difficulties in interpreting data collected in the monitoring programme. It was, therefore, not possible to include phytoplankton monitoring as part of the long term work. A research programme into the phytoplankton of one of the sampling sites in Ghana is under way and preliminary studies into the effects of various insecticides on phytoplankton reproduction is in progress at the University of Salford.

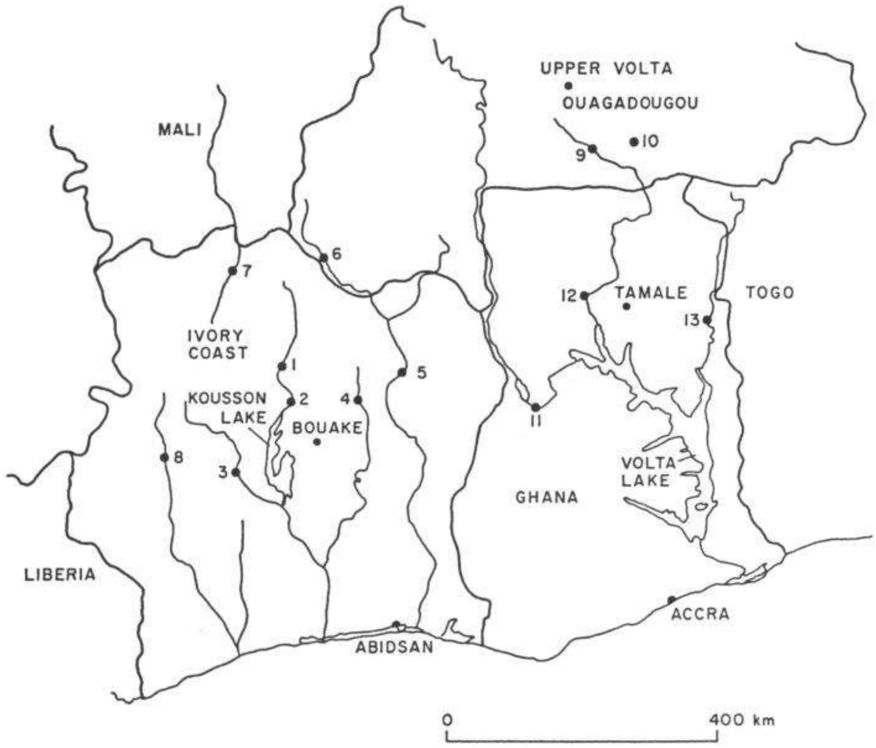

FIGURE 2. THE LOCATION OF THE MONITORING STATIONS. —, NATIONAL BOUNDARIES; —, RIVERS. 1. BANDAMA RIVER AT NIAKARAMANDOUGOU (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 2. BANDAMA RIVER AT MARABADIASSA (TREATED RIVER). FISH SAMPLES ONLY. 3. RIVER MARAOUÉ AT DANANGARO (UNTREATED RIVER). INVERTEBRATE SAMPLES ONLY. 4. RIVER N'ZI AT THE BRIDGE OF DABAKALA (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 5. RIVER COMOE AT THE GANSÉ FERRY (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 6. RIVER LÉRABA AT THE FRONTIER BRIDGE (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 7. RIVER BAGOÉ AT KOUTO (TO BE TREATED IN 1977). FISH AND INVERTEBRATE SAMPLES. 8. RIVER SASSANDRA AT SEMIEN (UNTREATED RIVER). INVERTEBRATE SAMPLES ONLY. 9. RED VOLTA RIVER AT PO BRIDGE (TO BE TREATED IN 1977). INVERTEBRATE SAMPLES ONLY. 10. WHITE VOLTA RIVER AT POURA (TO BE TREATED IN 1977). INVERTEBRATE SAMPLES ONLY. 11. BLACK VOLTA RIVER AT BAMBOI (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 12. WHITE VOLTA RIVER AT DABOYA (TREATED RIVER). FISH AND INVERTEBRATE SAMPLES. 13. RIVER OTI AT SABARI NEAR YENDI (TO BE TREATED IN 1977). FISH AND INVERTEBRATE SAMPLES.

14 The selection of sites was based on a preliminary field investigation carried out by Dr Stanley Frost from Salford University, Dr Christian Lévêque from Bouaké and Dr S. K. Prah and Mr J. Samman from Accra. The importance of this survey, by competent freshwater biologists, cannot be over-emphasised. It was possible to select sampling stations on the basis of practical knowledge of the sites rather than solely on the basis of theoretical desirability of map location. The sites were selected to do two main things. To sample a wide range of river types in Upper Volta and Ivory Coast and to sample as near as possible to Volta Lake, with its important fishery, in Ghana. In all seven sampling sites were selected in Ivory Coast, two in Upper Volta and three in Ghana. These are shown in Fig. 2. Of these rivers the Maraoué and Sassandra will not be treated and act as permanent Controls. The Rivers Oti and Bagoë will not be treated until 1977 and the Red Volta, a temporary river, was studied for one year/season, before treatment commenced. (Studied 1975, treatment commenced 1976.) Thus, there will have been precontrol studies for one season on the Red Volta and for 20 months on the Oti and Bagoë. When treatment of the latter rivers commences the data from them will be of great interest as there will be the possibility of a before and after study on the effect of abate on the river faunas.

MONITORING METHODS AND EQUIPMENT

15 As already stated, the methods of sampling were selected to be usable, as far as possible, in both wet and dry seasons. The apparatus was also selected for ease of maintenance and robustness, an important consideration because of the difficulty of replacement in the African bush.

16 Five sampling methods are used: drift net sampling for invertebrates; fish drift; surber samples; artificial substrates and fish of edible size sampled using commercial gill nets.

Drift net sampling for invertebrates

17 At dusk many riverine invertebrates leave the river bottom and drift downstream. These are sampled by placing nets across the flow of the river. The apertures of the nets were 25 x 25 cm, and the mesh aperture was 300 µm. In order to allow a large filtering surface the nets were 2 m long. The nets are arranged in several positions across the river and located so that the top of the frame is 2 cm below the surface. Samples are taken approximately one and one half hours before and after sunset. For the day drift samples, three nets are used for half an hour. For night samples a total of six nettings are used, the nets remaining in the river for three minutes. The river flow is measured at the time of sampling and the number of animais represented as the number of organisms per cubic metre filtered. In terms of actual numbers of animais per cubic metre, the numbers are only approximate but the data collected are comparable both within one site over successive months and for the different sites in the monitoring programme. The basic techniques of drift sampling are well established, but those in use in the programme are as described by Elouard & Lévêque (1975).

Fish drift

18 Small fish drift at night in a similar way to the invertebrates and as they are not caught by gill or other commercial nets, fish drift nets are used to catch small fish either as fry or as small species. These nets have a rectangular opening 40 x 70 cm with a mesh size of 1.5 mm. They are 3 m long. Two fish drift nets are used, the nets being placed in position at sunset and retrieved two hours later. Sampling of the fish drift usually occurs every two months. There are at present considerable taxonomic problems concerning the identification of the small fish, but these are being overcome by the fish biologists on the staff of the two hydrobiological institutes and by Dr B. Roman, a World Health Consultant based at Ouagadougou in Upper Volta. The details of this method were worked out by Dr C. Lévêque from whom information may be obtained.

Surber samples (Fig. 3)

19 A modified form of surber sampler developed by the O.R.S.T.O.M. team is used to sample known areas of river bed. Again, this is a simple method using robust apparatus. In this case, however, it cannot be used during the wet season. The apparatus cannot be used in water deeper than about half arms length. For comparative work the figures are represented as number of animais per square metre. (Although in fact the method is

unlikely to sample every animal in the area enclosed by the apparatus.) As the surber samples are standardised for all monitoring groups, it is possible to compare the data not only on successive months, but also for different stations. The area of river bottom covered by the sampler is 15 x 15 cm, and a metal mesh is placed across the upstream opening of the apparatus. This grid reduces the possibility of drifting organisms entering the sampler.

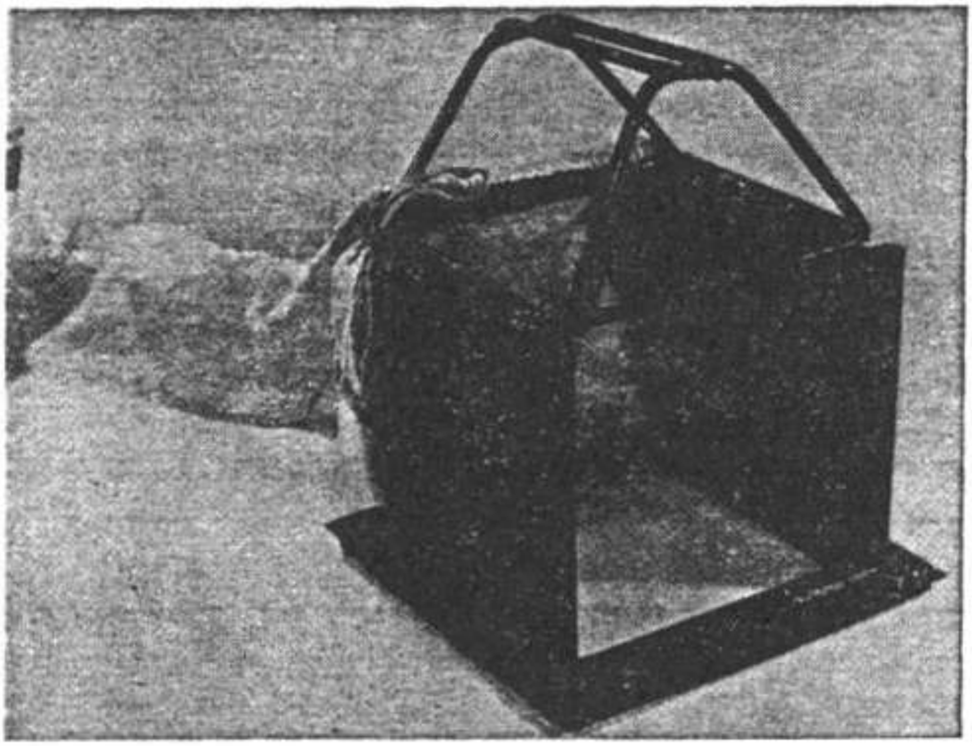

Figure 3. SURBER SAMPLER AS USED BY THE MONITORING TEAMS. THIS FORM OF SAMPLER WAS DEVISED BY THE O.R.S.T.O.M., TEAM AT BOUAKÉ AND IS MORE ROBUST AND RELIABLE THAN THE TRADITIONAL PATTERN. THE GRILL FROM THE UPSTREAM APERTURE OF THE APPARATUS HAS BEEN REMOVED IN THIS PHOTOGRAPH.

Artificial substrates

20 The team at Bouaké have developed a method of securing small concrete blocks in the rivers. These are colonised over three months and the animals counted. The blocks are colonised by some genera which are not caught in the drift. This method can be used during both low and high flow periods. Several artificial substrate methods were tried but this proved the best, principally because the blocks are less likely to be stolen than the bags and metal baskets used in the other trial methods. The apparatus as used in the monitoring programme consists of a metal rod 90 cm long. On this rod there are five hooks. At each end of the rod are large concrete weights. In use, small cernent blocks 7 x 7 x 4 cm in size are secured to the hooks and the whole apparatus is then securely anchored in the river. The blocks are left immersed for one month, after which the organisms are carefully removed and counted. The blocks are easily cleaned and are of a definite size, making it possible to represent the fauna as numbers of animals per square metre. This method, together with other artificial substrate techniques investigated for the monitoring programme are described in a paper by Dejoux & Venard (1976).

Fish of edible size sampled using commercial gill nets

21 These nets are 25 m long and 2 m deep. They are divided into five blocks of mesh size, 15, 20, 25, 30 and 40 mm. Each block is 5 m long. Such nets are used by the teams in Ghana and Ivory Coast, but labour is not available for fish sampling by this method in Upper Volta. The nets are left in position over night. The fish catch is represented as fish per unit effort, which is the number of fish caught per 100 m of net per night. Only that area of net which is in a position to fish is considered in the calculation. For instance in shallow water those parts of the net which are not usable are excluded from the calculation.

22 Finally in Upper Volta, Roman is making fish collections on the basis of hand net sampling for a fixed time per site. From these data species percentage composition is worked out.

THE STANDARDISATION OF METHODS

23 Six-monthly meetings of the monitoring groups have been held to discuss field methods and to try to ensure that the monitoring groups keep to the standard methods. To aid in the standardisation of methods they have been written up in the form of a 'Protocol for Aquatic Monitoring'which defines the various methods of sampling. Copies of this Protocol are held by the monitoring teams from Bouaké, from the Institute of Aquatic Biology and by the workers in Upper Volta. The field sampling programme has been running for twenty months and appears to have worked well. There has been no breakdown in the sampling programme and once standard equipment was available, identical collecting methods were used by all monitoring groups.

THE INTERPRETATION OF FIELD DATA

24 The data collected from the field is discussed at the six-monthly meetings and it is also sent regularly to WHO, at Geneva. Recent work at Salford by Drs Stanley Frost and Colin Fairhurst (1976) has indicated that similar river types have similar faunas and that data from them can be compared one with the other. Some of the rivers are not duplicated in type and can only be compared with themselves on previous occasions and at periods of similar river flow, the latter being a very important consideration.

25 Analysis of the data is as yet at a very early stage but we hope to look for changes in the species diversity index and changes in the proportion of pollution tolerant to pollution resistant species. This is based on the concept that abate is essentially a pollutant and will therefore affect pollution tolerant species less than resistant ones.

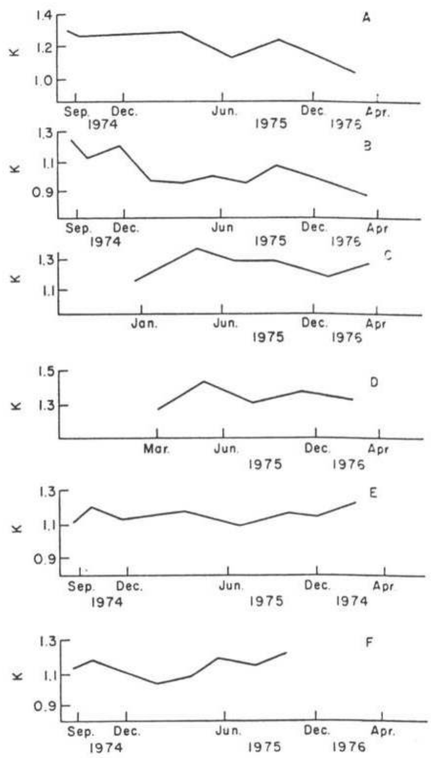

Figure 4a-d. Variation in the coefficient of condition for *Alestes baremose* in the treated rivers Bandama (A, B) and Comoé (C) and the untreated river Sassandra (D). There is no evidence to show that there is a change in the coefficient of condition due to treatment. *A. barcmose* is a general feeder. E, F. Coefficient of condition, for the cat-fish *Eutropius mentalis* in the treated Bandama. There is no indication that treatment is affecting the fish. Data are available for both male and female fish. Only data for female fish are presented in this figure. (From Lévêque, 1976.)

26 A literatura survey on the effects of organic and toxic Chemical pollution on the fauna of tropical and particularly of African rivers is at present being undertaken. Information gained from this survey will, it is hoped, help in thc interpretation of monitoring data.

27 With regards to the fish; in addition to data on the relative abundance of the various species, the Coefficient of Condition is being calculated by the formula:

$$K = \frac{10^5 P}{L^3}.$$

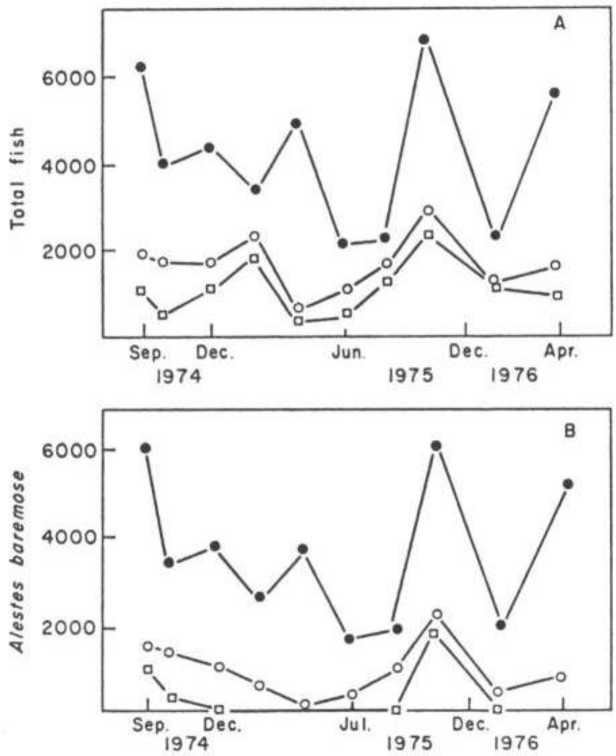

FIGURE 5. VARIATION IN THE NUMBER OF FISH CAUGHT PER UNIT EFFORT IN THE TREATED RIVER BANDAMA. THE MAJOR FLUCTUATIONS OCCUR BECAUSE OF FLUCTUATIONS IN RIVER FLOW. A. FISH CAUGHT PER UNIT EFFORT FOR ALL FISH. B. CATCHES FOR *ALESTES BAREMOSE.* (FROM LÉVÊQUE, 1976.) □ LARGE MESH NET; ○, MEDIUM MESH NET; ●, SMALL MESH NET.

28 Where *P* = weight in grammes, *L* = length in millimetres of fish from tip of head to end of caudal peduncle

29 The coefficient of condition is an indication of the general health of a fish.

30 The analysis and interpretation of data from the monitoring is only now being developed and one of the largest problems of interpreting results is ignorance of the biology of African rivers and of the behaviour of tropical riverine organisms to pollution. Ease of the interpretation is not aided by the absence of long-term control rivers in the programme area. There are considerable differences in the topography of the rivers outside the programme area to the south, as they are in rain-forest and not in savanna. To the north east most of the larger rivers drain into the Niger System and not into the Volta River Basin. The Niger differs both in catchment and water regime from rivers in the Volta System. This part of the work is, therefore, being developed on a progressive basis combining knowledge gained from the actual monitoring, from general field observations and from a search of the relevant literature. We have only now completed a full 12 months of sampling and thus have only just obtained complete coverage of an annual cycle of river conditions in the Programme Area. The analysis of data and it nterpretation is, therefore, now being given active consideration.

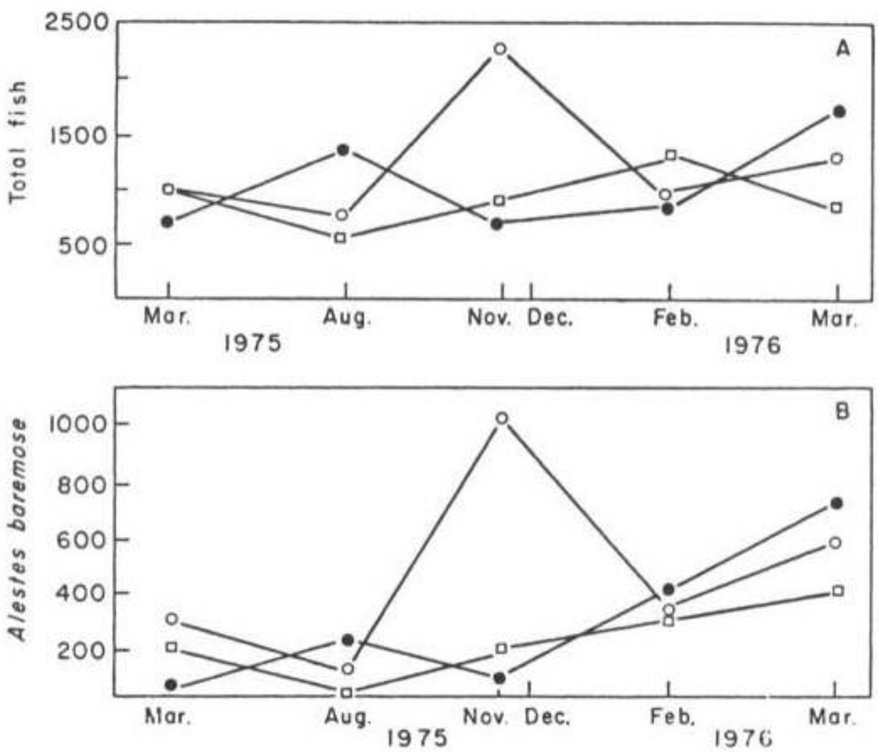

FIGURE 6. NUMBERS OF FISH CAUGHT IN THE UNTREATED RIVER SASSANDRA. A. TOTAL NUMBER OF FISH CAUGHT PER UNIT EFFORT. B. NUMBER OF *ALESTES BAREMOSE* CAUGHT PER UNIT EFFORT. IN GENERAL, THE SAME PATTERN OF FISH CATCHES CAN BE SEEN AS IN THE TREATED RIVER BANDAMA. (FIG. 5). □ LARGE MESH NET; ○, MEDIUM MESH NET; ●, SMALL MESH NET.

31 At this stage it is, however, possible to say that there are no discernable effects on the fish in the programme area on the basis of fish caught per unit effort, species composition of the fish fauna, or coefficient of condition, see Figs 4, 5 and 6.

32 For the invertebrates the situation is more difficult to interpret because of the rapid turnover of many invertebrate species. At present it is not possible to demonstrate any clear long term effects of abate on the invertebrates in treated rivers. Such changes as have occurred, could well be within the natural variations of the river faunas. When the Oti and Bagouë are treated in Phase III of the Control Programme, it will be possible to compare the two rivers before and after treatment. This comparison will be very interesting.

33 As stated above, data from the field is sent regularly to the World Health Organisation Headquarters in Geneva. Provision has been made there for the storage, analysis and print out of the field information. Expert statistical advise is also available on how the accumulated material may best be handled in the future.

DISCUSSION AND SUMMARY

34 The biological monitoring programme of the Onchocerciasis Control Programme is aimed at identifying major ecological change in the biology of the rivers in the control programme area. To achieve this, sites and methods have been selected which will allow the collection of data on a regular and standarised basis. These data can then be used to try to estímate changes in the river fauna due to the use of pesticide in the area.

35 There are 12 sampling sites in the monitoring programme. Two of these are permanently outside the *Simulium* control area and two of them have not yet been treated with abate (December 1976). By looking at data from all the sites it should be possible both to see and, therefore, discard natural changes in the fauna on one or two of the individual river catchments and at the same time check on long term changes in the fauna of the Onchocerciasis Control Programme region of the Volta River Basin as a whole. The size and difficulty of this task is not to be underestimated. There are 700,000 km^2 of land in the treatment area. Little is known about the biology of African Rivers and even less about their biology when polluted. The monitoring programme is rapidly increasing this knowledge. Even in temperate rivers the concept of significant ecological change is difficult to comprehend and define. The ecology of the fauna of the rivers in the Programme Area will certainly change if only because of the control of *Simulium damnosum* larvae. It is also unlikely that during the whole of the control period the non-target organisms will be totally unaffected.

36 The use of biological indicators of pollution to show changes in water quality as outlined by Wilhm (1975) may help to indícate ecological change in a theoretical or academic sense. In terms of the Onchocerciasis Control Programme, significant ecological change musí be related to 70,000 severely disabled people, to upland soil erosion and to the general deprivation of the population in the Volta River Basin. The concept of 'significant ecological change'is, therefore, being actively considered by the Ecological Panel.

ACKNOWLEDGEMENTS

37 The following have contributed to the setting up and running of the Aquatic Biology Monitoring Programme in the Onchocerciasis Control Programme Area: E. K. Abban, J. J. Albaret, J. S. Amakye, H. N. Appler, R. Bigorne, C. A. Biney, C. Dejoux, J. Duppenthaler, J. M. Elouard, C. P. Fairhurst, P. Forge, S. Frost, P. Herbinet, H. B. N. Hynes, A. litis, J. D. M. Marr, B. de Merona, D. Paugy, S. K. Prah, B. Roman, J. Samman, P. Venard, Frank Walsh and Brenda Walsh. They have all contributed, therefore, to the writing of this paper. The World Health Organisation finance the monitoring programme by contracts with the Institute of Aquatic Biology, Accra, Ghana, and the Laboratoire d'Hydrobiologie, Bouaké, Ivory Coast.

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Onchocerciasis Control Programme in West Africa: a 20-year monitoring of fish assemblages

Programme de lutte contre l'onchocercose en Afrique de l'Ouest: vingt années de surveillance des peuplements ichtyologiques.

Didier Paugy, Yves Fermon, Kofi Eddie Abban, Moussa Elimane Diop and Kassoum Traoré

1. INTRODUCTION

1 Human onchocerciasis, or river blindness, a filaria that causes major public health damage and is a great problem to the economic growth, has been the target of a widespread control campaign that started in the middle of the 1970s in West Africa. Under the auspices of the World Health Organisation (WHO), the Onchocerciasis Control Programme (OCP) was set up in December 1974 on the Volta basin, one of the more seriously affected areas. The control programme was initially planned for a 20-year period, and to include 11 countries comprising an area of 1 300 000 km^2. The control area initially included 50 000 km of rivers with about thirty million human residents. At the beginning of the programme, Burkina Faso, the western part of Niger, the northern parts of Benin, Ghana, Côte d'Ivoire, Togo and the south-eastern part of Mali were included *(figure 1).* Treatments started in February 1975 in the central part of the area covered by the OCP, and the programme progressively extended to the south, east and west [10, 29].

1.1. Strategy of larvicide applications

2 The only possibility to stem the disease transmission was to control the vector, and to avoid a major risk of pollution, it was decided to use pesticides to target the aquatic larval population living in the fast-flowing parts of the rivers.

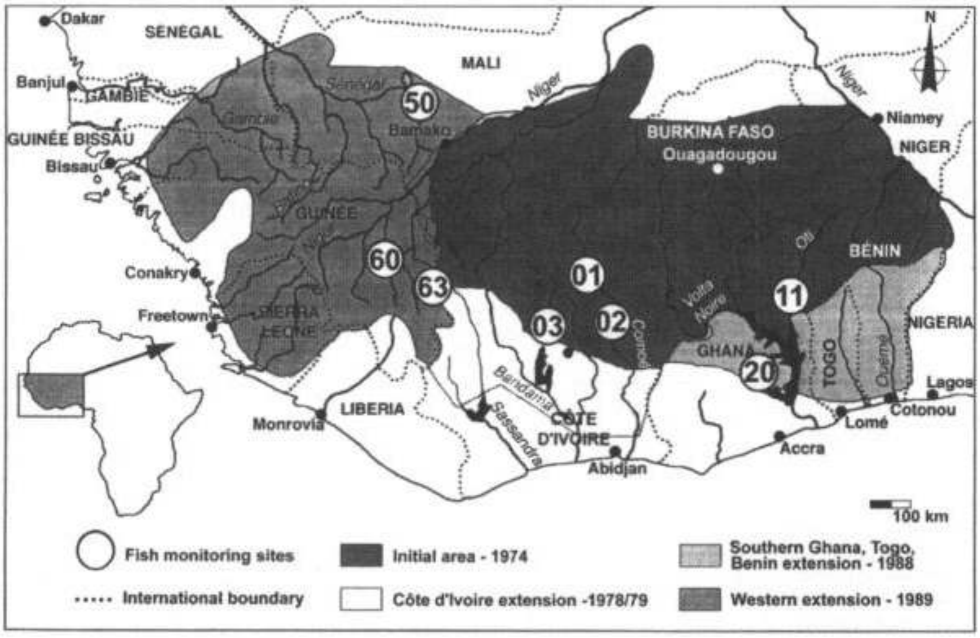

Figure 1. Maximum extension of the Onchocerciasis Control Programme in West Africa and location of the fish monitoring stations.

Table I. Amount (in thousands of litres) of commercialised pesticides used by the OCP.

Year	Temephos organo-phosphate	Chlorphoxim organo-phosphate	B.t. H14 'biological' insecticide	Permethrin pyrethroid	Carbosulfan carbamate	Pyraclofos organo-phosphate	Phoxim organo-phosphate	Etofenprox pyrethroid
1975	76							
1976	130							
1977	156							
1978	216							
1979	263							
1980	185	6	0.5					
1981	130	70	1.5					
1982	163	7	233					
1983	75	36	310					
1984	77	57	257					
1985	130	6	211	3	9			
1986	120	15	385	10	20			
1987	71	30	229	10	8			
1988	84	80	380	26	11			
1989	92	65	275	51	31			
1990	109	34	407	44	36	3		
1991	76		271	46	33	32	21	
1992	47		376	23	27	55	34	
1993	76		209	20	13	45	20	
1994	48		225	29	13	45	20	5
1995	41		237	15	21	38	18	17
1996	28		205	21	6	33	24	14

3 At an early stage of the programme, the OCP used a single organophosphorous compound called temephos. Then, the appearance of resistant strains of the vector to temephos necessitated the use of various kinds of pesticides belonging to compounds of several families *(table I).*

4 In order to insure optimum efficiency and to avoid any important or even definitive resistant strains, the current strategy of the OCP is complex [11]. First, the sections of rivers where blackflies still occur are the only ones treated. Second, strategies related to the discharge of the rivers are used to promote efficiency (rotation of molecules), and to be cheaper and less hazardous for the environment.

1.2. The environmental monitoring

5 Insofar as it has been proved that the fish were not really affected by a direct effect of the pesticides in the conditions of the nest control campaign [2], it was decided to monitor the variations in the fish communities in the long term.

6 The Onchocerciasis Control Programme was probably one of the first long-term pesticide programmes to take into account potential long-term environmental impacts of the pesticides. In order to evaluate the magnitude of the environmental risk, hydrobiologists have used consistent methods and protocols to monitor potential long-term effects of continuous use of larvicides on aquatic populations [4, 8, 18].

7 Fish monitoring was carried out with standard sets of gillnets of different mesh size. Despite the selective properties of this kind of fishing gear, it was the only method which could be used as a standard by all the teams on the whole OCP area. The following information was recorded: catch per unit effort (CPUE), measured as the quantity of fishes, the communities' structures and the degree of balance between them. For each fish we recorded: weight, length, condition, stomach contents, sex and sexual maturity.

8 The study we present here follows a synthesis made after 10 years of monitoring of potential long-term effects of the pesticides on the fish fauna [16]. Samples were collected by researchers from IRD-ex Orstom and from the national teams of the participating countries with the financial support of the OCP.

1.3. River hydrology

9 The OCP area covers a large number of river Systems, mostly savannah rivers with a water regime characterised by a flood period from July to November, with a peak in September and a long low water period from December to June. Many of the rivers are intermittent and may totally dry up. For permanent rivers, the discharge is very low during the dry season and the upper course is sometimes reduced to a few pools. Seasonal flood regimes strongly affect the ecology of fish populations [30].

10 Water discharge shows strong inter-annual variation *(figure 2).* During the major part of the monitoring period, a long drought occurred, not only in the OCP area, but also in all of West Africa [20]. Such a long drought greatly influences aquatic organisms because, even if the rainy season is normal for a particular year, lack of ground water will induce a very quick decrease of the discharge [23].

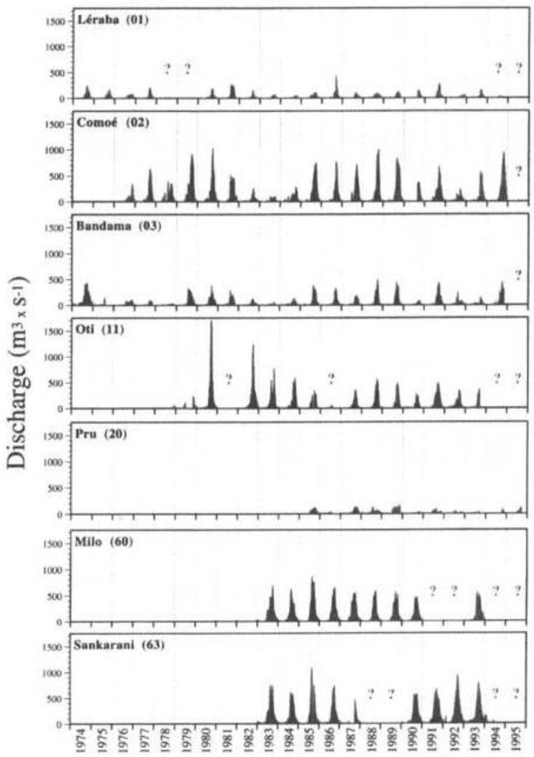

FIGURE 2. Discharge (m^3 x $s^{-1)}$ for lhe seven monitoring sites of the Onchocerciasis Control Programme (OCP) area.

2. MATERIALS AND METHODS

2.1. Selection of the stations

11 Initially, for each country three or four sites were selected both on treated and untreated rivers. But, later the OCP and the Ecological Group requested a decrease in monitoring sites, mostly for economical reasons. Therefore, the number of stations with available sampling data on a significant period was re stricted to seven (the eighth one, Baoule (Upper Senegal) in Mali (site 50) was never treated and therefore removed from analysis *(figure 1):*

12 — Leraba at boundary bridge (Comoe basin/Côte d'Ivoire), site 01;

13 — Comoe at Ganse (Comoe basin/Côte d'Ivoire), site 02;

14 — Bandama at Niakaramandougou (Bandama basin/Côte d'Ivoire), site 03;

15 — Oti at Sabari (Volta basin/Ghana), site 11;

16 — Pru at Asubende (Volta basin/Ghana), site 20;

17 — Milo at Boussoule (Niger basin/Guinea), site 60;

18 — Sankarani at Mandiana (Niger basin/Guinea), site 63.

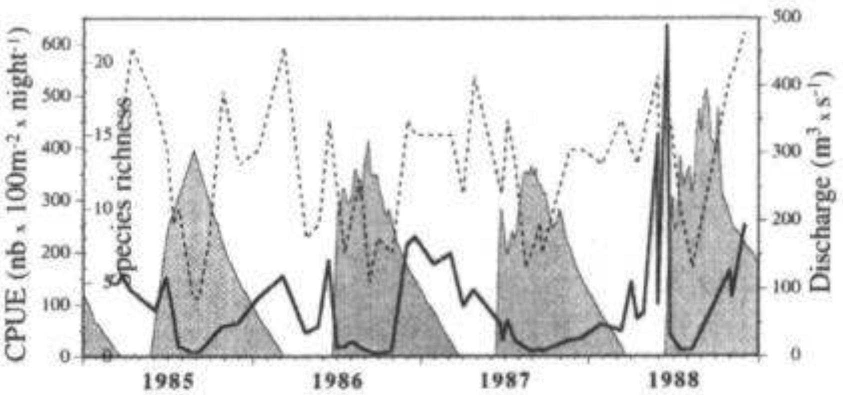

FIGURE 3. Baoule at Missira (site 50): effect of the flood on CPUE (nb x 100 m^{-2} x $night^{-1}$) and species richness. CPUE: —; species richness: —; discharge: ■

2.2. Sampling protocols

19 According to the protocol established before the experiment was started [18], the sampling was undertaken with a standard set of gillnets of different mesh sizes: 15, 20, 25, 30 and 40 mm (knot to knot distance). Gillnets were used for two consecutive nights, every 3 months.

20 We first checked that no bias was induced by the different teams' operational procedure.

21 The most effective mesh size proved to be 15 mm, then 20 mm. There were many small-sized species and many juveniles of the larger species.

2.3. Variables considered in the analyses

22 We consider catch per unit effort (CPUE) as the numbers of specimens caught each night for 100m^2 of gillnet area. Condition factor (K) was calculated according to the following formula:

$$K = \frac{W}{L^3} \times 10^5$$

23 where *W* is the weight (g) and *L* the standard length (mm).

24 Condition is an expression of health and, more generally, the physiological State of the fishes. It may reflect the direct effect of the pesticides on the physiology of the fishes (for example, the impact on reproductive potential) or for indirect effect of pesticides due to an impact on the aquatic invertebrate fauna which is the main feeding source for many species.

25 To detect community-level change, we calculated the dominance index of Berger-Parker *(d)* [19] and the Shannon diversity index *(I)* calculated using the formula: where *Nmax* is the number of individuals of the most abundant species caught, *N* is the total number of specimens, p_i the relative frequency of the species *i* and log p_i its decimal logarithm.

$$d = \frac{N_{\max}}{N}$$

$$I = \sum_{i}^{n} p_i \times \log p_i$$

26 Species richness and CPUE appear to be closely related to the hydrological conditions *(figure 3).* To reduce the effect of hydrology we have also used the variables described above considering only the sampling dates during the dry season. Dry season months were defined as those months with minimal discharge.

27 Generally, we have to consider that there is a link between the species richness, the size of the samples [19] and the number of fish caught. In order to avoid this problem, we have calculated the residuais of the regression log SR/log N (SR: species richness, N: number of fish). Whatever the sites and the season, we could never establish whether a significant decrease or increase in the residual richness exists. In each case, the slope is not significantly different from 0. Furthermore, whether we consider residuais or species richness alone, the general trends are more or less similar.

28 Finally, to check that the pesticides had not particularly affected any trophic group, the two indices described above were tested on seven well-defined trophic groups [25].

2.4. Analyses carried out

29 Among the different methods available, we chose to observe an ecological System and its variation throughout and after perturbation [12].

30 In order to establish the evolution of the structure of the fish assemblages, we carried out correspondence analysis (CoA) on log transformed data [Ln $(x + 1)$] so as to homogenise and normalise the variances. This analysis optimises the variability when dealing with numerous variables (here species are the variables). A first normalised principal component analysis (PCA) was performed considering the overall stations so as to look at the geographical variations. A UPGMA distance analysis was carried out on the factorial coordinates of this PCA to check the intra-and interbasin relationships. A number of CoA was made taking each station separately on the overall sampling dates and on the dates of the dry season only. The first four or five axes generally explain more than 50 % of the total variance. So, during the monitoring period, the evolution of these variables will let us know the structure change in fish assemblage.

Table II. Shared species ratio: A inter-basins; B inter-sampling sites.

A) Inter basins	n = 225	Comoé	Bandama	Volta	Up. Niger
	Comoé	100	71.1	38.3	25.9
	Bandama		100	39.2	25.5
	Volta			100	44.6
	Up. Niger				100

B) Inter-sampling sites	n = 118	ST01	ST02	ST03	ST11	ST20	ST60	ST63
	ST01	100	84.3	78.2	37.9	38.3	39.7	36.8
	ST02		100	71.9	37.9	40.0	36.3	35.2
	ST03			100	33.7	38.3	37.8	35.2
	ST11				100	81.1	55.3	62.5
	ST20					100	56.7	64.1
	ST60						100	74.0
	ST63							100

n: number of samples.

31 Concerning the factorial correspondence analysis, we considered for each site only the species with an occurrence of higher than 5 % of the total number of catches. We carried out the same analyses using different trophic levels.

32 For the coefficient of condition, for each site, we only considered the species for which the number of specimens was, at least, equal to 5. To compare sites, we also considered ecologically similar species of the same genera (e.g. *Schilbe mystus* and *S. mandibularis).*

3. RESULTS

33 In general, catches were higher during the low water period (from November to June) than during the flood period (from July to October). This relates in part to gillnet efficiency which is lower when comparing sites or years. For that reason, when analysing the data we considered all samples or those taken during the dry season. Indeed, we have noted some differences, but the general pattern is globally the same in the long term. That is why most of the time we considered all together the dry and rainy season samples.

3.1. Species characteristics

34 The sites were selected in four different basins belonging to two distinct 'ichthyoregions' [13, 27]:

35 Eburneo-Ghanean zone: Leraba (site 01), Comoe (site 02) and Bandama (site 03);

36 Sahelo-Sudanian zone: Oti (site 11), Pru (site 20) and Niger (sites 60 and 63).

37 The climates of the different regions are quite similar, but the fish fauna from each is quite distinct

38 *(table II)* and it is necessary to consider each sampling site separately. Although we observed differences between each station, the same 27 species were caught in all the rivers. Nevertheless, most of the species were caught at few sites only and 20 species were caught only at one site.

39 The numerically dominant species in the rivers of the Côte d'Ivoire was *Alestes baremoze,* and to a lesser degree *Schilbe intermedius,* which lives mainly in the Comoe basin (sites 01 and 02). The dominant species in the Oti (site 11) was *Schilbe mystus,* but many other species might sometimes co-dominate such as *Brycinus* spp. and *Synodontis schall.* In the

Pru river (site 20), which is a small tributary of the Volta basin, *Schilbe intermedius* was the dominant species, but *Brycinus* spp. and *Schilbe mystus* were also very abundant. In the Upper Niger basin, the situation is quite different according to the various sites. At Boussoule (site 60), mormyrids were very numerous as were *Brycinus* spp. and *Schilbe intermedius.* The situation was very different at Mandiana (site 63) where *Brycinus leuciscus* was the dominant species but *Schilbe mystus* and *Chrysichthys auratus* were occasionally also abundant. Each site obviously has a fairly different configuration. For example, the Milo, in the region of Boussoule-Kankan, is a rocky river, which is a good biotope for mormyrids. Conversely, the Sankarani near Mandiana, is a large and sandy river just at the mouth of the Selingue dam, which is favourable to pelagic species such as *B. leuciscus.*

40 For most species, seasonal and annual changes in abundance follow the fluctuations of total catches. Among frequent species we have not noted serious decline, except for two species of Mormyridae, *Hippopotamyrus pictus* which disappeared from the Milo river (site 60) since mid 1992, and *Marcusenius ussheri,* which became very scarce in 1990-1991 in the sites of the Côte d'Ivoire, but reappeared later in the years 1995-1996.

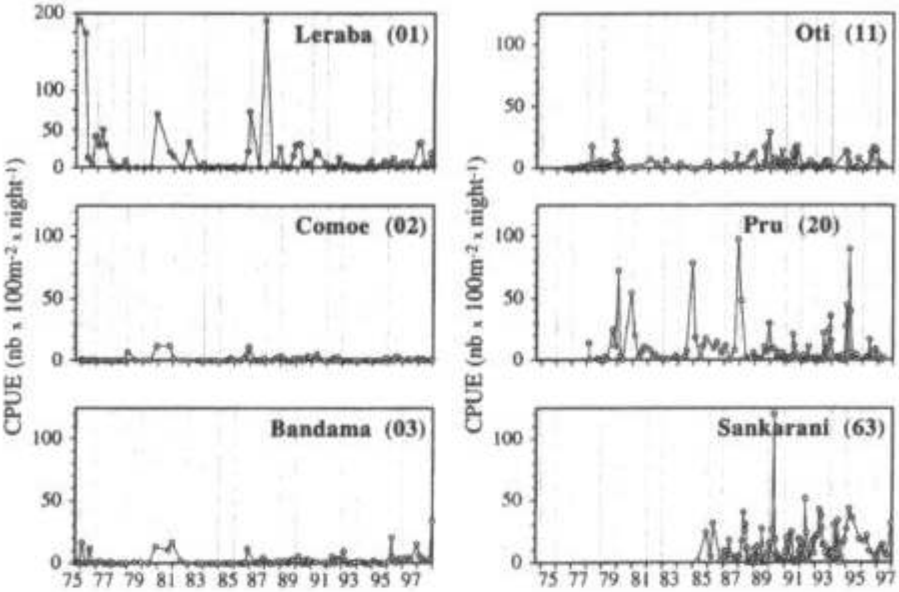

FIGURE 4. *Schilbe intermedius* (Schilbeidae): changes in CPUE (nb x 100 m^{-2} x $night^{-1}$) in six monitoring sites sampled with gillnets.

41 The case of *Schilbe intermedius* in the rivers of Côte d'Ivoire is distinct and deserves an explanation. There were cycles of 1-3 years during which we did not catch any member of that species. We initially attributed this disappearance to insecticides but as specimens became abundant again in spite of the continuous spraying of larvicides, the phenomenon appeared to be independent of insecticide applications. The absence of the species does not seem to be linked to any particular hydrological event. This should suggest that some natural fluctuations exist, but in the present State of our knowledge they are impossible to link with any precise external event [17]. The above phenomenon observed in the Côte d'Ivoire was not found in the Volta and Niger basins, where the catches were always more or less constant during the study *(figure 4).*

3.2. Species richness

42 In the Leraba (site 01) and the Comoe (site 02) rivers *(figure* 5), there was a decline in species richness until 1992-1993 (less than half of the species that were observed at the end of the 1970s), followed by an increase.

43 The situation was similar for the third station of the Côte d'Ivoire (site 03). The recovery started in 1995 and the values we observed in 1996-1997 are identical to those observed at the beginning of the study.

44 On the Volta basin, the situation differs between sites. On the Pru river (site 20), the species richness was lower during the early 1980s than after the first treatments (in 1985). However, since the early 1990s, there has been a regular decrease in species richness. Species richness in the Oti river station (site 11) was variable but with no obvious trends (see residuais *figure* 5).

45 In the Niger basin, both stations (sites 60 and 63) have shown an increase in species richness whether we take into account all samples or only dry season samples *(figure 5).*

46 To asses whether variation in CPUE and species richness were synchronised among sites, we carried out a PCA (principal component analysis on log transformed data [Ln *(x* + 1)] of the dry season) between 1988 and 1993. We only took into account the 20 most representative species for each station. As expected, stations in Côte d'Ivoire were more similar than between the stations of Côte d'Ivoire and others regions, even if these sites belong to the same basin *(figure 6).* This result shows that the correlation between geographical and/or climatic phenomena is higher than a possible impact of insecticides.

3.3. Experimental catches

47 At the Côte d'Ivoire stations, CPUE decreased until 1991 or 1993, after which time the number of catches in the three sites showed a clear rising trend *(figure T).* The treatments on the Leraba river (site 01) stopped in 1989. They stopped later (1993) on the two other rivers (sites 02 and 03). It is worth noting that the catches started rising again in 1995 in the three situations, after the end of the spraying operations. However, if the recovery of the number of catches occurred the same year, even through all the treatments did not end at the same time on the stations. These observations do not allow us to dismiss the idea of a possible effect of larvicides, but as the number of catches increased synchronously it is plausible that the improved situation is linked to better ecological conditions, as a result of improved hydrology and climate.

48 On the Pru river (site 20), CPUE seems to have been rising slightly from 1996 onwards. Trends in CPUE on the Oti river (site 11) are not evident. In this case, there may be an influence of the Volta lake which acted as a buffer against the flood that occurred in the region. In the case of the Pru river, the lake would play a smaller part because of the smaller size of the river.

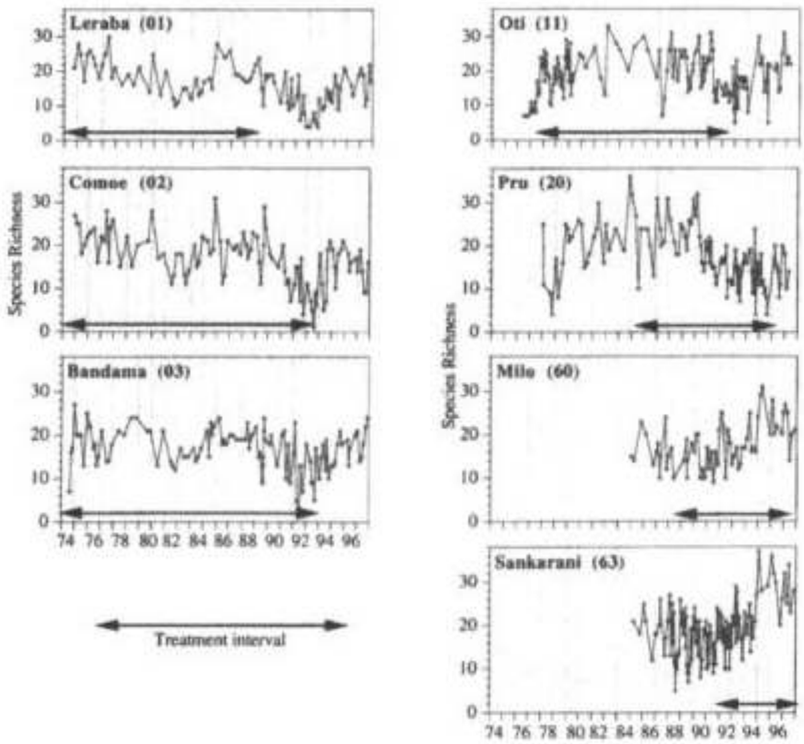

FIGURE 5. Changes in species richness per samples for the whole set of gillnets.

49 Generally speaking, the conditions in the Niger basin are good. In the Milo river the CPUE were stable until 1994 and then increased. At the Sankarani river, catches varied strongly with hydrological conditions. However, if we only consider the results of the dry season, no real decrease was observed *(figure 7).*

3.4. Structure of the fish catches

3.4.1. Correspondence analysis

50 In order to compare samples as well as species, the factorial analysis of correspondence (CoA) for which salient points have been described elsewhere [3, 15] was used. We used the software 'ADE-4' [5] for the whole analysis.

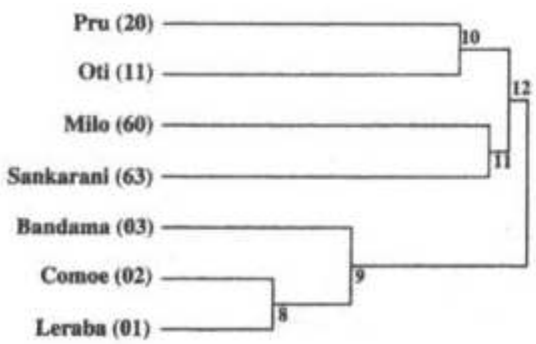

FIGURE 6. Hierarchical classification (average link on Euclidian distance) of the centre of mass of the factorial co-ordinates (PCA) of the sampling dates for each site.

51 Similarly to what can be seen for the species abundance and the CPUE, seasonal variations in the structure of catches are evident.

52 To illustrate the main results, three examples are considered, one for each region. A smooth change of structure in relation to first axis in the Côte d'Ivoire, particularly since 1989, is evident on the Bandama river (site 03) *(figure 8).* The diagram is approximately the same whether all the samples or only the dry season data are considered. The change in structure occurs especially in the case of rare species such as *Barbus macrops, Hemichromis bimaculatus* or *Raiamas senegalensis* which disappeared from the samples taken recently. Nevertheless, considering the most dominant species, there is a decrease in the total number of catches, conceming particularly *Alestes baremoze,* even if that species was clearly dominant in 1974-1976, it no longer dominates the catches to the detriment of others. In terms of abundance of species, the values are more or less the same, because only a few species are concerned. However, in terms of catches, because these species represented more or less 50 % of the total catches, the decrease in the CPUE is considerably influenced. The balance is different whether it is the abundance of species or the CPUE. It is unlikely that the situation has improved under the influence of insecticides, we can think for several reasons that we are facing a natural phenomenon. Following the perturbations due to different human impacts on the Bandama river (Kossou and Ferkesse dougou dams), it is likely that the System has attained a new improved equilibrium.

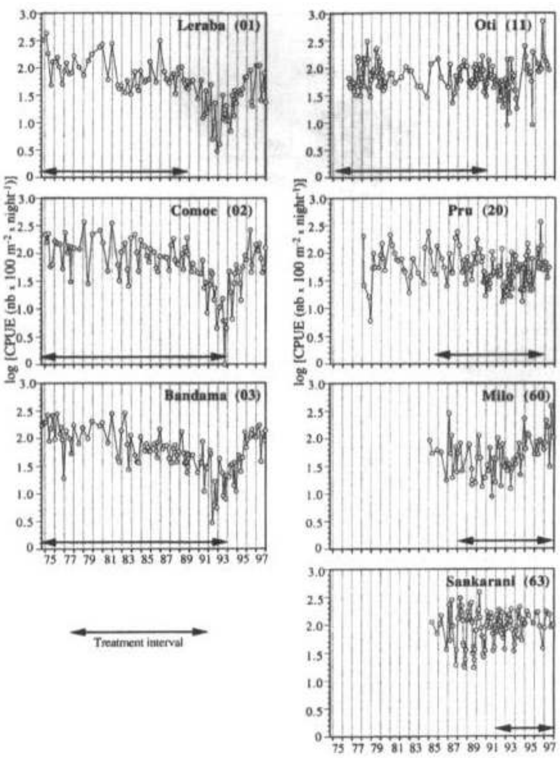

FIGURE 7. Changes in total CPUE (nb x 100 m^{-2} x $night^{-1}$) in the seven monitoring sites sampled with gillnets.

53 Our results suggest a slight, but regular change in the structure of catches in the Pru river. Some rare species such as *Heterotis niloticus, Mormyrus macrophthalmus, Gymnarchus niloticus, Clarias anguillaris* and *Auchenoglanis occidentalis* have completely disappeared front the catches. Conversely, species such as *Barbus macrops, Schilbe mystus, Siluranodon auritus* and *Synodontis violaceus* have started to appear or become more abundant since the beginning of 1990s onwards.

54 Concerning the Sankarani river (site 63) *(figure 9),* the first axis clearly separates the samples of the dry and the rainy seasons. More precisely, considering only the samples of dry season, there is a clear distinction between the samples taken in May and in June, and those of the other months. The rainy season samples are characterised by the presence of *Parailia pellucida,* a small Schilbeidae (i.e. *S. mystus* and

55 *S. intermedius),* which has to mígrate upstream from the lake during the flood to reach its breeding sites. This species does not accomplish as long a migration as do large schilbeids such as *Schilbe* [22]. The catches in May-June are dominated by the presence of *Schilbe mystus* which start their anadromous migration and leave the lake. At the end of the dry season and during the rainy season, the catches are characterised by the presence of three species of *Petrocephalus (P. bovei, P. soudanensis* and *P. ansorgii).* Apparently, in manmade lakes, migrations of mormyrids to the river mouths can occur several months before the flood starts [22].

3.4.2. Shannon diversity and evenness indexes

56 The dominant species in Côte d'Ivoire was *Atestes baremoze* and it was associated with *Schilbe intermedius* or *S. mandibularis (table III).* These species were dominant in more than 50 % of the catches. Since the monitoring started, the diversity has been relatively stable. However, events (1982-1984 and 1992-1994) sometimes lead to a decrease in diversity. In all of the cases, these events are related to a strong dominance of the species named before, mainly *Alestes baremoze.*

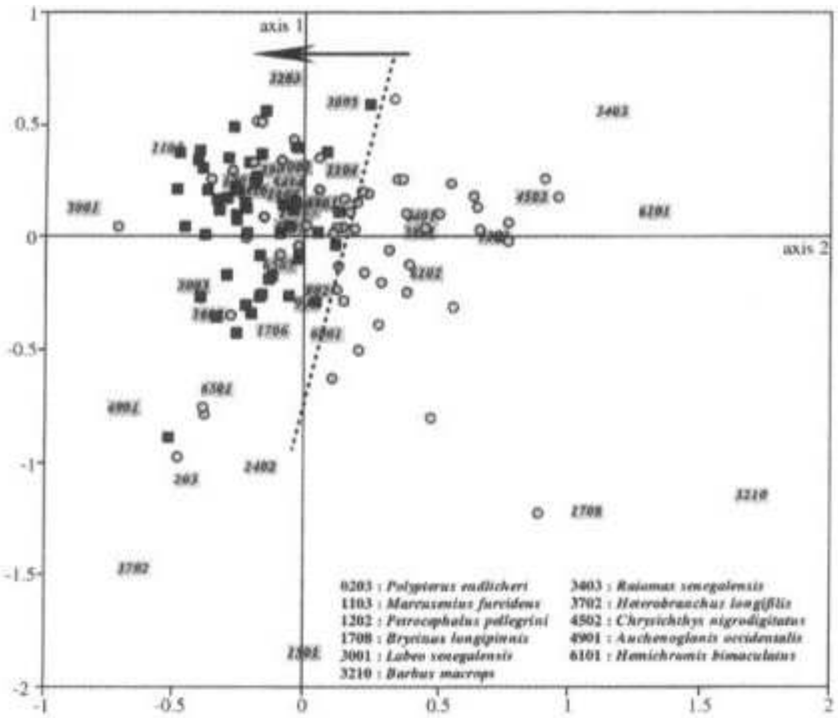

FIGURE 8. Bandama River (site 03): factorial plan I and II of the correspondance analysis (samples and species projection). Species listed are those which have an important contribution (axis 1) in the performed analysis (taxonomic classification). 1975-1988: ●; 1989-1997: ■

57 The structure and the diversity have not changed but these two or three species are 'naturally' dominant in the catches. The abundance of species and the total CPUE parameters confirm these observations.

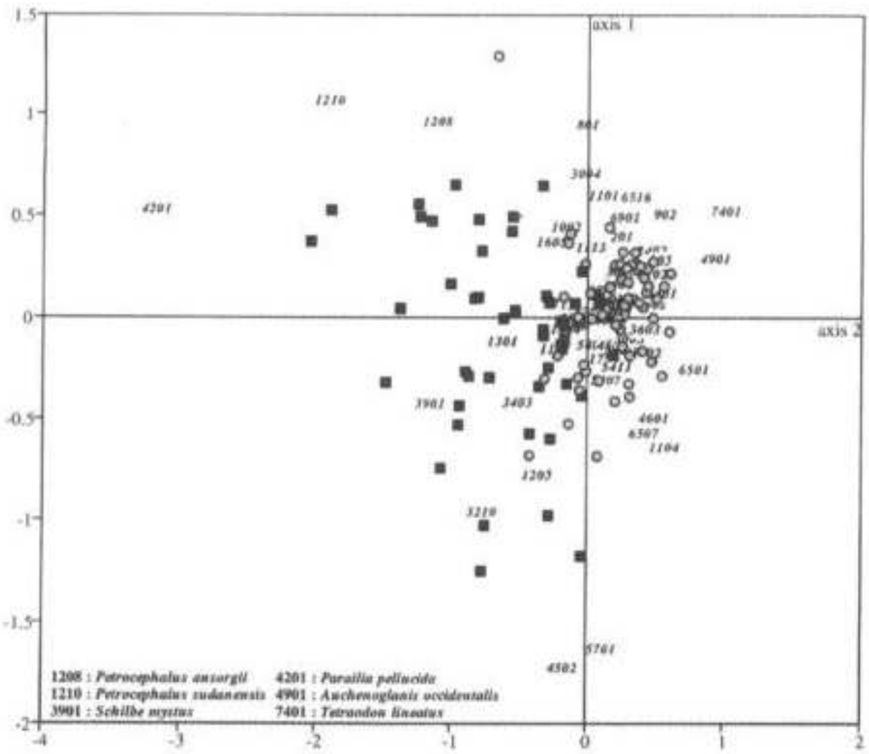

FIGURE 9. Sankarani (site 63): factorial plan I and **II** of the correspondence analysis (samples and species projection). Species listed are those which have an important contribution (axis 1) in the performed analysis (taxonomic classification). Dry season: ●; rainy season: ■

Table III. Dominance frequencies of the species in the seven monitoring sites for all the catches (AS) and for the dry season (DS) catches only.

	Site 01		Site 02		Site 03		Site 11		Site 20		Site 60		Site 63	
Species	AS	DS	AS	DS	AS	DS	AS	DS	AS	DS	AS	DS	AS	DS
Polypterus senegalus									5.0	4.3				
Polypterus endlicheri	1.1	2.3												
Mormyrus hasselquistii			1.0	2.1										
Hippopotamyrus pictus											3.2	2.6		
Marcusenius senegalensis							1.4	2.5	2.5	4.3				
Marcusenius cyprinoides							0.7	1.3						
Marcusenius ussheri	1.1													
Marcusenius mento											1.6	2.6	1.8	1.6
Petrocephalus bane							2.2	2.5						
Petrocephalus bovei	2.1	4.5	5.1	10.6	3.6	6.4	2.2		1.7	2.9	7.9	7.7	0.9	1.6
Petrocephalus ansorgii											11.1	7.7		
Petrocephalus pallidomaculatus							2.2		4.1	2.9	6.3	10.3		
Petrocephalus soudanensis											4.8	5.1	1.8	
Hippopotamyrus psittacus											4.8	7.7		
Pollimyrus isidori									0.8					
Hepsetus odoe									3.3	4.3				
Hydrocynus forskalii					1.8	2.1								
Hydrocynus vittatus							1.4	1.3						
Alestes baremoze	34.0	29.5	39.8	34.0	49.1	42.6								
Brycinus macrolepidotus	2.1	2.3	2.0	2.1	7.3	10.6	1.4	2.5	5.8	8.7	17.5	23.1	8.0	11.1
Brycinus imberi	2.1	4.5	4.1	6.4										
Brycinus nurse	3.2	4.5	4.1	4.3	18.2	21.3	7.9	13.8	9.9	15.9	7.9		1.8	3.2
Brycinus leuciscus							18.0	21.3	5.0	2.9	7.9	10.3	31.3	25.4
Distichodus rostratus							0.7	1.3	5.8	7.2				
Distichodus engycephalus													1.8	1.6
Labeo senegalensis							10.1	13.8						
Labeo coubie							2.2	3.8						
Labeo parvus									3.3	4.3				
Schilbe intermedius	28.7	29.5			2.7	4.3	2.9	2.5	17.4	11.6	11.1	2.6	13.4	15.9
Schilbe mystus							26.6	13.8	15.7	4.3	1.6		16.1	3.2
Schilbe mandibularis	10.6	2.3	25.5	23.4	9.1	4.3								
Chrysichthys auratus							0.7	1.3			9.5	15.4	18.8	30.2
Chrysichthys nigromarginatus			5.1	10.6	0.9	2.1	2.9	2.5					0.9	
Chrysichthys maurus	5.3	6.8	8.2	2.1	0.9									
Synodontis sorex							1.4	1.3						
Synodontis eupterus							0.7		2.5	2.9				
Synodontis filamentosus							2.2	3.8	1.7	2.9	1.6		2.7	4.8
Synodontis violaceus							0.7	1.3						
Synodontis ocellifer							3.6	3.8	0.8	1.4				
Synodontis velifer							1.4	1.3	1.7	1.4				
Synodontis schall	5.3	6.8			3.6	2.1	4.3	2.5	8.3	11.6				
Synodontis bastiani			1.0	2.1										
Synodontis comoensis	1.1	2.3	3.1	2.1										
Hemichromis fasciatus							0.7	1.3	0.8					
Chromidotilapia guntheri	2.1	4.5			1.8	4.3			4.1	5.8			0.9	1.6
Sarotherodon galilaeus					0.9									
Tilapia zillii			1.0				0.7	1.3						
Lates niloticus	1.1						0.7				3.2	5.1		

58 The catches in the Volta basin rivers are generally diverse but, sometimes, species such as *Brycinus nurse, B. leuciscus, Labeo senegalensis* and *Schilbe mystus* are particularly abundant. The case of the Pru river (site 20) requires few comments. The catches were more diverse between 1983 and 1992. The abundant species were less influent and, at the same time, a notable increase in the abundance of species occurred, mainly during the dry season. That period corresponds to a favourable hydrological balance. Because of the shape of the Pru (a small tributary of 6 500 km^2), we consider that the good flood conditions favoured the resettlement by species which remain scarce when the field conditions are less favourable. In that way, the Lake Volta played the role of a sanctuary when favourable conditions enabled its re-colonisation by a type of fauna close to that of the lake. For example, there is a decrease in the catches of species which are characteristic of small tributaires (e.g. *Polypterus senegalus, Hepsetus odoe* and *Hemichromis fasciatus).* On the contrary, the number of *Siluranodon auritus, Schilbe mystus* and *Synodontis violaceus,* which are characteristic of large rivers, increased in the catches of the Pru river.

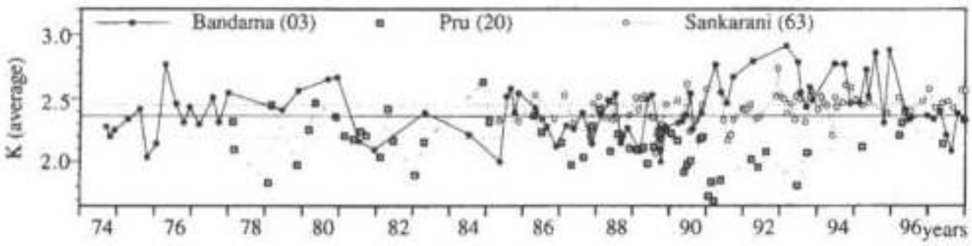

FIGURE 10. *Brycinus nurse* (Characidae): changes in coefficient of condition **(K)** for selected stations.

59 There is not much change in the index of diversity for the Niger basin. If *Brycinus leuciscus* is still dominant in the Sankarani (site 63), it is now often supplanted by *Schilbe intermedius.* This result is confirmed by the value of the evenness which has been higher since 1994. The bloom of the catches of *B. leuciscus* is likely to have been a consequence of the filling of the dam of Selingue. The fact that lower catches are returning is certainly the sign of a smooth equilibrium of the communities.

3.5. Condition factor

60 All results of the condition factor listed were calculated and interpreted for each single species and for each site. Condition factor can account for the direct effect of the pesticides on the physiology of the fishes (for example, the impact on the reproductive potential) or for indirect effects through an impact on the invertebrate fauna which is the main feeding source for many species. In order to analyse these possible effects, we selected species answering three criteria: abundance, strategies of reproduction and diet.

61 Considering all the populations of a species, a clear cycle of the condition factor according to the month or the season is not evident. Nevertheless, literature on the subject is full of data concerning the evolution of K according to the season. This depends mostly on the maturation of the gonads [1], but in a global analysis as is performed here where the average of the condition factors according to the month are calculated, the inter-annual variations seem to override any seasonal cycle.

62 On the contrary, in most cases, the average condition is linked to geography. That way, taking an ubiquitous species such as *Brycinus nurse,* nearly all the fish of the Volta basin have a lower condition than those of the Niger river or of the rivers of Côte d'Ivoire *(figure*

10). Further more, species of the SaheloSudanian group such as *Schilbe mystus* (formerly *Schilbe niloticus = Eutropius niloticus)* have a lower condition factor in the Volta than in the Niger basin. In general, the condition of the fish from the Oti river is less than that of the fish of the Pru river *(figure 11).* For the fish of Côte d'Ivoire alone, in most cases the K of the fish of the Leraba river is better than that of the fish of the other rivers.

63 Whatever the stations and whatever the species and their trophic group (insectivorous, carnivorous, omnivorous, etc.), the selected examples show that the values of the coefficient of condition are relatively irregular and fluctuate around an average. This does not appear to have been altered since the beginning of the treatments, except possibly for *Schilbe mystus* in the Oti *(figure 11)* and Black Volta (not in our analysis) rivers. In these two sites, the decrease in K for *S. mystus* more or less follows the introduction of treatments with permethrin and carbosulfan. These two drastic larvicides were not required that much in the Pru river where there was no decrease in K apart from the short period 1989-1990. So, although we are not able to demonstrate that there is an absolute correlation between the decrease in K in Oti (and Black Volta) and the application of toxic larvicides, we cannot dismiss the idea of a temporary effect of the insecticides on the condition of the fishes.

64 Other short-term effects were observed and seem to be independem of the insecticide spraying. For example, the condition of *Alestes baremoze* or *Schilbe mandibularis* which was initially low or medium in the same site eventually increased *(figure 12).* The low K values observed for these species at this site, beyond strict poor hydrological conditions, is probably due to the fact that this site is situated between two large dams (Kossou and Ferkessedougou). The harnessing of that river and the regulation of the flood must be a serious constraint for migratory species such as *A. baremoze* and *S. mandibularis.* The drop in K during the period 1976-1977 for *A. baremoze* was restricted to the course of the Bandama river situated between Kossou and Ferkessedougou. Below the Kossou dam and upstream from the Ferkessedougou dam, fishes show normal condition [24]. As the entire course of the Bandama was treated, it would appear that the decrease in condition is not due to the spraying of insecticide. These examples suggested that the effects of a single perturbation (dams) can interact with and multiply the consequences of other disturbances (low flood), with a cumulative impact on the ecosystem [9].

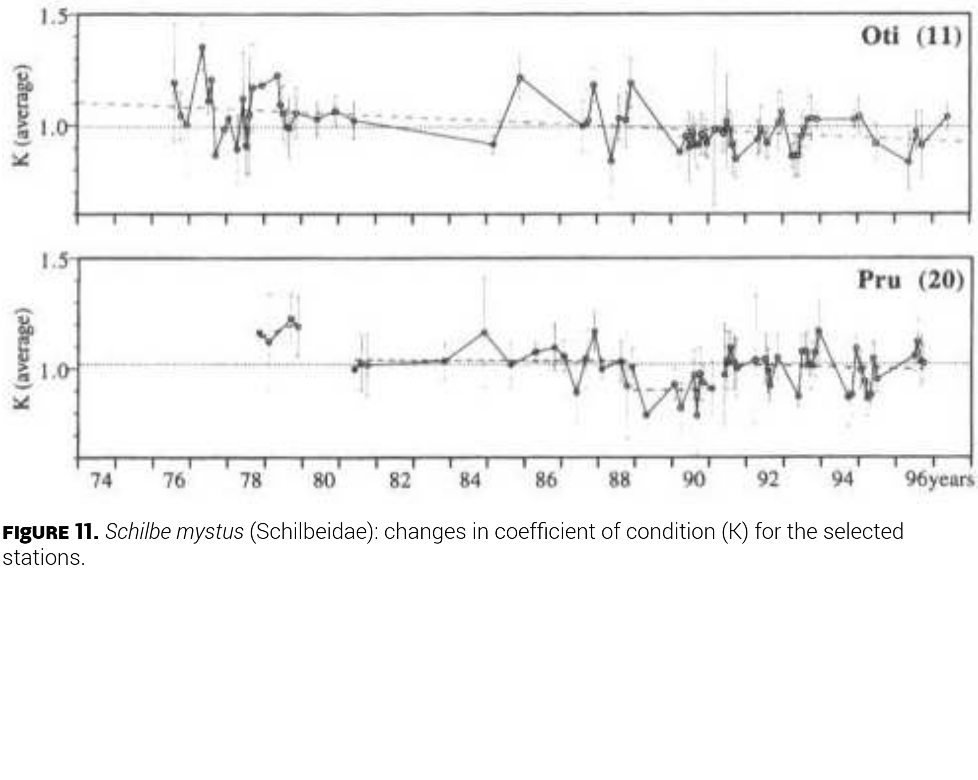

FIGURE 11. *Schilbe mystus* (Schilbeidae): changes in coefficient of condition (K) for the selected stations.

4. CONCLUSION

65 The species considered in this study reach maturation at the end of their first year and they rarely live more than 4 or 5 years [1, 21].

66 Therefore, for all the sites, the catches do not give more information concerning long-term variations, whether analysed as a whole or by trophic group (see above). Evidently, no trophic association, particularly the insectivorous group for which the main feeding item might be reduced by the treatments, seems to have been affected during the monitoring period. This compares favourably with other experiments carried out in other areas. For example, in the Victoria Nile (Uganda), the control of blackflies with the use of DDT induced a drastic change in the feeding resources of some of the species such as Mormyridae or a drastic lack of food for other species such as *Aethiomastacembelus frenatus* [6, 7].

67 The relative stability of the condition factor for each species indicates that their feeding was not unduly interrupted. Two hypotheses can be proposed. Either feeding sources are always available, or the fishes are able to change their feeding habits as has been demonstrated for other untreated rivers of the region [25]. Whatever explanation applies the fish do not seem to be affected biologically nor physiologically by larvicides [2]. That also indicates that the insecticides used do not have any detectable toxicity on the metabolism of species [28].

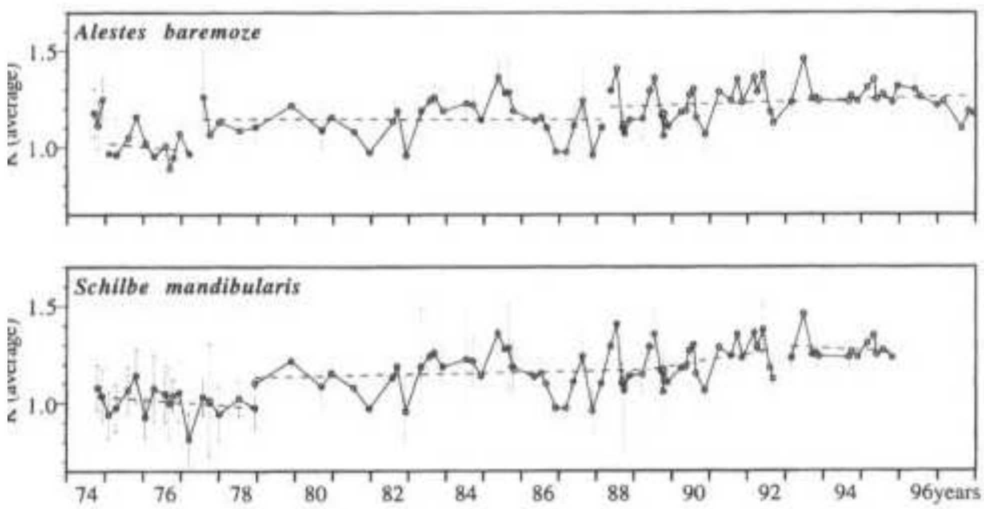

FIGURE 12. Bandama (site 03): changes in coefficient of condition (K) for the selected migratory species.

68 Considering the different variables used in this study, we did not find any detectable effects of pesticides on the CPUE, the abundance of species, as well as on the community and trophic structure and the fish health (condition factor and reproduction strategies). However, we noticed a number of trends which appear to be related mainly to climatic conditions, probably to hydrology. Thus, there was a regular decrease in CPUE from the beginning of the monitoring until 1995. The rivers were treated during that time until 1990 or 1993, depending on the stations *(figure T)*. The decrease persisted even after the treatments ended. As a result, we consider other factors to be the cause of that process. The average level of annual discharge in that region has been decreasing regularly from

the beginning of the 1970s *(figure 13).* A recent study (Hugueny, pers. comm.) showed that there was a correlation between discharge and CPUE during the first 10 years of monitoring (year^{-1}). The production of fish fluctuates in all the rivers according to the flood rate. Important floods inundate larger areas, making greater quantities of food available, and improving the conditions for reproduction [30]. In the central delta of the Niger (Mali), plots of annual catches and loss of water are highly correlated. The determinant factor of the ichthyological stock abundance appears to depend on the extern and durability of the flood. In fact, 69 % of fish under 1 year old are caught in nets of mesh sizes smaller or equal to 20 mm [14]. Those observations agree closely with our own observations. In our catches, the observed effect was not immediately clear but appeared a few years later as a cumulative effect of poor hydrological conditions. Conversely, the increase in the CPUE since 1996 has been related to better hydrological conditions (IRD, France, Hydrological Service, Internet data). In these latter years, there has been an intensification of the basic flow leading to a ground water renewal, although that phenomenon remains quite clearly smooth if we consider the whole basin (Sircoulon, pers. comm.).

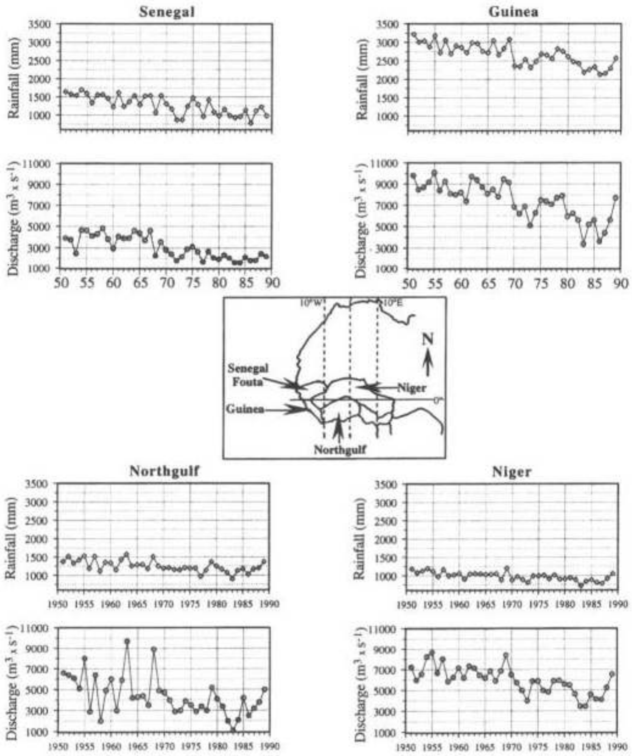

Figure 13. Average annual and regional discharge (m^3 x s^{-1}) and rainfall (mm) in four regions of West Africa (after Mahé, 1993).

69 Furthermore, for three of the stations investigated, it appeared that the hindrance of rivers (dams) induced different and/or antagonistic effects. In certain cases, notwithstanding poor hydrological conditions, some of the species were favoured by the presence of the dam. For example, in the Sankarani river just upstream from the Selingue lake, a bloom of pelagic species such as *Parailia pellucida* was recorded because of lacustrine conditions. At the same time, the condition factors of predators such as *Hydrocynus* spp. increased significantly. In a similar way, Lake Volta acted as a buffer for the rivers of the Ghanaian region under poor hydrological conditions. Conversely, the

damming of the river also has a negative effect on some species, particularly on the coefficient of condition of migratory fishes.

70 When compared with the results obtained after the first 10 years of monitoring [16], the fish structure has not changed and we can conclude that the pesticides sprayed by the OCP do not influence the structure of the main fish community, the species richness nor the fish biology. Finally, we record that no fish species has disappeared entirely. The influence of long-term monitoring must be considered before we interpret the results. Thus, climatic changes, which generally have a great influence on the population dynamics of fishes, need to be considered. This is why we have to understand and remove the natural fluctuations of species from the situation. In a short-term study, we could have ended up with the wrong conclusions, because natural fluctuations do occur and species (*Schilbe intermedius,* for example) can temporarily disappear.

71 Another important aspect is to evaluate the results in terms of the investment made. Apart from the results concerning the use of larvicides, the monitoring generated an important understanding of fish biodiversity [26]. We conclude that the OCP was a very successful control programme because, not only did it not have any drastic effect on the fauna, but we also know now that it prevented three million of children from going blind [29].

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ABSTRACTS

Onchocerciasis is a widespread disease in intertropical Africa, which, ultimately, causes irreversible blindness. The disease is transmitted by a small blackfly, *Simulium damnosum* (Diptera), which has aquatic larval and pupal stages. The breeding sites of the blackflies are riffles. These river reaches are the targets of the control campaign of the Onchocerciasis Control Programme in West Africa (OCP). An aquatic monitoring network covering the totality of the area exposed to the insecticide was set up to evaluate environmental impact. In this paper, we present results from the OCP 20-year period of monitoring of the ichthyofauna regularly exposed to larvicides. We do not record any measurable effects of pesticides on the CPUE, abundance of species, trophic structure, community structure or fish health. However, we detect the emergence of a number of medium-term tendencies. These tendencies may relate to climatic conditions that have a consequent effect on hydrology. Thus, we note a constant decrease in the CPUE from the beginning of the monitoring until 1995. The rivers were treated during that time until 1990 or 1993, depending on the station. But even after the treatments ended, the number of catches continued to decrease. As a result, we consider other factors to be the cause of that decline. The average level of annual discharges in this region has been decreasing regularly from the beginning of the 1970s. The production of fish fluctuates in all the rivers according to the flood rate. Important floods inundate larger areas, making greater quantifies of food available, and thus improving the conditions for reproduction. The determining factor of the ichthyological stock abundance seems to depend both on the extern and the duration of the flood. In our catches, the observed effect was not immediately evident but appeared a few years later as a cumulative effect of poor hydrological conditions. An increase in the CPUE since 1996 has been related to improved hydrological conditions. In these last few years, we have observed an intensification of the basic flow leading to a ground water renewal. Furthermore, on three of the stations investigated, it appeared that the impediment of rivers (dams) could induce different and/or antagonistic effects. In some cases, we have observed that in spite of unfavourable hydrological conditions, certain species appear to be favoured by the presence of the dam. But, the damming of the river has a negative effect on other species, particularly on the coefficient of condition of migratory fishes. The impact of these factors is enhanced by the fact they exist conjointly.

Résumé — Maladie largement répandue en Afrique intertropicale l'onchocercose est un fléau qui provoque, à son stade ultime, une cécité irréversible. La maladie est transmise par un petit Diptère, *Simulium damnosum,* qui présente une phase larvaire et nymphale aquatique. Ce sont les gîtes larvaires de ce vecteur, biefs à courant rapide des rivières, que le Programme de lutte contre l'onchocercose en Afrique de l'Ouest (OCP: *Onchocerciasis Control Programme in West Africa*) traite lors de ses campagnes de lutte. Comme toute lutte insecticide, OCP représentait une menace importante pour l'environnement. C'est pourquoi le programme s'est doté d'un réseau de surveillance des écosystèmes aquatiques, couvrant l'ensemble de la zone exposée aux épandages d'insecticides. Ce sont les résultats de vingt années de surveillance de l'ichtyofaune, régulièrement exposée aux traitements larvicides, qui sont présentés ici. Les différentes variables étudiées dans cette étude, ne permettent pas de mettre en évidence un effet décelable des pesticides sur la structure et la richesse spécifique des peuplements. De même, la composition trophique ou la santé des poissons ne semblent pas affectées. Cependant, nous observons parfois certaines tendances, à moyen terme, qui semblent être essentiellement sous l'influence des conditions climatiques, probablement hydrologiques. Ainsi, nous observons une diminution régulière des prises par unité d'effort (PUE) du début de la surveillance jusque vers 1995. Durant cette période, les rivières ont été, selon les stations, traitées jusque 1990 ou 1993. Mais, alors que les traitements étaient terminés, la diminution des captures s'est poursuivie. Nous pouvons donc estimer que d'autres facteurs en sont la cause. Si nous considérons les crues moyennes annuelles et régionales, nous observons une diminution régulière depuis le début des années 1970. Dans toutes les rivières, la production de poisson fluctue en fonction du régime d'inondation. Lorsque les crues sont favorables, elles inondent des superficies plus grandes, ce qui favorise la disponibilité en nourriture, et améliore donc les conditions pour la pérennité des espèces. En fait, le facteur déterminant de la production halieutique semble être lié à la fois à l'étendue et à la durabilité de l'inondation. Dans nos captures, l'effet apparaît avec quelques années de retard comme s'il y avait eu un effet cumulatif des mauvaises conditions hydrologiques. Inversement, l'accroissement des PUE depuis 1996 paraît bien corrélé aux meilleures conditions de crue observées. Ces dernières années, nous notons un renforcement de l'écoulement de base qui se traduit par une recharge des nappes phréatiques. Enfin, sur trois stations étudiées, les barrages peuvent induite différents effets, antagonistes ou non. Dans certains cas, nous observons qu'en dépit de mauvaises conditions hydrologiques, certaines espèces semblent être favorisées par le barrage et sa retenue d'eau. Inversement, le barrage du fleuve peut avoir un effet négatif sur certaines autres espèces, particulièrement sur celles qui effectuent des migrations longitudinales. Tous ces facteurs semblent montrer une pression d'autant plus importante qu'ils se produisent en synergie.

INDEX

Mots-clés: Peuplement de poissons, eau douce, insecticides, onchocercose, Afrique occidentale
Keywords: Fish assemblage, freshwater environment, insecticides, onchocerciasis, West Africa

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Laboratory Toxicity of Potential Blackfly Larvicides on Some African Fish Species in the Onchocerciasis Control Programme Area

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1 *Received June 5, 1990*

2 The Onchocerciasis Control Programme of the World Health Organization uses larvicides to fight against the aquatic stages of the vector *Simulium damnosum* s.l., and thereby interrupt transmission of the disease. Since the appearance of resistance to Abate and chlorphoxim in certain cytotypes of the vector, the efficacy of many possible replacement insecticides has been tested and the impact of the best of them (permethrin, cyphenothrin, pyraclofos, and carbosulfan) on the aquatic fauna evaluated. © 1991 Academic Press, Inc.

INTRODUCTION

3 A vast Chemical control operation was launched in 1974 to interrupt the transmission of human onchocerciasis in several West African countries. The aquatic larval stages of the *Simulium damnosum* Theobald complex, the vector, are eliminated by the weekly application of degradable insecticides on the breeding sites. Three insecticides have been utilized in the Onchocerciasis Control Programme area (Fig. 1) : temephos, chlorphoxim, and *Bacillus thuringiensis* H-14. Aware of the potential environmental risk of this insecticidal treatment, the Programme pays particular attention to the selection of larvicides and has developed monitoring facilities and protocols. An ecological group, composed of independent experts, Controls and directs the Programme's activities regarding environmental protection. The monitoring is geared mainly toward a regular study of the fish populations and the lotic benthic invertebrate populations on which many fish feed. In view of the results recorded during more than 10 years of larviciding, it

can be said that the larvicides utilized have little effect on the nontarget fauna in the tropical lotie environments (Lévêque *et al.,* 1988 ; Yaméogo *et al.,* 1988 ; Lévêque, 1989).

4 Since the appearance of resistance in some cytotypes of the *S. damnosum* s.l. complex to temephos and then chlorphoxim (Guillet *et al.,* 1980 ; Kurtak *et al.,* 1982), the screening of new blackfly larvicides has become one of the main fines of research in the Onchocerciasis Control Programme in West Africa.

5 Among the many larvicides and/or formulations tested, permethrin, cyphenothrin (pyrethroids), pyraclofos (organophosphorus compound), and carbosulfan (carbamate) have shown sufficient efficacy against the vector and present a very little risk of rapid development of cross-resistance with the organophosphorus compounds.

6 Trials were therefore carried out on the nontarget aquatic fauna to evaluate the toxicity levei of these larvicides. This paper reports the main results recorded during tank tests in laboratory on some fishes.

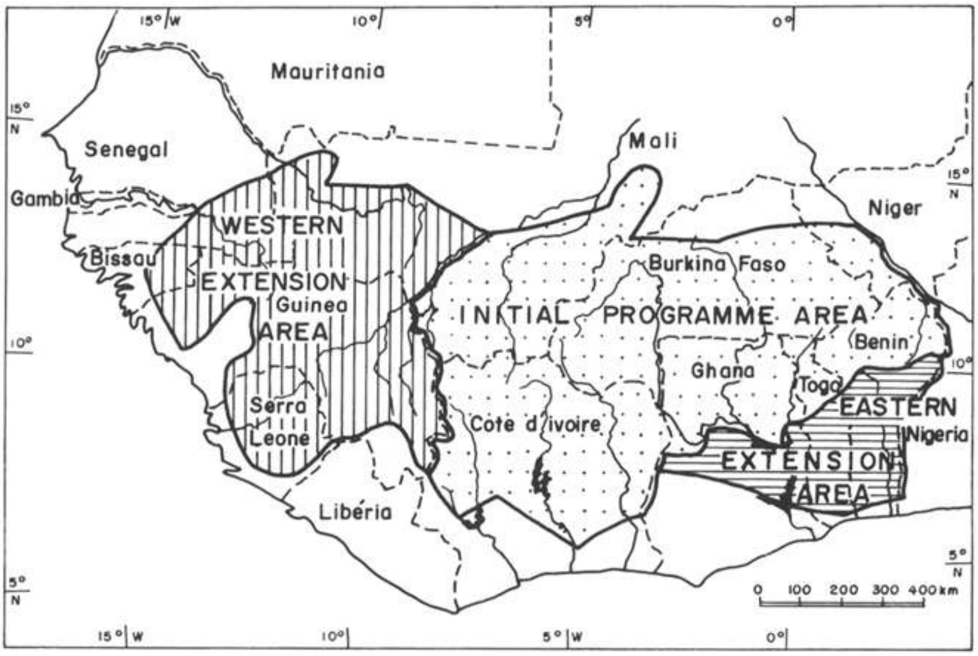

FIG. 1. Area covered by the Onchocerciasis Control Programme in West Africa (O.C.P.).

7 These tests were performed in difficult periods and field conditions. Therefore the results are not presented as a standard comparison at a fixed time and for the same fish species. It was, however, considered relevant to publish the results because of the scantiness of data on aquatic toxicity for African fish species.

MATERIALS AND METHODS

1. Fishes

8 A number of species that are members of families which are well represented in the Programme area were used. They were *Schilbe mystus, Pollimyrus isidori, Chrysichthys velifer, Chrysichthys nigrodigitatus,* and *Barbus macrops.*

9 *S. mystus (Schilbeidae)—Linné 1762.* This species is one of the most widespread African fish species, from the Zambesi and Kunene Rivers to the Senegal and Nile in the north

including the east coast rivers. The maximum standard length is about 300 mm. The species feeds on insects, shrimps, small fishes, and fruits but is, above all, ichthyophagous and this tendency increases with size. The average length of the individuals tested was about 50 mm.

10 *P. isidori (Mormyridae)—Valenciennes 1846.* Well known from the Niger, Gambia, Senegal, and Volta basins and from different Coastal rivers, *P. isidori* is a small species which feeds on insect larvae and on zooplankton. The average length of the individuals tested was about 47 mm.

11 *C. nigrodigitatus (Bagridae)—Lacépède 1803.* This species is known from the Gambia, Senegal, Niger, and Volta basins and from most of the Coastal rivers. The maximum standard length is 475 mm but the average length of the individuals tested was 65 mm. *Chrysichthys* feed mainly on insect larvae (Chironomidae) but also on small mollusca, zooplankton, and Hemiptera.

12 *C. velifer (Bagridae)—Norman 1923.* Reported only in the Bandama and Sassandra river basins, *C. velifer* presents the same feeding habits as *C. nigrodigitatus.* The average length of the individuais tested was 57 mm.

13 *Barbus macrops (Cyprinidae)—Boulenger 1911.* Widely distributed in the Programme area, the maximum standard length of this small species is 98 mm but the average length of the species tested was 46 mm. It feeds mainly on insect larvae, small crustaceans (Copepoda, Cladocera, Ostracoda), and plant debris.

14 Before the beginning of the trials the different fish species were kept in separate tanks in laboratory for a week. They were fed once daily and the water was oxygenated using diffusers.

15 The fishes were of comparable size for each of the species. They were carefully put one by one into the test tanks so as to have 10 individuals of the same species in each solution. The water utilized was from the same source as the fishes.

2. Tests

16 The acute toxicity tests were performed by means of the technique with periodic replacement of solutions (Ward and Parrish, 1983), which makes it possible to avoid the problems of decrease of the concentration of producís in aquariums during exposure time ; the solutions were replaced every 12 hr.

17 The test solutions were 10 liters for each tank and were oxygenated. The water temperatures were 27 ± 2°C. The end point was death. The lethal concentrations were calculated according to the probit analysis (Finney, 1952).

18 The Chemicals tested were : permethrin (3-phenoxybenzyl-(1 *RS)-cis, trans* 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) ; cyphenothrin (cyano(3-phenoxyphenyl)-methyl-(*1R)-cis, tans-2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropa-*necarboxylate) ; pyraclofos *((RS)-[O-1-(4-chlorophenyl)-pyrazol-4-yl-O-ethyl-S-pro-*pylphosphorothioate]) ; and carbosulfan (2,3-dihydro-2,2-dimethyl-7-benzofuramyl [(dibutylamino) thio] methylcarbamate).

19 The technical products were diluted taking into account the percentage of active ingredient and the results were expressed for each time, e.g., concentration of active

principie (µg/liter). The larvicide solutions were prepared 1 hr before the start of the trials.

RESULTS

20 The results are reported in Table 1 together with confidence limits and slopes. The toxicity curves are shown in Fig. 2. All the curves seem to be asymptotic and have normal trends ; therefore some missing data could be extrapolated. For example, the 72-hr LC50 for permethrin and *P. isidori* would be around 20 µg/liter, while the 72-hr LC50 for *C. velifer* exposed to cyphenothrin is about 150 µg/liter.

Permethrin

21 The trials were carried out only with *P. isidori.* No mortality was recorded in the control tanks and the behavior of the individuais was normal up to the end of the trials.

TABLE 1. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR DIFFERENT TROPICAL FISH SPECIES AND DIFFERENT INSECTICIDES

		Median lethal concentrations (µg/liter) Confidence limits Slopes		
	Larvicide used	24 hr	48 hr	72 hr
Pollimyrus isidori	Permethrin 20% EC	40 (30–63) 3.25	26 (19–31) 5.37	—
Chrysichthys velifer	Cyphenothrin 10% EC	630 (501–800) 4.39	220 (121–270) 5.47	—
Barbus macrops	Cyphenothrin 10% EC	15 (14–17) 5.73	12 (11–13) 4.26	10 (7–11) 4.44
Pollimyrus isidori	Pyraclofos TIA-230, 50% EC	170 (149–184) 5.27	70 (41–87) 3.71	40 (3–66) 2.95
Chrysichthys nigrodigitatus	Pyraclofos TIA-230, 50% EC	150 (113–632) 2.66	78 (53–95) 3.41	68 (48–82) 4.45
Pollimyrus isidori	Carbosulfan 25% EC	82 (71–81) 7.81	—	71 (61–82) 7.3
Schilbe mystus	Carbosulfan 25% EC	180 (124–26,970) 4.72	140 (105–162) 7.76	136 (108–152) 11.53

22 The fishes introduced into the permethrin solutions were a bit more active than those of the untreated tanks and less than 5 hr after the start of the trial some of them demonstrated disorderly movements followed by death.

23 The 24-hr LC50 for *P. isidori* (40 µg/liter) is less than with pyraclofos and carbosulfan. The mean lethal concentration for a 48-hr exposure time is 26 µg/liter.

24 A lot of data exist on the acute toxicity of permethrin in laboratory conditions on various fish species. However, they are quite scattered and different authors have given as 24-hr

LC50 on rainbow trout values ranging from 8 µg/liter (Mulla *et al.,* 1978) to 61 µg/liter (Coats and O'Donnel-Jeffrey, 1979) in comparable testing conditions. However, most of these values are in the low range : 12.5 ¿¿g/liter (Hill *et al.,* 1976a), 13.7 µg/liter (Abram *et al.,* 1980). Other species showed the same range of sensitivity. The 24-hr LC50 for *Pimephales prometas* is 15 µg/liter (Hill *et al.,* 1976b), 6 ¿¿g/liter for *Ictalurus punctatus* (Buccafusco, 1976), 8.6-21 µg/liter (Hill *et al,* 1976c, 1977). *Oreochromis aureus,* a *Tilapia* species, has a toxicity of 6 ¿¿g/liter as 24-hr LC50 (Herzberg, 1988), while *Oryzias latipes, Cyprinus carpio* and *Gambusia affinis* have 41 µg/liter, 98 µg/liter, and 100 µg/liter, respectively (Miyamato, 1976 ; Hill *et al.,* 1976c ; Mulla *et al.,* 1978).

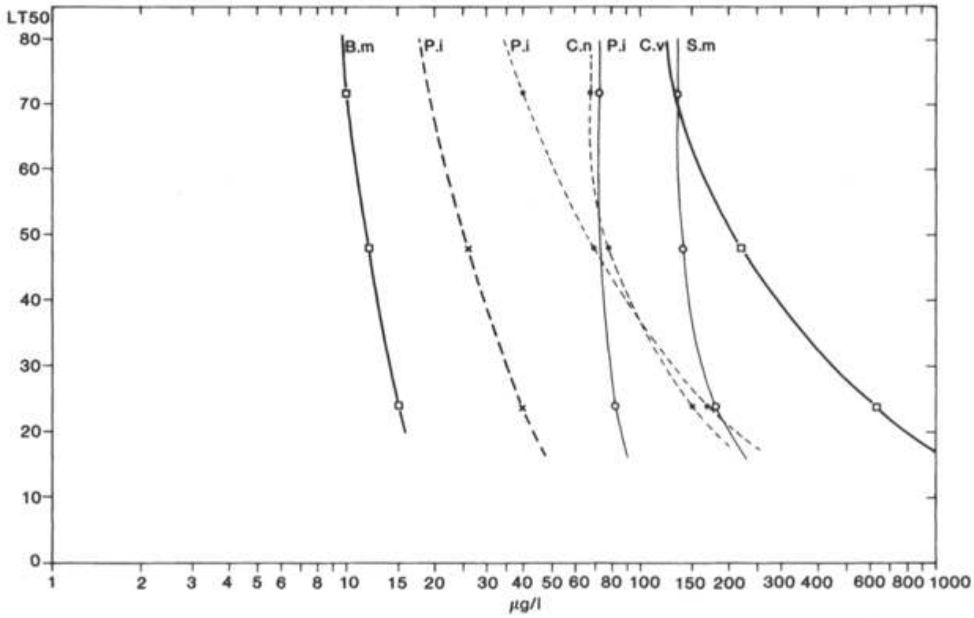

FIG. 2. Toxicity curves for the four insecticides tested. B.m, *Barbus macrops ;* P.i, *Pollimyrus isidori ;* C.n, *Chrysichlhys nigrodigitatus ;* C.v, *Chrysichthys velifer ;* S.m, *Schilbe mystus.* •, Pyraclofos ; O, carbosulfan ; X, permethrin ; □, cyphenothrin.

Cyphenothrin

25 During the trial no fish died in the control tanks, which were handled in the same way as the treated tanks. *B. macrops* and *C. velifer* were the species utilized for the trials.

26 For *B. macrops* the 24-hr LC50 is the highest toxicity observed, while for *C. velifer* more than 40 times this dose is necessary to obtain 50 % mortality within 24 hr.

27 For *C. carpio,* in a temperate climate, the 48-hr LC50 is 5.65 µg/liter (Sumitomo, 1983), a value that is very low compared with that recorded for *C. velifer* in a tropical environment.

28 The two species tested, therefore, presented different susceptibilities to cyphenothrin. *B. macrops* was the most affected species ; for the same exposure time (48 hr), the LC50 of *B. macrops* was 18 times less than for *C. velifer.* However, according to the results of Sumitomo Ltd., the *C. carpio* susceptibility is two times higher than that of *B. macrops.*

Carbosulfan

29 In the control tanks, only one Mormyridae died more than 48 hr after the beginning of the experiment.

30 In addition to the mortalities recorded in the tanks treated, which made it possible to calculate the lethal doses (Table 1), the quite particular behavior of the Schilbeidae should be mentioned. In fact, 36 hr after treatment, while most of the fish in the control tank remained towards the bottom, the individuais that had received the larvicide but were sufficiently energetic tended to move to the surface. They showed signs of weakness towards the 72nd hr. The Schilbeidae then became very black, a characteristic of the survivors through the remainder of the experiment.

31 The 24-hr LC50 of the species *P. isidori* for carbosulfan is less than that for *S. mystus,* being about half of the latter.

32 Few data are available on carbosulfan acute toxicity. Rand *et al.* (1985) reported, in a review for bluegill, rainbow trout, and carp, 96-hr LC50 of 15, 42, and 55 µg/liter, respectively, while Bayoumi and Ibrahim (1988) observed a much higher toxicity on *Tilapia zillii,* 0.29 µg/liter, in contrast with the other data.

Pyraclofos

33 Throughout the experiment no mortality was recorded in the control tanks for the two species used *(P. isidori* and *C. nigrodigitatus).*

34 The behavior of *P. isidori* in the treated tanks did not change almost 10 hr after the introduction of the individuais into the larvicide solutions. Following the first change of solution, i.e., 12 hr after the start of the trial, the individuais showed agitation in all the tanks and mortalities were recorded for concentrations above 80 µg/liter. At the dose of 249 µg/liter, all the surviving individuais were moribund less than 24 hr after the beginning of the experiment and died within 48 hr.

35 For *C. nigrodigitatus,* there were mortalities within 24 hr in the tanks treated with pyraclofos at 80 µg/liter. However, the situation became stable in all the solutions 72 hr after the start of the experiment and there were no additional mortalities up to the end of the trial 24 hr later.

36 The above indicates a difference in susceptibility between the two species tested. The longer the exposure time, the more the least increase in dose results in considerable *P. isidori* mortalities, while *C. nigrodigitatus* becomes less affected by slight concentration increases. *P. isidori* therefore tolerates long-duration exposures less than *C. nigrodigitatus* even if the concentration of the pyraclofos solution is relatively low.

37 The only available data for pyraclofos are those of the producer (Takeda, 1988), who gave the median tolerance limits (TL50) for an exposure time of 48 hr as 0.080 ppm for *Salmo gairdneri* and 0.044 for *C. carpio.*

Simulation Experiments

38 Spraying simulations were carried out with Cyphenothrin on *B. macrops.* Solutions were prepared at the following doses : 0.1,0.05, 0.02, 0.01,0.001, and 0.0005 mg/liter. The

individuais were then exposed to these doses successively for increasing times of 30 sec, 1 min, 3 min, 6 min, 3 hr, and 10 hr from the highest to the lowest doses. They were then put under observation in nontreated water. The experiment was repeated every week for 5 weeks and the tanks were visited regularly to remove dead individuais. The specimens were fed at least once every day in the observation tanks but this was stopped 24 hr before the start of each experiment.

39 *B. macrops* was selected for this trial because of its relatively great susceptibility to cyphenothrin. Nevertheless, no mortality due directly to the insecticide was observed after 5 weeks of weekly treatment. The individuais manifested signs of agitation but it should be noted that even in the absence of any insecticide, *Barbus* are active by nature.

DISCUSSION

40 As the first goal of this testing was to give an indication of the biological activity of these insecticides (which gave positive results on the target *S. damnoswn*) in terms of acute toxicity on fish, a comparison will be made between 24-hr LC50 for fish and the operational dose (OD).

41 An operational dose is the concentration of insecticide that will kill *Simulium* larvae with a carry of 2.5 km at 10m^3/sec and 10-15 km at 100m^3/sec and it is usually expressed in mg/liter/10 min. Obviously the concentration at the spraying point in the river is 10 to 20 times higher than the operational dose but dilution quickly occurs since the Chemical insecticides are used at high water while during low water the biological insecticide *B. thuringiensis* is applied most of the time.

42 It should be recalled as a guide that the 24-hr LC50 for *P. isidori* is 30.9 mg/liter with temephos (OD = 0.100) and that the lowest leveis found in literature data on other species are around 1 mg/liter. With the exception of this larvicide, which has been considered providential for the Programme for more than 10 years, all the insecticides currently utilized showed a high toxicity in laboratory on the fishes.

43 The operational dose for permethrin, cyphenothrin, carbosulfan, and pyraclofos are 0.015, 0.015, 0.050, and 0.100 mg/liter/10 min, respectively.

44 For permethrin, compared to the dose of 0.015 mg/liter utilized by OCP, the LC50 calculated are relatively higher while they correspond to those for cyphenothrin. Besides, the difference between the operational dose and the 24-hour LC50 is greater for permethrin than for carbosulfan.

45 In laboratory, *P. isidori* is susceptible to the effects of carbosulfan. The susceptibility of *S. mystus* is less but the doses which caused mortalities are not too far from that utilized in the control campaign.

46 For pyraclofos, the 24-hr LC50 of the species *P. isidori* is 1.5 times the operational dose and slightly greater than that of C. *nigrodigitatus.*

47 In order to avoid mass fish mortalities and not considering any other factor, a first levei of a basal assessment is made comparing the 24-hr LC50 with the operational dose, bearing in mind that the exposure in river is much less (10 min versus 24 hr).

48 Therefore, a worst-case situation is considered for the evaluation and a risk index for the ranking of the products is calculated.

49 The following table compares the lowest 24-hr LC50 for fish with the operational dose :

	Permethrin	Cyphenothrin	Carbosulfan	Pyraclofos
Operational dose Mg/liter	15.0	15.0	50.0	100.0
24-hr LC50 fish Mg/liter	40.0	15.0	82.0	150.0
Risk index	0.37	1.0	0.60	0.67

50 The highest score for risk was obtained by cyphenothrin ; it was medium for carbosulfan and pyraclofos, while the lowest was for permethrin. It can therefore be recommended to give low priority for further research to cyphenothrin in other experiments to be performed for more precise hazard assessments.

CONCLUSIONS

51 The question whether African fish species are more tolerant to Chemical poisoning than fish from temperate areas has for a long time been a matter of debate among aquatic toxicologists, but experimental data were not available.

52 Now, as a preliminary response, one can say from the above results that the toxicity of the insecticides tested is not extremely different for African fish species and fish from temperate areas and it seems that African fish react more or less like warm water fish with the exception of cyphenothrin, for which only a single record exists and, therefore, the possibilities of comparison are limited.

53 From the few data available, it seems also that *P. isidori* and *B. macrops* are less tolerant than other species and this could be an indication for future toxicity testing.

54 As a second resuit of this series of tests, one can give priority for further testing and investigation to permethrine, carbosulfan, and pyraclofos according to the ratio between operational dose and toxicity (0.37, 0.60, and 0.67, respectively).

55 This second phase of the hazard assessment will include a literature search for metabolism, bioaccumulation potential, and persistence as well as experimental toxicity testing on nontarget invertebrate fauna in minigutter.

56 The third phase will consist of the evaluation of the role of physicochemical parameters in the dispersion, taking into account river characteristics such as suspended solid/ organic matters, flood, and carrying capacity, and in carrying out pilot-scale treatments on selected rivers. All these steps should be followed before an insecticide is allowed to be used in the Onchocerciasis Control Programme.

57 Reports on the overall ecotoxicological evaluation are in preparation for pyraclofos (Yameogo *et al.,* 1990b) and permethrin (Yameogo *et ai,* 1990a).

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Long-term assessment of insecticides treatments in West Africa: aquatic entomofauna

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1. Introduction

1 *Onchocerca volvulus* is a parasitic worm giving rise to skin reactions and eventually severe ocular lesions followed by blindness (Zimmerman et al., 1992). This human disease had been a major public health problem in many fertile valleys of West African countries in which it was an obstacle to their social and economic development. The parasite is transmitted by the female blackfly of the *Simulium damnosum* complex (Philippon, 1977), particularly the savannah vectors *S. sirbanum* and *S. damnosum* s.s., whose larval instars, requiring a minimum flow of about 50 cm s^{-1} for survival, breed in the bedrock areas of fast flowing water courses.

2 In December 1974 the United Nations Development Programme under the aegis of WHO launched a 20-yr campaign for the control of the vector (Davies et al., 1978) and, being difficult to repress the adult of *Simulium*, it was decided to treat the larval stages whose distributions are limited to rapids.

3 The initial phase of the Onchocerciasis Control Programme (OCP) covers a vast area of 764,000 km^2 in which up to 18,000 km of rivers had been partly weekly sprayed with insecticides selected according to the criteria reported in Lévêque (1989) and on account of their efficacy against the larvae of the vector and their low toxicity for the non-target fauna.

4 Obviously, such an extensive and prolonged use of insecticides could have important environmental risks, therefore an aquatic monitoring programme as well as lab and on field toxicity tests have been set up from 1974 to evaluate the possible short-and long-term effects of the insecticides on the non-target fauna.

5 From 1975 to 1985 temephos (Abate®), chlorophoxim (two organophosphorous compounds) and a biological insecticide, *Bacillus thuringiensis* var. *israelensis* [*B.t.* H-14] ("Teknar") had been the insecticides used. After these first 10 yr of treatment, the data collected on aquatic insect larvae and on fish populations lead to review papers demonstrating that "... the insecticides employed had little effect on the non-target fauna (Lévêque et al., 1988; Yamèogo et al., 1988). Although the first application of temephos and chlorphoxim had a fairly strong impact on invertebrate communities in the short term, it would seem that these situations disappear fairly quickly after a year or less of successive applications".

6 From 1980, the appearing of certain forest cytotypes of the vector resistant to temephos (Guillet et al., 1980) and to chlorphoxim by 1982 (Kurtak et al., 1982) forced the search of new compounds and the implementation of a new treatment strategy based on the rotational use of different insecticides. The new compounds were searched from groups unlikely to produce cross-resistance and having different modes of action: permethrin (pyrethroid), carbosulfan (carbamate), pyraclofos (organophosphorus compounds) and vectron (pseudopyrethroid). To prevent Aies reinvasion, from 1989 the original programme was expanded to 1,235,000 km^2 controlling about 50,000 km of rivers.

7 After the above-mentioned review papers that provided a comprehensive evaluation of the first 10-yr monitoring of non-target aquatic fauna, the biological data collected within a wide area till 1998 allow to face new questions arising from the implementation of the OCP previously outlined.

8 Besides the main question addressed to the long-term changes of the invertebrate and fish populations with respect to their taxonomic composition as well their trophic structures, a better outlining of it is now possible:

I. the severity of each specific insecticide used during the programme,
II. the resistance of the communities to the induced stresses, and
III. their recovery capacity.

9 Analysing the invertebrate data collected in four countries during a period ranging from 1977 to 1996, this paper addresses the above questions.

10 With respect to the long-term changes of the invertebrate and fish populations it has to be outlined that the observed patterns by the end of treatments represent an addition of the effect of all the treatments. If such additive effect can bias the evaluation of the biological effects of each specific treatment, however it can be considered a minor problem in our case where the main question focuses on the overall biological effect of the programme.

11 All the ecological activities, encompassing the application of biological monitoring protocols, insecticides risk assessments and extensive impact evaluation studies, were recently reviewed in a synthetic paper by Calamari et al. (1998).

2. Materials and methods

2.1. Biological data and sampling methods

12 The study addresses the analysis of the invertebrates collected in four rivers located in West Africa during the time extents and within the sampling stations indicated in Table 1.

The rivers are savannah type showing high discharges from July to November and a low-water period from January to June, details on hydrological and physicochemical characteristics of the main West African rivers are reported by litis and Lévêque (1982) and by Moniod et al. (1977).

13 The biological data were collected from 1977 to 1996 after the larvicides application as well as during suspension and pre-treatment periods; the insecticides applied are detailed in Table 2 with the code adopted in this paper.

14 It is difficult to describe or to graphically show the overall treatment strategy but, to fully understand the presented results, the following aspects have to be clarified. The different larvicides were regularly applied during all the treatment period with a rotational strategy to avoid the appearing of resistant forms and the compounds were selected and applied according to the river flow. The biological samples were collected soon after the insecticides application and during the suspension periods, that mainly took place during the low-water season. In the first case the samples, other than to describe the "post-treatment status" of the rivers, mainly represent the effect of the last applied compound; thus, the insecticides labels are used to group the samples for the analyses as well as in the graphs identify such "last" applied compounds.

Table 1. Location of the sampling stations and sampling periods

River	Station	Country	Maximum sampling period
Maraoué	Entomokro	Ivory coast	December 1977 to February 1996
Pru	Asubende	Ghana	January 1980 to April 1995
Niandan	Sassambaya	Guinea	December 1984 to April 1994
Kaba	Outamba	Sierra Leone	March 1989 to April 1994

Table 2. Treatments for which biological samples had been collected

Code	Treatment
no	Pre-treatment
su	Suspension
bt	*Bacillus thuringiensis* (B.t).
	H-14)
ab	Temephos (Abate®)
ph	Phoxim
pe	Permethrin

ch	Chlorophoxim
py	Pyraclofos

15 A relative elevate number of pre-treatment observations is available for Niandan (25) and Pru rivers (17), on the contrary only 1 and 8 pre-treatment observations are available, respectively, for Kaba and Maraoué rivers.

16 The organisms were sampled by means of Surber net and as day and night drifts. Details of monitoring and sampling methods can be found in Lévêque et al. (1979) and Yamèogo et al. (1991).

2.1.1. Drift samples

17 Drift nets, 2 m long, 20 x 20 cm of diameter and 300 µm mesh size, were used to collect the drifting organisms. Considering that the densities of the drifting organisms in natural conditions are low during the daytime and maximum 1-2 h after the sun set, both these two phases had been sampled. Three samples were taken l½ h before sunset (day drift) and six samples 1½ h after sunset (night drift), the sampling time was 10-30 min for daytime drift and 3 min for night drift.

18 As the number of drift organisms is related to the volume of water filtered the data were standardised expressing the number of individuais captured per m^3 of filtered water (drift index).

2.1.2. Surber samples

19 The benthic invertebrates were collected in shallow riffles over rock substrate using a modified 15 x 15 Surber sampler. The sampling stations were chosen because, being the breeding sites of *S. damnosum*, these areas had been subjected to direct application of the insecticides.

20 Five samples were normally taken for each site and the mean number of individuais as well as the 95% confidence limits were calculated (Elliott and Décamps, 1973).

21 The three sampling strategies, Surber samples, day and night drifts, had been employed in order to collect biological data showing different information. Night drift, which is supposed to be mainly voluntary, reflects an active period while the number of the day drift organisms is related to their health condition. Schematically, an increase in the number of day drift organisms can be related to external factors stressing the invertebrates, by contrast a high night drift could reflect an increased activity of the benthic organisms.

22 As regards the use of the Surber net, this technique allows to sample, in a quantitative way, the organisms living in specific river areas and for this reason the collected organisms reflect the structure of the benthic communities, where this term indícate an assemblage of interacting individuals sharing at the same time the same space. Obviously, this concept of community is less applicable to the drift samples which represent “collections” of organisms turning up from a wider area located upstream the sampling sites.

23 All the sampled individuais were classified according to their family levei and their trophic role: predators, shredders, scrapers and filtering or gathering collectors, this second classification method is based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories (Cummins, 1973). To avoid the presence of rare taxa only the principal systematic units belonging to the Ephemeroptera, Tricoptera and Chironomidae were used for the analysis.

2.2. Numerical analysis methods

24 The invertebrate data collected in each river have been analysed independently, distinguishing, for each river, the three techniques adopted: Surber net (named invertebrate communities), day and night drifts (named invertebrate assemblages).

25 The numerical analyses strategy was selected in order to assess the long-term invertebrate structure variations with respect to the pre-treatment periods as well as the biological variation occurring during the suspension periods. The attention given to these two situations – treatment and suspension – is justified by the fact that they allow the evaluation of two main attributes of the biological communities, namely, the resistance (the capabilities of contrasting stress factors) and the resilience (the recover capabilities after a stress).

26 Since the invertebrates collected were classified with respect to their taxonomic leveis as well as to their functional feeding group (trophic role), the analysis of the biological variation was addressed to both these structural and functional attributes. Whereas the first aspect concern, besides the faunistic interest over loss of global biodiversity, the quality of the biomass available for the upper trophic leveis, the second one is related to the stability of the energetic flows. On account of the invertebrate position in the first levels of the river food webs, changes in this latter aspect can be an alert signal about greater detrimental effects on the ecological characteristics of the whole river System.

27 Because the trophic structure is a property of the living organisms emerging at community levei, only the Surber samples were used for the analysis of this biological property.

28 On the basis of the results of preliminary data inspection (Crosa et al., 1998), the following analysis techniques were applied:

29 • *Invertebrate taxonomic diversity.* The non-parametric index utilised was the Shannon heterogeneity index: $H'=-\Sigma pi$ In(pi). The deviation of the Shannon index from the pre-treatment situation was statistically tested by means of the Mann-Whitney U-test.

30 • *Relative abundance of the functional groups.* The relative abundance of the invertebrates classified as functional groups was estimated for each treatment.

31 • *Rank abundance models.* This graph approach to the analysis of the biological structures consists in a conventional form of presenting the importance of each taxon as abundance (y-axis) with the different systematic units concerned arranged in rank-order along the x-axis from the commonest to the rarest. The pattern of the line connecting the taxa of each sample allows a visual inspection of the invertebrate structures: Sshaped curves are related to high heterogeneity values, on the contrary high slopes indícate more dominated taxonomic structures.

32 The comparison of the curves pattern facilitates the inspection of the changes that can take place in the invertebrate structures during the different sampling periods.

33 In the graphs, the species abundance are represented by means of the median values occurring during the pre-treatment, treatment and suspension periods.

34 • *Multivariate analysis.* Due to the linear response of the invertebrate abundance the Principal Components Analysis (PCA) was preferred to the unimodal multivariate analysis approaches (i.e., redundancy analysis). The PCA was applied to the log-transformed abundance of the taxa collected by means of Surber net.

35 To avoid the biological variation due to the different environmental conditions of the sampled rivers, outlined by a preliminary factorial analysis applied to an all rivers data matrix, the invertebrates collected in each river were analysed independently.

3. Results

3.1. Invertebrate structures analysis

36 The results of the analyses of the invertebrate community structures are shown according to the type of sampling method used to collect the organisms: Surber samples, day and night drifts.

3.2. Surber samples

37 The gathering and filtering collectors were the most abundant trophic groups analysed in the sampled comcommunities; the remaining three functional groups show a low contribution to the overall invertebrate abundance and for this reason these guilds are not illustrated in the graphs (Fig. 1). More in detail the pre-treatment samples show a dominance of the gathering collectors in Pru, Niandan and Kaba rivers and a co-dominance of the gathering and filtering collectors in the communities sampled in Maraoué river.

38 With reference to the pre-treatment data, changes in the relative abundance of these two collector guilds are noticeable during the insecticides application in all rivers but Pru. These changes, that take place mainly during chlorophoxim, pyraclofos, phoxim and permethrin treatments, are the resuit of the increase of the gathering collectors, related to a decrease of the filtering collectors. A different variation occurs during *B.t.* treatments in Niandan and Kaba rivers for which the gathering invertebrates decrease and the filtering group increases.

39 Regarding the communities sampled during temephos treatments, no appreciable changes occur in the guild structures with respect to the pre-treatment periods. A similar absence of changes is outlined for the invertebrate functional structures identified during the suspension periods; in these occasions the gathering and filtering collectors show percentages similar to the pretreatment periods.

40 For the analysis of the diversity of the taxonomic invertebrate communities collected by means of the Surber net during the pre-treatment, treatment and suspension periods, the mean values of the Shannon heterogeneity index are shown in Fig. 2(a).

41 The communities sampled in the Niandan river show the most noticeable reductions in the taxonomie diversity during the treatment periods with the greatest changes occurring after permethrin and phoxim treatments. During these periods the Shannon index shows the lowest mean values. The communities sampled during the suspension periods are characterised by lower heterogeneity values compared with the pre-treatment ones.

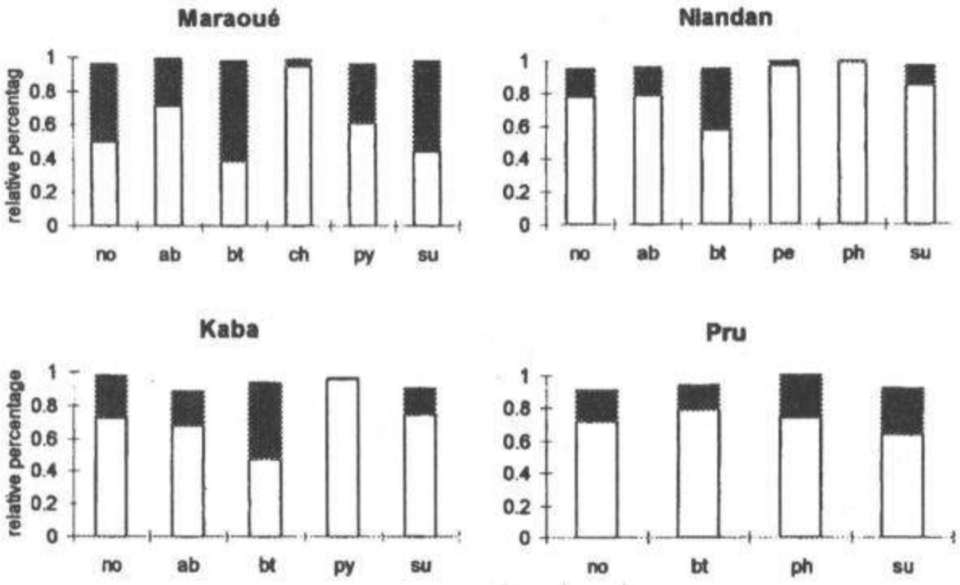

Fig. 1. *Surber samples.* Mean percentages of the functional groups sampled during the pre-treatment periods (no), at the end of the different treatment periods (x-axis) and during suspension periods (su); for the treatment code see Table 2. White bars gathering collectors, dark bars filtering collectors, the remaining three functional groups are not illustrated in the graphs because of their very low abundance.

42 Maraoué and Kaba rivers show appreciable reductions of the heterogeneity values only for the communities sampled during chlorophoxim and pyraclofos treatments.

43 Regarding the comparison of the Shannon index calculated for the biological data sampled during each treatment and those related to the pre-treatment situation, the Mann Whitney U-test reveals statistically lower values (95% confidence range) during temephos, *B. t.* and suspension periods for Niandan and during chlorophoxim for Maraoué.

44 Fig. 2(b) shows the comparison of the taxonomic structures related to each treatment period with respect to the pre-treatment ones by means of rank abundance models.

45 The greatest variations in the rank models occur in the Niandan and Kaba rivers for which the total abundance and heterogeneity of the sampled communities were generally reduced during the insecticides applications.

46 For these two rivers the ranking gradients with respect to the pre-treatment data can be outlined as folfollows: *B.t.*, temephos, pyraclofos, phoxim and permethrin; the models related to the suspension periods are located in an intermedíate position.

47 In Pru river, excluding the model related to the suspension period which shows the highest heterogeneity, no differences are appreciable for the remaining models.

48 In Maraoué, only the community structure sampled during chlorophoxim treatment shows a reduction both in heterogeneity and abundance.

3.3. Day drift

49 The diversity indices calculated for the day drift invertebrate assemblages show patterns similar to those detected for the Surber sampled communities: no changes appear in Pru river, on the contrary, changes are evident for the invertebrates sampled in the remaining three rivers (Fig. 3(a)).

50 With respect to the pre-treatment periods a clear decrease in the heterogeneity can be shown for the assemblages collected during pyraclofos (Kaba; *H'* approximately from 2 to 0.7), permethrin (Niandan; *H'* approximately from 1.6 to 0.8) and, in less degree, during temephos and chlorophoxim treatments. In all the sampled rivers the diversity indices calculated for the invertebrates collected during *B.t.* treatments show values similar to the pre-treatment ones. In the Niandan and Kaba rivers, for which the greatest variation of the heterogeneity indices was measured, the structures of the invertebrate assemblages collected during the suspension periods are characterised by low heterogeneity values. The comparison of the heterogeneity indices between pre-treatment and suspensions periods for the remaining two rivers points out no differences. With reference to the pre-treatment period, the Mann Whitney U-test reveals statistically lower values ($P < 0.05$) during temephos, *B.t.* and suspension periods for Niandan river and during temephos for Maraoué river.

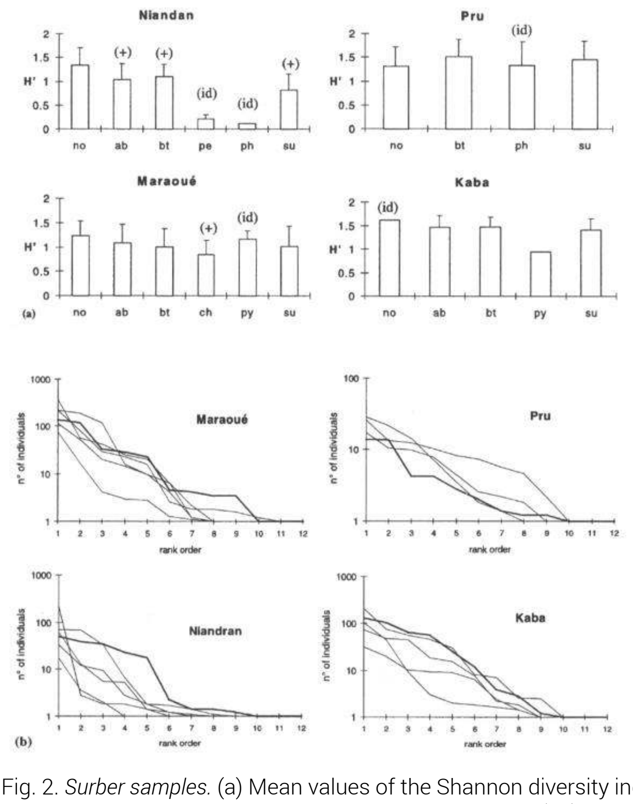

Fig. 2. *Surber samples.* (a) Mean values of the Shannon diversity index calculated for the invertebrate communities sampled during the pre-treatment periods (no), at the end of the different treatment periods and during suspension periods (su) (x-axis); for the treatment code see Table 2; (+) denotes a significant (95% confidence range) deviation from the pre-treatment situation according to the Mann Whitney U-test; id – insufficient data to apply the test; vertical bars desígnate 1 S.D. (b) Rank abundance models showing the departure of the invertebrate communities structures sampled at the end of the insecticides application from the pre-treatment condition (bold fines).

51 The rank abundance models related to the day drift assemblages sampled during the treatments periods in Kaba, Niandan and Pru rivers (Fig. 3(b)) show a small change in the taxonomic structures from pre-treatment periods mainly due to a general decrease of the taxa abundance; no differences in the structures are evident for the communities sampled in Maraoué river.

3.4. Night drift

52 As for the day drift, the mean values of the diversity indices calculated (Fig. 4(a)) for the night drift assemblages sampled in Pru river during the different treatments do not show appreciable variations for that the mean values remain close to 1.6. This value points out invertebrate assemblages characterised by a relatively high heterogeneity.

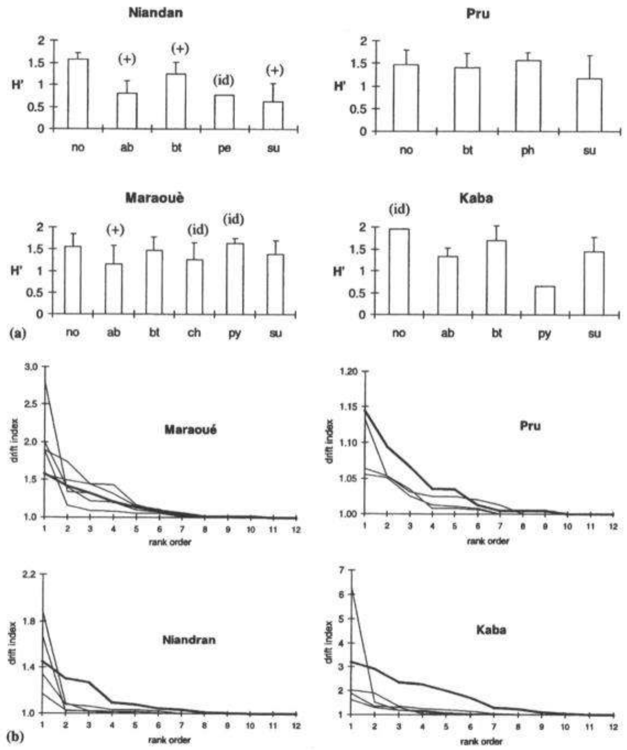

Fig. 3. *Day drift.* (a) Mean values of the Shannon diversity index calculated for the invertebrate communities sampled during the pretreatment periods (no), at the end of the different treatment periods and during suspension periods (su) (x-axis); for the treatment code see Table 3. (+) denotes a significant (95% confidence range) deviation from the pre-treatment situation aceording to the Mann Whitney U-test; id – insufficient data to apply the test; vertical bars designate 1 S.D. (b) Rank abundance models showing the departure of the invertebrate communities structures sampled at the end of the insecticides application from the pre-treatment condition (bold fines).

53 For the remaining rivers the lowest diversity values of the invertebrate assemblages occur in Niandan river during phoxim and permethrin applications.

54 According to the Mann Whitney U-test, significant difference from the pre-treatment heterogeneity, approximately $\Delta H'$= 0.4-0.5, is recognisable for the values related to *B. t.* and temephos treatments in Maraoué river.

55 Regarding the rank abundance models related to the night drift illustrated in Fig. 4(b), a general greater abundance of the systematic units can be outlined with respect to the drift invertebrate assemblages collected during the day. Only the invertebrate structures

sampled in Kaba and Niandan rivers present changes according to the different treatments with the wider heterogeneity reductions related to the first river.

56 Similar structure, in dominance, pattern and abundance values, is evident for the invertebrate assemblages sampled during the different treatment periods both in Maraoué and Pru rivers.

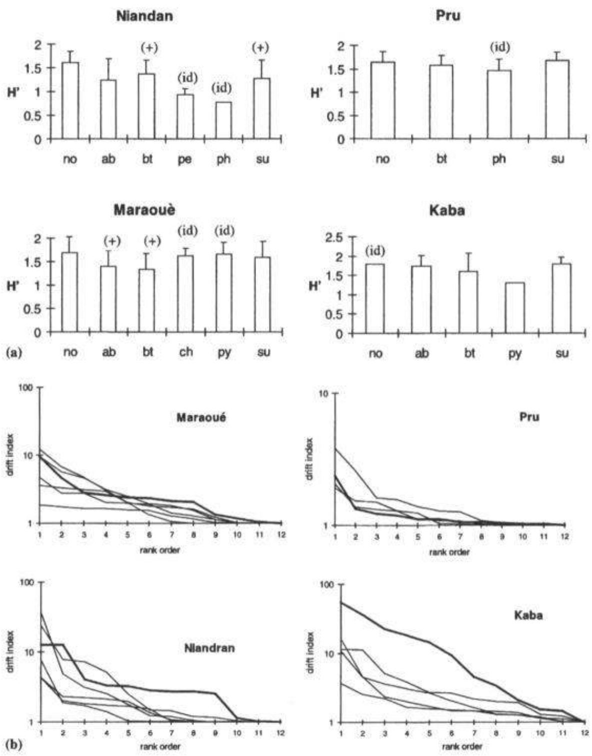

Fig. 4. *Night drift.* (a) Mean values of the Shannon diversity index calculated for the invertebrate sampled during the pre-treatment periods (no), at the end of the different treatment periods and during suspension periods (su) (x-axis); for the treatment code see Table 3. (+) denotes a significant (95% confidence range) deviation from the pre-treatment situation according to the Mann Whitney U-test; id – insufficient data to apply the test; vertical bars desígnate 1 S.D. (b) Rank abundance models showing the departure of the invertebrate communities structures sampled at the end of the insecticides application from the pre-treatment condition (bold lines).

3.5. Ordination

57 The results of the ordination analysis of the sampled invertebrate communities are illustrated by means of functional graphs which consist in representing the sample co-ordinates along with the sampling time (Figs 5-9).

58 For the description of the biological variation the first two components of the analysis have been used; in Table 3 the percentages of variance accounted for each axis are reported.

3.6. Niandan

59 The first PCA axis scores show a cyclic pattern with maximum values occurring approximately every 6 1/2 months. This pattern is quite evident during 1988-1995 and can be explained with reference to the periodic variation of the river discharges (Fig. 6).

The co-ordinate scores show the maximum values after the rain season then, as the dry season proceed, the values decrease and the following year the cycle repeats itself again.

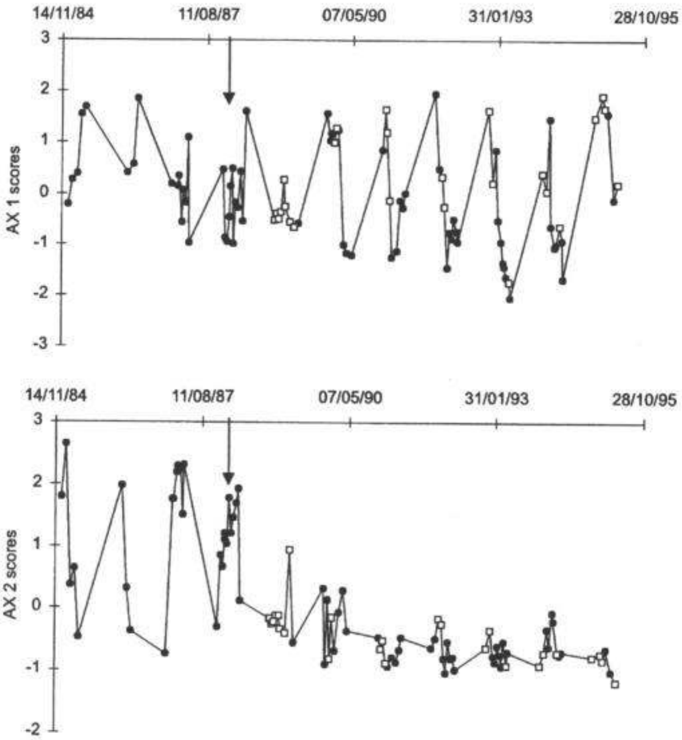

Fig. 5. *Niandan.* Functional graphical presentation of the first two axes PCA scores (after 45° axes rotation). Arrows mark the end of the pre-treatment observations; white squares show biological data collected during suspension periods.

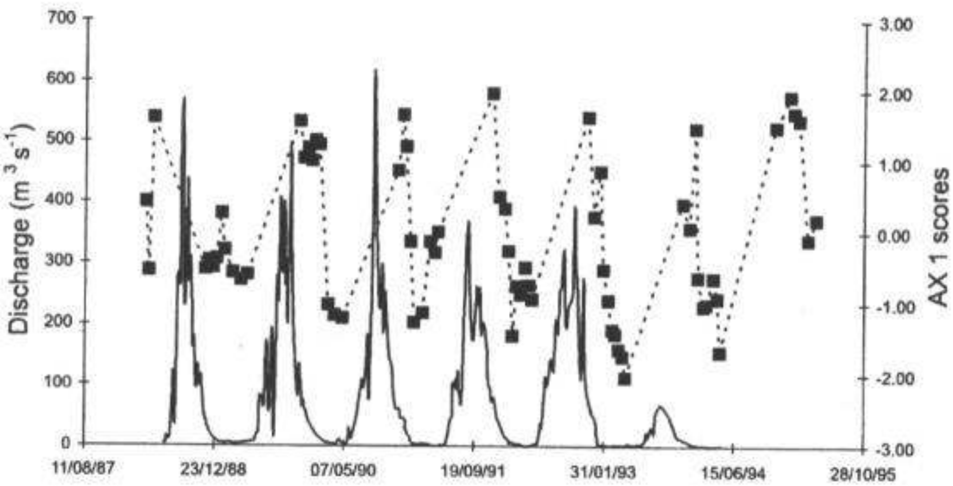

Fig. 6. *Niandan.* Functional graphical presentation of the first PCA axis scores after 45° axes rotation (dotted line) and river flows (lower line). Only the treatments period is shown.

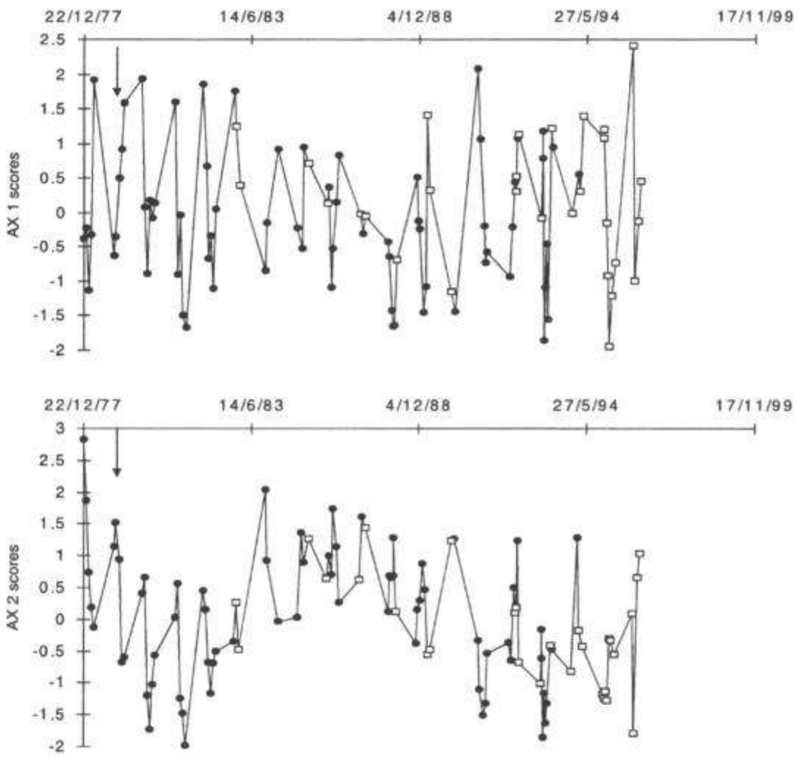

Fig. 7. *Maroué,* functional graphical presentation of the first two axes PCA scores. Arrows mark the end of the pre-treatment obobservations; white squares show biological data collected during suspension periods.

60 This regular variation of the invertebrate communities is not altered by the different treatments and the suspension period scores follow the cyclic pattern.

61 The second axis scores show a change of the taxonomic invertebrate structures occurring at the beginning of the treatments (Fig. 5). This difference is mainly due to the reduction of the relative abundance of the Tricorythidae, Leptoceridae and Chironomini. It has to be noted that these changes are mainly related to a decrease of the scores variation that is quite wide during the pre-treatment period. During the treatments no further patterns are evident and the sample scores of the suspension periods still show low values.

3.7. Maraoué

62 In Fig. 7, the first and second axes scores plotted along with the sampling time show no evident long-term trends or differences with respect to the pre-treatment scores. The invertebrate taxonomic structures show cyclic variation non-altered by the different insecticides applied during the investigated period.

63 The second axis scores, positively correlated to Tricorythidae and Baetidae and negatively to Orthocladiinae, describe a reduction in the abundance of the first two systematic units associated to the increase of the third one as the treatments take place. The decrease of the second axis scores occurring at the beginning of the treatments disappears after 5 yr.

3.8. Kaba

64 Although the unique pre-treatment observation does not allow a significant interpretation of the changes of the treated communities (Fig. 8), an increase of the first component scores does occur at the end the treatment period. The second component scores do not resuit structured along with the sampling time.

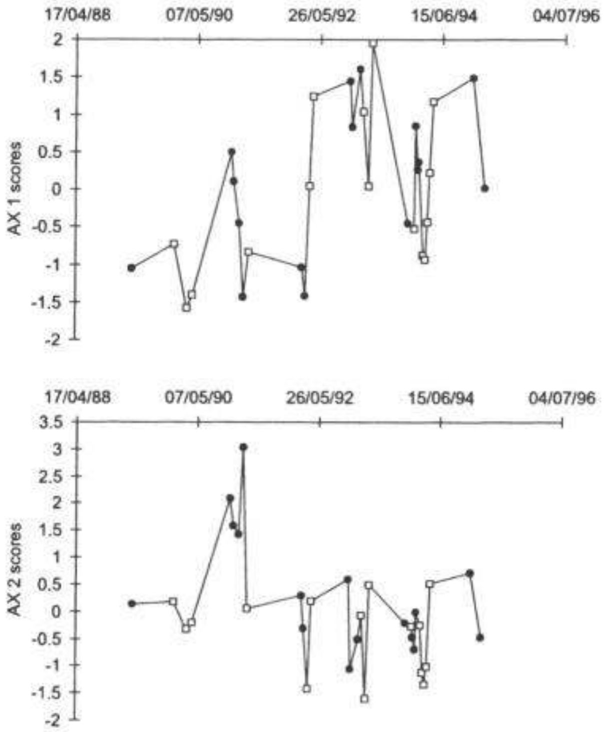

Fig. 8. *Kaba,* Functional graphical presentation of the first two axes PCA scores. In both graphs only the first point is a pre-treatment observation; white squares show biological data collected during suspension periods.

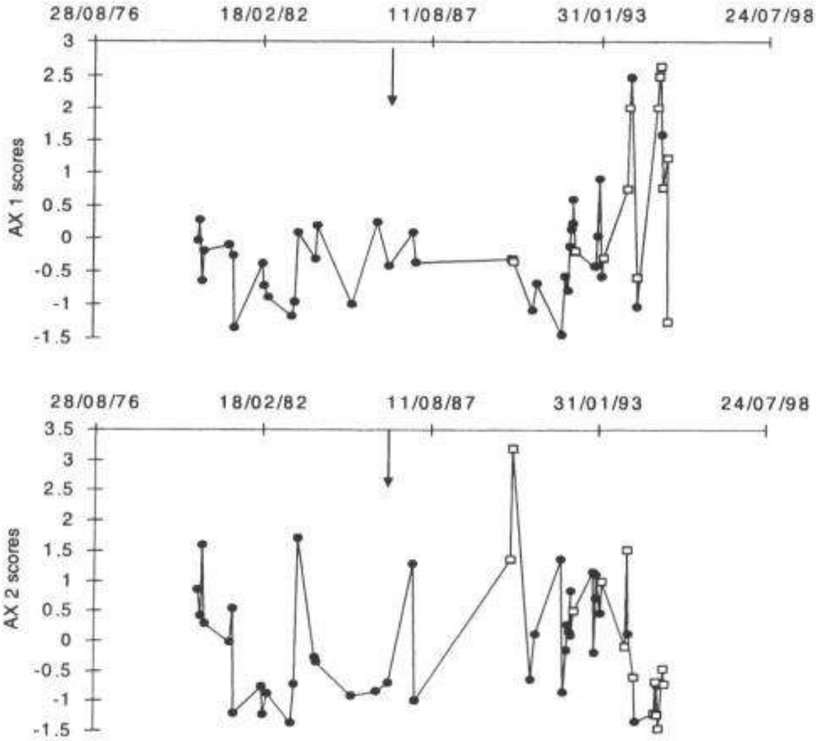

Fig. 9. *Pru.* Functional graphical presentation of the first two axes PCA scores. Arrows mark the end of the pre-treatment oberobservations; white squares show biological data collected during suspension periods.

Table 3. Percentages of variance accounted by each axis of the PCA

	Axis 1	Axis 2	Axis 3	Axis 4
Maraoué	38	18	11	9
Pru	40	15	12	10
Niandan	43	22	11	8
Kaba	52	16	11	7

3.9. Pru

65 The sample co-ordinates do not show changes as the treatments take place and no long-term variations resuit structured along with the sampling time (Fig. 9). With reference to the first component, it has to be noted that the wide variation of the suspension period scores at the end of the studied period.

4. Discussion

4.1. Comments on the invertebrate communities and assemblage structures

66 The results of the analyses allow the following general considerations:

67 • Although no absolute reference values exist for the Shannon index (in theory this index can increase to infinite but common values range between 1.5 and 3.5), the heterogeneity values and the rank-abundance models calculated during the pre-treatments periods are compatible with non-altered invertebrate communities.

68 • The most informative invertebrate collections were those related to the Surber samples. These allow a detailed examination of the community changes in terms of both taxonomic and functional structures.

69 With regard to the drift, the one collected during the night provides slightly better data for the analysis of the invertebrate structures than the day drift.

70 • Pru river shows the lowest changes of the invertebrate structures sampled during the different treatments, the greatest changes occur for the invertebrates collected in Niandan and Kaba rivers.

71 • Some of the insecticides show different efifects on the invertebrate structures depending on the river in which they are applied. For example, the diversity of the invertebrate assemblages collected in Niandan river was greatly reduced by phoxim, but no effect is recognisable during the same treatment in Pru river.

72 A different response also occurs in relation to the type of invertebrate collection analysed: during temephos treatment in Kaba river, a reduction of the heterogeneity indices is detectable only for the day drift assemblages but no or very little changes are shown for the night drift assemblages or for the invertebrate communities sampled by means of Surber net. These differences can be partially explained with the different selective capture of the taxonomic units by the different sampling techniques applied.

73 • Irrespective of the above-mentioned differences, the greatest reduction in the heterogeneity and abundance values of the invertebrate assemblages occur during phoxim, permethrin and pyraclofos treatments.

74 • During the suspension periods, the invertebrate communities and assemblages do not show structures similar to the ones typical of the pre-treatment periods for that the diversity values and the rank models present more or less evident differences. This suggests that, although the invertebrates show a recover trend, the time for this to be completed strongly depends on the treatments that take place before the suspension periods.

4.2. Comments on the PCA

75 The results of the PCA allow the following general considerations.

76 For all the investigated rivers, the component scores related to the suspension samples follow the cyclic pattern defined by all the scores that suggest a seasonal, flow-related variation of the invertebrate community structures. This is particularly evident for the

rivers showing cyclic variation of their samples'scores as Niandan (first axis in Figs. 5 and 6) or Maraoué (both axes in Fig. 7). In these situations the suspension samples are not altered by the treatments. Only for Niandan river the second axis scores clearly point out a relevant change in the community taxonomie structures occurring as the treatments took place and persisting during the suspension periods.

77 No changes are evident for Pru river, while for Kaba river the unique pre-treatment sample does not allow an evaluation of the taxonomie variations that take place during the treatment periods.

5. Conclusions

78 For the data analysis we faced different difficulties, among the sources of bias that can introduce additive variation to the biological data due to the natural environmental differences of the treated rivers and to the human factors, the following problems resuit more specific of the sampling protocol adopted:

79 • A natural factor that can bias the biological data collected can be identified in the different hydraulic conditions occurring during the treatments. Actually, the operational use of insecticides was set up according to the discharges of the river to be treated, for example, during low discharges (up to 1 m^3 s^{-1}) only *B.t.* was applied and above 450 m^3 s^{-1} only permethrin resulted appropriate. Because it is reasonable to assume that the drift and the benthic organisms react to the changing hydraulic conditions, this correspondent between discharges and treatment makes less comparable the biological data.

80 • For some rivers, the number of pre-treatment samples is not sufficient in defining the invertebrate taxonomic and functional structures as well as the natural biological variation occurring before the treatment campaigns. Obviously, this makes more difficult the judgement of the biological changes induced by the treatment and, as the underlying abiotic and biotic processes and functions of running waters are still largely unknown in subtropics rivers, this insufficiency in reference data becomes a more serious problem in the investigated areas.

81 • The rivers were differently treated with a rotational use of the insecticides. This cyclic sequence leads to difficulties in interpreting the biological data collected during specific periods because of the additional effects related to "what happend before". This is clearly the case of the suspension periods during which the invertebrate recovery tendency is related to the severity of the stresses taking place during the past treatments.

82 Since it is difficult to compare many graphs of taxa abundance against time, for the functional graphical presentation of the sample co-ordinates an appropriate technique has been revealed that it allows, for each sample, the inspection of the changes of the invertebrate structures with reference to the remaining ones in a single scatterplot, at the same time making it easy to relate each sample to the corresponding period.

83 • Biological data often show outlier values that can bias the statistical analyses being, in most cases, related to factors extemal than those in study. Usually a great number of replicates can smooth their effects but in the specific study for operational constrains the number of replicates is limited.

84 • The number of invertebrate collection replicates is not enough to show the distribution of the parent distributions sampled and this prevenís from applying parametric test for

the comparison of the samples. This is a common problem in the sampling programmes encompassing wide temporal or spatial extents in which the logistic difficulties constrain the field Works.

85 This forces the use of descriptive methods, like the rank abundance models, and quantitative ones, like the non-parametric Shannon diversity index and the PCA, to show and measure the biological variation without reference to the statistical descriptors of the data.

86 • The effects of each treatment on the biota are river specific, for example, the faunistic changes that occur during pyraclofos and *B.t.* treatments are greater in the Kaba river than in Maraoué river. The greater induced stress detected in Kaba river is partially explainable because of its pristine environmental condition compared to those of Maraoué for which a number of stressing factors are recognisable.

87 This example outlines the importance of considering the different anthropogenic pressures that have been taking place, with different time, in the treated rivers. Obviously, the induced stresses on the non-target fauna are less noticeable in those rivers in which the biological communities are altered because of extemal factors.

88 Finally, it has to be outlined that the analysis strategy employed allows itself to face the possible source of bias included in the biological data collected.

89 Considering that no unique standard analysis procedures are available for answering the question addressed in this paper or, more in general, in the longterm impact assessment studies, different numerical analyses are necessary to corroborate a comprehensive evaluation of the biological data.

90 Regarding the results of the analyses applied to the biological data collected during the 20-yr monitoring programme a first synthesis can be shown for the response leveis of the four sampled rivers. Niandan river presents the most relevant changes in the invertebrate taxonomie and functional structures. Pru river shows the lowest biological variation; Kaba and Maraoué rivers are located in an intermediate levei with the wider biological variation limited, respectively, during pyraclofos and chlorophoxim treatments. For these two rivers it has to be noted that the low amount of pretreatment observations does not allow a significant comparison of the biological variation that takes place during the treatment periods.

91 This synthesis is corroborated by the high similarity of the results obtained with all the analyses applied both to the taxonomie as well as to the functional classifications of the invertebrates.

92 The most informative data collections for the above conclusions can be identified in the Surber samples.

93 Regarding the leveis of the stressing factors induced on the aquatic fauna by the different treatments, the lowest have been revealed for temephos and *B.t.*, the highest for the remaining insecticides.

94 A tentative of classifying the applied insecticides on the basis of the stresses induced on the invertebrate communities can be drawn from the results of the taxonomic and functional structure analyses applied to the Surber samples, the gradient, ranking from low to high stress, results: temephos, *B.t.*, chlorophoxim, permethrin, pyraclofos and phoxim.

95 From a trophic point of view all the communities are dominated by the gathering collectors and, to a less extent, by the filtering collectors; the abundance of these feeding groups is a direct evidence of the availability of fine particulate organic matter (FPOM) that characterise the food resources within the studied rivers.

96 This dominated structure tends to increase with the application of all insecticides but the *B.t.* During this biological treatment the two guilds show the highest evenness.

97 The similar trophic structures shown by the communities sampled during the suspension periods with respect to the pre-treatment ones, as well as the changes occurring after the insecticide treatments, have to be positively considered, because they demonstrate the recovery potential of the guild structures analysed.

98 In conclusion the data analyses demonstrate community levei effects of the insecticides applied during the OCP on the invertebrate fauna as well as the maintaining of their flow-related cyclic variation. This second aspect is encouraging and, though we did not discuss population levei data of different species so that no concluding remark is possible with respect to biodiversity, it can be concluded that the taxonomic and functional biological structures examined are not greatly altered from the range of biological variation that would normally occur in these river Systems. In the natural situation these river invertebrate communities would rarely be in equilibrium (constant in taxonomic and trophic composition) because of the natural driving forces, like drought and spate events, which would occur with great frequency and regularity. When these factors, the high priority of the human health protection objective of the programme and the criteria indicated within the mandate of the ecological group: “temporary and seasonal variation in invertebrate populations other then *Simulium* could be accepted” are taken into consideration, the biological variations previously discussed can be considered ecologically acceptable. Finally, it has to be considered that the programme succeeds in protecting 30 million people from onchocercal disease and it is estimated that none of the 9 million children that has been born within the OCP area since operation began has ever run the risk of contracting onchocercal blindness (Samba, 1994).

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ABSTRACTS

For the control of the *Onchocerca volvulus* vector in West Africa, up to 18,000 km of rivers from 1975 and up to 50,000 km from 1989 had been partly sprayed weekly with insecticides as part of the Onchocerciasis Control Programme (OCP). To evaluate the possible short-term and long-term effects of the application of insecticides on the nontarget fauna, an aquatic monitoring programme was set up during the initial phase of the programme. By analysing the in vertebrate data, which were collected using various sampling strategies from four different countries between 1977 and 1996, this paper evaluates the long-term changes of the invertebrate populations with respect to their taxonomic composition as well as their trophic structures. The discussed results of the applied numerical analysis strategy suggest that neither the taxonomic nor the trophic structures are greatly altered from the range of biological, flow-related variation that normally occurs in the studied river Systems. This allows us to conclude that the biological variation found here is ecologically acceptable.

INDEX

Keywords: Insecticides assessment, River blindness, Blackfly, Aquatic insect larvae, West Africa

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Chapter 9. The Fish Monitoring Programme of the Onchocerciasis Control Programme in West Africa: a Model for Fish and Fisheries Preservation in the Face of Development

E.K. Abban, L. Yaméogo, D. Paugy, K. Traoré, M.E. Diop and E.M. Samba

9.1 Introduction

1 Human onchocerciasis, or river blindness, is a filarial disease caused by *Onchocerca volvulus* which, in West Africa, is transmitted by the adult female of the blackfly, *Simulium damnosum* s.l. (Zimmerman *et al,* 1992). It was common mainly along the fertile valleys of the watercourses and was not only a major public health problem but also an obstacle to the socioeconomic development of the infested areas.

2 In 1974, the concern of the governments of seven of the most affected countries in West Africa (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Niger, Mali and Togo), supported by the World Health Organization (WHO), the United Nations Development Programme (UNDP), the Food and Agriculture Organization of the United Nations (FAO), the World Bank and many donor countries and institutions, culminated in the launching of the Onchocerciasis Control Programme (OCP) (WHO, 1985). Following requests for membership made by four other countries in the sub-region (Guinea, Guinea-Bissau, Senegal and Sierra Leone), the Onchocerciasis Control Programme in West Africa has since 1984 involved 11 countries and has now been effectively controlling the reinvasion of the original area by exogenous blackflies.

3 The principal objective of the OCP has always been to reduce the incidence of the disease to a level at which it will no longer be a major public health problem or an obstacle to socioeconomic development. Because of the absence of an effective drug for mass treatment of infected humans, it was decided to apply larvicides on the fast-flowing sections of the watercourses where the aquatic larval stages of the vector develop. The very short larval life of the blackfly led to the choosing of weekly larvicide applications to eliminate transmission of the filarial worm by the blackflies.

4 It was also emphasized that this larviciding would need to last for 20 years (WHO, 1969; Davies *et al.*, 1978) to permit Virtual elimination of *O. volvulus* from the human population. With such an expanse of aquatic environment to be exposed to regular larviciding, the concerns of the participating countries and the donors were as numerous as they were diverse. Therefore, the sponsoring agencies instituted several checks at different stages of its operations. Principally, these included the criteria for selecting insecticides for operational use (Lévêque *et al.,*. 1979;Yaméogo *et al,* 1991a), the process of identifying which of the approved larviçides would be used in a particular week's treatment (Guillet, 1991; Hougard *et al.,* 1993) and the long-term monitoring of aquatic fauna (Dejoux, 1980; Lévêque *et al,* 1979, 1988; Paugy, 1983; Yaméogo *et al,* 1988; Yaméogo *et al,* 1991b; Hugueny, 1992; Yaméogo, 1994).

5 This chapter presents an overview of the current status of the fish monitoring component, its objectives, rationale, methodologies and results in order to show the role that different factors could play in the modification of the structure and composition of the fish populations.

9.2 The fish monitoring programme and practice

9.2.1 Basics

6 The concepts and issues considered in evolving the fish monitoring programme and protocol prior to the commencement of the OCP larviciding have remained valid till now. Lévêque *et al* (1979) outlined the issues and established the programme and protocol within two years of practice. However, the programme has been dynamic and refinements and standardization have continued as a consequence of accumulated data and a better understanding of the ecosystems involved. For example, Paugy (1983) made a comprehensive review of aspects of the monitoring practice and interpretation of data. However, the primary objectives of the monitoring have remained valid after 20 years of OCP operations. These briefly are:

1. To detect any indications of any long-term effect of larviciding on fish populations and communities, and thus provide warning to larvicidal activities of the OCP, should any adverse effect be noted
2. To assure all concerned that care was being taken not to unduly interfere with fisheries of the rivers as they form a major economic activity base for several communities in the OCP area

7 The rationale behind the objectives, as indicated by Lévêque *et al* (1979). and Lévêque (1989), are that long-term exposure of fish populations to even sub-lethal concentrations of insecticides could physiologically influence the life cycles of populations or affect eggs and juveniles. In either or both cases, fish abundance would be expected to decrease in

the long term. This could be for the whole fish community or particular species. Secondly, it could be possible that larviciding may affect the food chain of fish, leading to reduction. Such a situation could, in the long term, be observable by a change in the 'condition' of species affected (Lévêque, 1989; Yaméogo *et al.,* 1993a). It is for the above reasons that, with particular reference to fish, one of the criteria for the selection of a larvicide for operational use has been that the larvicide, at the operational dosage, should heve no direct or indirect impact on the life cycle or activities of fish (Lévêque *et al.,* 1979; Lévêque, 1989; Hougard *et al.,* 1993).

9.2.2 Manpower

8 For reliable data collection over the OCP area, appropriate human resources had to be available. Initially, the fish monitoring exercise was undertaken by two institutions: an ORSTOM hydrobiological laboratory in Bouaké, Côte d'Ivoire, and the Institute of Aquatic Biology, in Ghana. Over the years, the two institutions, with great assistance from the OCP, have provided training grounds for personnel from all programme countries to constitute national monitoring teams. The national teams continue to expand their knowledge through the OCP by mutual interactions and exposure to international experts. A group of international experts, constituting an independent 'Ecological Group' meets every year to evaluate the results of the national hydrobiology teams and advises the OCP on monitoring procedures and on safe insecticide screening and use (Cummins, 1985).

9.2.3 Selection of stations

9 The locations of the sites which have been used as regular monitoring stations are shown in Fig. 9.1. The major criteria for their selection were: accessibility all year round, suitability for the deployment of sampling gear and availability of hydrological data (Lévêque *et al,* 1979; Lévêque, 1989). While theoretical suitability of a site was based on map location, practical knowledge of stations was considered for their selection.

9.2.4 Sampling methods

10 Details of the methods used for OCP fish monitoring have been described by previous authors (WHO, 1976, Paugy, 1983).

11 A basic fish sample for OCP fish monitoring consists of fish caught in a standard battery of gill nets. Initially the battery consisted of five mesh sizes, 15, 20, 25, 30 and 40 mm). During the late 1980s, these were augmented to include three more mesh sizes (12.5, 17.5 and 22.5 mm). Each net has a surface area of 50 m^2 (2 m deep and 25 m long).

12 Usually a sample is the catch made by deploying two batteries of nets for two consecutive nights. For comparison and standardization, results are expressed as number of fish caught per 100 m^2 of gill net per night or catch per unit effort (CPUE). Standard data sheets are available to all teams to record CPUEs per mesh size of net, per species and a cumulative CPUE per sample at a station.

13 After individual identification, the length and weight of fish are measured as well as sexing and determination of gonad stage. Other biological parameters of individual

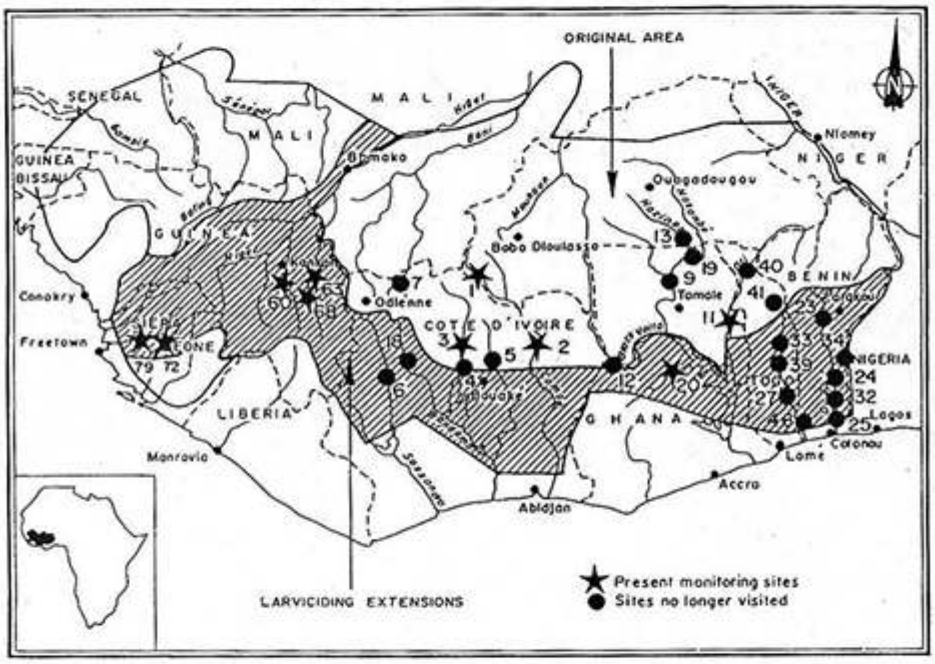

Fig. 9.1 The Onchocerciasis Control Programme in West Africa as accepted by the Joint Programme Committee (JPC) in 1984. Location of the fish monitoring sites as at 31 December 1993. (Key to monitoring stations: 1 = Léraba-bridge/Léraba; 2 = Gansé/comoé; 3 = Niaka/White Bandama; 11 = Sabari/Oti; 20 = Asubende/Pru; 60 = Boussoulé/Milo; 63 = Mandiana/Sankarani; 68 = Baranama/Dion; 72 = Matotoka/Pampana; 79 = Magburaka/Seli.)

14 species such as the coefficient of 'condition' factor of species, gonadosomatic index of fish, length at first maturity and fecundity are also estimated from the sample. The estimation of the 'condition' of species is a basic requirement from all monitoring teams. This is because the 'condition' (K) is a mathematical expression of the wellbeing of a fish. The factor is thus used to assess the ecological suitability of an environment to fish. With reference to OCP larviciding therefore, it is paramount to follow the 'condition' of species as a summary assessment of the influence of environmental conditions on fish. Data obtained in the field are recorded on standard designed forms and sent to the OCP headquarters in Ouagadougou, Burkina Faso, where they are fed into a computer for later analysis.

15 In addition to data collected by the regular monitoring, it was realized at the beginning of the programme that scientific information on fish and their biology in the OCP area was generally limited. Therefore specific special studies were and are still made to provide background information to and aid the interpretation of the monitoring results. These have included studies on the short-term effects of various larvicides on fish, feeding habits, fecundity and biology of specific species (e.g. Paugy, 1978, 1980; de Merona, 1981; Abban and Samman, 1982; Albaret, 1982; Antwi, 1983; Antwi, 1987; Yaméogo *et al.,* 1991c, 1993a, 1993b).

9.2.5 Sampling frequency

16 The concept at the beginning of the programme was that the sampling had to be sufficiently frequent to make it likely to differentiate between natural and artificially induced changes in the fish populations. Thus, basically, a monthly sequence was established for all stations (Lévêque *et al.,* 1979).

17 Since 1985, sampling frequency for basic OCP monitoring data at most of the operational stations has been reduced to once every two months. The number of sampling sites has also been reduced, justified by accumulated data on stations, comparability of geographically close stations, expansion of programme area and resource availability.

9.2.6 Hydrological regime

18 In the whole OCP area, hydrology of most of the rivers is mainly characterized by a flood period from July-August to October-November with a peak in September, and a low-water period from January to June. The flood period is correlated mainly with high turbidity and conductivity values, with a decrease in pH values.

19 The discharges of the savanna rivers in the original programme area indicated low values from 1975 to 1978 (Niaka on White Bandama) and from 1982 to 1984–85 (Niaka on White Bandama and Oti at Sabari), periods which corresponded to the drought (Fig. 9.2). In tropical conditions, water volume is a limiting factor of production compared with temperate zones where temperature is the limiting factor. Welcomme (1985) showed that fish reproduction tends to be highly seasonal and correlated primarily with flood. It is therefore assumed that poor flood years will be followed by poor recruitment and then poor catches by gill nets.

9.3 Results and discussion

20 The data which will be analysed here are those recorded by the national hydrobiology teams which work under contracts signed with the OCP programme.

9.3.1 Fish species richness

21 At most of the monitoring sites, the number of fish species caught shows seasonal fluctuations with a maximum at low-water periods. These periods correspond to the maximum efficiency of the gill nets. All rivers show no evidence of a reduction in species richness (Fig. 9.3) after almost 20 years of larviciding in the original area of the programme and some seven years of larviciding in Guinea. Lévêque *et al.* (1988) reached the same conclusions after ten years of larviciding with Abate®, Chlorphoxim and *Bacillus thuringiensis* H-14. The same authors suggested that the total number of species could mask changes in species composition. However, results from all rivers did not show disappearance of species after 20 years of larviciding.

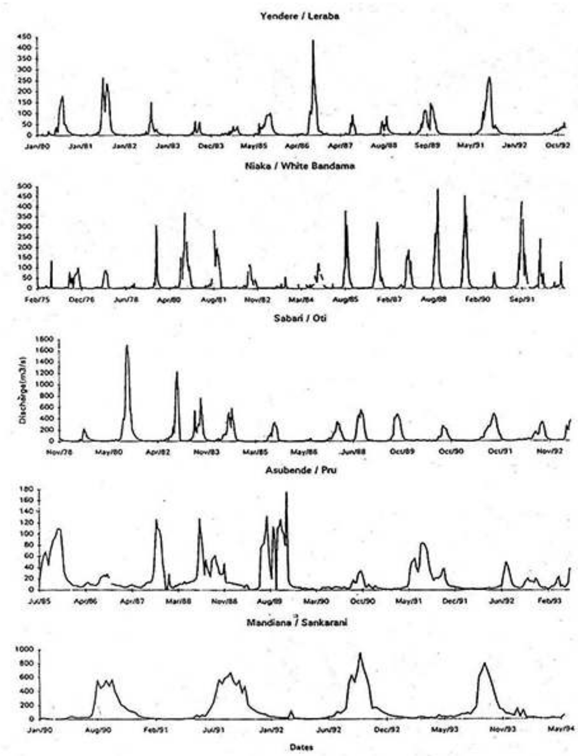

Fig. 9.2 Trend in discharge for some of the monitoring sites studied in this chapter.

9.3.2 Species composition

22 The data collected since the beginning of the monitoring make it possible to affirm that not all the stations are located in the same ecological zones or watercourses. The species composition and representative species therefore differ from one site to another (Fig. 9.4) but, on the stations covered by this report, *Petrocephalus bovei,*

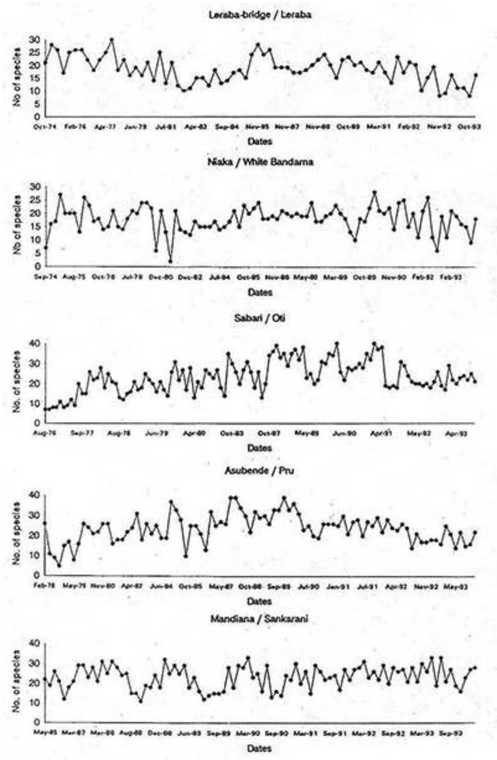

Fig. 9.3 Trend in the number of fish species per sample at different sampling sites during the monitoring period.

23 *Brycinus macrolepidotus, B. nurse, Schilbe intermedius* and *Synodontis schall* are the species which are encountered in most of the catches. *Brycinus leuciscus* and *Chrysichthys auratus*

are virtually absent from Niaka on the White Bandama and Lérababridge on the Léraba while they are represented at the other stations.

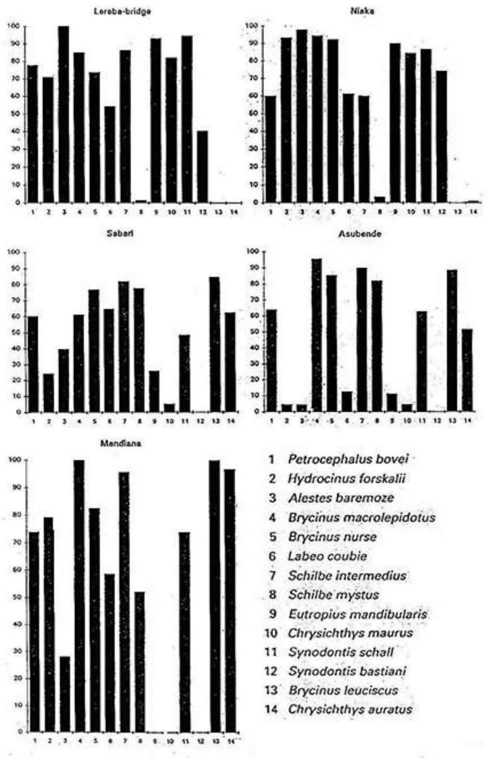

Fig. 9.4 Frequency of experimental catches of the principal species caught in gill nets at different monitoring stations.

9.3.3 Fish abundance in experimental catches

24 In the short term, the catches per unit effort (CPUEs) of fishing could be affected by the use of larvicides, the duration of the effect being dependent on the discharge and the nature and/or dosage of the larvicide (Abban and Samman, 1982; Yamégo *et al.,* 1993). It has been observed, just as for the richness, that the maximum CPUEs are recorded at the end of the high-water period (November to January).

25 Considering the experimental catches made at different monitoring stations and expressed as the mean CPUE for the standardized set of gill nets (Fig. 9.5), two phenomena are noted:

1. A seasonal pattern with higher catches at the flood subsidence and lower catches during the high-water period
2. In the long term, the catches indicate some fluctuations but the mean remains quite stable in most of the cases (Oti at Sabari, Pru at Asubende and Sankarani at Mandiana), a slight decrease is observed in the Léraba at Léraba-bridge and on the White Bandama at Niaka

26 The fluctuations in the abundance of fish in the rivers are related mainly to the hydrologic regime (Lévêque and Herbinet, 1980; Abban *et al.,* 1982; Lévêque *et al,* 1988). In recent years, the great pressure exerted by fishermen, who are increasingly using fishing gears that are not very selective, has contributed to the impoverishment of the watercourses. In fact, at Léraba-bridge, where the number of inhabitants increased from

zero in 1980 to 400 in 1993, the catches are less good (CPUE = 1482 g) than at 12 km downstream (CPUE = 7414 g) where there is still no village.

27 As regards catch composition, the data collected for almost 20 years do not indicate any disappearance of species. On the other hand, a dynamics of the fish, which is difficult to interpret, has been noted. The case of the species *Schilbe intermedius*, in the upper basin of the Léraba, is an illustration: after the start of the anti-blackfly control operations in 1975, this species decreased and, in 1978, disappeared from the catches (Fig. 9.6). This was thought to be the result of the temephos sprayings. However, while the programme activities had remained unchanged, in 1979 this species reappeared in abundance. A similar observation was made on the same species in the River Pru, Ghana, prior to the commencement of its treatment. *Alestes baremoze* presented a similar problem on the Bandama (Paugy, 1978). In these cases, it was observed that the population returned to their initial level when the hydrological regime became 'normal' after a period of disruption due to drought.

9.3.4 Coefficient of condition

28 Estimates generally show relative stability in the 'condition' (corpulence) of the principal fish species (Fig. 9.7). However, the values for individual species fluctuate around means which do not seem to have been appreciably altered over the years. The above suggest the availability of sufficient food and no alteration in the feeding habits of fish, both of which could be affected by the presence of 'excessive' amounts of insecticides (Abban and Samman, 1982). The fluctuations observed seem to be attributable to the influence of the reproductive cycle of the fish as well as the ecological effect of flood regimes (Paugy, 1978 and 1980; Lévêque and Herbinet, 1980; Lévêque *et al.,* 1988; Abban *et al.,* 1993). There is

thus no evidence of a long-term modification in the 'wellbeing' of fish attributable to OCP larviciding.

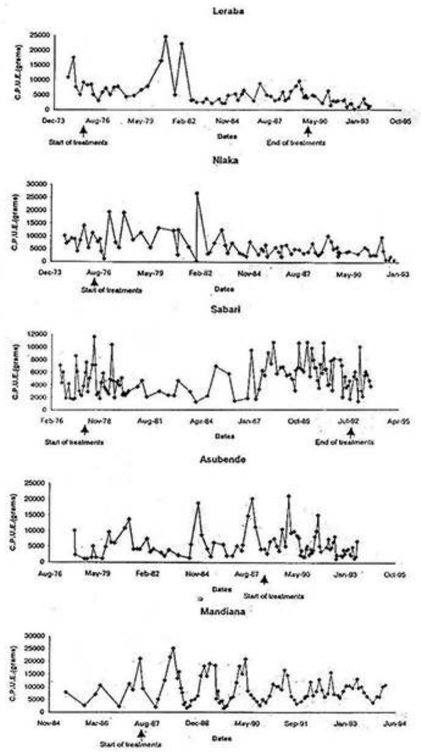

Fig. 9.5 Trend in mean catches per 100m^2 of gill nets per night (CPUE in grams) at different monitoring sites.

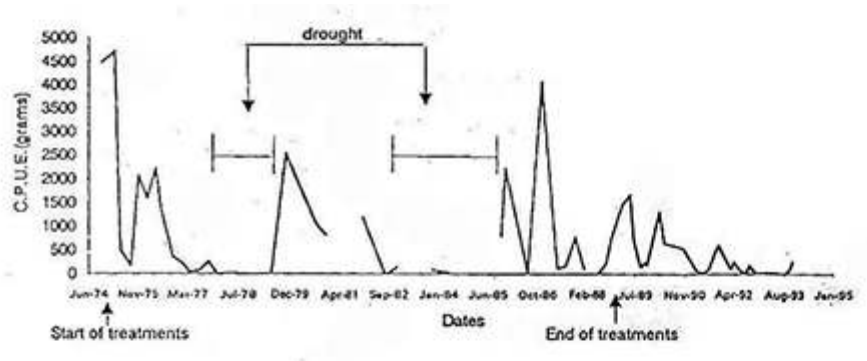

Fig. 9.6 Trend in catches per unit effort of *Schilbe intermedius* at Léraba-bridge/Léraba.

9.3.5 Special and one-off studies

29 Studies on a one-off basis are conducted on the biology of fish (especially feeding habits and brain cholinesterase activity of fish species) to ensure that there is no evidence of a strong impact of larvicides on these parameters.

30 As regards the food of fish, analysis of the stomach contents of different species from treated and untreated rivers did not provide evidence of an influence of larviciding on fish food (Vidy, 1976). For insectivorous species, this situation is attributed to the recolonization by entomic fauna of the treated sections of the rivers from untreated segments and tributaries (Yaméogo *et al.*, 1993a). Also, the capability of most of the fish

species to adjust their feeding habits to new conditions was considered an important factor.

31 Toxicity of organophosphorus compounds is due mainly to their effect on the brain cholinesterase activity (Lévêque, 1989). Antwi (1985) found no inhibition effect in the brain AChE activity of fish (*Brycinus nurse, Schilbe mystus* and *Tilapia* spp.) in rivers treated for many years. However, caged *Tilapia zillii* showed a 20% reduction in the brain AChE activity five hours after Chlorphoxim treatment.

32 These studies indicate therefore that there is no evidence of a long-term impact of larviciding on the biology of fish in the watercourses treated by the programme.

9.4 Conclusion

33 The biological monitoring programme developed and instituted by the OCP gives an overview of fish populations in the rivers affected by the larviciding activities. After almost 20 years of monitoring, the results show that apart from the larviciding, other different factors (hydrological regime, human activities) have to be taken into consideration in order to understand the situation.

34 In fact, there has been no evidence of fish mortalities due to the use of blackfly larvicides at operational doses but fluctuations are observed in the abundance and richness of fish attributable to hydrological conditions and to the pressure exerted by- local fishermen. As regards the condition of principal fish species, slight variations around a raean which remained quite stable have been observed, indicating the availability of food for the fish despite the use of different larvicides to control blackfly larvae. It is concluded therefore that the impact of human activities and that of drought seem to have more adverse effects on fish populations than that of the OCP's larviciding.

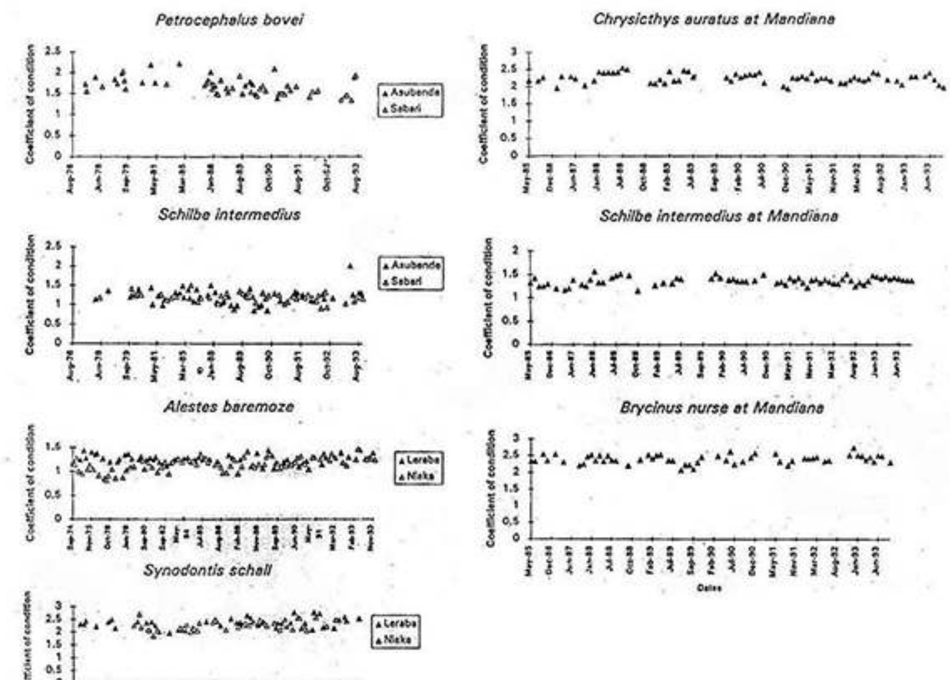

Fig. 9.7 Changes in the condition of the principal fish species at different monitoring stations.

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Effects of Aerial Spraying of Chlorphoxim on the Brain Acetylcholinesterase Activity of Fish from Three Rivers in the Ivory Coast, West Africa

L. A. K. Antwi

INTRODUCTION

1 Since 1974, rivers in the Volta River Basin of West Africa have been treated with temephos (*O, O, O′, O′*-tetramethyl-*O, O′*-thiodiphenylene phosphorothioate) to kill larvae of the *Simulium damnosum* complex, the vectors of river blindness (onchocerciasis), in connection with the ongoing Onchocerciasis Control Programme (OCP). However, from May 1980, it was detected that two forest species of the *S. damnosum* complex (*S. sanctipauli* and *S. soubrense*) had developed resistance to temephos on the lower Bandama river in the Ivory Coast (OCP, 1981). Later in the year, temephos resistance was observed on the rivers Marahoué and N'zi. These unexpected developments necessitated replacing temephos treatment of the three rivers with a series of weekly chlorphoxim (*O, O*-diethyl-2-chloro-α-cyanobenzylidene amino-oxyphosphonothioate) treatments, which began in October 1980.

2 Toxicity of chlorphoxim to African fish species has not been studied extensively, but Galleta (1968) had reported the 24 h LC_{50} of chlorphoxim to *Gambusia affinis* to be 2 mg $litre^{-1}$ while the 24h laboratory tests with 0.10 mg $litre^{-1}$ and 1.0 mg $litre^{-1}$ chlorphoxim on black bullhead gave mortalities of 10% and 80%, respectively (Anon., 1967). These studies did indicate that chlorphoxim might be more toxic to fish than temephos. Again, at the beginning of the OCP operations in 1974, some concern was expressed over the possible harmful effect such large-scale application of insecticide would have on the riverine fishery (Asibey, 1975, 1977). There was therefore a need to monitor closely the effect of

the chlorphoxim treatment on the fish species in the treated rivers. This paper reports one such study on fish from the rivers Marahoué, N'zi and Bandama in the Ivory Coast, which have all been treated with chlorphoxim.

MATERIALS AND METHODS

Description of the study area

3 The Marahoué and N'zi rivers are tributaries of the river Bandama and all flow from north to south. Details of the hydrology and the physicochemical characteristics of the rivers have been given by Iltis & Lévêque (1982). At Danangoro on the river Marahoué, where the cage experiment was carried out (see below), the breeding site of the blackfly *Simulium damnosum* s.l. is an expanded section of the river containing huge rocks (Fig. 1). At the time of the river treatment, the water level was high and the river flow fast, forming small rapids on the rocks.

Fish collection

4 Three commercial fish species, *Tilapia galilaea, Tilapia zilli* and *Alestes nurse,* were randomly caught daily for one week with a cast net from the treated rivers—the Marahoué at Danangoro, the N'zi at Dabakala and the Bandama at Marabadiasa—and from the untreated river Kadioni, which was used as the control.

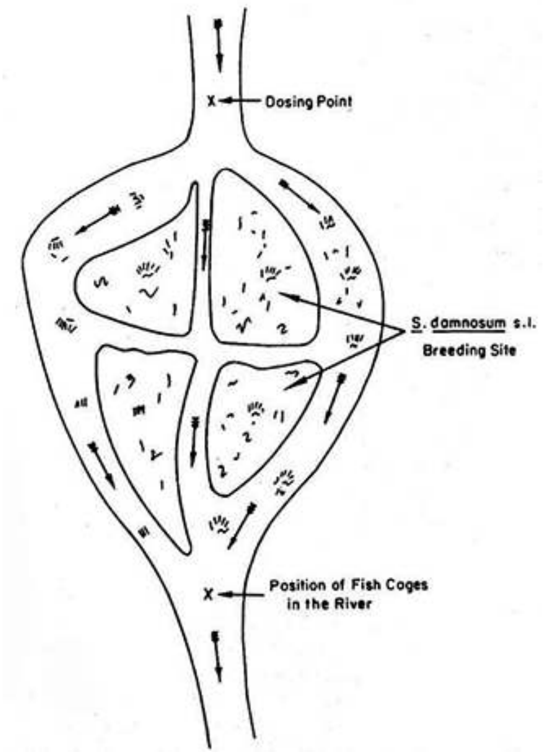

Fig. 1. Expanded section of river Marahoué at Danangoro.

Cage experiment with *Tilapia zilli* in the river Marahoué

5 To study the immediate impact of aerial applications of chlorphoxim to rivers on the fish brain acetylcholinesterase activity, a cage experiment was carried out using *T. zilli* in the river Marahoué during one of its weekly aerial sprayings with chlorphoxim.

6 On the day of the river treatment, *T. zilli* were obtained with a cast net from an untreated barrage at Loka near Bouaké. The fish were transported live in plastic bags filled with aerated water to the riverside, where they were divided into groups according to size and distributed among two 52 x 52 x 52cm cages, each holding about 10 fish ranging from 8 to 12 cm in length. A set of 10 fish was taken for the control measurement. The cages were next placed in the river at a point below the breeding/treatment site (Fig. 1) 2 h before treatment. At about 1200 h the larvicide was applied by the OCP team as part of their routine treatment of the river from a helicopter at a point about 200 m upstream of the *S. damnosum* s.l. breeding site. According to an OCP estimate, the concentration of the larvicide at the breeding site of the blackfly was 0 05 mg litre^{-1} for 10 min of river discharge (Davies *et al.,* 1978).

7 The first cage was removed from the river 2 h after spraying and the second cage was removed after 5 h. The fish in each cage were placed in a polythene bag, labelled and placed in an ice-box for transportation to the laboratory for enzyme analysis.

Acetylcholinesterase activity measurement

8 The colorimetric method of Ellman *et al.* (1961) was used to measure the acetylcholinesterase activity.

9 Each fish was decapitated and the operculum removed. The fish head alone was weighed and homogenised using a Potter–Elvehjin homogeniser in a 0 1 M phosphate buffer solution (pH 7 0) so as to make a 10% solution. The fish homogenate solution was next diluted with the phosphate buffer to make a 0 1% solution, 4 0 ml of which was measured into a photometric cuvette, and 0 1 ml dithiobisnitrobenzoic acid (DTNB) solution added and thoroughly mixed. The spectrophotometric zero was set with this solution, after which 0 1 ml acetylthiocholine (ASCh) solution (0 2 M) was added. Immediately after mixing, the rate of the yellow colour production was followed by measuring the absorbance *(A)* at 412nm every 30s for 120s using a Coleman 295 spectrophotometer. Duplicate measurements were made on each fish homogenate. At the end of each measurement, 0 004 ml anticholinesterase solution was added to check for any non-enzymatic hydrolysis. No increase in the yellow colour was recorded after the addition of the anticholinesterase solution, indicating that there was no other hydrolysis than that due to the acetylcholinesterase.

10 Change of absorbance per minute (ΔA min$^{-1)}$ was calculated and the rates converted to absolute units using the formula:

11 *µmol* ASCh min^{-1} g^{-1} fish head wt

$$= \frac{\Delta A \min^{-1} \times \frac{\text{Total vol. solution in cuvette (ml)}}{\text{Vol. of homogenate solution taken (ml)}}}{1{\cdot}36 \times 10^4 \times 10^3} \times \frac{100}{\%\ \text{homogenate}} \times 10^6 \times \text{head wt (g)}$$

12 where 1.36 x10^4 = extinction coefficient of DTNB,10^3 = conversion of mol $litre^{-1}$ to mol ml^{-1}, and 10^6 = conversion of mol ASCh min^{-1} g^{-1} head weight to *µmol* ASCh min^{-1} g^{1} head weight. The measurements were made at a room temperature of 25–30°C.

RESULTS AND DISCUSSION

13 The results of the AChE activity measurements are summarised in Tables 1 and 2. The enzyme activities are expressed as µmol acetylthiocholine (ASCh) hydrolysed per minute per gram fish head weight. Student's *t*-test (p = 0 5) was used to compare the enzyme activity of.the fish from the treated rivers with their respective Controls.

14 Table 1 shows that the brain AChE activity of the caged *T. zilli* was significantly reduced by 16.32% after 2 h (p <0.05) of the river treatment and by 19.06% after 5 h (p <0.05). These results indicate that during the river treatment with chlorphoxim the larvicide induces a significant reduction in the brain acetylcholinesterase activity of fish present in the river water just below the *S. damnosum* s.l. breeding site. Near the point of the chlorphoxim release, the level of enzyme inhibition would be greater than the observed 20% because of the higher larvicide concentration.

TABLE 1. Acetylcholinesterase (AChE) Activity of Caged *Tilapia zilli* Kept in the River Marahoué During Chlorphoxim Treatment

	No. of fish analysed	*Exposure time (h)*	*Fish head weight (g) (mean ± SD)*	*Total AChE activity in µmol ASCh min⁻¹ g⁻¹ head weight (mean ± SD)*	*% inhibition*
Experiment A	10	2	2·79 ± 1·20	16·86 ± 4·05	16·32
Experiment B	12	5	2·76 ± 1·18	16·31 ± 5·34	19·06
Control	11	—	3·05 ± 1·69	20·15 ± 6·15	—

15 The increase in the level of the enzyme inhibition from 16% after 2 h to about 20% after 5 h of river treatment might have been caused by either the residual chlorphoxim left in the river after the bulk of the larvicide had passed downstream or the appearance of some metabolites of chlorphoxim more toxic than the parent compound. Metabolites of malathion were found to continue inhibiting AChE activity for several weeks after exposure was discontinued and the parent compound had disappeared from the water (Weiss, 1961). Verma *et al.* (1979) have also reported that cholinesterase of vertebrates remained inhibited for several weeks after exposure caused by oxygen analogue metabolites of thiophosphates.

16 A similar cage experiment performed in the Black Volta river treated with temephos did not reveal any significant inhibitory effect on the fish brain AChE activity (Scheringa *et al.*, 1981), providing further evidence that chlorphoxim is more toxic to fish than temephos.

17 In contrast to the observed toxic effect of chlorphoxim treatment on the brain AChE activity of the caged *T. zilli* in the river Marahoué, the enzyme activity levels of *A. nurse, T. galilaea* and *T. zilli* randomly sampled with a cast net from the rivers Bandama and N'zi (Table 2) were not significantly different from their respective Controls ($p > 0.05$).

18 Both rivers had had weekly treatments with chlorphoxim for about 10 months. It appears then that during treatment the majority of the fish in the treated rivers avoid the impact of the Chemical by swimming downstream. Avoidance reactions of fish to Chemicals in water have beeri observed by many workers including Hansen (1969), Scherer (1975) and Abban & Samman (1980). The large volume of flowing river water, especially during the rainy season when the chlorphoxim treatment of the rivers in the Ivory Coast is undertaken, ensures a rapid dilution of the larvicide from the dosing point, so that downstream below the breeding site the chlorphoxim is barely detectable. The fish near the treatment site can swim safely into the waters downstream of the breeding site.

TABLE 2. Acetylcholinesterase (AChE) Activity of Three Fish Species Randomly Taken from Two Rivers which had been Treated with Chlorphoxim

River	*Fish species*	*No. of fish analysed*	*Fish head weight (g) (mean ± SD)*	*Total AChE activity in µmol ASCh min $^{-1}$ g $^{-1}$ head weight (mean ± SD)*
Bandama (chlorphoxim-treated)	*Tilapia zilli*	5	3·25 ± 0·73	20·41 ± 3·73
Barrage de Loka (control)	*Tilapia zilli*	11	3·05 ± 1·69	20·15 ± 6·15
Bandama (chlorphoxim-treated)	*Tilapia galilaea*	11	2·66 ± 0·79	18·20 ± 5·96
N'zi (chlorphoxim-treated)	*Tilapia galilaea*	9	2·73 ± 0·77	17·70 ± 4.21
Kadioni (control)	*Tilapia galilaea*	6	2·46 ± 0·82	17·65 ± 5·08
Bandama (chlorphoxim-treated)	*Alestes nurse*	12	2·12 ± 0·50	13·11 ± 4·63
N'zi (chlorphoxim-treated)	*Alestes nurse*	14	2·52 ± 1·35	11·81 ± 3·11
Kadioni (control)	*Alestes nurse*	7	1·91 ± 0·56	11·11 ± 4·68

19 Another factor which has contributed to the survival of the fish population in the treated rivers from the toxic effect of the larvicide is its low persistence in the environment (OCP,

1973). This property of chlorphoxim ensures that it does not accumulate to lethal levels in the treated rivers even after a series of prolonged treatments.

20 No fish kill was observed during the chlorphoxim treatment of the river. However, the recorded 20% reduction in the caged fish brain AChE activity might have cause! the fish to exhibit some toxic symptoms such as a reduction both in their ability to tolerate reduced oxygen tension (Eaton, 1970) and in their feeding activities (Verma *et al.,* 1979).

21 In the laboratory, fish exposed to sublethal concentrations of organophosphorus compounds recover their brain AChE activities to the normal levels usually within one month (Weiss, 1959; Benke & Murphy, 1974). However, in the fish's natural environment such recoveries could be much faster. It is therefore possible that in the treated rivers, fish that are exposed to chlorphoxim could recover their original acetylcholinesterase activity before the next river treatment.

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22 I wish to thank Professor J. H. Koeman, Dr J. J. T. W. A. Strik and Ms E. Scheringa, all of the Agricultural University, Wageningen, The Netherlands, for training experience in the measurement of AChE activity. The cage experiment was undertaken at the suggestion of Professor Koeman. Special mention must be made of Mr T. Sineyogo, at the OCP Headquarters, Ouagadougou, Burkina Faso, who collected the fish samples from the rivers. Finally, I thank Dr M. A. Odei of the Institute of Aquatic Biology, Achimota, Ghana, for making it possible for me to undertake this study, Funds for this study were provided by the OCP.

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ABSTRACTS

The effect of aerial treatment of rivers with chlorphoxim, in connection with the ongoing Onchocerciasis Control Programme (OCP) in the Volta River Basin, on the brain acetylcholinesterase (AChE) activity of three fish species Tilapia galilaea, Tilapia zilli *and* Alestes nurse *has been studied in the Ivory Coast. A cage experiment with* Tilapia zilli, *placed just below the breeding site of* Simulium damnosum *s.l. in the river Marahoué and aerially treated with chlorphoxim at a concentration of 0.05 mg litre^{-1} per 10 min of river discharge, showed a 16% reduction in the brain acetylcholinesterase activity of the caged* Tilapia zilli *after 2 h and about 20 % after 5 h of the river treatment. However, there was no reduction in the brain AChE activity of some* Tilapia galilaea, Tilapia zilli *and* Alestes nurse *randomly taken with a cast net from the rivers Bandama and N'zi, which had been similarly treated with chlorphoxim for about 10 months. It appears that during river treatment the fish avoid the impact of the larvicide by swimming downstream.*

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Progress in controlling the reinvasion of wind borne vectors into the western area of the Onchocerciasis Control Programme in West Africa

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EDITOR'S NOTE

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1. INTRODUCTION

1 Since vector control started in 1975, waves of *Simulium sirbanum* and *S. damnosum s.str.* the principal vectors of severe blinding onchocerciasis in the West African savannas, have reinvaded treated rivers inside the original boundaries of the Onchocerciasis Control Programme in West Africa (OCP) (Le Berre *et al.* 1979). Larviciding of potential source breeding sites has shown that these 'savanna' species are capable of travelling and carrying *Onchocerca* infection for at least 500 km northeastwards with the monsoon winds in the early rainy season (Garms *et al.* 1979; Magor & Rosenberg 1980; Walsh *et al.* 1981; Johnson *et al.* 1985; Garms & Walsh 1987). Annual Transmission Potentials (ATP), as defined by Walsh *et al.* (1978), in communities near the original western borders of the Programme have remained above tolerable levels and human infection indices have decreased much more slowly than in central areas where this reinvasion has been controlled. Vector control has, therefore, been extended progressively westwards.

2 Garms *et al.* (1979) documented how experimental treatments of the Marahoué and Sassandra Basins in western Côte d'Ivoire during 1977 and 1978 markedly reduced the reinvasion of the Leraba and Upper Bandama Basins in the northeast of that country. In 1979, year-round control activities were extended to these river basins and substantial reductions in biting and transmission were recorded, although the Marahoué and Sassandra continued to be reinvaded (Walsh *et al.* 1981). In 1984, the Programme embarked on a major western extension into Guinea, Sierra Leone, western Mali, Senegal, and Guinea-Bissau. The first significant impact on reinvasion occurred in 1985 when the Upper Sassandra Basin in southeastern Guinea was treated with larvicides for the first time. Biting and transmission by re-invading flies in the Sassandra, Marahoué, Bandama and Leraba Basins decreased by over 90% (Baldry *et al.* 1985).

3 The Baoulé, Bagoé and Banifing tributaries of the River Niger in southeastern Mali and the extreme northwest of Côte d'Ivoire have been treated since 1977 but have proved to be more difficult to protect. The treatment of the Sankarani and Fié, the easternmost river basins in Guinea, had only a marginal effect on reinvasion (Baker *et al.* 1986). Further west, but still downwind and within 300 km of the re invaded areas, the Milo, Niandan, Kouya, Mafou and Niger Rivers were found to contain very large *S. sirbanum* breeding sites and flies collected from these sites were similar in size to those invading. Once the Inter-Tropical Convergence Zone (ITCZ) had been established to the north of the reinvasion zone, the periodicity of reinvasion could be related to changes in the height of these rivers, starting soon after their first rise at the end of the dry season and ending when they began to fiood after the onset of the main rainy season. It was concluded that these rivers must be the principal sources of the reinvading flies (Baker *et al.* 1987).

4 This paper describes progress in discovering the sources, elucidating the migration pathways and controlling reinvasion in the western area of the OCP during 1987–1989. Progress in containing the reinvasion of the eastern area of the OCP has been presented by Garms *et al.* (1982); Cheke & Garms (1983) and Walsh (discussion after Le Berre, this symposium).

2. STUDY AREA

5 The area covered by this study (figure 1) extends through five countries and includes the Upper Niger River Basin in Mali and Guinea, the Sassandra, Bandama, Comoé and Black Volta River Basins in Côte d'Ivoire and Burkina Faso, together with Coastal river basins in Sierra Leone such as the Great and Little Scarcies and Seli (Rokel). Descriptions of these areas have been given by Baldry *et al.* (1985) and Baker *et al.* (1986, 1987) and, for Sierra Leone, by Post & Crosskey (1985).

3. METHODS

6 The graduai westward extension of OCP's vector control operations was preceded by extensive helicopter and ground prospections to determine the geographical and seasonal distribution of breeding sites for each vector species. A network of points (only a few of which are shown in figure 1) for 11-hour day collections of biting flies was progressively established along all the main rivers. Flies were dissected to determine parity and infection following a standard protocol (Walsh *et al.* 1979). Water-level gauges

were installed or recalibrated on all the main watercourses, the most representative being fitted with ArgosR beacons, which transmitted river heights every 20 min to the aerial bases via satellite, to enable the accurate calculation of discharge rates.

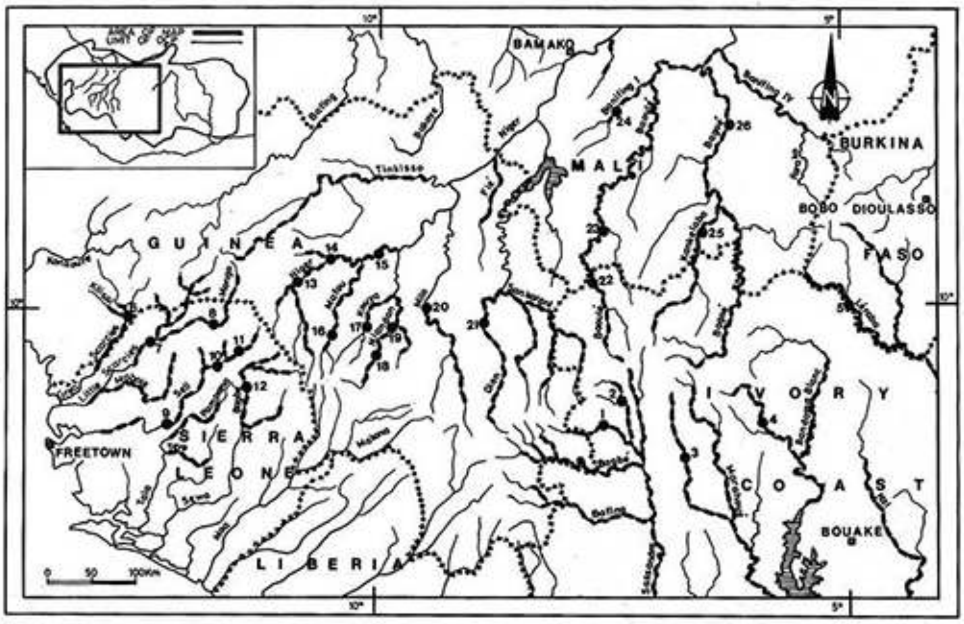

FIGURE 1. The Study Area showing: country boundaries (+ + + + +), the main rivers (thin lines), the river lengths (at maximum) treated in 1989 (thick dashed lines), and the capture points mentioned in the text (1, Niamotou; 2, Massadougou; 3, Kato, 4, Gîte 3; 5, Pont Leraba; 6, Badi Kanti; 7, Kaba Ferry; 8, Musaia; 9, Makpankaw; 10, Arfanya; 11, Yirafilaia; 12, Yifin; 13, Balandougou; 14, Banfarala; 15, Diaragbela; 16, Yalawa; 17, Kouya Laya; 18, Yradou; 19, Sansanbaya; 20, Morigbedougou; 21, Téré; 22, Madina; 23, Madina Diassa; 24, Mpiela; 25, Kankela; 26, Metela).

7 Cytotaxonomy of the larval polytene chromosomes (Vajime & Dunbar 1975) was the only unequivocal method of identifying savanna vectors, but adult females could generally be distinguished from 'forest'forms of the *S. damnosum s.l.* complex in this area by their pale wing tufts (Kurtak *et al.* 1981), pale fore-coxae and short, pale, compressed antennae (Garms *et al.* 1982; R. H. A. Baker *et al.,* unpublished). In Sierra Leone, *S. squamosum* were distinguished by electrophoresis (M. C. Thomson, unpublished).

8 Maps showing the distribution of each cytospecies were prepared and compared with graphs showing the strength and periodicity of reinvasion in the areas under vector control. In this way, the critical river stretches requiring priority treatment to prevent reinvasion were selected. To minimize the costs of vector control, only river stretches with rapids colonized by the savanna vector species were subjected to weekly aerial larviciding. Kurtak *et al.* (1987) have reviewed the insecticides, application methods and strategies involved. One biological (*Bacillus thuringiensis* serotype H14) and four Chemical insecticides (temephos, chlorphoxim, permethrin, and carbosulfan) were carefully alternated to maintain effective control and minimize the development of insecticide resistance while preserving the ecological balance in the treated rivers. The insecticides were generally applied by helicopter, although a fixed-wing aircraft was sometimes used when river discharges were high.

9 In the Upper Sassandra Basin of southeastern Guinea, where anti-invasion larviciding had been so successful (Baldry *et al.* 1985), the same treatment limits were maintained, enlarging as each large tributary began to flow and contracting with the disappearance of savanna species.

10 As a result of the limited effects of vector control in the Sankarani and Fié Basins in 1984 and 1985 (Baker *et al.* 1986) and the discovery of the very large savanna breeeding sites along the lowland stretches of the other main rivers in the Upper Niger Basin in eastern Guinea (Baker *et al.* 1987), control operations had to be extended to these rivers. During 1986, *B. thuringiensis (B.t.)* H14 larviciding was carried out in the Sankarani Basin to contain insecticide resistance, while in the Upper Niger Basin, the network of capture points to monitor control became operational, base-line data were collected and the river gauges installed and calibrated. All stretches of rivers in the Upper Niger Basin harbouring savanna flies (figure 1) were included in anti-reinvasion treatment circuits from April–July in 1987, 1988 and 1989. In Sierra Leone, the collection of baseline data, the installation of river gauges and the training of the National Onchocerciasis Team began in 1988, and these results were used to determine the river stretches requiring priority treatment when vector control was initiated in April 1989 (figure 1).

11 To evaluate the achievements of an anti-invasion vector control campaign, the source breeding sites of any flies still biting within the treated area must be interpreted from the vector monitoring data. The possibility of local breeding within the treated area must first be excluded either directly by prospections of breeding sites or indirectly from the parous rates, with nulliparous rates above 20%, usually implying a control failure. The existence of additional re-invasion sources can be inferred when significant numbers of flies with high parous rates are collected at capture points near the westward treatment limits. Movements of these flies may be tracked northeastward by comparing graphs of biting densifies per day at each capture point on their path (Johnson *et al.* 1985). Our incomplete knowledge of the factors initiating and terminating such long-distance movements, coupled with uncertainty as to the times of day and heights of travel in the air stream (reviewed by Garms & Walsh (1987)) make it difficult to back-track reinvasions accurately to their source and to predict their strength from year to year. Detailed interpretation was also limited by the incompleteness of the meteorological data from Guinea and Sierra Leone. These difficulties were compounded in this study by the limited sampling programme, with captures at most points restricted to one day per week. Capture frequencies were increased at heavily reinvaded sites but some points on the apparent reinvasion path, particularly those not situated close to large breeding sites, rarely recorded the passage of migrant flies.

4. RESULTS

(a) Reinvasion of Côte d’Ivoire *1986–1989*

12 Table 1 shows that since 1985, the treatments of the Upper Sassandra in southeastern Guinea have maintained their impact on biting and transmission during the critical April-June reinvasion period with 75–95% reductions in Monthly Biting Rates (MBRs) and 95–99% reductions in Monthly Transmission Potentials (MTPs) in comparison with the same period in 1982–84, when the treatment strategy in the reinvaded zones was most comparable. Annual Transmission Potentials (ATPs) (figure 2) were first reduced in 1979 when year-round vector control was undertaken in the Marahoué and Sassandra Basins and again when the Upper Sassandra in Guinea was added in 1985. The community microfilarial load in man, the geometric mean number of *O. volvulus* microfilariae per skin

snip in adults of 20 years or older, for-Massadougou was dropping rapidly (figure 3), and was approaching the trend predicted by a computer model (Remme *et al.* 1990).

(b) Reinvasion of southeastern Mali *1987–1988*

(i) Biting and transmission in Guinea and Mali

13 Vector control in both 1987 and 1988 greatly reduced biting and transmission in Guinea at those points where collections were made in 1986 (table 2). Over 80% reductions in biting rate were recorded at Téré and Morigbedougou in the Sankarani and Milo Basins, and over 60% at Sansanbaya on the River Niandan. Despite these reductions, considerable numbers of parous flies were caught. At Sansanbaya an MBR of 24830 was obtained in May-July 1986, but 7579 and 10436 were still recorded in 1987 and 1988.

14 The 1987–1988 reinvasion in Mali, as represented by cumulative May-July MBRs, constituted a 72% reduction at Madina Diassa and a 36% reduction at Mpiela, but only a 10% reduction at Kankela and Madina, when compared with 1977–1983 when no treatments were conducted in Guinea (table 3). At Metela there was a 35% increase. However, estimates of the reductions in biting and transmission achieved by spraying source breeding sites can only be very approximate, because MBRs and MTPs have varied considerably from year to year (figures 4 and 5) and it has not been possible to predict the strength of the annual reinvasion.

(ii) Effectiveness of treatments in Guinea and Mali

15 Parous rates at capture points beside treated river stretches in Guinea and Mali remained very high in both 1987 and 1988. In 1987, during the period when flies were invading Mali, however, some local breeding was reported in several of the complex rapid Systems on the River Niandan and River Niger in Guinea, which had been treated with *B.t.* H14. Formulations of this insecticide could have a relatively short ‘carry’ and treatment failures could occur when complex rapid Systems were treated for the first time. With greater experience and more accurately calibrated river gauges this problem was largely eliminated in 1988.

TABLE 1. CUMULATIVE APRIL-JUNE MONTHLY BITING RATES AND CUMULATIVE MONTHLY TRANSMISSION POTENTIALS IN THE CÔTE D’IVOIRE REINVASION ZONE 1982–1989, TOGETHER WITH MEAN VALUES FOR 1982–1984 AND 1985–1989 FOR BOTH INDICES AND PERCENTAGE REDUCTIONS BETWEEN THE TWO PERIODS

capture point and map number (figure 1)	1982	1983	1984	1985	1986	1987	1988	1989	mean 1982–84	mean 1985–89	percentage reduction
					Monthly biting rates (MBR)						
Niamotou (1)	16892	14536	6919	808	230	270	1082	516	12782	581	95.5
Massadougou (2)	12372	3762	4613	209	1372	1068	711	173	6916	707	89.8
Kato (3)	13782	3540	7905	189	224	1094	946	1387	8409	768	90.9
Gîte 3 (4)	7102	1233	2761	111	288	744	1106	1599	3699	770	79.2
Pont Leraba (5)	2423	259	705	20	459	269	351	323	1129	284	74.8
					Monthly transmission potentials (MTP)						
Niamotou (1)	808	986	551	20	0	0	15	0	782	7	99.1
Massadougou (2)	476	66	179	2	8	15	6	0	240	6	97.4
Kato (3)	410	154	211	1	0	17	5	2	258	5	98.1
Gîte 3 (4)	452	30	177	11	0	22	13	9	220	11	95.0
Pont Leraba (5)	161	18	36	0	4	3	0	0	72	1	98.0

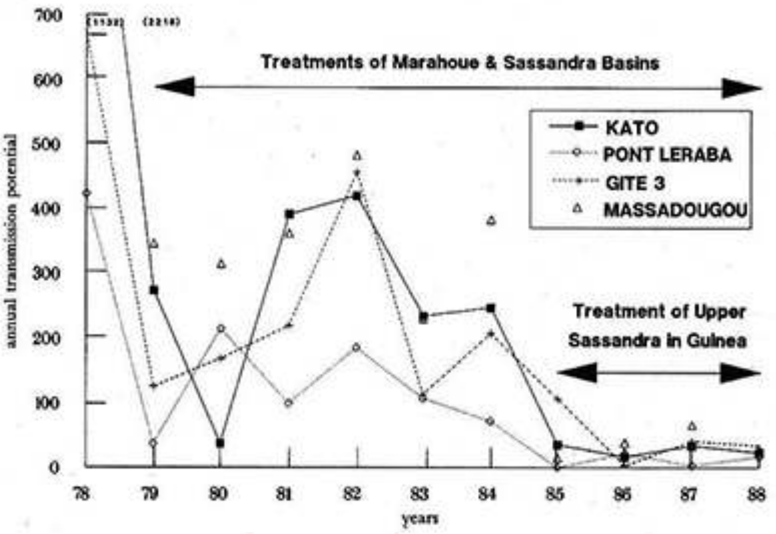

FIGURE 2. The effect of insecticide treatments of the Marahoué and Sassandra Basins followed by the Upper Sassandra in Guinea, on Annual Transmission Potentials at four capture points in the Côte d'Ivoire reinvasion zone.

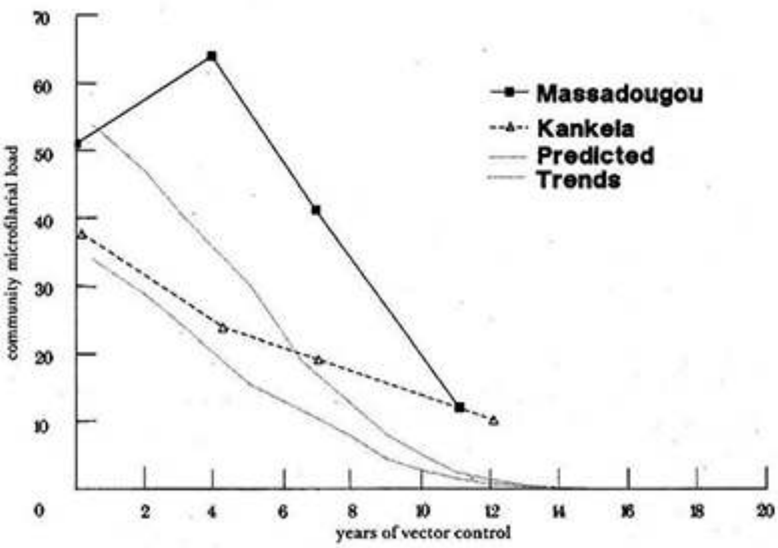

FIGURE 3. Epidemiological trends in the reinvasion zones. The predicted trends are those of Remme *et al.* (1990).

TABLE 2. CUMULATIVE APRIL-JULY MONTHLY BITING RATES AND CUMULATIVE MONTHLY TRANSMISSION POTENTIALS IN THE UPPER NIGER BASIN IN GUINEA 1986–89

capture point and map	pre-treatment 1986	all Upper Niger Basin treated 1987	1988	1987–88	Sierra Leone added 1989	percentage reduction 1987–88 and 1986	1989 and 1986	1989 and 1987–88
			Monthly biting rates (MBR)					
Tere (21)	28097	3373	2647	3010	1466	89.3	94.8	51.3
Morigbedougou (20)	23675	4026	3315	3671	2144	84.5	90.9	41.6
Sansanbaya (19)	24830	7579	10436	9008	2276	63.7	90.8	74.7
Yradou (18)	—	3881	7424	5653	2262	—	—	60.0
Kouya Laya (17)	—	7471	8769	8120	2587	—	—	68.1
Yalawa (16)	—	6986	8489	7738	3069	—	—	60.3
Diaragbela (15)	—	5978	2320	4149	470	—	—	88.7
Banfarala (14)	—	8140	15945	12043	716	—	—	94.1
Balandougou (13)	—	4955	8459	6707	1336	—	—	80.1
			Monthly transmission potentials (MTP)					
Téré (21)	559	116	14	65	9	88.4	98.4	86.2
Morigbedougou (20)	636	176	78	127	41	80.0	93.6	67.7
Sansanbaya (19)	1168	242	363	303	106	74.1	90.9	65.0
Yradou (18)	—	205	154	180	23	—	—	87.2
Kouya Laya (17)	—	204	71	138	93	—	—	32.4
Yalawa (16)	—	126	151	139	133	—	—	4.0
Diaragbela (15)	—	370	102	236	14	—	—	94.1
Banfarala (14)	—	420	347	384	0	—	—	100.0
Balandougou (13)	—	39	407	223	69	—	—	69.1

TABLE 3. CUMULATIVE APRIL-JULY MONTHLY BITING RATES AND CUMULATIVE MONTHLY TRANSMISSION POTENTIALS IN THE MALI AND NORTHWESTERN CÔTE D'IVOIRE REINVASION ZONE 1977–89

capture point and map number	mean MBR pre-Guinea treatments 1977–83	mean MBR Sankarani treatments 1984–86	mean MBR Upper Niger treatments 1987–88	percentage reduction after Sankarani treatments	after Upper Niger treatments	after Sierra Leone treatments
			Monthly biting rates (MBR)			
Madina (22)	4798	7115	4278	(a)	10.8	83.6
M-Diassa (23)	8965	12699	2497	(a)	72.1	71.1
Mpiela (24)	1864	1176	1189	36.9	36.2	86.1
Kankela (25)	5120	4372	4586	14.6	10.4	65.2
Metela (26)	1768	1243	2387	29.7	(a)	68.7
			Monthly transmission potentials (MTP)			
Madina (22)	107	54	41	49.2	61.6	87.8
M-Diassa (23)	425	387	66	9.1	84.6	83.3
Mpiela (24)	220	110	28	50.0	87.5	95.5
Kankela (25)	409	229	168	43.9	59.0	83.1
Metela (26)	23	7	15	67.9	34.4	86.9

(a) No reduction.

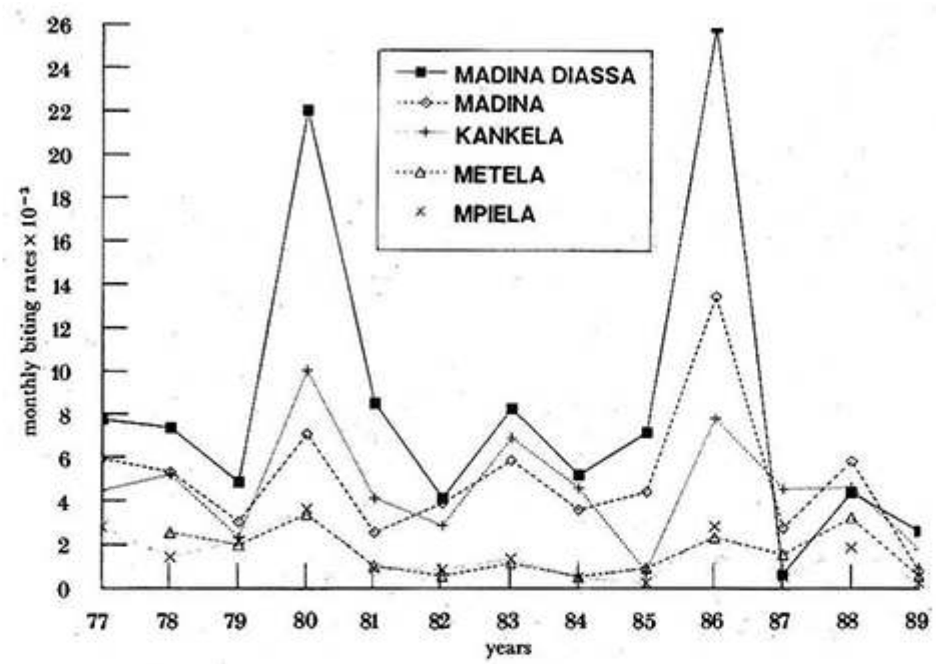

Figure 4. May-July cumulative Monthly Biting Rates at five capture points in the Mali/northwestern Côte d'Ivoire reinvasion zone from 1977–1988.

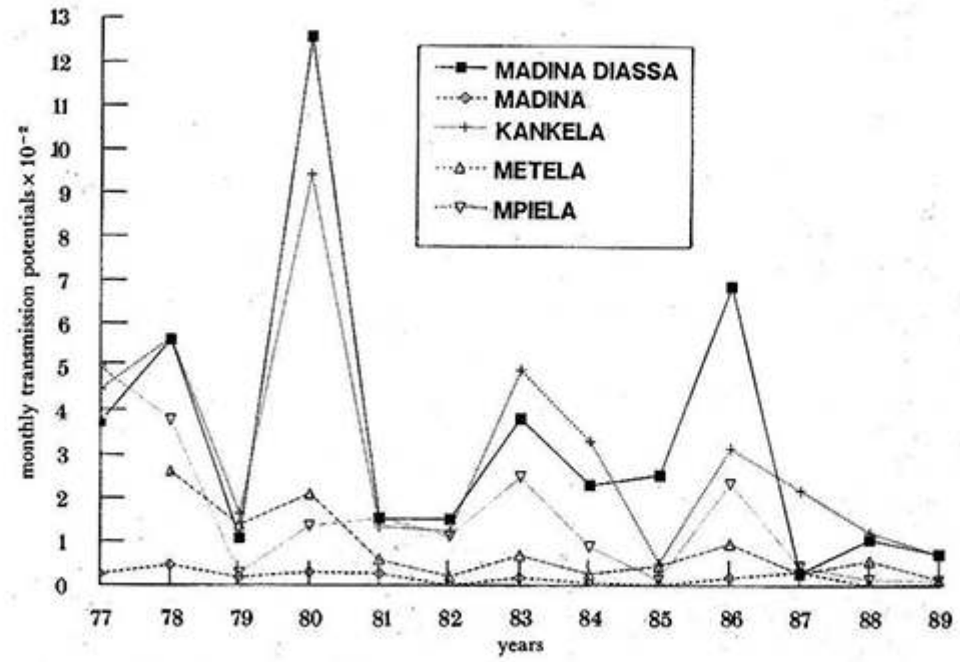

Figure 5. May-July cumulative Monthly Transmission Potentials at five capture points in the Mali/ northwestem Côte d'Ivoire reinvasion zone from 1977–1989.

(iii) Sierra Leone savanna populations and reinvasion of Mali

16 During 1988, the search for additional sources of reinvasion further upwind in Sierra Leone was intensified. Post & Crosskey (1985) showed that savanna vectors were mostly limited to the extreme north of the country, but in May 1988, breeding populations of *S. sirbanum* were found in all the main river basins, though northern rivers were more

heavily colonized (figure 6). Biting savanna flies were recorded at all capture points, with a maximum of 650 per day on the River Seli at Arfanya in late May. During June, *S. soubrense* B began to dominate in biting collections until, by the end of July, biting savanna flies were virtually restricted to the Little and Great Scarcies Basins in either side of the border with Guinea (figure 7).

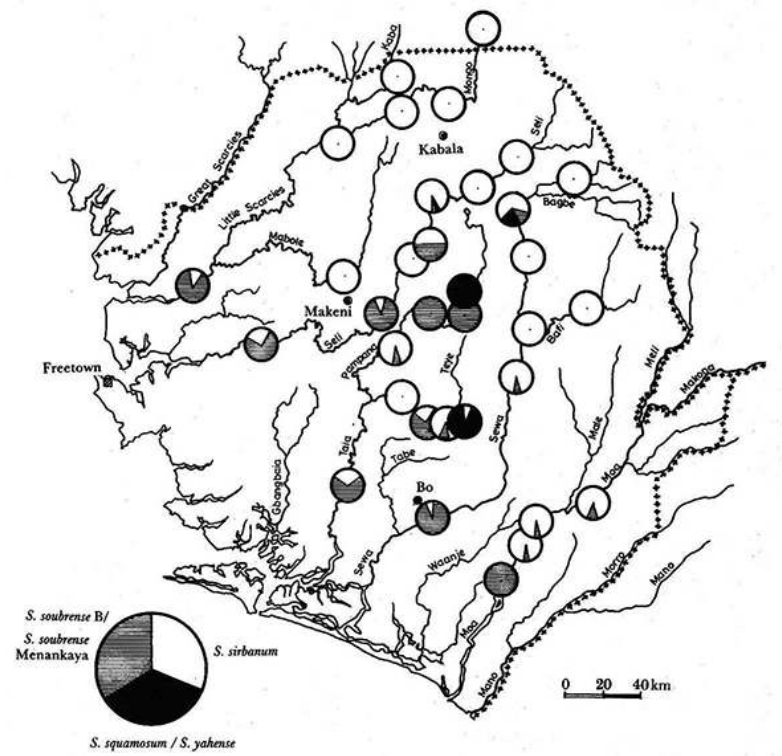

FIGURE 6. The distribution of different members of the *Simulium damnosum* species complex in Sierra Leone in April-May 1988, identified by larval cytotaxonomy.

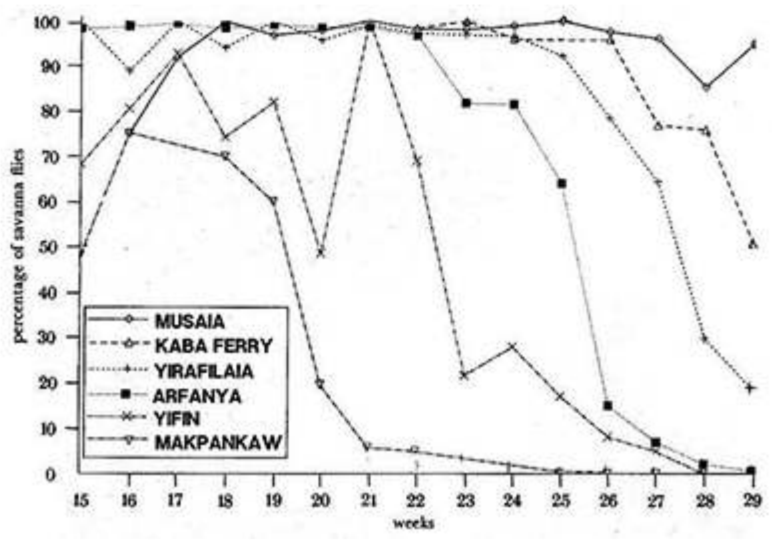

FIGURE 7. Percentage of savanna biting flies in weekly catches at six capture points in Sierra Leone during calendar weeks 15–29, 1988.

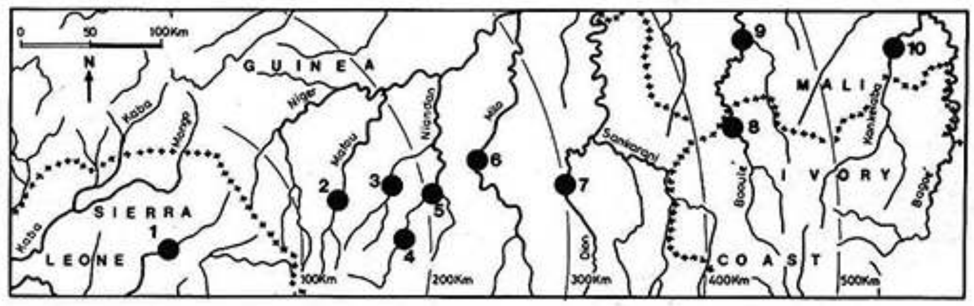

FIGURE 8. Map situating the capture points (1, Arfanya; 2, Yalawa; 3, Kouya Laya; 4, Yradou; 5, Sansanbaya; 6, Morigbedougou; 7, Téré; 8, Madina; 9, Madina Diassa; 10, Kankela) on the reinvasion axis between Sierra Leone and Mali as used in figures 9 and 10.

17 When the 1988 mean daily biting rates per week at capture points in Sierra Leone, Guinea and Mali (figure 8) were plotted on the same graph (figure 9), a wave of savanna flies could be followed northeastwards from Arfanya through Yalawa (101–150 km) on the R. Mafou, Kouya Laya on the River Kouya and Yradou on the River Niandan (151–200 km), Sansanbaya on the R. Niandan and Morigbedougou on the River Milo (201–250 km), Téré on the R. Dion (251–300 m) and reaching Madina in northwestern Côte d'Ivoire (401–450 km), Madina Diassa (451–500 km) and Kankela in Mali (501–550 km) in calendar week 24–25. Another wave was observed two weeks later passing through Balandougou,

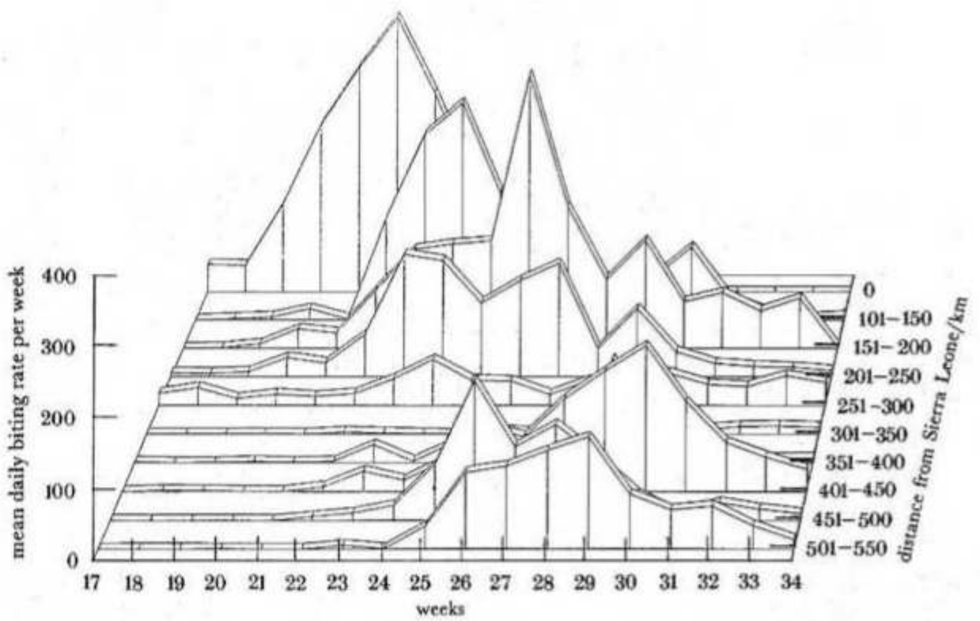

FIGURE 9. Three-dimensional representation of the mean daily biting rate per week at increasing distances from Sierra Leone in 1988.

18 Banfarala and Diaragbela on the River Niger some 100 kms further north. Flies were thus apparently taking 2–3 weeks to reach Mali from the Mafou and Niandan Basins in Guinea and 4–5 weeks to travel the approximately 500 km from Sierra Leone to Mali, a rate of one day per 15–20 km. This is within the estimate of 7–35 km made by Johnson *et al.* (1985) for migrating flies in Côte d'Ivoire and Burkina Faso.

(c) Reinvasion of southeastern Mali *1989*

(i) Biting and transmission in Sierra Leone, Guinea and Mali

19 Figures 4 and 5 and table 3 show that the principal points in Mali and northwestern Côte d'Ivoire were still reinvaded in 1989 despite the treatment of all known savanna breeding sites upwind. Significant numbers of parous invading flies were recorded at Madina Diassa, Kankela and Mpiela with maximum mean daily biting rates of 145, 91 and 94, respectively. However, very few flies were caught at Madina (maximum mean daily biting rate per week of 31, and a May-July MBR of 788), the best resuit ever recorded. In 1989, May-July MBRs were 65–86% and MTPs 83–95% less than means for 1977–1983, before any control was undertaken in the Western Extension.

20 The 1989 treatments also further reduced biting and transmission in the Upper Niger Basin (table 2). This effect was most noticeable at Sansanbaya where the cumulative May-July MBR of 2276 was 78% lower than that recorded in 1988, 70% lower than 1987 and 91% lower than 1986. MBRs on the River Niger were reduced by over 80% compared to the 1987–1988 mean and, at all points where 1986 data were collected, MBRs in 1989 were also reduced by at least 80%. On the Kouya, Upper Niandan and Mafou, MBRs remained at between 60–70% of the 1987–1988 mean.

21 MTPs followed the same trend. At Sansanbaya, the MTP of 106 was 70% lower than 1988, 55% lower than 1987 and 91% lower than 1986. Over 65% reductions compared to the 1987–1988 mean were recorded in the Sankarani, Milo, Niandan and Niger Basins, but there was little or no change on the Kouya and Mafou. In May, ivermectin, a microfilaricidal drug potentially capable of reducing transmission by up to 75% in isolated endemic communities (Remme *et al.* 1989), was delivered to hyperendemic communities in the Milo, Niandan, Mafou and Niger Basins, but no consistent effect on vector infectivity was recorded.

22 Vector control also greatly depressed biting and transmission rates in northern Sierra Leone. At Arfanya, the May-July MBR dropped by 97% (from 25420 to 680, with forest vectors making up 27% of the residual MBR) and the MTP by 98% (from 1810 to 1833). Low savanna vector biting rates and high parous rates were observed at all points except those in the Upper Great and Little Scarcies Basins near or north of the Guinea border.

(ii) Effectiveness of treatment in Sierra Leone, Guinea and Mali

23 Parous rates remained high at virtually all capture points beside treated river stretches in Sierra Leone, Guinea and Mali. However, some local breeding may have occurred for a short time in Mali near Madina Diassa because on the two days when the highest numbers of flies (238 and 200) were caught, only 76% and 67% were parous although by the next day the parous rate had reverted to 98%. This suggests that reinvasion at Madina Diassa was less important than the biting levels imply. Parous rates at Kankela and Madina were very high throughout and these two points may be better reflections of the reinvasion events.

24 Savanna vector breeding was not, however, completely eliminated in Sierra Leone. This was again partly because of difficulties in applying *B.t* H14 to complex breeding sites for the first time, but mainly to the problems encountered in treating tributaries of the Great and Little Scarcies which, at a critical time, were dry near their confluence with the main

river, but flowing productively upstream. Biting rates on the Great Scarcies River at Badi Kanti increased rapidly from week 23, well before the river began to flow. Substantial breeding by *S. sirbanum* was found two weeks later in the River Kilissi, a major tributary in Guinea, which was then included in the treatment circuit. However, catches at Badi Kanti peaked at 230 per day and were clearly an important potential source of invading flies.

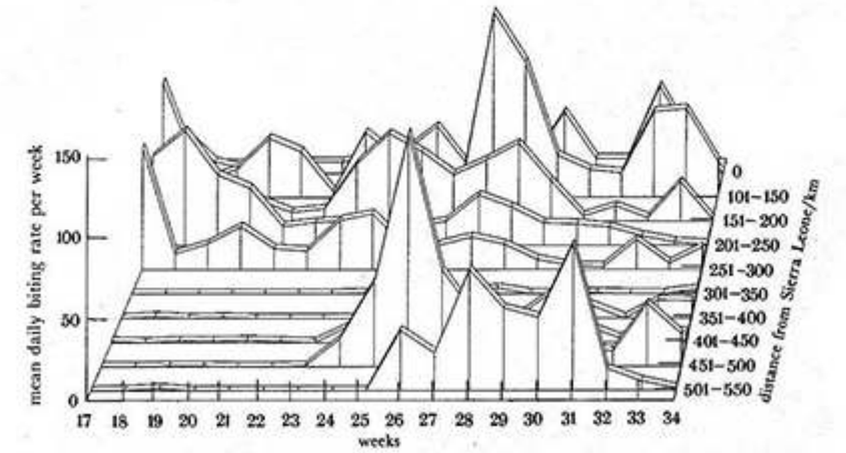

FIGURE 10. Three-dimensional representation of the mean daily biting rate per week at increasing distances from Sierra Leone in 1989.

(iii) Sources of reinvasion in *1989*

25 A graph of biting rates along the reinvasion axis for 1989 (figure 10) was much less clear cut than for 1988 (figure 9). In week 22 the situation looked excellent with about 10 flies per day at all points (25 at Sansanbaya). Then in weeks 23 and 24 daily biting trebled or quadrupled at Yalawa, Yradou, Kouya Laya and Téré, though remaining stable at Sansanbaya and Morigbedougou. Such rises could be correlated with the increases in Mali during weeks 25 and 26, but it is not clear why they were not detectable at Sansanbaya and Morigbedougou, both points where reinvasion had been very important in previous years. An additional rise in weeks 27 and 28 can, however, be detected at all points on the Southern Guinea axis.

26 Along the Niger River fly numbers gradually increased at Balandougou in weeks 25–27, peaking at Balandougou, Banfarala and Diaragabela in week 27. A second peak at Balandougou was observed in week 31.

5. DISCUSSION

(a) Côte d’Ivoire

27 Since treatments began in the Upper Sassandra Basin in Guinea, May-July MBRs in the Sassandra, Marahoué, Bandama and Leraba Basins in Côte d’Ivoire have been maintained at 4–25% and MTPs at 1–5% of pre-1985 levels. Epidemiological indices (figure 3) have fallen rapidly and, with ATPs below 100 since 1985 (figure 2), this trend should continue.

28 Potential reinvasion sources still exist to the Southwest of the Côte d’Ivoire reinvasion zone. Garms (1987) and discussion after this paper has collected adults and larvae of both savanna vector species from several rivers in Liberia. A large population of breeding and

biting savanna flies (maximum 335 per day in late May 1988) was found on the River Moa in southeastern Sierra Leone in both 1988 (figure 6) and 1989. However, since biting rates and transmission potentials have now been maintained at low levels for five reinvasion seasons and epidemiological indices are rapidly improving, it would appear that reinvasion from Liberia or southeastern Sierra Leone does not play an important role in the transmission of onchocerciasis in Côte d'Ivoire.

(b) Mali and norlhwestern Côte d'Ivoire

29 In 1989, for the first time, biting and transmission at all points were consistently low throughout the Mali and norlhwestern Côte d'Ivoire reinvasion zone and over 65% less than recorded before OCP began insecticide treatments in Guinea. In previous years, the strength of reinvasion had varied unpredictably (figure 4), with up to sixfold differences in biting rates between the years. Until 1987, reinvasion biting rates had always been highest at Madina Diassa, but in that year 72% less than the 1977–1983 mean were caught. However, in 1987 the River Baoulé only started to flow on 6 July, six weeks later than usual. Capture points beside slow Aowing river stretches rarely caught migrating flies and if, as seems likely, these flies, which were believed to migrate gravid (Bellec 1976), were attracted to the vicinity of rapids then this behaviour could explain the low catch at Madina Diassa in 1987. If years with unusually late fiow by the capture points are excluded (together with Kankela in 1985, which may have been affected by treatments of the Sankarani Basin (Baker *et al.* 1986)), the 1989 reinvasion was lower than ever before at all points. In 1989, cumulative May-July MTPs (figure 5) were below 100 for the first time. Epidemiological indices, which have only been slowly decreasing in this area (figure 3 for Kankela) should improve rapidly if this trend of low MTPs is maintained.

(c) Sources of reinvasion in *1989*

30 The key questions would appear to be to determine the source of the flies invading Mali during the main peak, which started in week 24 and was at a maximum 2–3 weeks later. If we accept that flies do take at least 4 weeks to travel the 500 km from northeastern Sierra Leone to Mali, then the flies breeding in the poorly treated tributaries of the Great and Little Scarcies, 100–150 km further west, should take even longer. This makes it unlikely that these flies, which first appeared in week 23, were the main contributors to this invasion.

31 An alternative hypothesis is based on the increase in biting rates observed in the Mafou, Kouya, Upper Niandan and Dion Basins during weeks 23–24, but this is confounded by the stability of the biting rates at Sansanbaya and Morigbedougou during the same period. Could the occasional poorly treated breeding site in Sierra Leone and Guinea have been responsible for the 30–40 flies per day in the Southern part of the Upper Niger Basin and could these flies have produced 150 per day at Madina Diassa? If we assume that the Madina Diassa peak was inflated by local breeding, because of the relatively low parous rate in week 26, and if wind conditions favoured long distance movement on particular days, thus increasing densities in the reinvaded zones, we may have a partial explanation. There were no other obvious potential source rivers.

32 Breeding sites in the Upper Great and Little Scarcies were probably responsible for the wave of invading flies that passed through capture points in the Niger River in weeks 26–

27 and maintained relatively high biting rates in Mali and northwestern Côte d'Ivoire until weeks 34–35.

33 With the experience obtained in treating complex breeding sites and partially flowing rivers in Sierra Leone during 1989 and with the evidence for the existence of source areas there, future reinvasions of Guinea and Mali should be further minimized by the appropriate insecticide treatments.

34 In a programme of the scale of OCP it is impossible to acknowledge all the individuals who contributed to the work described here. Special thanks are extended to: Mr I. Sesay, Entomologist of the Sierra Leone National Onchocerciasis Team and his staff; Messrs R. Lama, A. Sagno and Dr K. Kaba, entomologists of the Guinea National Onchocerciasis Team; Dr A. Akpoboua, OCP Bouaké, Sector Chief, his Sub-Sector Chiefs and their staff; Messrs I. Diallo and Y. Diarra, OCP Sub-sector Chiefs at Bamako and Bougouni, and their staff, Mr M. Kassambara, OCP Bobo Dioulasso, and his staff; Mr. S. Dramane, Dr K. Doucouré, Dr L. Toé, and Mr B. Tele for detailed surveys in Sierra Leone; Dr M. C. Thomson for electrophoretic identifications; Mr A. Soumbey and Dr S. Doumbia for data analysis; Dr C. Back for comments on the manuscript; Drs G. De Sole, K. Y. Dadzie and J. Remme for epidemiological information; Messrs A. Sib and Y. Coulibaly for cytotaxonomic assistance; Mr A. Belli and the administrative services for logistic support; the pilots of Viking Helicopters Ltd. and Evergreen Helicopters Inc. and the OCP aerial operations staff.

35 We acknowledge the key roles played by Dr B. Philippon and Dr D. Quillévéré, past and present Chiefs of the Vector Control Units; Dr D. A. T. Baldry and Mr P. Kaboré, past and present Chief Aviation Officers; Dr J. Grunewald, Research Coordinator; D. G. Zerbo, Chief Entomological Evaluation and Dr H. Agoua, Chief Central Zone.

36 Finally, we are grateful to Dr E. M. Samba, Programme Director for his continuing encouragement and permission to publish.

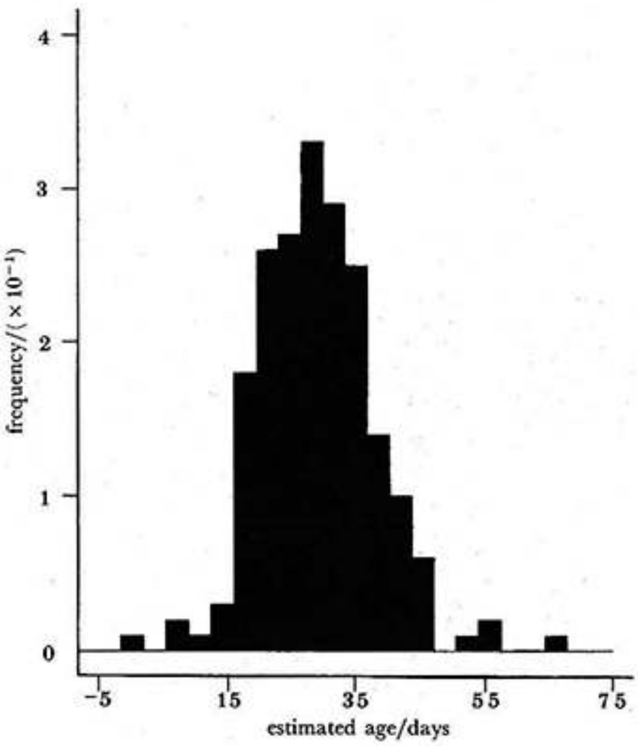

FIGURE 1. A frequency histogram of the estimated ages of *Simulium sirbanum*, caught at Kanibougoula on 22–23 June 1988. (Average age, $\bar{x}$ = 29.08 days; s.d. = 8.99.)

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Discussion

R. GARMS,[1] R. A. CHEKE[2] AND R. SACHS[1] ([1] Bernhard Nocht Institutefor Tropical Medicine, BernhardNocht-Strasse 74, D-2000 Hamburg 36, F. R.G.;[2] Overseas Development Natural Resources Institute, Chatham, U.K.). Baker et al. described movements in a northeastward direction of savanna members of the Simulium damnosum species compiex from uncontrolled areas. Litde is known about whether savanna flies also move in the opposite direction from north to south, from the savanna into the forest. Some recent observations in Liberia are therefore of interest.

Savanna species were known to occur and occasionaily breed in northem Liberia (Garms & Vajime 1975), but they were not found elsewhere in the country untii the dry season of 1985, when a few flies were caught biting man at sites beside the St Paul River in the evergreen rainforest zone. They were thought to have arrived from northem sites assisted by the northeasterly harmattan winds (Garms 1987). Three years later, during the dry season of 1988, residents of the Bong iron ore mine complained about a serious nuisance caused by blackflies biting them within the mine's concession area as much as 10 km away from the St Paul River, where flies had been extremely rare in previous years. Fly catches in May and June 1988 confirmed that biting densities were very high (more than 500 in 7 h). Morphological identiAcations showed that practically all of these flies were savanna members of the S. damnosum species complex.

This phenomenon of mass biting did not recur in the dry season of 1989 when regular Ay catches were performed at four sites within and Ave sites outside the Bong Mine concession area. However, some savanna flies were present, but they were only caught in small numbers within the concession area. Their source could be traced to the Yea Creek, a small stream emerging from the Bong Mine's tailings pond. Larvae collected on 20 March, 21 and 22 April were cytologically identified as 39 S. damnosum s.str., 19 S. sirbanum and 2 S. soubrense (G. K. Fiasorgbor, personal communication). Interestingly, they were associated with S. adersi, another savanna species, which had not been recorded from the area before. It is probable that the savanna species had invaded the region from the north with the harmattan winds, had encountered favourable breeding conditions and were able to establish themselves in the area for at least a few generations. They were not found in any of six streams and rivers around the concession area which were regularly checked.

It is not known why the outlet of the tailings pond, a unique and highly artiAcial environment, is such an attractive breeding site for the savanna species but not for the local forest species. Most of the water originates from the St Paul river from where it is pumped through a pipe line ca. 10 km long to the concentrator of the Bong Mine. From there the water, heavily loaded with inorganic material, is passed to a large impondment area, the tailings pond, where the solid wastes settled prior to the release of clean water to the river. The water leaving the tailings pond is characterized by constant high temperatures of 30°C and several Chemical characteristics, in particular hardness, which are quite distinct from those of the natural watercourses. It is very rich in microorganisms, probably produced by the drowning forests and decaying trees within the impondment area.

It is not yet clear whether or not the invasion by savanna species and their colonization of the Bong Range will have any epidemiological consequences.

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R. H. A. BAKER. Despite the potential threat of savanna flies re-invading the Sassandra, Marahoué and Bandama Basins from isolated seasonal breeding sites in Liberia, described by Dr. Garms *et al.* and Southern Sierra Leone (figure 6), biting and transmission rates in the reinvasion zones have been maintained at low levels for the past five years (since the treatment of the Upper Sassandra in southeastern Guinea began). This was true even in 1988, when the savanna flies were most numerous at the Bong Mine in Liberia. Rivers in Southern Sierra Leone will be included in treatment circuits from 1990, further diminishing this threat.

R. A. CHEKE[1], M. A. HOWE[2], M.J. LEHANE[2], A. L. MILLEST[2], T. KONE[3] R. H. A. BAKER[3] ([1] *Overseas Development Natural Resources Institute, Chatham, U.K.;* [2] *Sckool of Animal Biology, University College of North Wales, Bangor, Gwynedd U.K.;* [3] *WHO Onchocerciasis Control Programme. B.P.* 549, *Ouagadougou, Burkina Faso*). Baker *et al.* suggested that the migrant *Simulium sirbanum* reaching Madina Diassa in Mali during the 1988 wet season were 4–5 weeks old. The basis for their conclusions were analyses of the time sequences of waves of flies traversing a series of sites between Madina Diassa and their likely sources, 450–500 km away in Sierra Leone. We have recently estimated the ages of a sample of *S. sirbanum,* caught near Madina Diassa in June 1988, by analysis of their pteridine concentrations. Our results, based on this biochemical technique, agree very well with the conclusions of Baker *et al.*

Pteridine concentrations are known to increase with age and Ay size in different members of the *S. damnosum* species complex (Cheke *et al.* 1987, 1989). Such variation was established for Malian populations of *S. sirbanum* by experiments with flies emerged from pupae, collected at Tienfala (12° 44'N, 08° 00'W; 40 km E of Bamako) in the Niger River. The flies were maintained at different temperatures (20–33 °C), killed at daily intervals, measured for size, and pteridine concentrations estimated by Auorescence spectrometry. Although, surprisingly, temperature had no effect (P = 0.99), a significant multiple regression was obtained relating pteridine concentrations in the flies' heads to fly age and size, as follows: Log PTHD = 0.0113 age (days) +0.2158 fly thorax length (mm) + 0.53 (P <0.0001), where PTHD is the pteridine concentration in the head capsule. This equation was then used to estimate the ages of 199 flies caught at Kanibougoula, 10 km upstream of Madina Diassa, on 22 and 23 June 1988. A histogram of the results is shown in figure 1. The mean of this distribution was 29.08 days (s.d. = 8.99), within the range of 4–5 weeks suggested by Baker for his flies caught nearby during the same period. A few flies were estimated as being less than two weeks old and some others more than six weeks, with a maximum of 67 days. This maximum is more than twice the previous longevity record, estimated by pteridines, of 27 days for a female *S. damnosum s.str.* (Cheke *et al.* 1989). However, it is recognized that conclusions about individual flies must be treated with caution as the method is best restricted to populations

(Cheke *et al.* 1989). As such a population analysis, the results in figure 1 provide good supportive evidence for the conclusion of Baker *et al.* that *sirbanum* take about a month to migrate 500 km.

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J. B. DAVIES (*Medical Research Council and School of Tropical Medicine, Liverpool, U.K.*). I am extremely interested in Dr Baker's observation that flies were taking 4–5 weeks at average speeds of 15–20 km per day, to travel 500 km or so, and note how this is supported by Dr Cheke's estimates of age from pteridine concentrations and how closely both sets of results agree with earlier estimates for similar migrations (Johnson *et al.* 1985). I have always found Dr Johnson's earlier estimates of survival difficult to accept, although I have never questioned the fact that the flies were covering these distances. However, although the data showed the journeys took 30–60 days to accomplish, I found it hard to conceive that sufficient numbers of flies were surviving so long to provide such high biting rates at the furthest destinations. With this new corroborating evidence it seems that under the right conditions *S. damnosum* is a better survivor than some of us supposed, and certainly better than laboratory survival experiments show. I for one, will have to change my opinion of this fascinating little insect. This new evidence is very welcome.

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Johnson, C. G., Walsh, J. F., Davies, J. B., Clark, S. J. & Perry, J. N. 1985 The pattern and speed of displacement of females of *Simulium damnosum* Theobald s.l. (Diptera: Simuliidae) across the Onchocerciasis Control Programme area of West Africa in 1977 and 1978. *Bull. ent. Res.* 75, 73–92.

R. H. A. BAKER. Regarding the corroboration between fly longevity, as estimated by pteridine concentrations, and the time taken for waves of invading flies to travel 500 km from Sierra Leone to Mali, how can this information be used operationally? If longevity can be directly related to distance flown, a retrospective study could try to identify the source breeding sites of the invading flies. It is possible, however, that some flies would take a much shorter time to Ay this distance than the 15–20 km per day suggested from comparisons of the waves of moving flies.

The value of data from satellites for forecasting outbreaks of locusts and other pests has been discussed by various speakers at this meeting, but satellite imagery could also be of benefit to OCP if it could provide information at the river or even breeding site level. At the beginning of the wet season, spraying helicopters could be targetted more accurately if it were possible to identify tribu taries where there had been rain and which were likely to have started to Aow. Ideally, it would be possible to detect the presence of Aowing (white) water.

M. D. WILSON (*Department of Medical Entomology, School of Tropical Medicine, Liverpool, U.K.*). There is a lot of deforestation taking place in the lowland rainforest outside the Onchocerciasis Control Programme area of West Africa. Will this phenomenon resuit in the creation of habitats permanently suitable for colonization by the dangerous savanna vector species? This would have obvious implications for the future control strategy and boundary of the Programme.

R. H. A. BAKER. This is a very important point. In recent years, the savanna vector species have begun to colonize deforested parts of the Programme area and this situation can only deteriorate.

ABSTRACTS

Since vector control began in 1975, waves of *Simulium sirbanum* and *S. damnosum s.str.,* the principal vectors of severe blinding onchocerciasis in the West African savannas, have reinvaded treated rivers inside the original boundaries of the Onchocerciasis Control Programme in West Africa. Larviciding of potential source breeding sites has shown that these 'savanna' species are capable of travelling and carrying *Onchocerca* infection for at least 500 km northeastwards with the monsoon winds in the early rainy season. Vector control has, therefore, been extended progressively westwards. In 1984 the Programme embarked on a major western extension into Guinea, Sierra Leone, western Mali, Senegal and Guinea-Bissau. The transmission resulting from the reinvasion of northern Côte d'Ivoire and Burkina Faso has been reduced by over 95%, but eastern Mali has proved more difficult to protect because of sources in both Guinea and Sierra Leone. Rivers in Sierra Leone were treated for the first time in 1989 and biting and transmission rates in Sierra Leone and Guinea fell by over 90%. Because of treatment problems in some complex rapids and mountainous areas, flies still reinvaded Mali, though biting rates were approximately 70% lower than those recorded before anti-reinvasion treatments started. It was concluded that transmission in eastern Mali has now been reduced to the levels required to control onchocerciasis.

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Environmental Impact Assessment of Settlement and Development in the Upper Léraba Basin

Burkina Faso, Côte d'Ivoire, and Mali

David Baldry, Davide Calamari and Laurent Yaméogo

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Foreword

Jean-Louis Sarbib

1 This volume presents the results of an environmental impact assessment of land settlement in one river valley on the Burkina Faso-Côte d'Ivoire border. The methodology used for the assessment is quite simple and inexpensive and grew out of the environmental monitoring conducted by the Onchocerciasis Control Programme (OCP).

2 OCP is one of the most successful health projects in Africa. The program was launched by seven West African countries and nine donors, including the World Bank, and has expanded to include eleven countries and twenty-three donors. Thirty million people are now protected from the disease and 25 million hectares of arable land have been made available for settlement.

3 What has been less widely publicized than the health benefits of the Programme is the fact that, although the OCP strategy relies on the repeated application of Chemical insecticides and biological control agents to the aquatic breeding sites of the blackfly vectors, there have been no significant disturbances of the aquatic environment throughout the Programme area. The environmental soundness of the OCP strategy has been demonstrated by an extensive environmental monitoring System supervised by an independent group of environmental experts. However, despite the care taken to prevent damage to the OCP rivers, there have recently been indications that other factors might be having a negative impact. To investigate this possibility the Committee of Sponsoring Agencies (CSA: comprised of FAO, UNDP, WHO, the World Bank), the statutory body that oversees OCP, undertook to organize this assessment, with funding from the Government of the Netherlands.

4 The findings of the assessment are important in that they indicate that the rapid settlement occurring in many of the areas where riverblindness has been controlled may have negative consequences for the aquatic environment even when there is very little use of Chemical fertilizers and pesticides. There is a need, therefore, for proactive policies by the concerned governments to ensure that when settlement occurs it does so in a manner that will not harm the environment.

AUTHOR

JEAN-LOUIS SARBIB

Director Western Africa Department

Acknowledgments

1 The Upper Léraba pilot study was commissioned by the Committee of Sponsoring Agencies (CSA) of the Onchocerciasis Control Programme in West Africa (OCP), and jointly funded by the Government of The Netherlands and The World Bank. The original design for the study was formulated in January 1992 by the Ecological Group (EG) of the OCP's Expert Advisory Committee (EAC), in response to a request from the CSA. After internal review and reformatting into a plan of action, the proposal was submitted by the CSA to the OCP Joint Programme Committee (OCP 1992), which endorsed it during its thirteenth session (Geneva, December 1992).

2 In January 1993, the plan of action for the pilot study was revised to reduce its geographical scope (in its original form the plan made provision for a parallel study in Côte d'Ivoire), and implemented by a team of three senior biological scientists; Dr. David Baldry, formerly a biologist with WHO and FAO, Professor Davide Calamari, Professor of Applied Ecology, Institute of Agricultural Entomology, University of Milan, and Dr. Laurent Yaméogo, OCP Hydrobiologist.

3 Aerial photographic surveys and fact-finding missions in the OCP area could not have been conducted so effectively without the support of Dr. Ebrahim M. Samba, former OCP Programme Director, and his staff, who placed their facilities at the disposal of the study team while its members were in West Africa, and who provided important archival information on the Upper Léraba Basin.

4 Likewise, it would have been much more difficult for the study team to accurately assess agrochemical impacts without the collaboration of officials of the Government of Burkina Faso, who kindly provided climatic, hydrological and agricultural data on the Upper Léraba Basin.

5 The members of the study team would also like to express special thanks to Mr. Manuel Bravo, pilot of Evergreen Helicopters, Inc., for safe aerial operations in the OCP area, and to Mr. Bruce Benton, Dr. John Elder and Dr. Bernhard Liese, The World Bank, for their unstinting support and encouragement during the implementation of the Pilot Project.

Acronyms/Abbreviations

ABR	Annual Biting Rate (of Blackflies)
ATP	Annual Transmission Potential (of Blackflies)
CSA	Committee of Sponsoring Agencies of the OCP
CPUE	Catch Per Unit of Effort (of Fish)
DI	Drift Index (of aquatic invertebrates)
EAC	Expert Advisory Committee of the OCP
EG	Ecological Group of the OCP Expert Advisory Committee
FAO	Food and Agriculture Organization of the United Nations
GUS	Groundwater Ubiquity Score
JPC	Joint Programme Committee of the OCP
LUA	Land Utilization Analysis Area
L.oc	Léraba Occidentale: the main western tributary of the main River Léraba
L.or	Léraba Orientale: the main eastern tributary of the main River Léraba
L.ss	Léraba *sensu stricto:* the main River Léraba, downstream of the junction of the Léraba Orientale and Léraba Occidentale
OCP	Onchocerciasis Control Programme in West Africa
PEC	Predicted Environmental Concentration
WHO	World Health Organization

Abstract

1 The great successes achieved by the Onchocerciasis Control Programme in West Africa (OCP) in the large-scale control of the blinding savanna form of onchocerciasis (river blindness) have allowed vast tracts of previously abandoned valleys to be resettled and developed. Among the many issues and problems that have to be addressed, to ensure the most effective long-term management of settlement and development in these onchocerciasis-controlled areas, is that which relates to the potential impact of those development activities on the environment.

2 Despite the fact that for many years anti-blackfly larvicides were regularly applied to the rivers of the OCP area, the quality of the aquatic environment was preserved, partly because of the precautions taken by the OCP, but largely because of the absence of human population pressure in the areas most severely affected by onchocerciasis. However, the situation is now changing—rapidly in parts of the OCP area—and there is increasing concern for the welfare of both aquatic and terrestrial environments.

3 In this context, it was recognized that there was a need for the formulation of an appropriate methodology for environmental impact assessment. In 1991 the OCP Committee of Sponsoring Agencies (CSA) started to address this issue, and in 1993 launched a pilot project in one selected basin (the Upper Léraba Basin), the results of which are described in this report.

4 The primary objectives of the Pilot Project were i) assessment of the present environmental situation in the Basin, with a view to determining potential sources of impact on the aquatic environment, ii) quantification of Chemical loads, and assessment of modification to the physical environment, and iii) identification of simple study methods which would be applicable to other areas and which could be offered as examples to predict environmental impacts of settlement and development, and offer ways to minimize those impacts.

5 Through the collection of geographic information and data on land utilization, as well as by an assessment of loads (fertilizers, pesticides, human and livestock wastes), an ecotoxicological impact evaluation of human activities was made, at basin scale, in the Upper Léraba Basin. More detailed evaluations of changes in the physical environment were made at medium and local scales of resolution, with special attention being given to

analyses of changes in land utilization patterns between 1972 and 1993, and to determining the ecological significance of the observed changes.

6 It was concluded that organic loads and nutrients were only of relevance along limited stretches of the main Léraba rivers, where settlements were located close to the river banks and provided point sources of contamination. The contamination of river water by pesticides used for cotton protection was calculated by means of simple models which also permitted the theoretical concentrations of pesticides in river water to be estimated. Although the predicted concentrations of pesticides in river water were not at levels that could give cause for alarm, they could however be considered as early warning signals.

7 With regard to physical changes in the environment, it was concluded that about 75 per cent of the original savanna woodland had been cleared for settlement and for agricultural development (most of it over the last decade). The riverine forests of many of the smaller rivers and streams had been destroyed, and on some cleared river banks the first signs of soil erosion were detectable.

8 On the positive side, it was concluded that there had not been any significant disturbance of the riverine forests and associated floodplain grasslands of the main rivers.

9 The overall conclusion from the Pilot Project was that, on the basis of studies which were limited both in time and scope, it was possible to make valid and meaningful assessments of the Upper Léraba Basin, in terms of Chemical contamination of river water, of localized changes in the biological condition of the aquatic ecosystem, and of physical degradation of the woodland savanna component of the terrestrial environment. In relation to the Pilot Project's third main objective, the investigations clearly demonstrated that the techniques employed—using input data that were very basic/but readily available from national agricultural and development authorities, and by the application of standard hydrobiological techniques and simple models—can be used as examples of quick and reliable environmental impact assessment methodologies, which are applicable to other, similar areas.

1. Introduction

1 Since its inception in 1974, the Onchocerciasis Control Programme in West Africa (OCP) has implemented a strategy of onchocerciasis (river blindness) control which has been aimed at the long-term interruption of the transmission of the causative agent (the filarial worm *Onchocerca volvulus*), in all areas where there was a human reservoir of infection of the severe blinding form of the parasite. In operational terms, this has required the weekly application of insecticides (mainly by aircraft) to the fast-flowing stretches of rivers which constitute the habitats of the larval stages of the blackflies which are the onchocerciasis vectors.[1]

2 Although the OCP commenced its vector control operations with only a single Chemical larvicide, the organophosphate compound temephos,[2] it was recognized from the outset that the possible impact of larviciding operations on the aquatic fauna would need to be carefully monitored. During the early years of the OCP a small Ecological Panel fulfilled this function. In 1980, when the OCP structure was reorganized, the Ecological Panel became the Ecological Group of the OCP Expert Advisory Committee, with a mandate to study "the ecological impact on the environment of the use of insecticides in the Programme" (Samba 1994).

3 Repeatedly, the Ecological Panel and the Ecological Group were able to demonstrate to the outside world that there had been no significant disturbances of the aquatic environments of the OCP area which could be attributed to OCP larviciding operations. In fact, the environmental soundness of the OCP strategy was repeatedly demonstrated by routine aquatic monitoring and assessment activities; the results of which were published in scientific literature (see, for example, Lévêque *et al.* 1988, Yaméogo *et al.* 1993).

4 Despite the proven environmental soundness of the OCP control strategy, the last few years have witnessed growing concern that human settlement and development activities in onchocerciasis-controlled areas could impose new threats to both aquatic and terrestrial ecosystems. Increases in agrochemical use and in other potentially pollutant loads, and physical change to the environment could all, directly or indirectly, have adverse effects on the quality and productivity of the valleys concerned.

5 From the analysis of aquatic monitoring data collected in the original OCP area during the early 1990s, the Ecological Group concluded that strong scientific evidence was available to substantiate these concerns. For example, environmental studies of some watercourses

which were no longer larvicided by OCP showed that faunal recolonization was in progress, but that in certain cases, human activities had clearly contributed to a degradation of the environment, resulting in an impoverishment of the fauna. The Upper Léraba Basin (covering parts of Burkina Faso, Côte d'Ivoire and Mali) was considered to be a good example of where this type of situation existed (OCP 1992).

6 In 1991 these matters were discussed by the OCP Committee of Sponsoring Agencies (CSA) and later by the OCP's governing body, the Joint Programme Committee (JPC), in the context of the CSA's future work programme of support in socioeconomic development to the OCP Participating Counties. At its 12th session (December 1991) the JPC endorsed a CSA work programme which, inter alia, would provide for the CSA to address some of the broader environmental aspects of settlement and development in the OCP area, and in the first instance, to conduct an environmental impact assessment of agrochemicals. Members of the Ecological Group who attended that JPC session welcomed this new initiative, and stressed the importance of taking a broader view of environmental issues, especially as they related to water resource conservation and management.

7 During 1992, after discussions between the Ecological Group, the CSA and staff of the OCP, a detailed "Draft Proposal for a Pilot Project on Environmental Impact Assessment in the OCP Area" was prepared (OCP 1992), and submitted to the 13th session of the JPC (December. 1992) for approval. This proposal made provision for pilot studies in two river basins, the Upper Léraba Basin and the White Bandama Basin (in Côte d'Ivoire). However, it was decided that, in the first instance, it would be prudent to embark upon a study in only one of the basins, with preference being given to the smaller of the two; the Upper Léraba. Accordingly, in early 1993, The World Bank, on behalf of the CSA, launched a Pilot Project for an environmental impact assessment in the Upper Léraba Basin (hereafter referred to as the Basin), in accordance with the detailed terms of reference that were prepared and approved at the end of 1992 (OCP 1992).

Objectives of the Pilot Project

8 The primary objectives of the Pilot Project were:

- assessment of the present environmental situation in the Basin, with a view to determining potential sources of impact on the aquatic environment,
- quantification of Chemical loads, and assessment of modification to the physical environment,
- identification of simple study methods which would be applicable to other areas, and which could be offered as examples to predict environmental impacts of settlement and development, and to furnish guidelines to minimize those impacts.

9 Although several methodologies for regional environmental impact assessments have been proposed (see, for example, proposals for thematic maps for regional ecotoxicological risk assessment by Herrchen 1994), very few impact assessments have actually been conducted worldwide (one of the few examples being that described by Di Guardo *et al.* 1993a). One of the few assessments so far made in sub-Saharan Africa, a study conducted by Calamari et al. (1992) in the Winam Gulf Basin of Kenya, had some relevance to the Léraba investigation. Although the ecological differences between the two basins were such that direct comparisons could not be made, some elements of the Kenyan experience provided guidance during the planning of the Léraba Pilot Project.

Thus, to the best of the present authors' knowledge, the Léraba environmental impact assessment conducted by them is unique for West Africa, if not for sub-Saharan Africa as a whole. The authors have therefore prepared this account in such a way as to present the Léraba investigation as a model which is applicable to assessments that may be required in other parts of Africa, where environmental conditions are the same or similar.

Project activities

10 The Pilot Project commenced in January 1993, with the collection of geographical, meteorological and hydrometric data, with the assembly of quantitative information on agricultural practices, fertilizers and pesticides, and with the preparation of ecotoxicological profiles of the pesticides known to be used in the Basin. In May 1993, an OCP helicopter was used to make aerial reconnaissance and photographic flights over the Basin, and some adjacent areas. The aerial photographs that were taken were then used to make maps of pre-selected parts of the Basin, and to construct vegetation profiles. Details of these latter activities are given in Annex 1.

11 During the latter months of 1993 and during early 1994, the main Project activities were:

- quantification and assessment of organic loads, nutrients and fertilizers;
- pesticide risk assessment, employing the SoilFug model to predict pesticide runoff;
- computerization and interpretation of cartographic land utilisation data;
- localized vegetation mapping around Léraba Bridge, and assessment of the status of riverine forests;
- sampling of fish and aquatic invertebrates at Léraba Bridge and at two other nearby sites;
- overall assessment of the environmental situation in the Basin, and reporting.

Other sources of information

12 Information on the general geography and climatology of the Basin, as well as technical information on agrochemicals, were taken from the published literature and sources are acknowledged accordingly in the text. Quantitative data on rainfall, hydrometry, soil properties, human and livestock populations, crops, fertilizers and pesticides, were obtained from the appropriate Government offices in Burkina Faso.

13 Historical data on land utilization (especially two maps: "Carte II6-B-24. Situation en 1972. OCP/ECO. J. C. Clanet & P. H. Somé, 1985", and "Carte II6-B-25. Occupation du sol 1983. OCP/ECO. J. P. Hervouet, 1985") were provided by OCP Headquarters in Ouagadougou.

NOTES

1. See Samba 1994 for a recent Overview of the OCP.

2. With the appearance of blackfly resistance to temephos in the early 1980s, the OCP arsenal of operational insecticides was progressively increased. By 1994, six compounds were in regular, rotational use; temephos, *Bacillus thuringiensis* (B.t. H-14), permethrin, carbosulfan, phoxim and pyraclofos (Samba 1994).

2. Description of the Pilot Project

1 The Basin, with a catchment area of approximately 5 376 km^{2}, was located between latitudes 10°-11°N and longitudes 4°55-5°50 W (Figure 2.1), and involved the following countries:

- **Burkina Faso** to the North: the Agro-Pastoral Zones of Sindou (44 500 ha), Loumana (60 000 ha) and western Niangoloko (297 250 ha, of which only about 99 080 ha were located in the Basin), drained mainly by north-bank tributaries of the River Léraba s.s. (L.ss) and the Léraba Occidentale (L.oc), and by the Léraba Orientale (L.or);
- **Côte d'Ivoire** to the South: the Niellé, Diawala and Ouangolodougou Sub-Prefectures of Ferkessédougou Department, drained by south-bank tributaries of the L.ss and the L.oc;
- **Mali** to the West: Kadiolo Cercle, Région de Sikasso, drained by the western tributaries of the L.oc.

2 That part of the Basin subjected to a mediumscale, longitudinal land utilization analysis, was located between latitudes 10°7-10°23 N and longitudes 5°01-5°13 W, in Burkina Faso and Côte d'Ivoire. It was drained mainly by L.ss, but also by L.oc and L.or in the North.

3 The short stretch of L.ss used for local, largescale vegetation mapping was situated at approximately latitude 10°10 N and longitude 5°04 W, in Burkina Faso and Côte d'Ivoire.

Soils

4 The ferralitic/ferruginous soils of the Basin were of two main types (Anon, 1976):

- category 8d: shallow to medium depth soils (<40 cm), gravelly and/or sandy to clayish in the savanna, with danger of erosion;
- category 12: deep soils (>100 cm), alluvial/clayish near the surface and clayish below, immediately adjacent to the large watercourses (broader on the deposition banks of the rivers and narrower on the erosion banks).

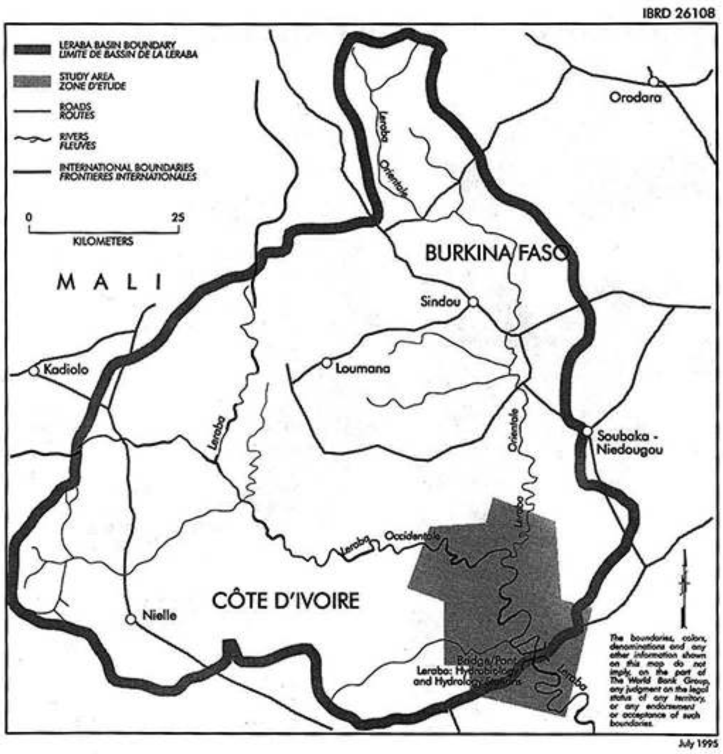
Climate

Prior to the onset of the recent drought, which started in the early 1970s, the mean annual rainfall of the basin was from 1 100 to 1 400 mm. The rainfall statistics presented in Table 2.1, clearly show that in 1992 rainfall was still below the predrought average.

Table 2.1 Rainfall Patterns at Sindou, Loumana and Niangoloko, Burkina Faso, 1992 (mm).

Month	Sindou	Loumana	Niangoloko
I	1.5	7.2	0
II	0	0	0
III	0	0	10.3
IV	13.1	18.8	41.9
V	129.5	152.3	25.2
VI	176.9	226.6	149.7
VII	223.7	243.1	272.4
VIII	205.2	221.6	134.3
IX	126.9	145.8	145.4
X	48.2	80.2	95.7
XI	9.2	3.5	20.8
XII	0	0	0
Totals:	934.2	1 099.1	895.7

2.1 Location and Basic Geographical Features of the Upper Léraba Basin. The Inset Labelled "A" Denotes the Boundaries of the Medium-scale Land Utilization Assessment Area. BURKINA FASO / CÔTE D'IVOIRE LERABA BASIN / BASIN DE LA LERABA



6 The wet season, from April/May to September/October, was characterized by a unimodal precipitation pattern. The mean annual temperature was about 27°C.

Hydrometry

7 Hydrometric data collected at Léraba Bridge hydrology station during 1990 and 1991, are presented in Table 2.2, and are listed on a monthly basis.

Vegetation

8 The Basin was located in the dry, Sudanian vegetation zone (Péron & Zalacain 1975; Vennetier 1978; Traoré & Monnier 1980), which was characterized by savanna/woodland mosaics.

9 The principal climax vegetation types were, fire-tolerant savanna woodland, floodplain grassland, and fire-tender, riverine forest. Where the woodland joined the floodplain grassland, and around the rocky upland plateaus, there were densifications of the vegetation, known as ecotones. A vegetation profile taken across the main Léraba valley is presented in Figure 2.2, to illustrate the relationships between these different vegetation communities, in the absence of human interference. More precise descriptions of each of these main communities are given below.

Woodland Savanna

10 The woodland savanna biotope occupied the largest surface area, and constituted the one most suitable for human settlement and cultivation.

Table 2.2 Mean River Discharge Rates (m^3/s) at Léraba Bridge, 1990 and 1991

Month	1990	1991
I	1.70	1.27
II	0.85	0.85
III	0.47	0.74
IV	0.43	0.61
V	0.96	0.91
VI	3.55	2.74
VII	29.99	32.90
VIII	135.86	147.00
IX	110.58	166.00
X	36.14	30.90
XI	6.69	7.52
XII	3.06	3.12
Annual Means:	27.52	32.88

11 The most typical tree species of the undisturbed woodland, which rarely exceeded a height of 20 m, and seldom created a completely closed canopy, were *Isoberlinia doka, I. dalzielii, Burkea africana* and *Detarium microcarpum.* Other common species included *Uapaca somon, U. togoensis, Erythrophleum guineense, Pterocarpus erinaceus, Afzelia africana,* and *Daniella oliveri.* Species of economic importance and thus common in cultivated areas, included *Borassus aethiopum, Butyrospermum parkii* and *Parkia biglobosa.* In the northern part of the area it was not uncommon to find *Ziziphus mauritiana* and species of *Acacia,* which were more typical of the Sahelian savanna. Likewise in the south of the area some species that were typical of the more southerly Guinea savanna were also encountered, e.g. *Uapaca togoensis, Lophira lanceolata* and *Cussonia barteri.*

12 Perennial grasses included species *of Hyparrhenia, Cymbopogon, Ctenium* and *Loudetia,* while annuals included species of *Andropogon, Pennisetum, Eragrostis* and *Ctenium.* On the top of arid, rocky plateaus the dominant grass was *Sporobalus pectinellus.*

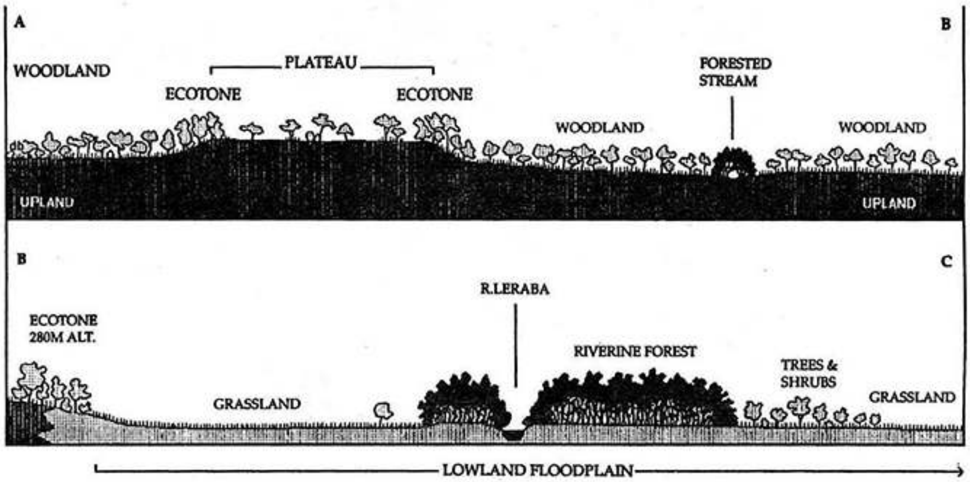
Floodplain Grassland

13 Where the ground sloped downwards below the 280 m contour line, the woodland was replaced by shallow depressions, which constituted the floodplain grasslands. Those grasslands were most extensive along L.ss, but also occurred along L.or and L.oc. and some of their larger tributaries. Being prone to flooding in years of heavy rainfall, or to becoming marshy during years of less heavy rainfall, those grasslands were generally devoid of tree/shrub cover (the tree D. *oliveri* and some fragmented parts of the riverine forest were exceptions) and supported a very limited grass flora; *Vetiveria nigritana* being the only common grass species. Largely because of these factors, the floodplain grasslands were unsuitable for the cultivation of many crop species. However, they were suitable for rain-fed rice, e.g. along the lower L.or, and provided good pasture for livestock.

Riverine Forests

14 The slightly higher ground that formed the banks of the larger rivers supported tall (up to at least 30 m), closed-canopy, evergreen forest. Just north of Léraba Bridge those forests were up to 165 m wide on the deposition banks of the river and as narrow as only a few meters on the erosion banks.

2.2. Schematic Vegetation Profile (Transverse Section) of the Southern Part of the Upper Léraba Basin



15 The most common trees and shrubs found in the riverine forests of the main watercourses (especially L.ss.) were *Berlinia grandiflora, Parinari polyanda, Ficus platyphylla, Khaya senegalensis, Syzygium guineense, Cola cordifolia, Carapa procera, Pentadesma butyracea, Adina microcephala* and *Mucuna pruriens.*

16 Smaller rivers and streams also supported riverine forests, but they were much less extensive and composed of a much smaller number of tree species.

Ethnography

17 Traditionally, the greater part of the Basin was occupied by farmers of the Senoufo ethnic group, with some significant populations of Toussian and Gouin peoples in the extreme eastern part of the area. However, in recent years there had been an influx of other peoples from much further afield in Burkina Faso and Côte d'Ivoire. Lérabadougou village was a case in point. Although this village was dominated by the Senoufo, Toussian and Gouin, there were significant numbers of people of Bobo and Mossi origin (coming from as far away as Ouagadougou).

Agricultural Practices

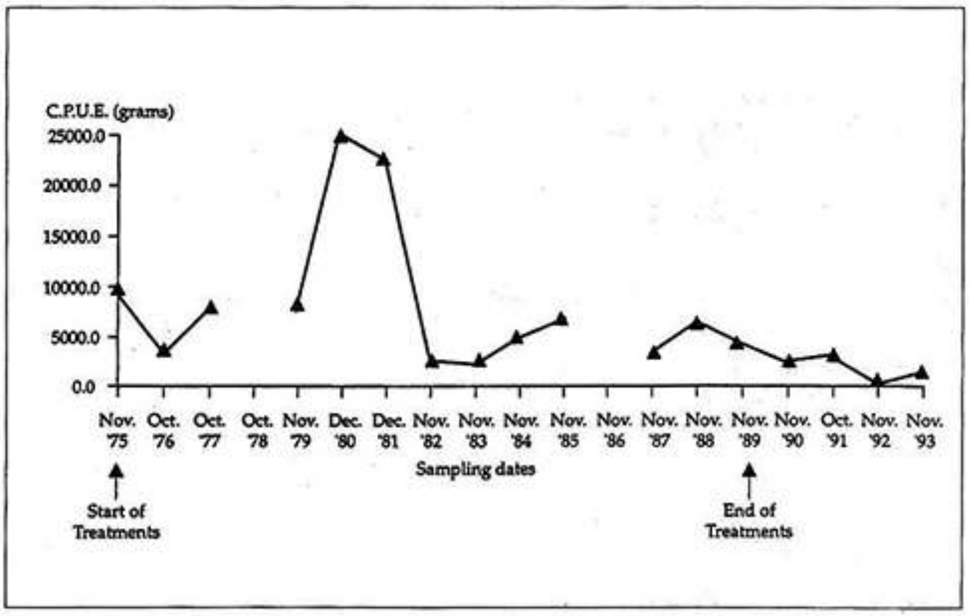
18 The most common subsistence crops were sorghum, maize, millet and yam. Secondary and/or localized crops included manioc, groundnuts, sweet potato and rain-fed rice. Since 1972, cotton had become an increasingly important cash crop, and the only one for which pesticides were used.

19 Livestock included cattle, sheep, goats, pigs and fowl.

Onchocerciasis and its Control

20 In the early 1970s, prior to the introduction of control measures, onchocerciasis was endemic throughout the Pilot Project area. The disease was hyperendemic (prevalence rate>60%) in the north of the Basin, associated with the headwaters of the L.or and L.oc tributaries and in the southeast along L.ss. Between these two important foci, it was mesoendemic (prevalence rate 40-60%) in the west of the L.oc valley, and around the L.oc/L.or/

2.3. Evolution of the Catch Per Unit of Effort of Fish (by Weight) at Léraba Bridge, 1975 to 1993

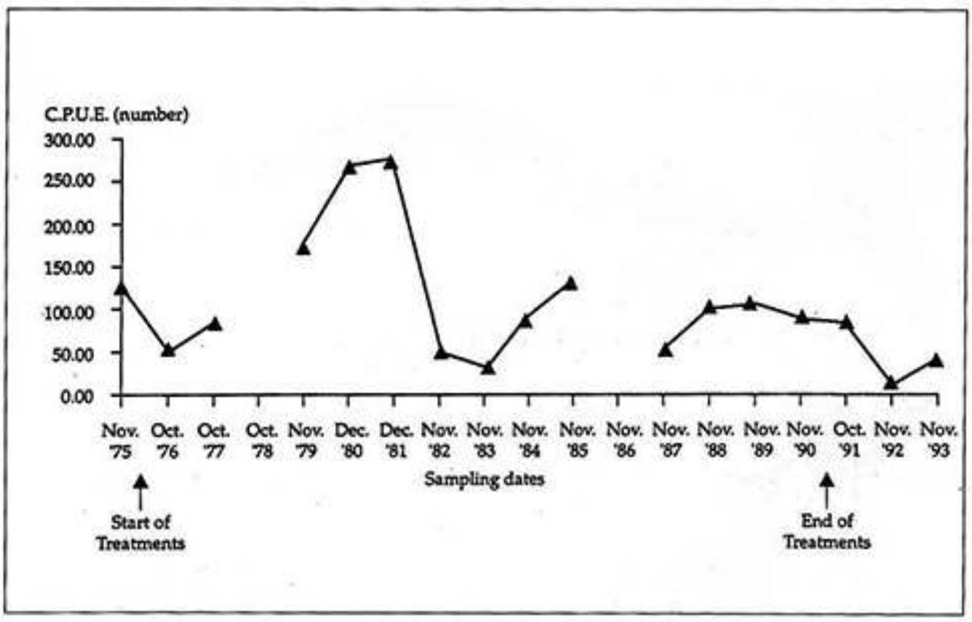


21 L.ss junction. In all other parts of the basin it was hypo-endemic (prevalence rate <40%).

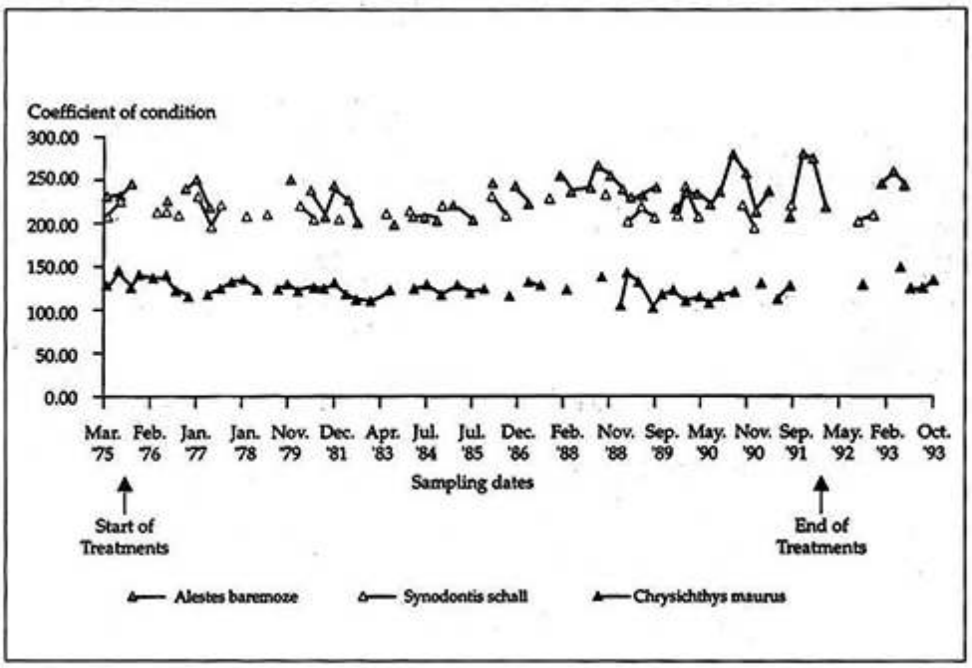
22 In 1974 the OCP came into existence, and the following year onchocerciasis control, through large-scale vector control, commenced in the Pilot Project area and in adjacent parts of Burkina Faso, Côte d'Ivoire, Ghana and Mali. By 1988 (14 years after the commencement of vector control) onchocerciasis had been so successfully controlled in the Basin that it was possible to phase out vector control operations over the next two years.

23 Regular monitoring of the aquatic environments of the OCP area since 1975 showed that, overall, vector control activities had had a very limited impact on the aquatic environments. However, at the Léraba Bridge monitoring station, there was no indication of a rapid recovery of the fish fauna following the cessation of vector control, according to the recorded values of the Catch per Unit of Effort (Figures 2.3 and 2.4). However, the relatively constant levels of the Coefficient of Condition and of the Species Richness over the period 1975 to 1993 (Figures 2.5 and 2.6) implied that the aquatic ecosystem was in a healthy State. These somewhat conflicting views were interpreted as suggesting that, although the ecosystem was in a State of general well-being, there were stress factors exerting an influence, which were not associated with OCP vector control operations. This State of affaire raised important environmental questions which eventually resulted in the decision being taken to conduct the Pilot Study.

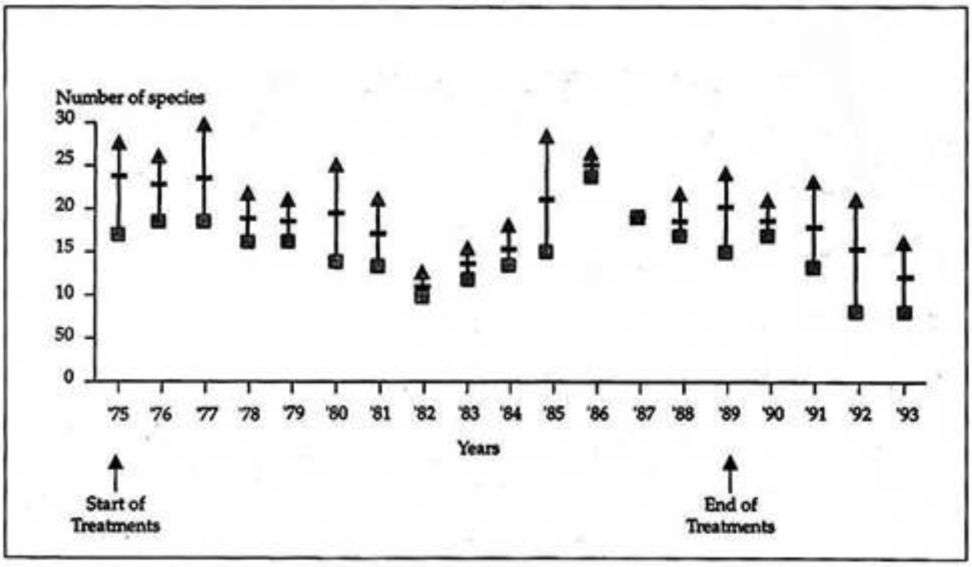
2.4. Evolution of the Catch Per Unit of Effort of Fish (in Numbers) at Léraba Bridge, 1975 to 1993



2.5. Evolution of the Coefficient of Condition of the Principal Species of Fish at Léraba Bridge, 1975 to 1993



2.6. Evolution of the Species Richness of Fish at Léraba Bridge, 1975 to 1993



3. Ecotoxicological Impact of Human Activity at the Basin Scale: Assessment of Chemical Loads

1 The statistics on human and livestock populations, crops, fertilizers and pesticides in the Burkina Faso sector of the Basin, which were required for the hazard assessments, were grouped at source according to agro-pastoral zone (Sindou, Loumana and Niangoloko). While the first two of these zones fitted completely into the upper Léraba basin, the third did not. Only about one third of the Niangoloko zone was actually inside the Basin. Therefore, all the figures received for the Niangoloko zone, were divided by three before being entered into the accompanying tables and before being used for the assessments.

Human and Livestock Populations

2 The numbers of humans and of livestock in the Burkina Faso sector of the Basin, recorded in 1991 and 1989 respectively, are given in Table 3.1.

Crop Types and Surface Areas Cultivated

3 The surface areas (ha) cultivated by the main crops in the Burkina Faso sector of the Basin in 1992/93, are given in Table 3.2. Cotton, the only important cash crop for which pesticides were used, was normally sown at the end of May, and harvested in October.

Use of Fertilizers

4 The fertilizers commonly used in the Basin were "NPK" (a product rich in nitrogen, phosphorus and potassium) and urea. The quantities (kg) of those products used in the Burkina Faso sector of the Basin in 1993, are given in Table 3.3.

Pesticides Used and their Ecotoxicological Profiles

5 Throughout the Basin, pesticides were only used for the protection of cash crops, of which cotton was the only one grown in significant quantity. In general, pesticides were applied twice during the cotton-growing season, to plants which were attacked by aphids (usually attacking in July or in September), fruit-eating caterpillars (usually attacking in September or October), arachnids and whitefly.

Table 3.1 Numbers of Humans and of Livestock in the Burkina Faso Sector of the Basin, 1991 and 1989, respectively

Zone	*Humans*	*Bovines*	*Ovines*	*Caprines*	*Porcines*	*Poultry*
Sindou	27 187	10 000	7 000	12 000	—	50 000
Loumana	35 379	10 000	7 000	4 000	—	55 000
Niangoloko	15 000	8 330	6 000	1 330	1 330	40 000
Totals:	77 566	28 330	20 000	17 330	1 330	145 000

Table 3.2 Main Crop Types and Surface Areas Cultivated (ha) in the Burkina Faso Sector of the Basin, 1992/93

Zone	*Sorghum*	*Millet*	*Maize*	*Total cereals*	*Cotton*
Sindou	5 336	3 304	2 369	12 302	408
Loumana	3 268	1 861	3 184	9 484	4 908
Niangoloko	3 067	880	2 143	6 090	597
Totals:	11 671	6 045	7 696	27 876	5 913

6 The two most commonly used pesticides for cotton protection were:

7 • **Sherdiphos**: containing 30g/litre cypermethrin, 150g/litre dimethoate and 240g/litre triazophos; application rate 30g/ha, 150g/ha and 240g/ha a.i., respectively, 1-2 applications/year.

8 • **Fastac D**: containing 7g/litre alpha-cypermethrin and 133g/litre dimethoate; application rate 15 g/ha and 400 g/ha a.i., respectively, 2-3 applications/year.

9 In addition, **Ciperthion CK** (containing alphacypermethrin and isoxathion) was used on a small scale in some localities (2-3 applications/year).

10 The quantities (litres) of **Sherdiphos and Fastac D** dispensed in the Burkina Faso sector of the Basin in 1993, are given in Table 3.4.

11 Ecotoxicological profiles of the active ingredients of these products were synthesized, on the basis of physico-chemical properties, application rate, toxicology, terrestrial/aquatic/atmospheric fate, environmental distribution, groundwater ubiquity score (GUS), etc. Summaries are given below.

12 • **Cypermethrin**: According to its half-life and GUS values, cypermethrin (a synthetic pyrethroid) appears to be a non-persistent and non-leachable molecule. Its low vapour pressure suggests that it does not volatilize significantly, while its high log Kow value and its low solubility explain its scant affinity for water. Despite its high lipofilicity, it is not bioaccumulative, as the molecule is rapidly metabolized. Cypermethrin exhibits high toxicity to some non-target organisms, such as honey-bees, fish and aquatic crustaceans, but is considered to be only slightly toxic to birds and mammals. Once the molecule has entered the environment, it is reasonable to expect that it will be almost completely absorbed by soil, in which it will degrade in a few days.

13 • **Dimethoate**: This organophosphate compound has very low vapour pressure and log Kow values, which cause it to have a high affinity for water. The molecule can therefore be considered as leachable. It is also non-persistent. Its low lipofilicity excludes the possibility of bioaccumulation. While it is only slightly toxic to mammals and birds, it is highly toxic to non-target invertebrates. It is to be expected that, once introduced into the environment, dimethoate will be readily leached into running water by the first rain.

14 • **Triazophos**: This organophosphate has a low to medium level of persistence, and can be expected to volatilize moderately from soil. However, in view of its limited lipofilicity, triazophos could leach through the soil profile, thus increasing the probability that it could be transported by runoff from rainfall. The molecule is slightly toxic to mammals and birds, moderately to highly toxic to fish, and highly toxic to crustaceans and bees. Once introduced into the environment, it is expected that triazpophos will most readily enter the water compartment, within which degradation will occur. Because of its limited lipofilicity, small quantities of triazophos may also be found in river sediments.

15 From these preliminary assessments, it was concluded that alpha-cypermethrin, dimethoate, and triazophos were non-bioaccumulative and relatively short-lived, with a predicted limited impact on the aquatic environment. However, dimethoate and triazophos could, through leaching and runoff, reach flowing water and have some impact. Accordingly, a more refined evaluation of these compounds should be made with a view to improving this prediction.

Table 3.3 Quantities of Fertilizers (kg) Used in the Burkina Faso Sector of the Basin, 1993

Zone	*NPK*	*Urea*
Sindou	14150	10 000
Loumana	5 000	500
Niangoloko	3 666	3000
Totals:	22 816	13 500

Table 3.4 Quantities (litres) Sherdiphos and Fastac D Dispensed in the Burkina Faso Sector of the Basin, 1993

Zone	*Sherdiphos*	*Fastac D*
Sindou	—	490
Loumana	1960	—
Niangoloko	753	—
Totals:	2 713	490

Table 3.5 Chemical Properties of the Pesticides Entered into the SoilFug Model

Chemical	*Molecular weight*	*Water solubility (mg/L)*	*Vapour pressure (Pascal)*	*Log-Kow*	*Half-life (days)*
Cypermethrin	416.3	0.01	1.60E-5	4.5	20
Dimethoate	229.2	2.5E+4	2.29E-4	-0.47	7
Triazophos	313.3	3.50E+01	1.30E-02	2.93	20

The SoilFug Model and its Application for Estimating Pesticide Runoff

16 For all the pesticide runoff simulations, the SoilFug model of Di Guardo *et al.* (1994a) was used.

17 SoilFug is a model for the prediction of potential surface water contamination derived from pesticide use on agricultural fields. It uses the fugacity approach to a basin scale soil environment and calculates the partition of the Chemical applied to the soil phases and its possible contamination of surface water during the rain events, periods of time starting with a rainfall and ending with the return to the background water level in the adjacent streams. It requires a limited amount of Chemical and environmental data, and it furnishes an average concentration of pesticide in outflowing waters. SoilFug is essentially an unsteady-state but equilibrium event model. This is because it takes into account the disappearance of the Chemical according to different phenomena (degradation, volatilization, runoff), but then calculates the partition among the different phases of the soil according to a Level 1 fugacity calculation (Mackay 1979; Mackay & Paterson 1981) in the rain event periods. It is derived from previously published models (Mackay 1991; Mackay & Stiver 1991; Di Guardo *et al.* 1993b).

18 Briefly, the model considers four different compartments in the soil: soil air, soil water, organic matter and mineral matter. For each of these compartments a capacity (Z) can be calculated and therefore the fugacity can be worked out, once the volume and the Chemical input are known. From the fugacity, Chemical amounts and concentrations in each compartment can be calculated.

Input Data for the SoilFug Model

19 Input data for the Soilfug model fell under four headings—Chemicals, soil properties, water input/output balance, and pesticide treatments—each of which is elaborated below.

Chemicals

20 Physico-chemical properties of the pesticides, together with their half-life in soil, were the first data to be entered into the SoilFug model. The values actually employed are given in Table 3.5. They were drawn from the recent literature (Worthing & Hance 1991, Howard 1991a and 1991b), with the exception of the half-life figures, which were arbitrarily selected on the basis of the extremes of the ranges of variability encountered under different conditions, and taking into account the high temperatures of the Léraba Basin.

Soil Properties

21 The input data required for the soil scenario description of the model were temperature, depth, volume fractions of air and of water in soil at field capacity, organic carbon content, area of the Basin, and the number of simulations, i.e. the number of rain events. The actual values of these parameters, as used for the simulations, are given in Table 3.6. They were selected on the basis of agronomical considerations, e.g. soil depth was derived from data in the BUNASOL Technical Report (N° 85, Ouagadougou, 1993), while the soil itself was assumed to be "clay, structured soil" with a field capacity of pF = 2.5.

Table 3.6 Soil Properties Data Entered into the SoilFug Model

Temperature	28°C
Soil depth	0.2 m
Air volume fraction	0.25
Water volume fraction	0.42
Organic carbon fraction	0.01
Basin area	537 600 ha
Rain events	10

Water Input/Output Balance

22 The mass balance of the water was calculated on the basis of rain events and outflow. The basic data for the water input calculation were the number of rain events, the interval between rain events, the duration of the rain events and the quantity of rain.

23 A rain event was arbitrarily considered to be a period during which consecutive instances of rainfall were separated by no more than 3 days, and each event had to be sufficient to produce a measurable outflow, since the model was required to estimate the quantities of the pesticides in advective water moving out of the basin. In addition, each rain event had to allow for the repartition and the re-equilibration of the Chemicals between the soil phases and the rainwater.

24 Outflows were estimated on the basis of the river discharge rates recorded at Léraba Bridge hydrology station, taking into account the estimated amount of rainfall over the whole basin, extrapolated from the Niangoloko rainfall data, which were considered to be reasonably representative of the Basin.

25 The actual values used for the simulations, based on the Burkina Faso official records for Niangoloko for the year 1992, are given in Table 3.7.

Pesticide Treatments

26 The next parameters necessary for the running of the model were the periods and numbers of pesticide applications, pesticide dosage, days between pesticide applications and rain events, area treated, and the half-life of the pesticides. The actual values of these parameters that were used for the simulation, are given in Table 3.8.

27 In assembling these values for the SoilFug model, it was assumed that:

- cotton was sown at the end of May, that the main growing season was from June to September, and that harvesting was done in October,
- the first pesticide treatment was around 20 July, and the second treatment in late August or in early September,
- treatments with Sherdiphos were made throughout the Basin, as this was the product which accounted for more than 80% of total pesticide consumption,
- there were about 10,000 ha of cotton in the Basin, since there were about 6,000 ha in the Burkina Faso sector, the area of which accounted for about 70% of the total basin.

Table 3.7 Rain Event Data Entered into the SoilFug Model, Niangoloko, 1992

Rain event N°	*Days to rain event*	*Duration of rain event (days)*	*mm "in" (rainfall)*	*mm"out" (outflow)*
1	3	13	149	75
2	4	5	26	13
3	7	6	71	36
4,	4	3	30	15
5	4	1	39	20
6	5	1	16	8
7	6	1	23	3
8	8	5	56	6
9	4	12	80	8
10	19	2	21	2

Table 3.8 Pesticide Treatment Data Entered into the SoilFug Model

Rain event N °	*Area treated (ha)*	*Days before rain event*	*Dosage (kg/ ha)*		*Half-life (days)*
1	10 000	3	0.03	Cypermethrin	20
			0.15	Dimethoate	7

			0.24	Triazophos	20
2–4	—	—	—		—
5	10 000	6	0.03	Cypermethrin	20
			0.15	Dimethoate	7
			0.24	Triazophos	20
6–10	—	—	—		—

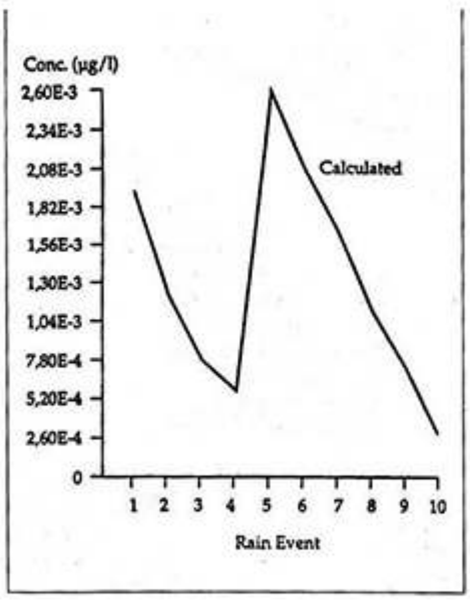
Output of the SoilFug Model

28 From the sets of data described above, the model was able to calculate the average concentrations of the different pesticides in water during each rain event, taking into account not only the partitioning phenomena between soil and water, but also the estimated persistence of each molecule (i.e. its half-life). The final output was a series of graphs showing the predicted concentrations of the different pesticides at the basin outlet which, in this case, was represented by the main River Léraba at Léraba Bridge. These graphs are presented in Figures 3.1, 3.2 and 3.3, and are discussed below.

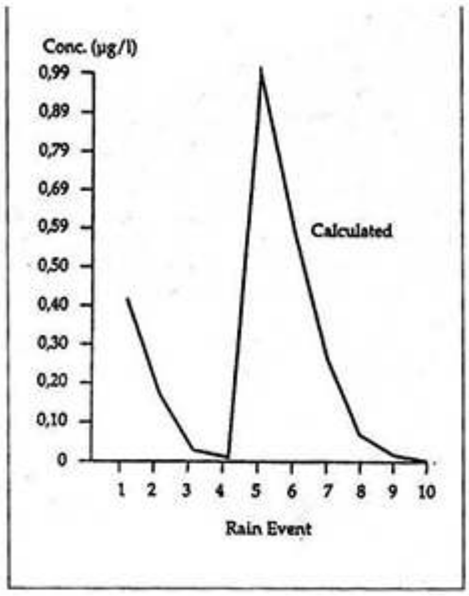
Estimated Impact of the Pesticide Loads

29 The results of the SoilFug model calculations demonstrated that certain quantities of the most common cotton protection pesticides (none of which have bio-accumulation potential) could have been present at the Léraba Bridge outflow, during the period July to November. The ecotoxicological significance of the estimated concentrations of the different Chemicals at the Léraba outflow, is discussed below.

3.1 Graph Showing the Concentration of Cypermethrin at the Léraba Bridge Outlet, Predicted by the SoilFug Model



3.2 Graph Showing the Concentration of Dimethoate at the Léraba Bridge Outlet, Predicted by the SoilFug Model

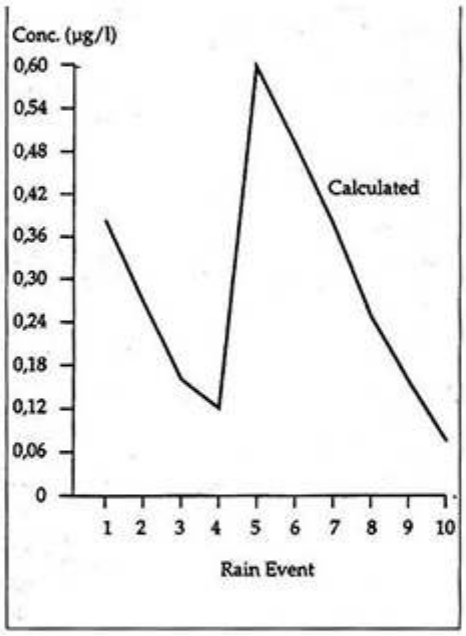


30 **Cypermethrin**: The concentration of cypermethrin peaked at approximately 0.002 µg/litre immediately after the first treatment, and then rapidly declined to 0.0006 µg/litre. After the second treatment, the concentration peaked at about 0.0026 µg/litre, and subsequently declined to 0.0003 µg/litre.

31 According to the literature on cypermethrin, most of the acute toxicity concentrations for the aquatic fauna are on the order of a few µg/litre, i.e., at significantly higher concentrations than those given by the SoilFug model.

32 **Dimethoate**: The concentation of dimethoate peaked at about 0.41 µg/litre immediately after the first treatment, and then declined to 0.015 µg/litre.

3.3 Graph Showing the Concentration of Triazophos at the Léraba Bridge Outlet, Predicted by the SoilFug Model



33 After the second treatment, the concentration peaked at 0.99 µg/litre, and then rapidly declined to 0.001 µg/litre, due to the short half-life of this molecule.

34 Data in the literature on dimethoate indicate that its acute toxicity for the aquatic fauna is in the order of a few mg/litre, i.e. at significantly higher concentrations than those given by the model.

35 **Triazophos**: The concentration of triazophos peaked at approximately 0.39 µg/litre immediately after the first treatment, and then rapidly declined to 0.12 µg/litre. After the second treatment, the concentration peaked at about 0.60 µg/litre, and subsequently declined to 0.07 µg/litre.

36 Data in the literature on triazophos indicate that its acute toxicity for fish is on the order of a few mg/litre, and for aquatic crustaceans in the order of a few µg/litre.

37 If a risk assessment is made, by examining the three molecules simply on the basis of a comparison between the predicted environmental concentrations (PEC) and acute toxicity data for the aquatic fauna, the results are quite positive and give no immediate cause for concern. Even at the highest peaks of concentration, which lasted only a few days, the distances between the two sets of values were in **orders** of magnitude. If, however, a more severe criterion is used to evaluate the model predictions, e.g. by applying the water quality objective for aquatic life established by the EEC Scientific Advisory Committee on Toxicology and Ecotoxicology (CSTE 1994) as 0.01 µg/litre for several organophosphate compounds, one can observe that this more stringent level of concentration could have been exceeded for several days after each pesticide treatment.

38 It can therefore be concluded that on the basis of the acute toxicity criteria considered in the first of the two types of assessment, there is no immediate cause for concern with

regard to the aquatic fauna of the Léraba. However, if the more severe EEC criterion is applied to the assessment, the fact that the criterion was theoretically exceeded for several days has to be accepted as proof of contamination, and should therefore be viewed as an early warning.

Impact of Organic Loads and Nutrients

Overall Conclusions

39 Taking into consideration that there were no sewage Systems, no formal Systems of pit latrines and septic tanks, generally dispersed settlement infrastructures (especially in the Burkina Faso sector), and only moderate human and livestock population densities, it is reasonable to assume that by far the greater proportion of organic materials entering the environment were being degraded at the soil compartment level, over a large surface area, and that the nutrient component of organic waste would be taken up directly by vegetation, before it had a chance to be conveyed into the aquatic environment.

40 Furthermore, from the limited data available on the use of proprietary fertilizers, it would appear that over the Basin as a whole, those products were dispensed in relatively small quantities.

41 For these reasons, it was concluded that there was insufficient justification for making detailed quantitative calculations of the impact of organic matter and nutrients in the Léraba river System.

Point Sources of Organic Loads and Nutrients and their Impact on the Aquatic Environment

42 Early on in the implementation of the Pilot Project the authors realized that although there was insufficient justification for making quantitative estimations of organic substances and nutrients, they should not overlook the possibility that there would be some situations where these substances could have localized impact; for instance, where a village was located close to a main river. In such situations, the habits of bathing, washing clothes and culinary utensils and the watering of livestock at the water's edge, could provide direct point sources of organic substances and nutrients, to such an extent as to modify the quality of the water and consequently, to exert negative effects on aquatic life.

43 In March 1994 the opportunity arose to study this assumption in more detail, by making hydrobiological measurements of the biological condition of the Léraba at Léraba Bridge and comparing the results with those obtained from further downstream and from a comparable nearby river. In the first investigation, fish samples were collected from the Léraba at Léraba Bridge, from the Léraba near Léraba Railway Station (a less densely populated area, some 10 km downstream from Léraba Bridge), and from the River Comoé (near Folonso in a sparsely populated section of the Comoé valley, and some 55 km south-east of Léraba Bridge), for the purpose of calculating CPUE values. The second investigation, conducted at these same sampling points, involved the daytime and night-time collection of drifting aquatic invertebrates, and the calculation of the corresponding

Drift Indexes (number of individuals collected per cubic meter of filtered water during the daytime and night-time).

44 The results of the two investigations are presented in Table 3.9.

Table 3.9 Data on the Biological Condition of Fish and Invertebrates at Three Sampling Sites (Léraba Bridge, Léraba Railway Station and R. Comoé)

	Fish,CPUE[1]		*Invertebrates,D.I.*	
Sampling site	*No.*	*Weight (g)*	*Day*	*Night*
Lér. Bridge	67	1 482	1.4[2]	9.72[2]
Lér. Station	197	7 414	4.53[3]	21.73[3]
R. Comoé	294	12 881	2.93[3]	37.83[3]

1. Data for 1994.
2. Mean data for 1990-1994.
3. Data for 1994.

45 It is evident from these data that, for almost every parameter, the R. Comoé was richer in fish and invertebrates than the Léraba, which was to be expected considering that there was very little human activity around the Comoé sampling site.

46 However, by far the most informative of the data are those relating to the two Léraba sampling sites. In all respects, the fish and invertebrate populations of the Léraba at the Léraba Railway Station site were richer than those at Léraba Bridge, even though the two sampling sites were separated by only about 10 km of river. The explanation proposed for these differences is that the fish and invertebrate populations of the Léraba at Léraba Bridge were influenced by the very close proximity of Lérabadougou village, whose western perimeter was no more than 300 meters from the sampling site. Evidently, the human and livestock populations of Lérabadougou constituted a point source of organic substances and nutrients which had a very localized impact on the aquatic environment, since the biological condition of the river was so much better only a few kilometers downstream at Léraba Railway Station; a much smaller settlement, twice as far from the river as Lérabadougou, and presumably having little or no adverse effect on the adjacent stretch of the river.

4. Ecological Impact of Human Activity using Medium and Local-Scale Assessments

Medium Scale Land Utilization Analyses, 1972, 1983 and 1993

1 From the maps presented in Figures 4.1, 4.2 and 4.3, it is to be noted that there have been appreciable extensions of settlement and of cultivation in the Land Settlement Analysis area (LUA) since 1972, and corresponding decreases in the savanna woodland. More detailed analytical information on these developments is presented below.

The Situation in 1972

2 From the data presented in Figure 4.1, it is evident that in 1972, before onchocerciasis control measures were introduced, farming communities were largely confined to villages on the higher ground, 10 km or more from the center-line of the Léraba valley: Nadera, Létiéfesso, Katierla, Nafona, Mambiré, Titédougou and Danguandougou in Burkina Faso; Mambiadougou, Katierkpon and a village whose name is not known (denoted by NNN on the map) in Côte d'Ivoire. From an epidemiological viewpoint, many of these settlements were classified as "front-line villages," and some of them (such as Danguandougou) suffered the devastating consequences of unabated blinding onchocerciasis.

3 However, there were some isolated plots of cultivation, or smallholdings closer to the main rivers, especially in the hypo-endemic areas that flanked the L.or; much less so in the Southern (hyper-endemic) part of the area where vector Annual Biting Rates (ABR) and onchocerciasis Annual Transmission Potentials (ATP) were extremely high. In this context, it has to be recalled that, according to WHO (1987), an area under vector control could only be considered safe for resettlement if the ABRs were less than 1000 and the ATPs less than 100 for two consecutive years.

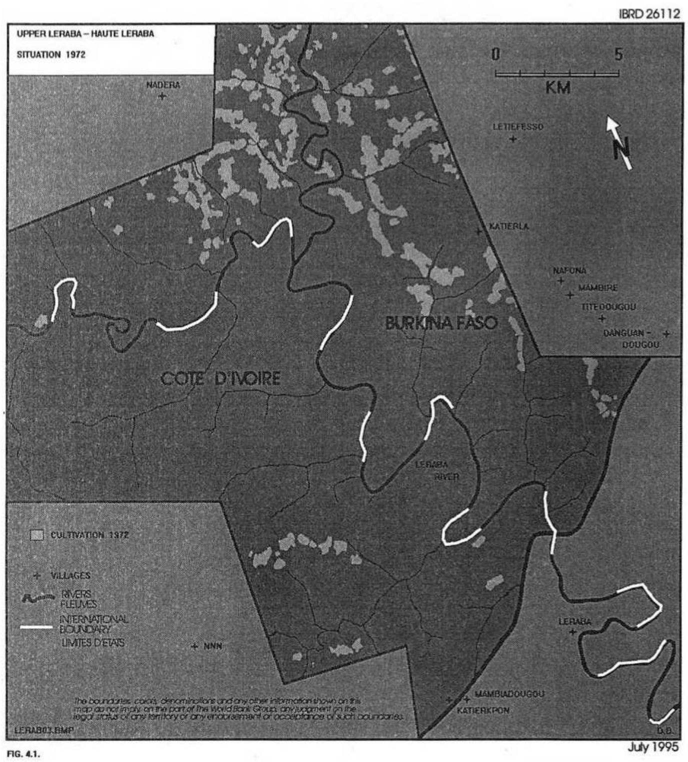
4 Not surprisingly, there were no settlements at the sites of these smallholdings, and we must conclude that, either from fear of contracting onchocerciasis or because of the intensity of blackfly and tsetse bites, these fields were cleared, sown and harvested by farmers who commuted to them from the relative safety of the villages located well away from the rivers.

Table 4.1 ABR and ATP values at Léraba Bridge, 1975-1993

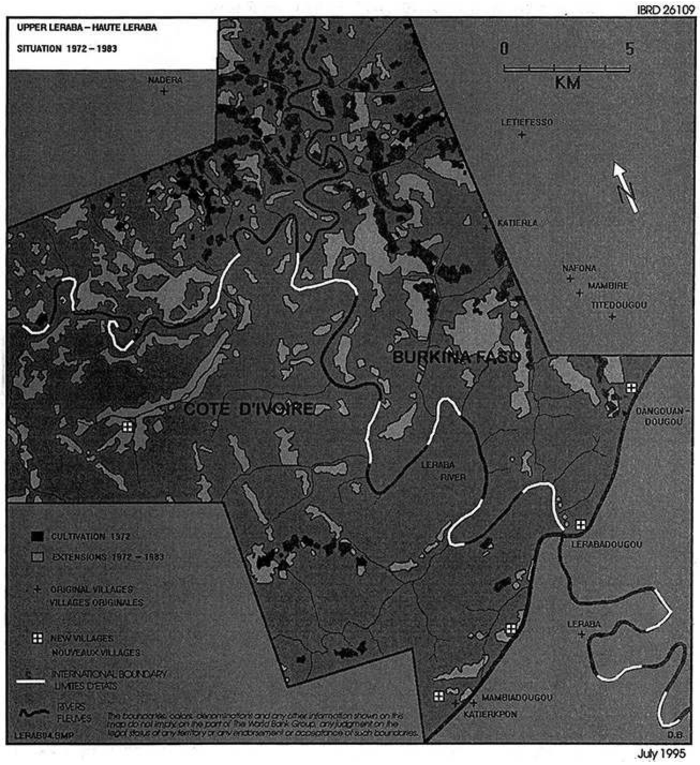
Year	*ABR*	*ATP*	*Year*	*ABR*	*ATP*
1975	26 314	1 263	1984	3 238	71
1976	16 519	1 329	1985	616	0
1977	8 490	1 030	1986	915	21
1978	5 184	422	1987	634	3
1979	3 034	36	1988*	1 033	16
1980	3 485	213	1989*	3 418	4
1981	5 806	98	1990*	14 613	10
1982	2 680	184	1991	21 842	5
1983	1 258	106	1992	31 025	16

• PHASING OUT OF VECTOR CONTROL OPERATIONS.

4.1 Map of the LUA Area Showing Areas Cultivated in 1972. BURKINA FASO/COTE D'IVOIRE. LERABA BASIN / BASSIN DE LA LERABA1972



4.2 Map of the LUA Area Showing Areas Cultivated in 1983. BURKINA FASO/COTE D'IVOIRE. LERABA BASIN / BASSIN DE LA LERABA1972-1983



The Situation in 1983

5 By 1983 the LUA had been subjected to nine years of OCP vector control operations, which had significantly reduced vector density and onchocerciasis transmission. Although ABR and ATP values did not meet the specified requirements for safe resettlement (WHO 1987) until 1986 (see Table 4.1), there had clearly been significant developments between 1972 and 1983, both with regard to settlement and to agriculture (see Figure 4.2).

6 With regard to human settlement, it was noted that over the decade 1972-1983 four new villages came into existence: three in Côte d'Ivoire and one in Burkina Faso (Lérabadougou, which was established in 1981). In addition, Danguandougou had moved to a more southerly location. With one exception, all these villages were located along the main surfaced road connecting Burkina Faso with Côte d'Ivoire. They were all at the center of local zones of more or less intensive cultivation (which also extended to the east of the main road in some cases).

7 The exception was a village located in the headwaters of a southbank tributary of L.oc in the western part of the LUA, about 3 km from L.oc, and about 19 km north of the town of Kaouara (located to the south on the main surfaced road, and too far south to be shown on the maps), to which it was connected by an unsurfaced road. Motorable tracks connecting upstream settlements and cultivated areas with that road were noticeable features of the increased settlement infrastructure in the area.

8 With regard to agricultural development, there had been a significant extension of land under cultivation over the decade preceding 1983; mainly in the savanna woodland, but

also on the banks of some of the Léraba tributaries. The area of land utilized in 1983 was at least 300% larger than that utilized in 1972.

9 It was also noted that smallholdings, distant from any village, still continued to account for the greater proportion of land under cultivation in 1983. However, not all of this land was being managed on a commuter basis, which appeared to have been the case in 1972. By 1983, many of the smallholdings were being tended by family groups living on-site, in small homesteads, interconnected by networks of footpaths.

10 The evidence suggests that one of the factors contributing to the great expansion of agricultural land during the pre-1983 decade, was the introduction of cotton as a cash crop.

The Situation in 1993

11 OCP vector control operations in the LUA ceased in early 1990, meaning that the land utilization pattern observed in early 1993 reflected the situation in the third post-control year. Although the blackfly population of the Léraba Bridge area had returned to its pre-control level of density, the ATP remained at an acceptably low level.

12 Figure 4.3 clearly illustrates that over the period 1983-1993 there had been further significant developments, both with regard to settlement infrastructure and land utilization.

13 It was very noticeable that over the decade preceding 1993, there had been a marked expansion of the area supporting homestead settlements and associated smallholder farms, particularly on the eastern side of L.ss (in Burkina Faso). It was equally apparent that on the western side of L.ss in Côte d'Ivoire, there had been a considerable increase in the number of clearly-defined villages surrounded by zones of intensive cultivation, and an expansion of the road network (not shown on the map). Ten years previously, there had been only one village in the upstream part of this area, i.e. away from the main road. In 1993, there were six.

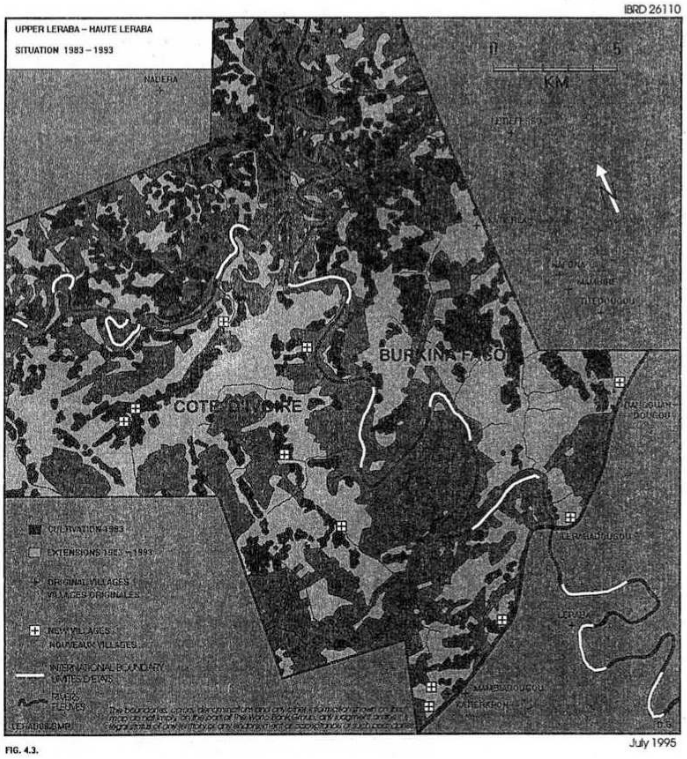
14 It was also noteworthy that some of the villages that were considered as "new" in 1983, had expanded very considerably over the following decade, and in this respect it is pertinent to digress by briefly describing the evolution of Lérabadougou.

15 The first settlers (only a few small family groups) arrived at the Lérabadougou site in 1981, and occupied a surface area of less than one hectare. In early 1993 the village housed about 400 people and covered an area of at least 5 ha. Initially, farmers were only concerned with subsistence crops, but since 1984 became more committed to cotton-growing.

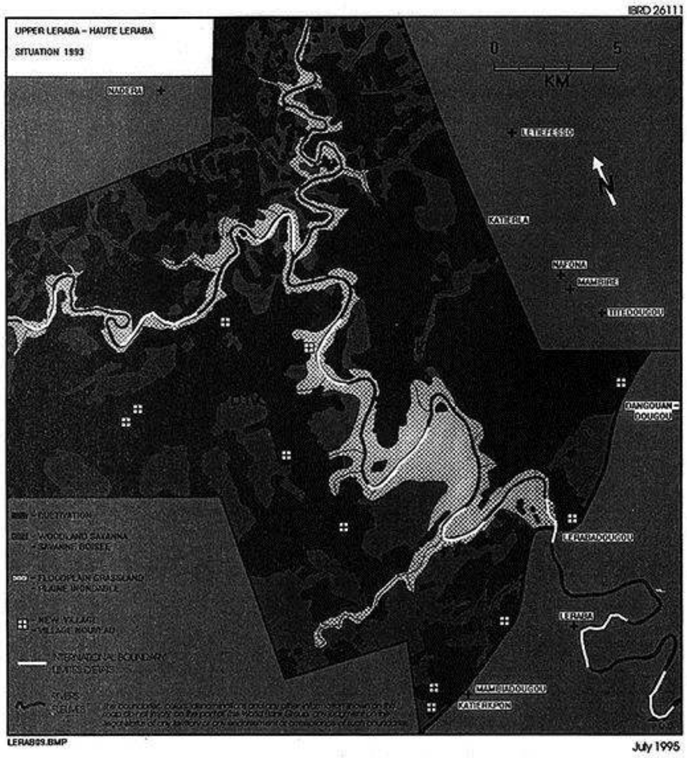
Ecological Significance of the Observed Changes in Land Utilization

16 It is now evident that progressively increasing demands for new farmland, for land for settlements, for wood (for building timber and as domestic fuel) and for improved pasture (to provide thatching material and better grazing for livestock), have resulted in some major ecological changes in the LUA over the last two decades.

4.3 Map of the LUA Area Showing Areas Cultivated in 1993. BURKINA FASO/COTE D'IVOIRE. LERABA BASIN/BASSIN DE LA LERABA1983-1993



4.4 Map of the LUA Area Showing Areas Cultivated, Remnants of Savanna Woodland and Floodplain Grassland, 1993. BURKINA FASO/COTE D'IVOIRE. LERABA BASIN / BASSIN DE LA LERABA1993 LAND USE



17 Physical changes in the two most extensive components of the terrestrial environment, woodland savanna and floodplain grassland, are elaborated below.

18 **Woodland savanna.** From the data presented in Figure 4.4 it is evident that by 1993 as much as 75% of the original savanna woodland had been destroyed or was in the process of becoming deforested, and it is reasonable to suppose that in the not too distant future, the woodland resource will become totally depleted, with three important consequences:

- overall environmental deterioration and a diminution of the carrying capacity of savanna land,
- threatening of the riverine forests of the main Léraba rivers, through settlers' further search for wood,
- increased albedo.

19 **Floodplain grassland.** As illustrated in Figures 4.4 and 4.5, there had been little agricultural incursion into the floodplain grasslands of the main Léraba rivers, except in the case of L.or, where some rice was grown. Certainly in the Southern part of the area there had been only minor incursions, which were not likely to have been of any major environmental consequence.

Medium and Local-Scale Assessments of the Status of Riverine Forests

20 The detailed vegetation analysis presented in Figure 4.6, constitutes the baseline for the stretch of the main Léraba river immediately above and below the OCP Hydrobiology Station at Léraba Bridge, and has been made in accordance with guidelines established by the Ecological Group of the OCP (WHO, 1992). By comparing this map with data to be collected during future vegetation surveys, it will be possible to monitor accurately any changes which may have taken place over the intervening years, and assess the impact of those changes on the aquatic environment.

21 At this juncture, it is appropriate to recall the important roles played by riverine forests in maintaining the ecological well-being and productivity of large perennial rivers (such as the Léraba and its two principal tributaries) and their adjacent floodplain grasslands. Although some (albeit, limited) publicity has been given to the ecological importance of the riverine forests of Africa—see Balk & Koeman (1984), Koeman & Dejoux (1990), and WHO (1992)—in general the reasons for attempting to conserve Africa's riverine forests are not always fully appreciated, either by the general public, or by authorities concerned with agricultural and natural resource development.

22 It cannot be overemphasized that riverine forests, as exemplified by the Léraba, constitute the most concentrated vegetal biomass of the whole river basin ecosystem, arranged like close-fitting protective shields along the rivers. And, it is now well known that, on a regional scale, loss of riverine forest has contributed to increased albedo, with resultant reductions in rainfall, water tables and duration of river flow, and to increased river flow velocity and soil-surface evaporation. The more specific regulative functions of the arboreous and subterranean components of riverine forests are summarized in Annex 2.

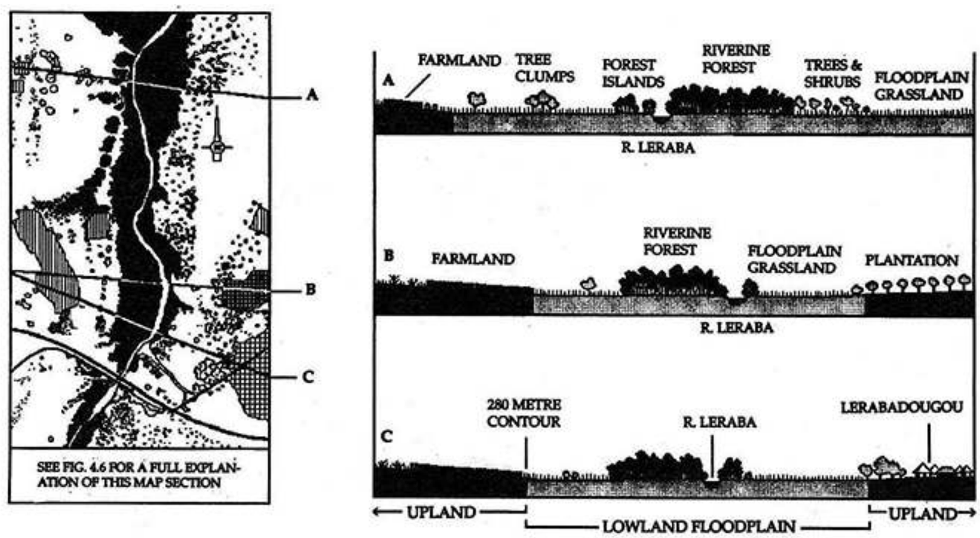
23 From the vegetation analysis presented in Figure 4.6 and on the basis of aerial surveys of the main R. Léraba, it appeared that there had been no significant disturbance of riverine

forest vegetation, except where limited forest clearing had been necessary to make space for the new road bridge to the west of Lérabadougou. This general impression is probably equally applicable to L.or and L.oc.

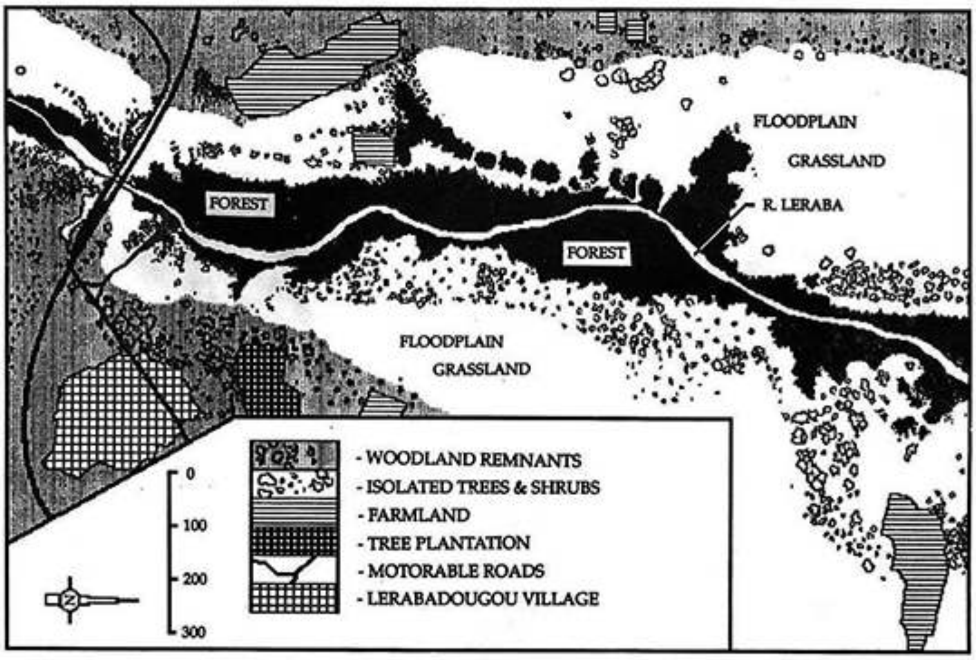
24 The practical implication of these observations is that greater quantities of leachable substances, e.g. agrochemicals, can be safely tolerated in the savanna, than if these forests had been significantly reduced, or destroyed, and it is only to be hoped that a similar situation will remain in ten years time.

25 Unfortunately, the same encouraging conclusion does not apply to the smaller rivers and streams in the LUA. It is clearly discernible in some of the maps that in many parts of the LUA, preference has been given to the cultivation of the banks of the tributaries that drain into the main Léraba rivers. What is not portrayed by the maps, but can easily be seen on aerial photographs, is that some of these tributaries have been so completely cleared of their natural vegetation and are now being so intensively cultivated, that it is now barely possible to discern their courses; at least under dry season conditions. In such instances, there are immediately increased risks of riverbank soil erosion, which can already be detected in a few places, and of agricultural chemicals being conveyed more directly into the main Léraba rivers, during the wet season.

4.5 Schematic Vegetation Profiles of the Léraba Valley Immediately to the North of Léraba Bridge



4.6 Map Showing the Vegetation of the Léraba Valley Adjacent to Léraba Bridge



Conclusions

1 It is concluded that, at least for the time being, organic loads and nutrients in the upper Léraba basin are only of relevance along limited stretches of the main Léraba rivers, where settlements are located close to the river banks and provide point sources of contamination, with localized effects on river water quality and thus on fish and aquatic invertebrates.

2 Assuming that the input data for the SoilFug model were reasonably correct, it can be concluded that contamination of river water by pesticides used for cotton protection, could be clearly detected. From an ecotoxicological viewpoint, it is deduced that the predicted concentrations of pesticides in river water are not yet at levels that could give cause for alarm. However, if for example, the predicted concentrations are considered in relation to the stricter EEC standards for river water quality, one is obliged to take a more serious view of the fact that acceptable levels of concentration were exceeded for several days each year.

3 With regard to the physical environment it can be concluded that about 75% of the original savanna woodland has now been cleared for settlement and for agricultural development (most of it during the last twenty years), or is in the process of being destroyed. Most probably the remaining woodland will disappear in the not too distant future, resulting in overall environmental deterioration and the risk of increased albedo. Likewise, the riverine forests of many of the smaller rivers and streams have been or are being destroyed, resulting in greater risks of increased runoff of chemical substances (pesticide, nutrients, etc.) into the main rivers.

4 On the positive side, it is concluded that there has not been any significant disturbance of the riverine forests and associated floodplains of the main rivers. However, it is to be expected that before long, when wood becomes progressively less readily available in the woodland savanna, the main riverine forests will become threatened.

5 The overall conclusion from the Pilot Project is that on the basis of studies that were limited in both time and scope, it was possible to make valid and meaningful assessments of the Upper Léraba basin in terms of Chemical contamination of river water, of localized changes in the biological condition of the aquatic ecosystem, and of physical degradation of the woodland savanna component of the terrestrial environment.

6 By inference, it is concluded that the first two main objectives of the Pilot Project have been attained, it being understood that it was never the intention of the Project to conduct very precise and exhaustive quantitative surveys of the study area, but rather to identify and briefly explore those elements of the Léraba situation that were most pertinent for further, more intensive investigations, and for management purposes.

7 While it cannot be claimed that the SoilFug runoff model was expected to provide very precise information on river water contamination, the authors concluded that the output data from the model enabled them to obtain a good overall impression of river water contamination in relation to local pesticide use. And here it is important to report that in a very recent study, Di Guardo *et al.* (1994b) provided evidence to support the authors' conviction that their choice of the SoilFug model was well-founded. In a detailed investigation, in which the model was applied to a farm situation, Di Guardo and his colleagues clearly demonstrated that there was a close correlation between **modelled** and **measured** concentrations of several pesticides in surface water.

8 Finally, the authors consider that in relation to the Pilot Project's third main objective, their investigations have clearly demonstrated that the employed techniques, using input data that were very basic, but readily available from national agricultural and development authorities and from the application of standard hydrobiological techniques, can be used as examples of quick and reliable environmental impact assessment methodologies for use under similar circumstances in other areas.

Annex 1. Summary of Cartographic Activities and Vegetation Profile Construction

Medium-scale Mapping: Land Utilization Patterns in the Upper Léraba Pilot Study Area in Burkina Faso and Côte d'Ivoire

1 The first map prepared, concerning L.ss and the lower stretches of L.oc and L.or, was at scale 1: 50 000, and in black/white. It covered an area of *ca* 400 km^2 upstream of Léraba Bridge, and embraced total valley and channel lengths of 36.3 km and 75.5 km, respectively. It displayed the cultivated/deforested areas discernible in 1993, together with information on the land utilization situations in 1972 and 1983.

2 Partly because it was impracticable to include such a large map in this paper, and partly because there was an unavoidable loss of definition in photocopy reductions of the original map, all land utilization data were computerized according to year, so that they were in a more manageable format for analysis and for inclusion in this paper (maps in Figures 4.1 to 4.6). Although those maps are presented in black/white, the originals were constructed in color, so that large format prints could be used for demonstration and training purposes.

Local-scale Mapping: Vegetation of the Léraba Valley Upstream of the Road Bridge

3 This map was originally constructed at scale 1:2 500, to facilitate longitudinal studies on vegetational changes, especially with regard to riverine forest. The map covered 1.86 km of valley within an area of approximately 1.5 km^2. River channel lengths were, 1.73 km above Léraba Bridge, and 0.26 km below it. The map boundary was purposely extended towards the southeast, to allow the whole of Lérabadougou village to be included.

4 The main feature of the map, the zonation of vegetation communities (generally parallel to the center line of the watercourse), which was a typical feature of rivers in the Sudanian savanna vegetation zone (Molyneux *et al,* 1978), were clearly depicted. The map also showed some areas of cultivation; generally located in woodland above the 280m contour line, and not usually extending into the floodplain grasslands.

5 This map was also reduced and re-drawn to enable it to be included in this paper, as Figure 4.6.

Vegetation Profiles

6 On the basis of the aerial photographs that were used to prepare the maps described above, some schematic vegetation profiles were developed across and at right angles to the course of the main Léraba river. These are depicted in Figures 2.2 and 4.5, and discussed in the main body of the paper.

7 In each of the illustrated profiles, the lower, shaded part represents the substrate. Its upper margin represents the surface of the ground, which supports various types of vegetation (trees, shrubs, grasses, etc) and which form different types of botanical communities. For the sake of clarity, the vertical scale has been exaggerated.

8 The transects used for the construction of the profiles presented in Figure 4.5 are marked as straight lines (A-A, B-B and C-C) on the map shown in Figure 4.6.

Annex 2. Summary of the Regulative Functions of Riverine Forests

Arboreous component

1 The sheer size of the trees and the compactness of the forest canopy reduce the direct impact of solar radiation onto the surface of the river and the river bank, which has four beneficial effects:

- helps to maintain river water at the temperature which is optimal for the aquatic fauna and flora,
- contributes towards the maintenance of an appropriate level of dissolved oxygen in the water,
- reduces the evaporation rate of the river water,
- assists in maintaining optimal temperatures and relative humidifies in the understory part of the forest, thus creating ideal habitats and refugia for many animal species; both invertebrates (including important insect predators and parasites) and small vertebrates (especially birds, small mammals and reptiles).

2 Fall-out of organic debris (leaves, bark, flowers and fruits) and other biological material (terrestrial invertebrates, especially insects), provides organic nutrients, upon which fish and aquatic invertebrates are directly or indirectly dependent.

Subterranean component

3 The concentrated mesh of root Systems has two main functions:

- it provides physical stability to the river bank, which prevents bank erosion and protects the adjacent floodplain,
- it filters ground water that seeps from the savanna into the river, and by so doing, retains nitrates, other inorganic compounds and some organic agrochemicals.

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A pictorial guide to the chromosomal identification of members of the Simulium damnosum Theobald complex in West Africa with particular reference to the Onchocerciasis Control Programme Area

Daniel Adjei Boakye

Introduction

1 *Simulium damnosum* Theobald the main vectors of human onchocerciasis in Africa has been found to be a complex of sibling species based on the analysis of the banding sequence on the polytene chromosomes from the larval salivary glands (Dunbar, 1966; Vajime and Dunbar. 1975; Quillévéré, 1975). Eight species were formally described from West Africa: *S. squamosum* Enderlein, *S. yahense, S. sanctipauli, S. soubrense, S. damnosum* s.s., *S. sirbanum, S. sudanense* and *S. dieguerense* (Vajime and Dunbar. 1975). Out of these the specific status of *S. sudanense* was not unequivocally accepted (Bedo, 1977) and recently, Vajime (1989) has synonymised it with *S. sirbanum. S. sanctipauli* and *S. soubrense* have been reclassified under a single sub-complex *S. sanctipauli* s.l. (Post, 1986). The *S. sanctipauli* sub-complex comprises of four main species *S. sanctipauli, S. soubrense, S. leonense* and *S. konkourense* and their forms (Boakye et al.. 1993). All the West African species are vectors of onchocerciasis but with varying vectorial capacities hence any control based on selective larvicidal treatments require reliable species identification.

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2 Methods of identifying adults have been beset with many difficulties (Dang and Peterson, 1980; Garnis et al., 1982) such that larval cytotaxonomy remains the most reliable means of identification. However, since the pioneering work of Vajime and Dunbar (1975) and Quillévéré (1975), nothing comprehensive has been published. Other authors have concentrated only on the variations encountered in specific groups and reclassifying some of the inversions of earlier works (Quilévéré et al., 1982; Meredith et al., 1983; Post. 1982.1986; Boakye and Mosha, 1988; Surtees et al., 1988; Boakye et al., 1993). This array of information is scattered in different journals and makes it difficult for students and beginners in *S. damnosum* s.l. cytotaxonomy to correctly identify diagnostic inversions for the different cytospecies. It has also resulted in the proliferation of different modes of naming inversions and their interpretation. Furthermore, information has been collected over the years in the Onchocerciasis Control Programme in West Africa (OCP) which has not been published.

3 This presentation collates information from various publications and new data on the various inversions used in the identification of the different cytotypes of the *S. damnosum* s.1. in West Africa with particular reference to the OCP area. Pictures of larval polytene chromosomes showing the important inversions in the different cytospecies are presented to serve as a training guide for *S. damnosum* s. 1. cytotaxonomy.

The chromosomes

4 The members of the *S. damnosum* complex have a chromosome complement of three pairs (n = 3). From the longest and decreasing in size, they are numbered; one (I), two (II) and three (III) each with distinct long (L) and short (S) arms based on the position of the centromere (Fig. **1 a**). From the tip of the short arm of chromosome one (IS) to the tip of the long arm of chromosome three (IIIL) is referred to as the total complement length (TCL) and given a value of one hundred. Each of the three chromosomes is arbitrarily subdivided into units based on its proportion relative to the total complement length. Chromosome I is 42% TCL and hence divided into 42 sections numbered 1 – 42; Chromosome II is 30% TCL. divided into 30 sections (43–72) and chromosome III 28% TCL (73–100) (Vajime and Dunbar. 1975).

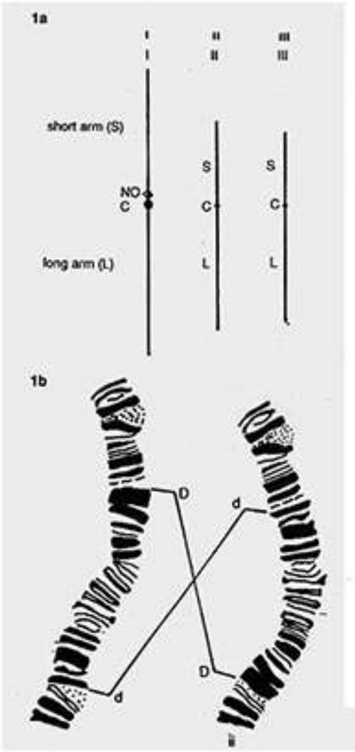


Fig. 1 A Idiogramatic representation of the chromosome complement of members of the *S. damnosum* s.l. in West Africa.
Fig. 1 B Diagramatic representation of an inversion.

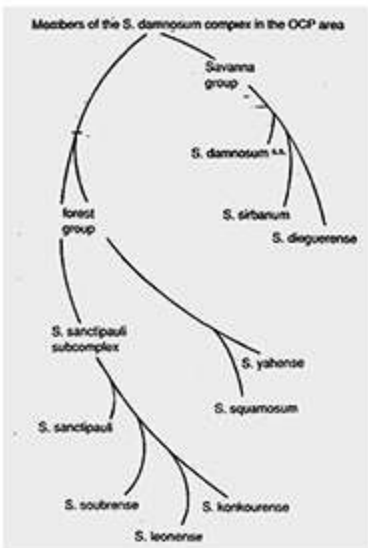


Fig. 2 Cytospecies of the *S. damnosum* complex in the OCP area.

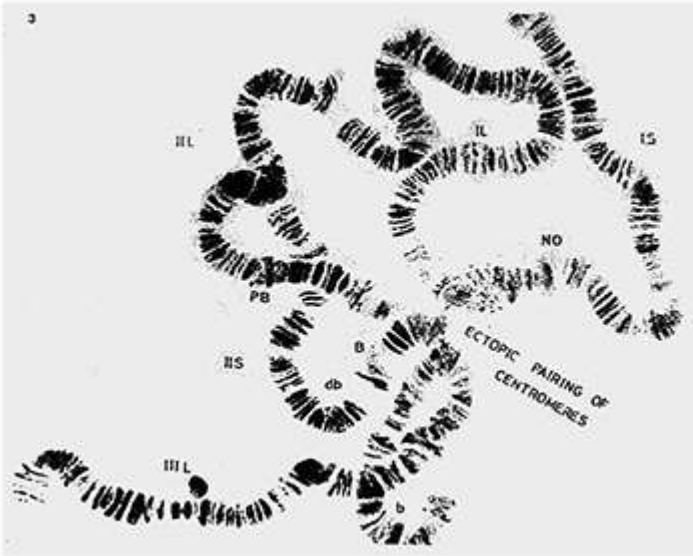


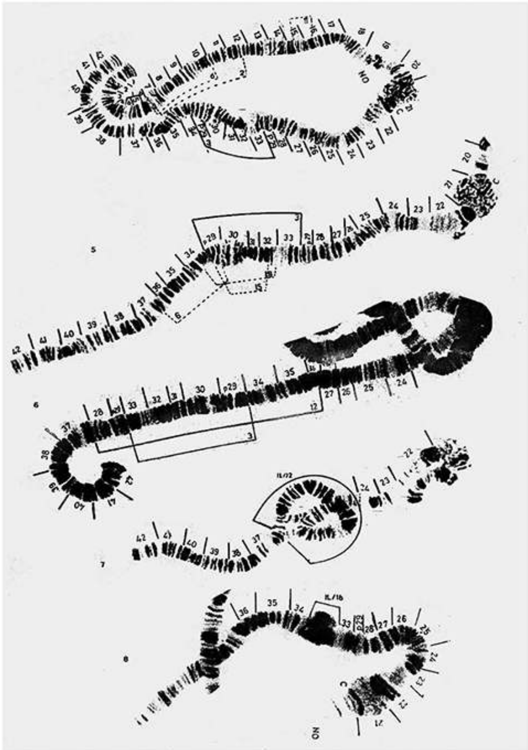
Fig. 3 Full chromosome complément of S. *squamosum* showing the ectopic pairing of the centromeres. B = Ring of Balbiani. db = double bulb. PB = para-Balbiani. b = blister. C = centromere.

5 The giant chromosomes show morphological detail in their banding patterns suitable for use in identification. Members of the *S. damnosum* species complex recorded so far have essentially the same chromosomal bands but these can be rearranged in fixed inversions, complement of floating inversions, sex determining Systems and micromorphological characters such as band dimorphisms.

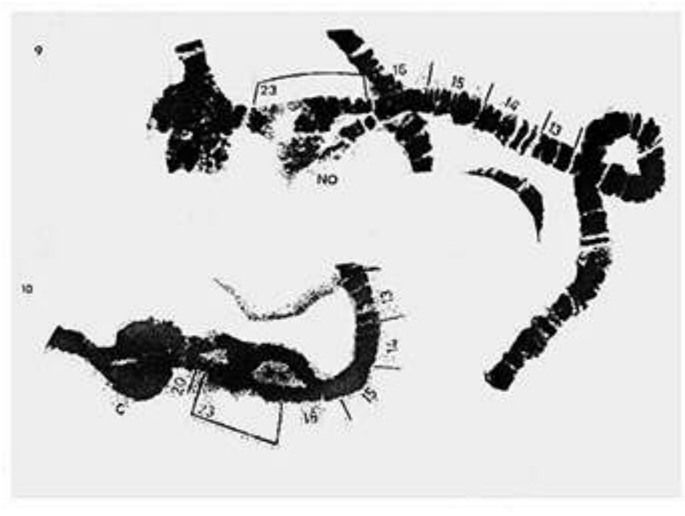
Inversions

6 These are chromosomal mutations characterized by the reversai of a chromosome segment (Ayala and Kiger, 1984) (Fig. **1b**). In *S. damnosum* s.l. cytotaxonomy, the inversions are given numbers, for example, 1IL-7 (Vajime and Dunbar, 1975) or letters, IIL-A (Post, 1986) based on an arbitrarily chosen standard sequence. The chromosomal sequence of *S. squamosum* is taken as the standard for members of the *S. damnosum* complex in West Africa.

7 Inversions may occur in a homozygous or heterozygous condition. The latter is more easily seen due to either a local non-pairing or reversed loops of the normally paired double structure (e.g. IL/12, Fig. 7). Inversions are termed fixed or floating. Fixed inversions are inter-specific and diagnostic to a particular species. Therefore, all the members of the same species have a particular fixed inversion. Floating inversions are intraspecific being absent, present homozygously or heterozygously in different individuals of the same species.



Figs. 4–8 Chromosome 1 (*S. squamosum/S. yahense).* (**4**) Standard sequence for members of the *S. damnosum* s.l. in west Africa. S-1/1, IL-3/3. (**5**) Long arm of chromosome I; IL-3/3. (**6**) Long arm of chromosome I; IL-3.12/3.12. (**7**) Long arm of chromosome I; IL-3/3.12. (**8**) Long arm of chromosome I; 3/3.18. C = centromere. NO = nucleolar organiser.



Figs. 9–10 Chromosome IS of *S. squamosum* showing IS-23 and altered centromere region.

Chromosomal markers

8 The morphology of the chromosomes presents characters useful in determining

1. the various chromosomes (I, II and III)
2. the long and short arms of each chromosome and
3. different inversion sequences.

9 Whilst some markers such as the centromeres are obvious, others require a knowledge of the subdivisions of a particular chromosome. A very important observation is that a chromosome may be stretched out, bent over itself or contracted during preparation thus giving the impression that the inversion markers differ on chromosomes from different cells.

1. Chromosome I

10 This is very-often easily identified by the length It is the longest chromosome but does not always appear as such due to preparation procedures. The long and short arms are of almost equal length. Another character of use is a large bulbular area about halfway between the long and short arms within which is located the centromere (C) (Fig. 3). The short arm (IS) is differentiated by the presence of a nucleolar organiser (NO). Generally, the chromosome appears to be weak or broken at the NO (Fig. 3). The bands forming the sub-division number 12 on IS (Fig. 4) and numbers 34–35 and 38–39 on IL (Fig. 5) are good landmarks for recogmzing most of the inversions on chromosome I.

Chromosome II

11 An easily identifiable centromere separates the chromosome into a definite short and long arm-acrocentric chromosome. On the short arm is located a pufiy area usually in the form of two superinposed circles. These are shown on Fig. 3; the ring of Balbiani (B) and an euchromatic section termed double bubble (db). Also on the short arm but very close to the centromere are two prominent dark bands (segment 54, Fig. 11).

12 The long arm (IIL) contains a marker which is very important or routine species identification-the para-Balbiani (PB) in the sixty-first segment (Fig. 11). Also important in identifying the different cytotypes are the series of bands comprising the segments 64, 65, 66 and 67. Most of the inversions-on IIL include these bands. Other landmarks are the segments 70 and 72.

13 The short arm of chromosome II shows very little variation in inversions between the various cytospecies in the *S. damnosum* complex.–It is therefore-not very useful in-routine identifications.

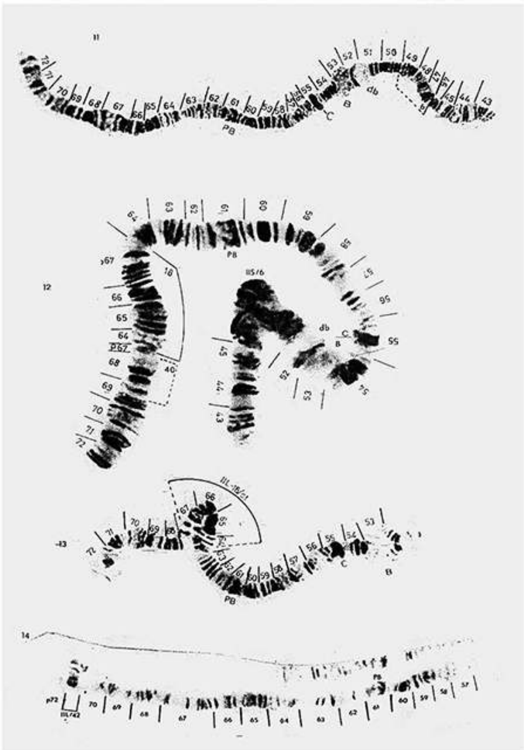
3. Chromosome III

14 This is also acrocentric with an easily iden tifîahle centromere that separates the short and longarms. A recognisable pufiy euchromatic region termed the blister (b) is found towards the end of short arm (Fig. 3). Important segments are; 86–87 and 94–95–96 (Fig. 16). Similar to the second chromosome, the short arm of chromosome III shows little variation, appearing uniform in all of the cytospecies.

Description of cytospecies

15 The members of the *S. damnosum* complex described below are shown in Fig. 2. They all share the fixed inversions IS-1 and IL-3. The designation of forest and savanna is only an indication of the areas where the cytotypes are usually found. The descriptions given below should not be considered as definitive for each species along its entire

distributional range. As more samples are examined, new inversions continue to be recorded as either fixed or polymorphic in various populations. On the figures, only break-points of the rare inversions are given and not the actual inversions except where they happened to occur on the same chromosome with a more common inversion. When only break-points are given, they are denoted by broken lines.



Figs. 11–14 Chromosome II of *S.squamosum/S.yahense* (**11**). Standard sequence of Chromosome II for West.African *S. damnosum* s.l. as found in *S. squamosum.* (**12**) Chromosome II of S. *yahense;* IIL-18/18, IIS/6. (**13**) Long arm of *S. yahense,* IIL/18. (**14**) Long arm of *S. squamosum;* IIL/42. B = Ring of Balbiani, db = double bulb. PB = para-Balbiani. C = centromere.

Fig. 15 Long arm of chromosome II of *S. yahense* heterozygous for IIL-61 (IIL-18/18.61). C = centromere.

Simulium squamosum Enderlein

16 This is the chosen standard for the West African members of the *S. damnosum* complex. It is the closest of this group to the East African forms from which it is differentiated by the

inversions IS-1 and IL-3 (Fig. 4 and 5). IL-3 has been found to be polymorphic in some populations of this species in Nigeria and Guinea (Boakye, unpubUshed; Dunbar, unpublished information to Dr. S. E. O. Meredith).

17 Chromosomes I, II and III of *S. squamosum* have been found to be joined together at their centromeres (ectopic pairing) in most areas (Fig. 3). Vajime and Dunbar (1975) reported-sex determination associated with an alteration of the centromere region of chromosome.I in Cameroon and Burkina Faso. However, in Guinea. Sierra Leone and Western Côte d'Ivoire, sex determination is related to a band dimorphism of the centromere of chromosome III (SCIIL Fig. 17). About 80% of males show this band polymorphism while the majority of females lack it In certain populations of this species identified from Ghana, the band dimorphism is present simultenously with an inversion designated IIIL-41 (Fig. 18). Meredith (unpublished report to the WHO/OCP, 1987) also reported different sex chromosomes in *S. squamosum* from Central Africa. Thus sex determination is associated with different chromosomes in different populations.

18 Common inversions recorded in this species are; IS-2, IS-6, (Fig. **4**); IS-23 (Figs. **9** and **10**), IL-6 (Fig. **5**), IL-12 (Figs. **6** and **7**), IIS-6 (Figs. **11** and **12**), IIL-18 (Figs. **12** and **13**), IIL-41 (Fig. **14**), IIIL-28, IIIL-22 (Fig. **16**), IIIL-82 (Fig. **19**) and 1IIL-42 (Fig. **20**). The centromeres for the second and third chromosomes are much prominent in size in this species and *S. yahense* than for the other members of the complex in West Africa. The proportions of floating inversions are not uniform along the entire distributional range of the species.

19 *S. squamosum* can be found in the forest zone breeding in small rivers. However, it has occasionally been identified from some medium to big rivers in the savanna zone.

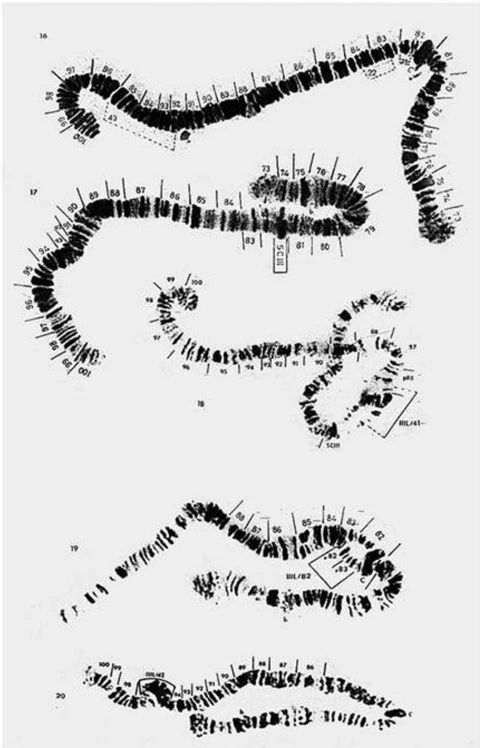
Simulium yahense Vajime and Dunbar

20 This species is found breeding in small rivers in the forest zone. It is rarely found in the savanna except in forested and mountainous enclaves. Its distribution is therefore pacthy across the OCP area. Recently, its distribution has been found to be extending in Southern Ghana due to the insecticidal control of the other cytospecies by the OCP (Fiasorgbor et al., 1992).

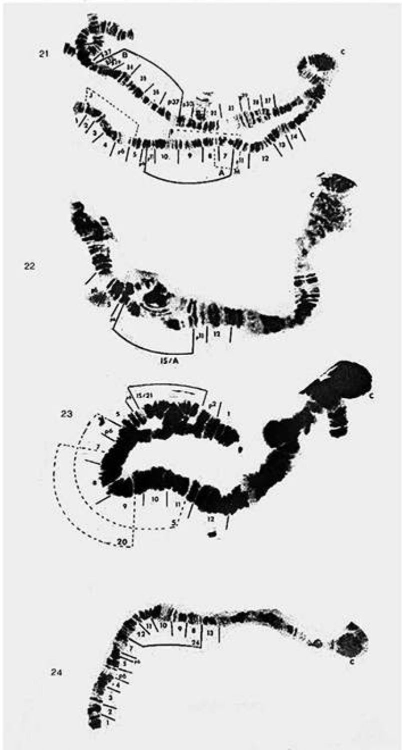
21 This is the closest of the West African *S. damnosum* s.l. to the chosen standard *S. squamosum.* The two species do not show any fixed inversion differences (Quillévéré, 1975) casting some initial doubt as to their separate specific identity. However, their specific status has been confirmed by iso-enzyme electrophoresis (Meredith and Townson, 1981; Garms and Zillmann, 1984, Thompson et al., 1990).

22 Chromosomally, *S. yahense* is distinguished from *S. squamosum* by the inversion IIL-18. About 97% of the females and 54% of males were found homozygous for the inversion IIL-18 (i.e. IIL-18/18) and the remaining 3% females and 46% males were IIL/18 (i.e. heterozygous; IIL-st/18) (Vajime and Dunbar, 1975). The homozygous and heterozygous conditions of IIL-18 are shown in Fig. 12 and 13. This situation has been found to differ in various populations. In Togo, Benin, Ghana and parts of the Côte d'Ivoire, both males and females are essentially IIL-18 homozygotes. In the Fouta Djallon areas of Guinea, males are 100% IIL-st/18 and females IIL-18/18. In Eastern Guinea and Sierra Leone, some populations are found with IIL/18 occurring equally in males and females. This creates an identification problem when this species is found sympatric with S. *squamosum* since the latter has IIL-18 as a floating inversion.

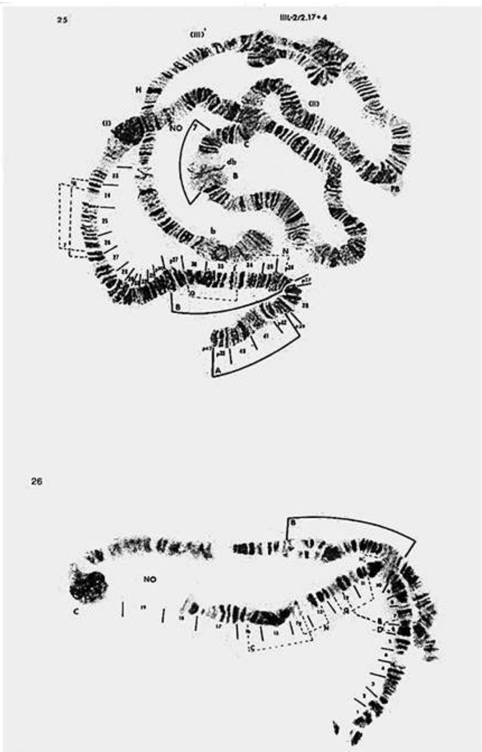
23 Other inversions found in *S. yahense* populations are; IS-11 (Fig. **4**), IL-12 (Figs. **6** and **7**), IL-15, IL-18 (Figs.**5** and **8**), IIS-6 (Figs. **11** and **2**), IIL-40 (Fig. **12**), IIL-61 (Fig. **15**), SCffl (Fig. **17**), IIIL-22, IIIL-43 and IIIL-28 (Fig. **16**). Some populations also show ectopic pairing of the centromeres of the three chromosomes (Fig. **3**).



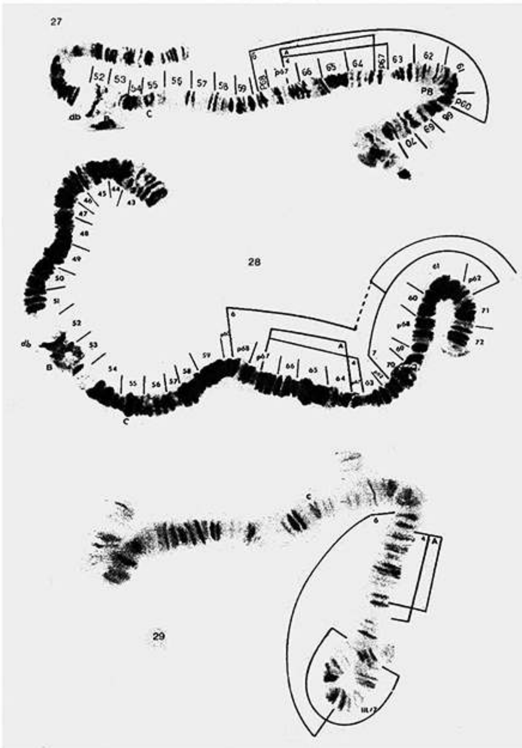
Figs. 16–20 Chromosome III of *S. sqamosum/S. yahense.* (**16**) Standard sequençe of Chromosome III for West African *S. damnosum* s.l. as found in *S. squamosum/S. yahense.* (**17**) band dimorphism of contromere of chromosome III; SCI. (**18**) Chromosome III of *S. squamosum* showing band dimorphism of the centromere and a heterozygous inversion-IIIDL/41. (**19**) Chromosome III of *S. squamosum;* IIIL/82. (**20**) Chromosome III of *S. squamosum;* IIIL/42. b = blister. C = centromere.



Figs. 21–24 Chromosome I of *S. sanctipauli* subcomplex. (**21**) Chromosome I with IS-A and IL-B homozygous; IS-A/A, IL-B/B. (**22**) Short arm showing IS-A heterozygous. (**23**) Short arm with IS-21 heterouzygous. (**24**) Short arm with IS-24. C = centromere.



Figs. 25–26 (**25**) Full karyotype of *S. leonense* (S. *soubrense* B); IS-A/A, IL-B.A/B.A, IIS-7/7. IIL-4.6.D.7/4.6.D.7. IIIL-2/2.17.4. (**26**) Chromosome I of *S. sanctipauli* s.l.; IL-B/B. B = Ring of Balbiani. db = double bulb. PB = para-Balbiani. b = blister. C = centromere. NO = nucleolar organiser.



Figs. 27–29 Chromosome II of *S. sanctipauli* s.l. (**27**) Chromosome II of *S. sanctipauli:* IIL-4.6A/4.6.A. (**28**) Chromosome II of *S. sanctipauli;* IIL-4.6.A.7/4.6.A.7. (**29**) Chromosome II of *S. sanctipauli;* IIL-4.6.A/4.6.A.7. B = Ring of Balbiani, db = double bulb. PB = para-Balbiani. C = centromere.

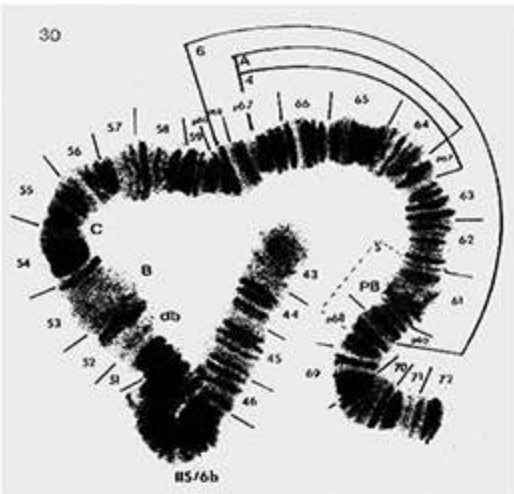


Fig. 30 Chromosome II of *S. sanctipauli* IIS/6b, IIL-4.6.A/4.6.A. B = Ring of Balbiani. db = double bulb. PB = para-Balbiani. C = centromere

Simulium sanctipauli s.l.

24 The descriptions of *S. sanctipauli* and *S. soubrense* (Vajime and Dunbar, 1975) have been revised by Post (1986) and Boakye et al. (1993). While formerly thought to consist of two cytospecies, Boakye et al. (1993) now describe four species under this sub-complex. These are; *Simulium sanctipauli, Simulium soubrense, Simulium leonense* and *Simulium konkourense.*

25 In the initial description of *S. sanctipauli* and *S. soubrense,* the two species were separated from *S. squamosum* by the Inversions IL-6, IIL-4.6 and the triple inversion IIIL-2.17.4. They were separated from each other by the inversion IIL-7 (Vajime and Dunbar, 1975). Quillévéré (1975) was the first to raise objection to the separation of the two species due to the presence of high proportions of IIL-7 heterozygotes in some populations in the Côte d'Ivoire. Similar observations were made in Togo (Meredith et al., 1983). Post (1986) in his revision maintained the inversions IIL-4.6 and IIIL-2.17.4 as diagnostic for the sub-complex but reported that the breakpoints of the inversion IL-6 were wrongly demarcated and that a double inversion designated IL-PQ was rather present. He further described new inversions that could be used to separate the two cytospecies from each other. Boakye et al. (1993), State that the break-points for IL-6 is incorrect but maintain that a single inversion difference and not the double inversion IL-PQ normally occurred in *S. sanctipauli* s.l. This inversion is apparently similar to IL-B of Post (1986) and it is therefore designated as such. However, the inversion IL-B as described by Post (1986) does not exist just as the inversions $_{\text{IIIL-C1.C2-}}$The new breakpoints of IL-B are shown on Fig. **21**, **25** and **26**.

26 Furthermore, various populations in Guinea, Sierra Leone, Liberia and Côte d'Ivoire show polymorphisms for the inversions IL-B (Figs. **21** and **25**), IIL-6 (Figs. **27–32**) and IIIL-2.17 + 4 (Fig. **38**). Thus, these inversions are not fixed for all members of the sub-complex. In view of these fmdings, the only fixed inversion diagnostic for the members of *S. sanctipauli* s.l. is IIL-4 (Boakye et al., 1993). Each member in addition to this has some fixed inversions, which differentiate it from the other species of the sub-complex.

Simulium sanctipauli s.s. Vajime and Dunbar (sensu Boakye et al., 1993)

27 This species is differentiated from the other members by the presence of the intraspecifi̇c inversion IIL-A (Post, 1986) which is shown on Figures **27–30**. Other fixed inversions are; IIL-6 and IIIL-2. Floating inversions recorded are IS-A (Fig.s**21** and **22**), IS-24 (Fig. **24**), IL-1 (Fig. 46), IIL-7 (Figs. **28** and **29**), IIIL-B (Figs. **38–40**), IIIL-4 and IIIL-17. The rest are; IS-21, IS-5 and IS-20 (Fig. **23**), IS-F (Fig. **21**), IIS-7 and IIS-A (Fig. **32**), IIL-S (Fig. **30**). IIIL-M and IIIL-A (Fig. **37**). Some individuals also show a micromorphological alteration of the centromeres of chromosome II or chromosome III.

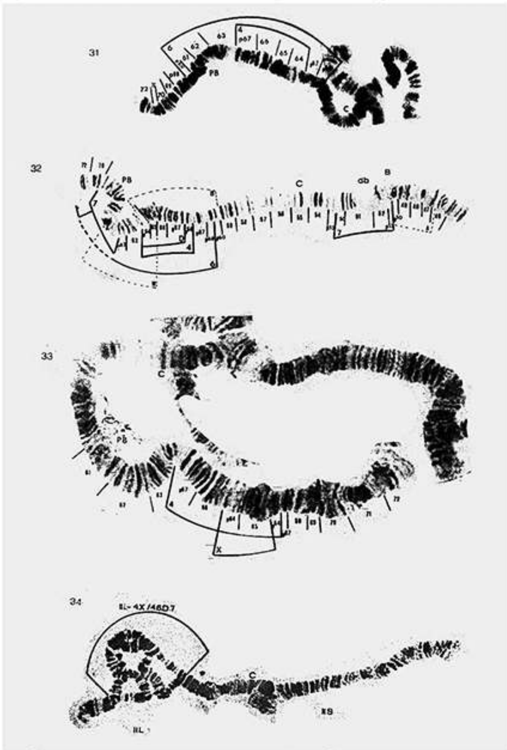
28 A geographical variant of this species designated as *S. sanctipauli* Djodji form was found in Togo and Ghana. This form has no fixed inversion differences from other populations of *S. sanctipauli* except that sex determination is associated with the inversion IS-2L (Fig. **23**), wrongly labeled as IS-α (Surtees, 1986). Males of the Djodji form are heterozygous for the inversion and it is absent in females (Surtees et aL, 1988). The diagnostic character relates to a population and therefore is not useful for identifying individuals.

29 *S. sanctipauli* has been recorded predominantly from the Côte d'Ivoire and Southern Ghana. Occasionally, it has been identified from rivers in Guinea. Sierra Leone and Liberia. One record of this species is known from Mali. Since the extension of larviciding to rivers in the south of Togo and Ghana in 1988, the Djodji form has not been found in larval samples from the area (OCP, unpublished reports).

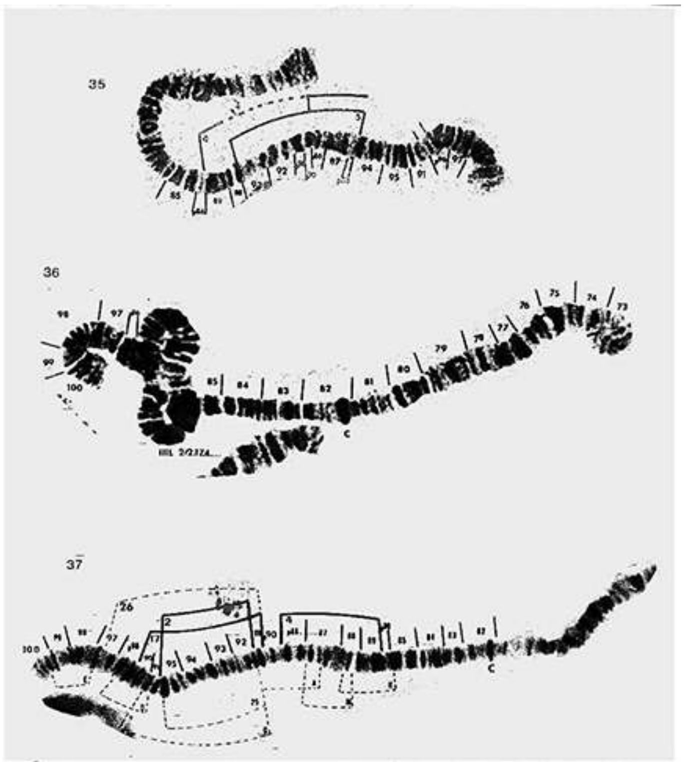
Simulium leonense Boakye, Post and Mosha (originally described by Post, 1986, as S. soubrense B)

30 This species is characterized by the inversions IL-A (Fig. **25**), IIS-7 (Fig. **32**) and IIIL-2 (Figs. **37** and **38**). There are eight floating inversions; IS-A (Figs. **21** and **22**), IIL-6, IIL-7, IIL-D, and IIL-X (Figs. **32–34**), IIIL-4 and IIIL-17 (Figs. **35–40**). Sex-linkage is found to be associated with IIIL- 4.17 in most populations. Rare inversions recorded in this species are IS-M (Fig. **21**), IS-N and IS-C (Fig. **26**), I L-T (Fig. **25**), IIS-A, IIL-W and IIL-B (Fig. **32**), IIIL-K and IIIL-D (Fig. **3**7) and IIIL-I (Fig. **41**).

31 Distribution of this cytospecies is limited mostly to Sierra Leone and its borders with Guinea. However, records of its presence in Liberia exist (Güzelhan and Garms, 1987).



Figs. 31–34 Chromosome II of *S. sanctipauli* s.l. (**31**) Chromosome II of *S. soubrense',* IIL-4.6/4.6. (**32**) Chromosome II typical for *S. leonense. S. konkourense* Menenkaya form and *S. soubrense* St. Paul form; IIS-7/7, IIL-4.6.D.7/4.6.D.7. (**33**) Chromosome II of *S. konkourense* Konkouré form; IIL-4.X/4.X. (**34**) Chromosome II of *S. konkourense* Konkouré form; IIL-4.X/4.6.D.7. B = Ring of Balbiani. db = double bulb. Pb = para-Balbiani. C = centromere.

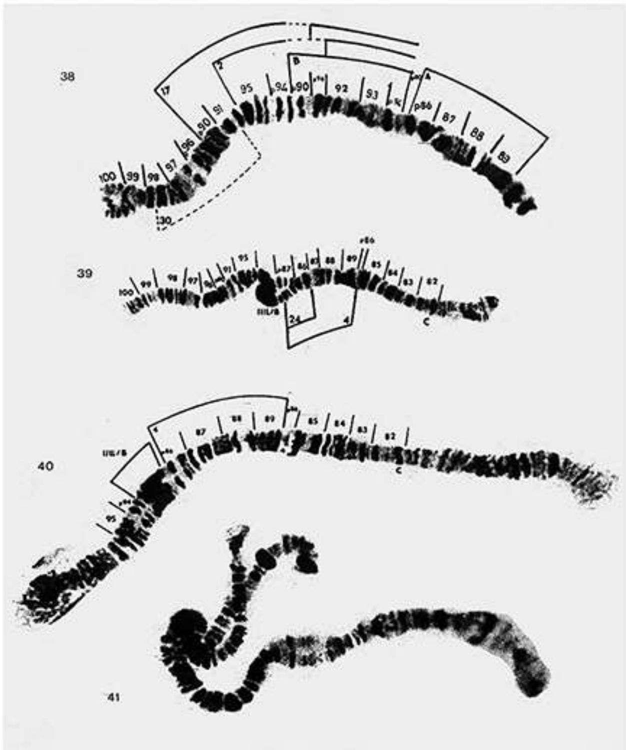


Figs. 35–37 Chromosome III of *S. sanctipauli* s.l. (**35**) Chromosome III homozygous for IIIL-5 (IIIL-2.17.4.5/2.17.4.5). (**36**) Chromosome III with IIIL-17 and IIIL-4heterozygous; IIIS-st/st, IIIL-2/2.17.4. (**37**) Chromosome III found commonly in all members of the *S. sanctipauli* subcomplex; IIIL-2.17.4/2.17.4. C = centromere.

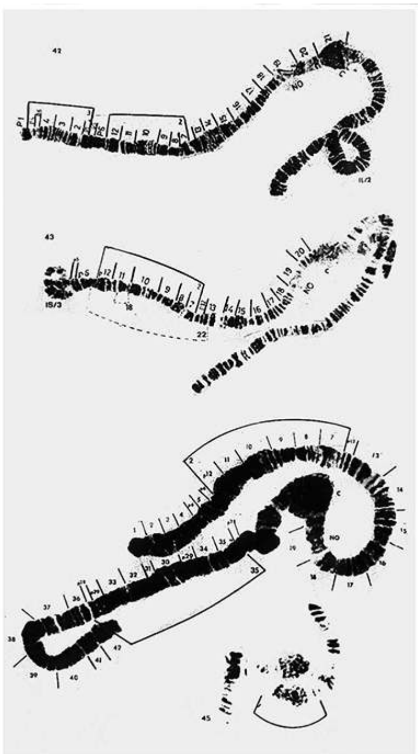
Simulium sourense Vajime and Dunbar (sensu Boakye et al., 1993)

32 The original classification of this species was reviewed by Post (1986). He recognised all members of the *S. sanctipauli* sub-complex lacking the inversions 1L-A and IIL-A as *S. soubrense.* Recent observations have however_ shown that there are lots of fixed inversion differences between various populations which are sometimes found breeding in sympatric situations. Hence some have been raised to specific status and others, are at present, considered as geographic variants designated as forms. Those regarded as different species are included in the *S. konkourense* (Boakye et al., 1993; see below). The various forms described under *S. soubrense* are; Beffa form from Togo and Benin (Meredith et al., 1983), Farmington and St. Paul forms from Liberia (Kashan and Garnis, 1987) and Chutes Milo form (Boakye et al., 1993).

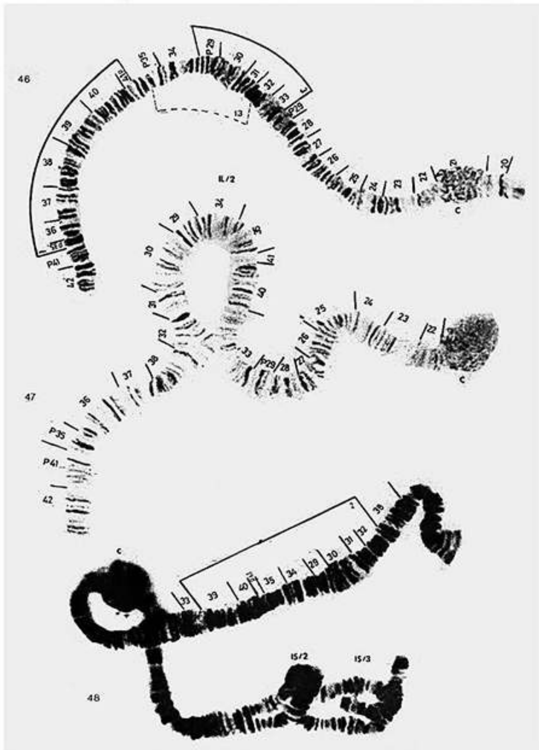
33 Chromosomally, all these forms and others not formally designated as such but belonging to *S. soubrense* lack_the inversions IL-A and IIL-A. Inversions IIL-6 and IIIL-2 are fixed in ail populations. The following common inversions are recorded floating in some populations but fixed in others; IS-A. IS-J, IS-G, IS-H, 1L-B, IIS-7, IIL-D, IIL-7, IIIL-4, IIIL-17, IIIL-5, IIIL-24, IIIL-B, IIIL-26, IIIL-E, IIIL-25 and IIIL-I. these inversions are shown on Figs. **21–41**.



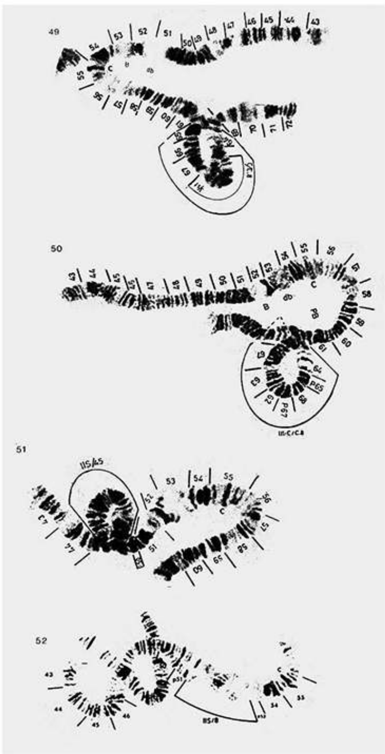
Figs. 38–41 Chromosome III of *S. sanctipauli* s.l. (**38**) Long arm of chromosome III homozygous for IIIL-2.17.B.4. (**39**) Long arm of Chromosome III; IIIL-2.17.B.4.24/2.17.4.24. (**40**) Chromosome III; IIIS-st/st, IIIL-2.17.B.4/2.17.4. (**41**) Asynapsis of homologous arms of Chromosome MIL due to an inclusion (arrow). C = centromere.



Figs. **42–45** (**42**) Chromosome I recorded for *S. damnosum* s.s.*l. S. sirbanum:* IS-2.3/2.3, IL-1/1.2, IL-1 not shown). (**43**) Chromosome I of *S. damnosum* s.s/S. *sirbanum* with IS-3 heterozygous; IS-2/2.3. (**44**) Chromosome I of *S. dieguerense:* IS-2/2, IL-35/35. (**45**) Altered centromere region of Chromosome I (arrow) found in-*S. dieguerense* males. C = centromere. NO = nucleolar organiser.



Figs.46–48 Chromosome I of *S. damnosum* s.s./*S. sirbanum.* (**46**) Long arm of Chromosome I typical of *S. damnosum s.s./S. sirba-num;* IL-3.1/3.1. (**47**) Long arm heterozygous for IL-2. (**48**) Chromosome I homozygous for IL-2 and heterozygous for IS-2 and IS-3 (IS/2.3, IL-2/2). C = centromere.



Figs. 49–52 (**49**) Chromosome II with standard sequence for IIS and heterozygous for inversions on the long arm typical of both *S. damnosum* s.s. and *S. sirbanum;* IIS-st/st, III-C/C.8.3. (**50**) Chromosome II with the inversions IIL-C/C.8 common to *S damnosum* s.s. males. (**51**) short arm of chromosome II of *S. sirbanum* heterozygous for IIS-45. (**52**) Short arm of Chromosome II of *S. sirbanum* heterozygous for IIS-8. B = Balbiani ring, db = double bulb. C = centromere, PB = Para-Balbiani.

Beffa form

34 Populations of this form show a distinctive sex-linkage associated with an inversion designated IIS-6b (Fig. 30) (Meredith et al., 1983). Males are heterozygous for the inversion which is absent in females. Floating inversions include IS-A, IS-J, IS-G, IS-H, IL-B, IIL-D, IIL-7, IIIL-4, IIIL-17 and IIIL-24.

Chutes Milo form

35 Chutes Milo form seems to be typical *S. soubrense,* almost identical to Vajime and Dunbar's Type material from the River Leraba (Boakye et al., 1993). IS-A, 1IL-6, IIIL-4, IIIL-17 and IIIL-24 are fixed. The inversions IIS-7, IIL-7, IIL-D and IIIL-5 are absent. Specific to the Chutes Milo form but different from other populations in Côte d'Ivoire and Ghana that share similar inversions, is the association of IIIL-B with sex determination.

Farmington and St. Paul forms

36 The Farmington form is characterised by the lack of any sex-linked inversion and absence of IIL-D. Polymorphic inversions recorded are IIS-7, IIL-7, IIIL-4, IIIL-17, IIIL-5 and IIIL-26.

37 The St. Paul form is identified by the fixation of IIL-D.7, IIIL-4.17 and sex-linkage to IIIL-5. Float ing inversions are IL-1 and IIS-7.

Simulium konkourense Boakye, Post, Mosha and Quillévéré

38 This species includes two cytotypes; Konkouré form (Quillévéré et al., 1982) and Menankaya form (Boakye et al., 1993) formerly considered as forms of *S. soubrense* because of the absence of the inversions IL-A and IIL-A. These two forms show clinal variation in the proportion of. floating inversions along their distributional range with two extremely different chromosomaL forms at the ends. Populations of the two forma are reproductively isolated from the Chutes Milo form in the areas of sympatry confirming their specific identity from that species. Populations considered as Menankaya form have a fixation of the inversion IIL-D.7 and those considered Konkouré have larvae with the inversion IIL-4.X/4.X or IIL-4.X/4.6.D.7. Hence the criteria used can be applied to individual larvae and not necessarily populations. Common polymorphie inversions reported for this species are; IS-A, IL-1, IIS-7, IIIL-4, IIIL-2, IIIL-17 and IIIL-5. Rare inversions are IS-J, IS-X, IS-N, IL-T, IL-X, IL-D, IIIS-H, IIIL-X, IIIL-Y, IIIL-E, IIIL-Z, IIIL-G and IIIL-I.

Simulium damnosum s.s. Theobald (sensu Vajime and Dunbar)

39 *S. damnosum* s.s. was cytotaxonomically diagnosed by Vajime and Dunbar (1975), as being distinct from *S. squamosum* by three independent inversions; IL-1, IIL-3 and IIIL-2. A re-examination of these inversions by Quillévéré (1975) revealed that the chromosome IIL inversion difference was not a single and simple one but rather a complex rearrangement. Post (1982) confirmed this finding and showed that some four different inversions were involved which he grouped together and termed IIL-C (Fig. **53**). Sex-

determination is related to IIL-8 in most populations of this species. Most males were found with the inversion IIL-C/C.8 (Fig. **50**) and females IIL-C/ (Fig. 53).

40 Recently, Vajime and Gregory (1990) have reported that the predominant cytotype in populations described as *S.damnosum s.s.* (Vajime and dunbar, 1975) in the OCP area is different from the type species identified from Uganda and should therefore be called Volta form (Dunbar and Vajime, 1981). Cytotypes considered similar to the type material were called Nile form (Dunbar and Vajime, 1981) but have been re-designated as *S. damnosum* s.s. with sex-linkage to the inversions IS-3.2. The predominant cytotype in the OCP area is therefore considered a separate taxon but without any formai specific name. Fiasorgbor et al. (pers. comm.) suggests the two cytotypes as sub-species of a single species *S. damnosum* s.s. Descriptions of inversions given in this text refer to both forms.

41 Fixed inversions are IL-1 (Fig. **46**), IIL-C and IIIL-2 (Fig. **57**). Routine identification is based on the presence of IIL-C. Common polymorphic inversions in this species are; IS-2, IS-3 (Figs. **42**, **43** and **48**), IL-2 (Figs. **42**, **47** and **48**), IIL-8, IIL-3, IIL-2, IIL-15 (Figs. **49, 50** and **53–56**), IIIL-6 and IIIL-7 (Figs. **58–61**). Some rare inversions are IS-18, IS-22, IL-13, IIL-35 and IIL-15.

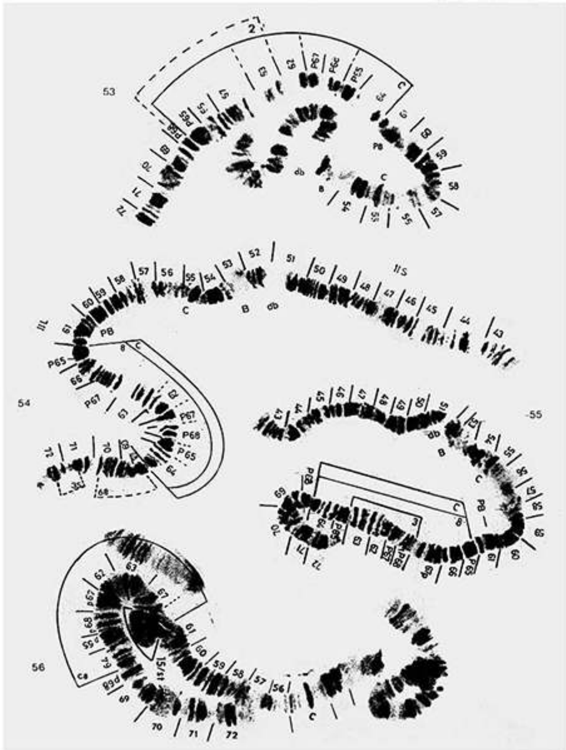
42 This species is found mainly in the transition zone between the forest and the savanna. It has been recorded from all the eleven countries of the OCP. It is the predominant cytotype of the savanna group found in the forest areas of Ghana, Togo, Benin and Côte d'Ivoire.

Simulium sirbanum Vajime and Dunbar

43 Two cytotypes originally considered as different species, *S. sirbanum* and *S. sudanense,* by Vajime and Dunbar (1975) are now included in this species (Vajime, 1989). The individuals of this species are separated from *S. squamosum* by the fixed inversions IL-1, HL-C.8 and IIIL-2. Thus, it differs from *S. damnosum* s.s. by fixation of HL-8. Sex-determination is related to IS-3 in both cytotypes. In one cytotype (former *S. sirbanum),* females do not possess the inversion whilst females of *the* other cytotype are found homozygous for the inversion. Most males of both forms are heterozygous (IS\3) and therefore indistinguishable (Vajime and Dunbar, 1975; Vajime, 1989).

44 Routinely, this species is diagnosed by the sequence IIL-C.8/C.8, IIL-C.8.3/C.8.3 or IIL-C.8/C.8.3. Floating inversions observed are; IS-2, IS-3 (Figs. **42**, **43** and **48**), IL-2, IL-13 (Figs. **42**, **47** and **48**), IIS-45 (Fig. **51**), IIS-8 (Fig. **52**), IIL-3, IIL-15, IIL-35 (Figs.**49** and **53–56**), IIIL-6 and IIIL-7 (Figs. **58–61**), IIIL-27 (Fig. **57**). Inversions such as IL-13, IIS-45, IIS-8, IIL-15, IIL-35 and IIIL-27 were not commonly observed.

45 *Simulium sirbanum* is the most widely distributed and important vector of human onchocerciasis of the *S. damnosum* s.l. in the OCP area. It has been been reeorded from all the river basins in the area although it occurs predominantly irr the savanna zones. The presence of this species in the forest areas is usually during the dry season. However, deforestation has resulted in savanna enclaves in the forest zone which do harbour *S. sirbanum* populations all year round.



Figs. 53–56 (**53**) Chromosome II of *S. damnosum* s.s.; IIS-st/st, III-C/C. (**54**) Chromosome II of *S. sirbanum/S. dieguerense:* IlS-st/st, IIL-C.8/C.8. (**55**) Chromosome II of *S. sirbanum;* IlS-st/st, IIL-C.8.3/C.8.3. (**56**) Chromosome II of *S. sirbanum:* IlS-st/st. IIL-C.8./C.8.64. B = Balbiani ring, db = double bulb. C = centromere. PB = Para-Balbiani.

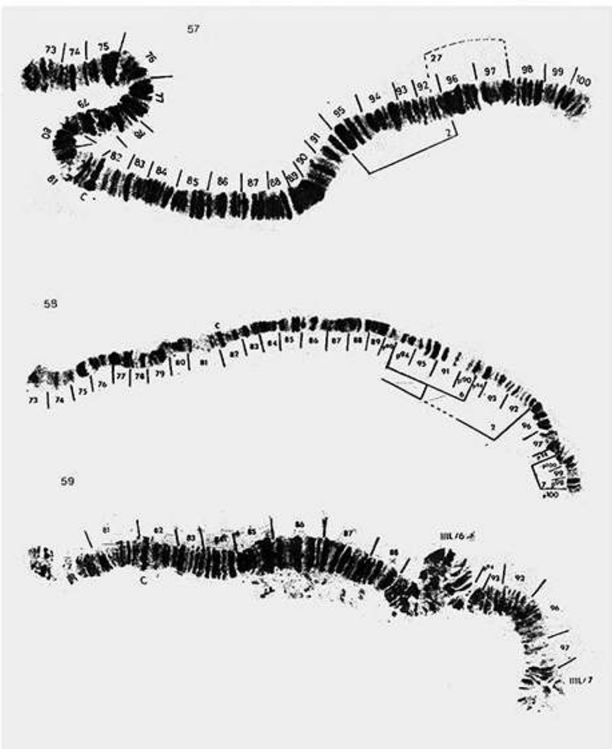
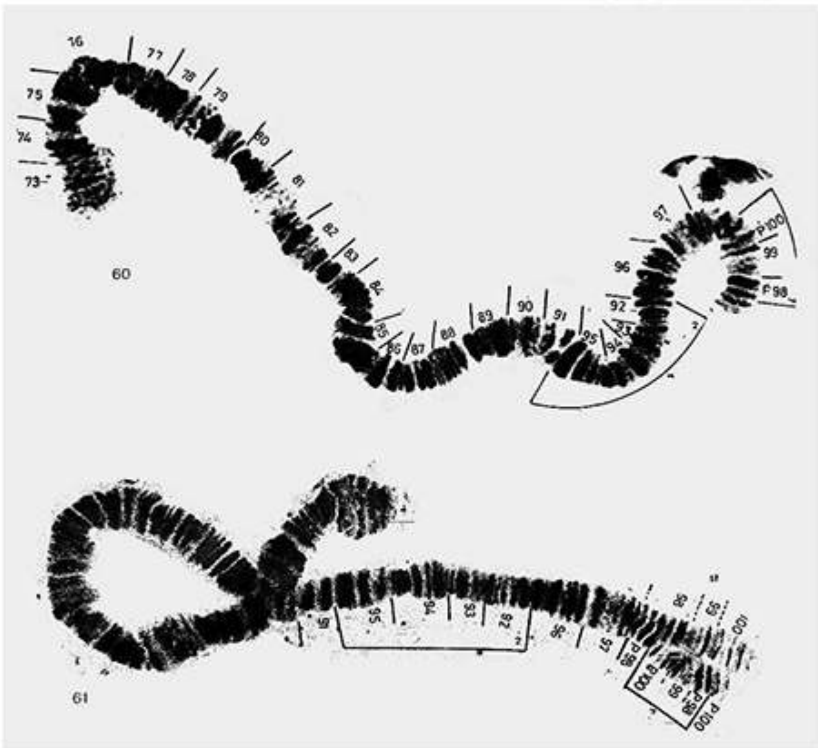


Fig. 57–59 (**57**) Chromosome III of *S. damnosum* s.s./*S. sirbanum/S. dieguerense,* IIIS-st/st, IIIL-2/2. (**58**) Chromosome III of *S. damnosum* s.s./*S.* sirbanum; IHS-st/st, IIIL-2.6.7/2.6.7. (**59**) Chromosome III of *S. damnosum* s.s./*S. sirbanum;* IHS-st/st, IIIL-2/2.6.7. C = centromere.



Figs. 60–61 Chromosome III of *S.-damnosum* s.s./*S. sirbanum;* (**60**) IIIS-st/st, IIIL-2.7/2.7. (**61**) IIIL-2/2.7. C = centromere.

Simulium dieguerense Vajime and Dunbar

46 Vajime and Dunbar (1975) described this species as having four inversion differences from *S. squamosum.* These were IS-2, IL-12, 1IL-3.8 and IIIL-2. Post (1982) revised the diagnostic criteria replacing IL-12 and IIL-3 by IL-35 (Fig. **44**) and IIL-C respectively. In this species IL-12 and IIL-3 are absent. Later, Boakye and Mosha (1988) reported sex-linkage to an altered segment of the centromere region of chromosome I (Fig. **45**) similar to that reported for *S. squamosum* (Vajime and Dunbar. 1975). About 90% of males and 17% of females have the altered segment.

47 Routine identifications based on only IIL-C.8 could resuit m mis-identifications with *S. sirbanum* since these inversions are common to both species. Floating inversions found in this species are IIL-68 (Fig. **54**) and IIIL-28 (Fig. **16**).

48 *Simulium dieguerense* has a limited distribution. It has been recorded breeding mostly on the Bakoye and Bafing river basins in Mali and Guinea. Occasionally, it is found breeding in other rivers in Guinea and Sierra Leone.

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ABSTRACTS

Simulium damnosum Theobald is made up of a complex of sibling species. Nine species are described in the area covered by the World Health Organisation's Onchocerciasis Control Programme in West Africa (OCP). These are, *S. squamosum, S. yahense, S. canctipauli, S. soubrense, S. leonense, S. konkourense, S. damnosum* s.s., *S. sirbanum* and *S. dieguerense.* All of them are vectors of human onchocerciasis, albeit to different capacities. Reliable species determination presently depends on larval cytotaxonomic criteria. The diagnostic chromosomal inversions and other micromorphological characters used in the identification of the species found in the OCP area are presented with figures for their recognition, some distributional information is also given.

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Cytotaxonomic revision of the *Simulium sanctipauli* subcomplex (Diptera: Simuliidae) in Guinea and the adjacent countries including descriptions of two new species

D.A. Boakye, R.J. Post, F.W. Mosha, D.P. Surtees and R.H.A. Baker

Introduction

1 Human onchocerciasis is a severely debilitating blinding disease caused by infection with the parasite *Onchocerca volvulus* (Nematoda: Filarioidea). In West Africa it is transmitted exclusively by members of the *Simulium* (*Edwardsellum*) *damnosum* Theobald complex (Diptera: Simuliidae). The sibling species within this complex have been distinguished by chromosomal (cytotaxonomic) features, and their accurate identification remains difficult. Nevertheless it is known that they can show profound differences in their epidemiological importance (Post & Boakye, 1992) and much effort has been invested in the taxonomy of the complex. However, the taxonomic description of the complex remains largely dependant on larval chromosome data, which has sometimes proved difficult to interpret. This is especially true for the *S. sanctipauli* Vajime & Dunbar subcomplex, and the present work was undertaken to clarify this confused subcomplex.

2 The *S. damnosum* complex in West Africa consists of eight named cytospecies, described on the basis of the interspecific inversion differences in the banding sequences of the larval silk gland polytene chromosomes (Vajime & Dunbar, 1975). *Simulium sanctipauli* and *S. soubrense* Vajime & Dunbar, which are both vectors of human onchocerciasis, share a host of floating inversions but were distinguished from each other by the inversion IIL-7. However, this inversion was subsequently found to be polymorphie extensively across West Africa (Quillévéré, 1975; Quillévéré *et al.,* 1982; Meredith *et al.,* 1983), and therefore was not suitable as a spedes-diagnostic inversion. More recently Post (1986) used two

previously unrecognized fixed inversions (IL-A and IIL-A) to distinguish three spedes, namely S. *sanctipauli, S. soubrense* and *S. soubrense* 'B'. 'Beffa' form (Meredith *et al.,* 1983) was considered as an intraspedfic variant within *S. soubrense,* and forme 'Konkouré' (Quillévéré *et al.,* 1982) remained unassigned to any particular species within the *S. sanctipauli* subcomplex (Post, 1986).

3 Previous distribution maps of *S. sanctipauli* and S. *soubrense* based solely on IIL-7 (e.g. Garms & Vajime, 1975; Quillévéré *et al.,* 1982) cannot easily be redrawn according to the new identification criteria proposed by Post (1986). However, in the Onchocerdasis Control Programme (OCP) Western Zone specimens identified since June 1984 from Guinea and surrounding countries were karyotyped-for IL-A and IIL-A as well as IIL-7 and other inversions. A reclassification of the subcomplex in this area has been possible through the analysis of karyotype data from these old records, and from recent chromosome analysis carried out from various localities both within and outside the OCP Western Zone.

Materials and methods

4 Larvae were fixed in Carnoy's solution during breeding site prospections in Senegal, Guinea, Mali, Sierra Leone and Liberia (table 1). Guinea Bissau was not prospected, and Côte d'ivoire is considered outside the scope of this paper. Most collections were fixed in cold Camoy's solution by OCP staff, and subsequently stored at 4°C. Specimens were identified morphologically as belonging to the S. *damnosum* complex according to Crosskey (1973). Larval silk gland polytene chromosomes were usually prepared according to a modification of Dunbar's (1972) method.

5 The larval abdomen was split open in Camoy's fixative along the mid-ventral line, and larvae were rinsed three times in distilled water. Larval bodies were blotted dry and hydrolysed in 3.5% HCl (Analar grade) at 60–65°C for seven minutes. After hydrolysis larvae were again rinsed three times in distilled water, blotted dry and stained in Feulgen for one to three hours. Larvae were sexed according to the shape of the gonadial rudiment and the silk glands mounted in lacto-acetic orcein. When required, preparations were subsequently made permanent by careful removal of the cover slip without freezing, drying both cover slip and slide in absolute ethanol and remounting in Euparol.

6 Inversions were recorded and species identified with reference to the chromosome maps published by Post (1986), who illustrates most of the inversions found in this study. However, a few new inversions and chromosome map revision of old inversions, are described below, and illustrated in figures 5 to 16. In practice most larvae were examined only for the diagnostic inversions for identification, although a small number of larvae from each sample were also fully karyotyped. In some samples all larvae were fully karyotyped to obtain estimates of the frequencies of polymorphic inversions. Data were analysed in two ways. Firstly by classical analysis of geographic and karyotypic (Hardy-Weinberg) distribution of individual inversions, and secondly for *S. konkourense* sp. n. by multivariate analysis of the sample frequencies of all inversions considered simultaneously. Two multivariate analyses were used. Firstly, principle component analysis of the dispersion (variance/covariance) matrix (Seber, 1984), using the programme SAS PROC PRINCOMP. This is an ordination technique that seeks to account

for the maximum variance in a multivariate data set into a small number of dimensions which can be represented graphically. Secondly, the programme SAS PROC CLUSTER was used to derive a minimum spanning tree from the similarity matrix between samples (Gower & Ross, 1969). The minimum spanning tree connects all samples (plotted on a map or an ordination) according to their chromosomal similarity, with no loops and minimum overall length.

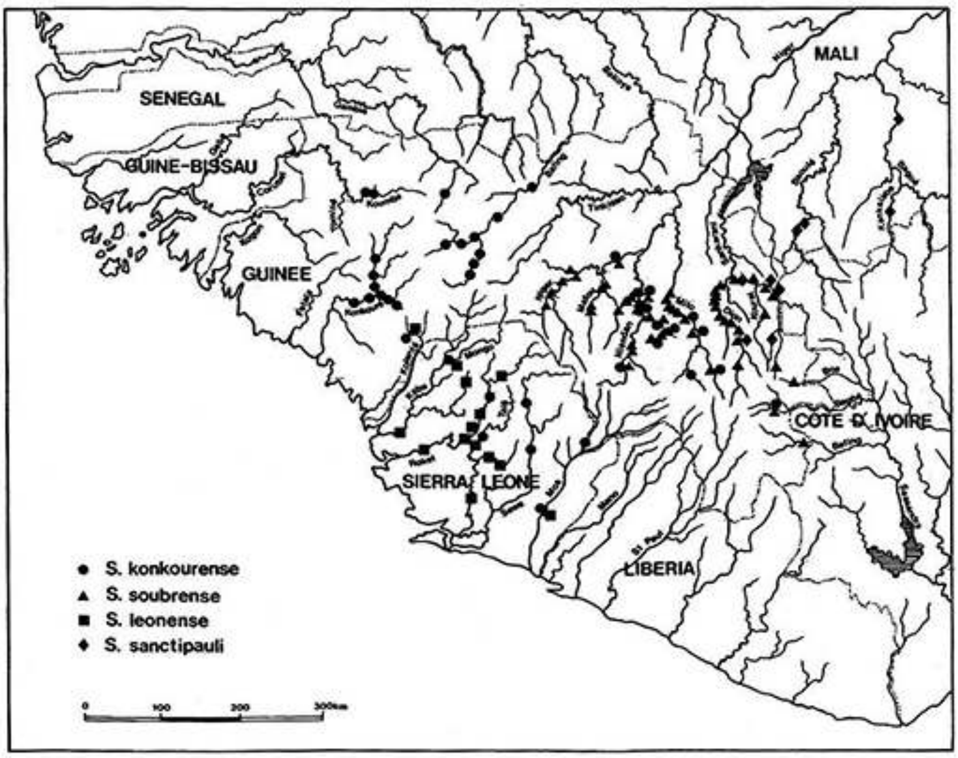

Fig. 1. The distribution of members of the *Simulium sanctipauli* subcomplex in Guinea and the surrounding countries.

Table 1. Sample data and diagnostic inversion karyotypes.

River basin Sample & river	Locality	Lat°/Long°	Date	*Simulium sanctipauli*	*S. leonense*	*S. soubrense*	*S. konkourense*			Other karyo types
				IL st/st IIL A/A IIL st/st	A.A st/st 7.D/7.D 7.D/X X/X	st/st st/st st/st	7.D/7.D	st/st st/st X/X	7.D/X	
Bani										
1 Kankélaba	Kankela	10°49'06°40'	13/12/85	4	0	0	0	0	0	0
2 Kankélaba	Kankela	10°49'06°40'	14/12/85	13	0	0	0	0	0	0
3 Kankélaba	Kankela	10°49'06°40'	19/12/85	19	0	0	0	0	0	0
4 Kankélaba	Kankela	10°49'06°40'	20/12/85	16	0	0	0	0	0	0
5 Bagoe	Metela	11°56'06°24'	10/01/86	22	0	0	0	0	0	0
6 Baoulé	Bouloumba	09°08'09°15	12/11/85	0	0	0	2	0	0	1 x 7.D/D
7 Baoulé	Madina Diassa	10°40'07°40'	15/01/86	14	0	0	0	0	0	0
8 Baoulé	Madina Diassa	10°40'07°40'	16/01/86	17	0	0	0	0	0	0
9 Baoulé	Madina Diassa	10°40'07°40'	18/01/86	3	0	0	0	0	0	0
10 Baoulé	Madina Diassa	10°40'07°40'	25/01/86	79	0	0	0	0	0	0
11 Baoulé	Madina Diassa	10°40'07°40'	26/01/86	68	0	0	0	0	0	0
12 Baoulé	Kanibougoula	10°45'07°46'	15/01/86	2	0	0	0	0	0	0
13 Baoulé	Kanibougoula	10°45'07°46'	27/01/86	81	0	0	0	0	0	0
Sankarani										
14 Diémouni		09°03'08°03'	08/10/85	0	0	1	0	0	0	0
15 Gbanhala	Hemakono	09°21'08°08'	20/11/85	4	0	0	0	0	0	0
16 Gbanhala	Kalafilia	09°57'08°07'	05/01/86	0	0	4	0	0	0	0
17 Kourai	Lemouroubo	09°40'08°17'	15/06/84	0	0	1	0	0	0	0
18 Kourai	Lemouroubo	09°40'08°17'	05/03/86	0	0	10	0	0	0	0
19 Kourai	Lemouroubo	09°40'08°17'	11/06/86	0	0	2	0	0	0	0
20 Kourai	Lemouroubo	09°42'08°19'	05/11/85	0	0	1	0	0	0	0
21 Sankarani	Kalafilia	09°57'08°07'	15/01/86	0	0	1	0	0	0	0
22 Sankarani	Kalafilia	09°57'08°07'	25/01/86	0	0	12	0	0	0	0
23 Sankarani	Kalafilia	09°57'08°07'	07/03/86	1	0	2	0	0	0	0
24 Sankarani	Kalafilia	09°57'08°07'	08/03/86	2	0	1	0	0	0	0
25 Sankarani	Kalafilia	09°57'08°07'	11/06/86	0	0	3	0	0	0	0
26 Sankarani	Kalafilia	09°57'08°07'	12/06/86	0	0	1	0	0	0	0
27 Sankarani	Oulouko	10°02'08°15'	20/02/86	1	0	1	0	0	0	0
28 Sankarani		10°09'08°34'	04/03/86	1	0	0	0	0	0	0
29 Sankarani		10°09'08°34'	11/06/86	0	0	1	0	0	0	0
30 Sankarani	Koimana	10°10'08°37'	11/02/87	0	0	4	0	0	0	0
31 Dion	Kamandougou	09°13'08°39'	26/03/85	0	0	1	0	0	0	0
32 Dion	Kamandougou	09°13'08°39	28/04/86	0	0	13	0	0	0	0
33 Dion	Sékoro	09°31'08°37'	05/11/85	1	0	5	0	0	0	0
34 Dion	Sékoro	09°31'08°37'	19/11/85	0	0	25	0	0	0	0
35 Dion	Banankoro	09°36'08°39'	05/01/86	0	0	7	0	0	0	0
36 Dion	Banankoro	08°36'08°39'	24/04/86	0	0	2	0	0	0	0
37 Dion		09°39'08°42'	26/02/85	0	0	1	0	0	0	0
38 Dion		09°39'08°42'	28/04/86	0	0	7	0	0	0	0
39 Dion		09°44'08°46'	30/04/86	0	0	7	0	0	0	0
40 Dion	Conf. Béni	09°41'08°42'	13/06/86	0	0	5	0	0	0	0
41 Dion		09°44'08°41	02/03/87	0	0	1	0	0	0	0
42 Dion		09°45'08°48'	25/02/85	0	0	2	0	0	0	0
43 Dion		09°45'08°47'	13/06/86	0	0	12	0	0	0	0
44 Dion	Sékoro	09°48'08°51'	24/04/86	0	0	11	0	0	0	0
45 Dion		09°50'08°52'	28/04/86	0	0	9	0	0	0	0
46 Dion	Téré	09°52'08°52'	26/05/85	0	0	1	0	0	0	0
47 Dion	Téré	09°52'08°52'	19/11/85	2	0	18	0	0	0	0
48 Dion	Téré	09°52'08°52'	05/03/86	0	0	5	0	0	0	0
49 Dion	Téré	09°52'08°52'	20/02/86	1	0	0	0	0	0	0
50 Dion	Téré	09°52'08°52'	05/03/86	3	0	2	0	0	0	0
51 Dion		09°53'08°45'	15/11/86	0	0	1	0	0	0	0
52 Dion		09°54'08°49'	26/03/86	0	0	1	0	0	0	0
53 Dion		09°54'08°49'	12/06/86	0	0	24	0	0	0	0
54 Dion		09°54'08°49'	12/06/86	0	0	43	0	0	0	0

Table 1 (*cont.*)

River basin Sample & river	Locality	Lat°/Long°	Date	*Simulium sanctipauli*	*S. leonense*	*S. soubrense*	*S. konkourense*			Other karyo types
				IL st/st IIL A/A IIL st/st	A.A st/st 7.D/7.D 7.D/X X/X	st/st st/st st/st	7.D/7.D	st/st st/st X/X	7.D/X	
55 Dion	Conf. Seremou	09°56'08°48'	05/11/85	0	0	4	0	0	0	0
56 Dion		10°01'08°49	02/03/87	0	0	5	1	0	0	0
57 Dion	Morissanako	10°13'08°42'	19/02/86	1	0	0	0	0	0	0
58 Béni		09°39'08°44'	20/05/86	0	0	14	0	0	0	0
59 Béni		09°39'08°44'	23/05/86	0	0	11	0	0	0	0
60 Béni		09°39'08°44'	04/07/86	0	0	32	0	0	0	0
61 Béni	Conf. Dion	09°39'08°43'	05/11/85	0	0	7	0	0	0	0
62 Béni	Conf. Dion	09°39'08°43'	22/11/85	0	0	1	0	0	0	0
63 Béni	Conf. Dion	09°39'08°43'	13/06/86	0	0	18	0	0	0	0
64 Béni	Conf. Dion	09°40'08°42'	23/05/86	0	0	11	0	0	0	0
Milo										
65 Milo	Tiékouradougou	08°58'08°59'	12/11/85	0	0	0	8	0	0	0
66 Milo	Tiékouradougou	08°58'08°59'	21/11/85	0	0	0	73	0	0	8x7.D/D
67 Milo	Tiékouradougou	08°58'08°59'	14/01/86	0	0	0	5	0	0	0
68 Milo	Tiékouradougou	08°58'08°59'	14/02/86	0	0	0	1	0	0	0
69 Milo	Tiékouradougou	08°58'08°59'	24/04/86	0	0	0	4	0	0	0
70 Milo	Tiékouradougou	08°58'08°59'	23/05/86	0	0	0	20	0	0	3x7.D/D
71 Milo	Tiékouradougou	08°58'08°59'	26/07/86	0	0	0	11	0	0	6x7.D/D
72 Milo	Tiékouradougou	08°58'08°59'	22/08/86	0	0	0	51	0	0	1x7.D/st
73 Milo	Tiékouradougou	08°58'08°59'	28/09/86	0	0	0	16	0	0	0
74 Milo	Tiékouradougou	08°58'08°59'	14/04/88	0	0	1	2	0	0	0
75 Milo	Tiékouradougou	08°58'08°59'	17/06/88	0	0	0	35	0	0	0
76 Milo	Gbe Milo	09°44'09°12'	21/03/86	0	0	1	0	0	0	0
77 Milo	Balandougou	09°46'09°14'	17/01/87	0	0	5	2	0	0	0
78 Milo	Balandougou	09°46'09°14'	08/02/87	0	0	50	0	0	0	0
79 Milo	Balandougou	09°47'09°14'	25/02/87	0	0	18	1	0	0	1x7.D/st
80 Milo	Chutes Milo	09°47'09°15	28/08/84	0	0	52	0	0	0	0
81 Milo	Chutes Milo	09°47'09°15'	14/09/84	0	0	10	0	0	0	0
82 Milo	Chutes Milo	09°47'09°15'	28/11/84	0	0	15	0	0	0	0
83 Milo	Chutes Milo	90°47'09°15'	08/01/85	0	0	8	0	0	0	0
84 Milo	Chutes Milo	09°47'09°15'	19/02/85	0	0	12	0	0	0	0
85 Milo	Chutes Milo	09°47'09°15	11/10/85	0	0	15	0	0	0	0
86 Milo	Chutes Milo	09°47'09°15'	20/02/86	0	0	2	0	0	0	0
87 Milo	Chutes Milo	09°47'09°15	25/02/86	0	0	17	0	0	0	0
88 Milo	Chutes Milo	09°47'09°15'	19/03/86	0	0	8	0	0	0	1xD/D
89 Milo	Chutes Milo	09°47'09°15'	19/03/86	0	0	6	0	0	0	0
90 Milo	Chutes Milo	09°47'09°15'	24/04/86	0	0	7	0	0	0	0
91 Milo	Chutes Milo	09°47'09°15'	22/05/86	0	0	30	0	0	0	0
92 Milo	Chutes Milo	09°47'09°15'	05/07/86	0	0	12	0	0	0	0
93 Milo	Chutes Milo	09°47'09°15'	26/07/86	0	0	19	0	0	0	0
94 Milo	Chutes Milo	09°47'09°15	28/09/86	0	0	13	0	0	0	0
95 Milo	Chutes Milo	09°47'09°15'	03/03/88	0	0	6	0	0	0	0
96 Milo	Dalanian	09°48'09°17'	15/11/84	0	0	19	0	0	0	1x7/st
97 Milo	Dalanian	09°48'09°17'	28/11/84	0	0	23	0	0	0	0
98 Milo	Dalanian	09°48'09°17'	15/07/85	0	0	4	0	0	0	0
99 Milo	Dalanian	09°48'09°17'	04/12/85	0	0	15	0	0	0	0
100 Milo	Dalanian	09°47'09°17'	20/12/85	0	0	21	0	0	0	0
101 Milo	Dalanian	09°48'09°17'	17/01/86	0	0	2	0	0	0	0
102 Milo	Dalanian	09°48'09°17'	18/01/86	0	0	3	0	0	0	0
103 Milo	Banankoro	09°54'09°22'	18/01/87	0	0	10	0	0	0	0
104 Milo	Banankoro	09°54'09°22'	08/02/87	0	0	5	0	0	0	0
105 Milo	Banankoro	09°54'09°22'	25/02/87	0	0	8	0	0	0	0
106 Milo	Bafalé	10°01'09°27'	20/12/85	0	0	9	0	0	0	0
107 Milo	Morigbédougou	10°36'09°26'	17/01/87	0	0	20	0	0	0	0
108 Milo	Morigbédougou	10°36'09°26'	07/02/87	0	0	17	0	0	0	0
109 Milo	Morigbédougou	10°40'09°26'	22/01/86	0	0	3	0	0	0	0
110 Milo	Morigbédougou	10°40'09°26'	03/03/88	0	0	1	0	0	0	0
111 Milo	Morigbédougou	10°40'09°26'	24/11/87	0	0	1	0	0	0	0

112 Balé	Conf. Magba	09°35'09°04'	26/07/86	0	0	1	1	0	0	0
113 Balé	Conf. Magba	09°35'09°04'	10/02/87	0	0	3	0	0	0	0
114 Balé	Conf. Magba	09°35'09°04'	25/02/87	0	0	10	0	0	0	0
115 Balé	Conf. Magba	09°35'09°04'	25/11/87	0	0	1	0	0	0	0
Niandan										
116 Balé	Menankaya	09°33'09°36'	15/01/86	0	0	2	2	0	0	0
117 Balé	Menankaya	09°33'09°36'	13/09/84	0	0	8	10	0	0	0
118 Balé	Menankaya	09°33'09°36'	16/07/85	0	0	5	22	0	0	1x7.D/st
119 Balé	Menankaya	09°33'09°36'	14/11/85	0	0	3	18	0	0	0
120 Balé	Menankaya	09°33'09°36'	25/02/86	0	0	0	1	0	0	0
121 Balé	Menankaya	09°33'09°36'	23/03/86	0	0	5	5	0	0	0
122 Balé	Menankaya	09°33'09°36'	22/04/86	0	0	0	1	0	0	0
123 Balé	Menankaya	09°33'09°36'	02/07/86	0	0	8	10	0	0	1x7.D/D
124 Balé	Menankaya	09°33'09°36'	26/07/86	0	0	2	6	0	0	0
125 Balé	Menankaya	09°33'09°36'	22/08/86	0	0	8	11	0	0	1x7.D/st
126 Balé	Menankaya	09°33'09°36'	28/09/86	0	0	3	0	0	0	0
127 Balé	Menankaya	09°33'09°36'	25/11/87	0	0	0	4	0	0	0
128 Balé	Menankaya	09°33'09°36'	03/03/88	0	0	17	0	0	0	0
129 Balé	Férémamoria	09°41'09°36'	15/01/87	0	0	4	1	0	0	0
130 Balé	Dinba	09°44'09°37'	25/11/87	0	0	2	1	0	0	0
131 Balé	Sasanbaya	09°49'09°45'	23/07/86	0	0	3	0	0	0	0
132 Kourai		09°33'09°41'	01/03/88	0	0	1	0	0	0	0
133 Niandan	NiandanScierie	09°18'10°01'	13/11/85	0	0	2	0	0	0	0
134 Niandan	NiandanScierie	09°18'10°01'	21/12/85	0	0	10	0	0	0	0
135 Niandan	NiandanScierie	09°18'10°01'	02/07/86	0	0	2	0	0	0	0
136 Niandan	Bambaforia	09°18'10°02'	26/02/86	0	0	2	0	0	0	0
137 Niandan	Dendadou	09°18'10°02'	14/01/87	0	0	6	1	0	0	0
138 Niandan	Boria	09°26'09°58'	14/02/86	0	0	8	0	0	0	0
139 Niandan	Boria	09°28'09°57'	15/01/87	0	0	5	0	0	0	0
140 Niandan	Bessekoro	09°31'09°55'	13/11/84	0	0	4	0	0	0	0
141 Niandan	Bessekoro	09°31'09°55'	22/04/86	0	0	1	0	0	0	0
142 Niandan	Bessekoro	09°31'09°55'	28/09/86	0	0	9	0	0	0	0
143 Niandan	Yradou	09°33'09°54'	21/12/85	0	0	20	0	0	0	0
144 Niandan	Yradou	09°33'09°54'	26/02/86	0	0	4	0	0	0	0
145 Niandan	Yradou	09°33'09°54'	16/01/87	0	0	58	0	0	0	0
146 Niandan	Yradou	09°33'09°54'	25/11/87	0	0	4	0	0	0	0
147 Niandan	Conf. Balé	09°47'09°47'	27/11/87	0	0	4	0	0	0	0
148 Niandan	Sanaanbaya	09°49'09°45'	23/02/85	0	0	2	0	0	0	0
149 Niandan	Sanaanbaya	09°49'09°45'	13/11/85	0	0	9	0	0	0	1x7.D/D
150 Niandan	Sanaanbaya	09°49'09°45'	21/12/85	0	0	11	1	0	0	0
151 Niandan	Sanaanbaya	09°49'09°45'	22/08/86	0	0	10	0	0	0	0
152 Niandan		09°53'09°44'	21/02/85	0	0	24	0	0	0	0
153 Niandan	Mamouria	09°54'09°45'	19/07/84	0	0	4	0	0	0	0
154 Niandan	Mamouria	09°54'09°45'	13/09/84	0	0	10	4	0	0	0
155 Niandan	Mamouria	09°54'09°45'	23/02/85	0	0	11	0	0	0	0
156 Niandan	Mamouria	09°54'09°45'	07/08/85	0	0	3	0	0	0	0
157 Niandan	Mamouria	09°54'09°45'	13/11/85	0	0	1	0	0	0	0
158 Niandan	Mamouria	09°54'09°45'	21/12/85	0	0	11	0	0	0	0
159 Niandan	Mamouria	09°54'09°45'	26/02/86	0	0	12	0	0	0	0
160 Niandan	Mamouria	09°54'09°45'	27/02/87	0	0	4	0	0	0	0
161 Niandan	Mamouria	09°54'09°45'	15/03/87	0	0	4	0	0	0	0
162 Niandan	Chutes Niandan	09°55'09°45'	19/07/84	0	0	1	0	0	0	0
163 Niandan	Chutes Niandan	09°55'09°45'	29/03/86	0	0	4	0	0	0	0
164 Niandan	Rapide Panpan	10°01'09°41'	10/01/85	0	0	21	0	0	0	0
165 Niandan	Rapide Panpan	10°01'09°41'	22/02/85	0	0	11	0	0	0	0
166 Niandan	Rapide Panpan	10°01'09°41'	22/02/85	0	0	3	0	0	0	0
167 Niandan	Rapide Panpan	10°01'09°41'	13/11/85	0	0	17	1	0	0	0
168 Nianden	Rapide Panpan	10°01'09°41'	21/12/85	0	0	18	0	0	0	0
169 Niandan	Rapide Panpan	10°01'09°41'	13/01/87	0	0	63	0	0	0	0
170 Niandan	Rapide Panpan	10°01'09°41'	10/02/87	0	0	9	0	0	0	0
171 Niandan	Rapide Panpan	10°01'09°41'	11/02/87	0	0	30	0	0	0	0
172 Niandan	Rapide Panpan	10°01'09°41'	24/03/87	0	0	12	0	0	0	0
173 Niandan	Rapide Panpan	10°01'09°41'	24/11/87	0	0	9	1	0	0	0
174 Niandan	Rapide Panpan	10°01'09°41'	04/03/88	0	0	2	0	0	0	0
175 Niandan	Rapide Panpan	10°02'09°42'	23/02/85	0	0	8	0	0	0	0
176 Kouya	Kouya Laya	09°53'10°03'	11/01/85	0	0	17	0	0	0	0
177 Kouya		10°03'09°57'	15/11/85	0	0	5	0	0	0	0

Table 1 (cont.)

River basin Sample & river	Locality	Lat°/Long°	Date	*Simulium sanctipauli*	*S. leonense*	*S. soubrense*	*S. konkourense*			
				IL st/st IIL A/A IIL st/st	A.A st/st 7.D/7.D 7.D/X X/X	st/st st/st st/st	7.D/7.D	st/st st/st X/X	7.D/X	Other karyo types
178 Kouya		10°04'09°54'	15/11/85	0	0	8	0	0	0	0
179 Kouya		10°06'09°51'	28/02/86	0	0	15	0	0	0	0
180 Kouya		10°06'09°52'	06/03/85	0	0	2	0	0	6	0
181 Kouya		10°07'09°49'	05/03/85	0	0	3	0	0	0	0
182 Kouya		10°07'09°49'	07/03/85	0	0	6	0	0	0	0
183 Kouya	Vieux Pont	10°07'09°49'	26/11/87	0	0	23	1	0	0	0
184 Kouya	Kouya	10°07'09°49'	22/12/85	0	0	14	0	0	0	0
Mafou										
185 Mafou			26/11/84	0	0	6	0	0	0	1xD/D
186 Mafou	Erako	09°45'10°25'	14/11/85	0	0	18	0	0	0	0
187 Mafou	Erako	09°45'10°25'	28/09/86	0	0	1	0	0	0	0
188 Mafou	Banankoro	09°53'10°20'	21/12/85	0	0	19	0	0	0	0
189 Mafou	Banankoro	09°53'10°20'	27/02/86	0	0	1	0	0	0	0
190 Mafou	Douma	10°19'10°15'	02/10/84	0	0	2	0	0	0	0
191 Mafou	Douma	10°19'10°15'	11/01/85	0	0	8	0	0	0	0
192 Mafou	Douma	10°19'10°14'	01/03/85	0	0	2	0	0	0	0
193 Mafou	Conf. Kinkoun	10°19'10°11'	14/11/85	0	0	13	0	0	0	0
194 Mafou	Sérékoroba	10°19'10°11'	21/12/85	0	0	6	0	0	0	0
195 Mafou	Sérékoroba	10°19'10°11'	23/07/86	0	0	1	0	0	0	1x7/7
196 Mafou	Conf. Niger	10°32'10°07'	01/03/85	0	0	2	0	0	0	0
Niger										
197 Balé	Koula	10°08'10°51'	15/11/85	0	0	1	0	0	0	0
198 Balé	Koula	10°08'10°51'	22/12/85	0	0	6	0	0	0	0
199 Niger	Conf. Balé	10°15'10°49'	14/02/86	0	0	1	0	0	0	0
200 Niger	Balandougou	10°17'10°46'	11/01/85	0	0	7	0	0	0	0
201 Niger	Balandougou	10°17'10°46'	15/11/85	0	0	8	0	0	0	0
202 Niger	Balandougou	10°17'10°46'	06/03/85	0	0	3	0	0	0	0
203 Niger	Balandougou	10°18'10°46'	27/02/86	0	0	1	0	0	0	0
204 Niger		10°26'10°30'	01/03/85	0	0	2	0	0	0	0
205 Niger	Rapides Niger	10°32'10°07'	16/11/85	0	0	3	0	0	0	0
206 Niger	Rapides Niger	10°32'10°07'	21/12/85	0	0	6	0	0	0	0
207 Niger	Rapides Niger	10°32'10°07'	25/09/86	0	0	9	1	0	0	0
208 Niantan	Banfarala	10°31'10°27'	30/11/85	0	0	1	0	0	0	0
Bafing										
209 Bafing	Socotoro	10°38'11°45'	13/11/86	0	0	0	11	0	10	0
210 Bafing	Socotoro	10°40'11°45'	20/08/86	0	0	0	1	0	0	0
211 Bafing	Socotoro	10°40'11°46'	22/09/86	0	0	0	7	0	0	0
212 Bafing	Socotoro	10°40'11°46'	13/09/87	0	0	0	2	1	2	1xD/D
213 Bafing	Socotoro	10°40'11°46'	18/09/87	0	0	0	7	0	0	0
214 Bafing	Nduria	10°45'11°45'	13/02/86	0	0	0	8	0	4	0
215 Bafing	Haut Bafing	10°47'11°44'	07/03/88	0	0	0	3	0	1	0
216 Bafing	Lago	10°50'11°35'	09/03/88	0	0	0	24	1	11	0
217 Bafing	Lago	10°51'11°36'	15/11/85	0	0	0	3	0	0	0
218 Bafing	Lago	10°51'11°36'	22/12/85	0	0	0	8	0	4	0
219 Bafing	Lago	10°51'11°36'	30/09/86	0	0	0	15	0	7	0
220 Bafing	Lago	10°51'11°36'	13/11/86	0	0	0	7	0	4	0
221 Bafing	Conf. Kioma	11°17'11°25'	30/09/86	0	0	0	14	0	5	0
222 Bafing	Conf. Kioma	11°17'11°25'	24/03/87	0	0	0	0	0	1	0
223 Bafing	Yagui	11°36'10°54'	30/09/86	0	0	0	4	0	0	0
224 Bafing	Yagui	11°34'10°52'	22/11/86	0	0	0	7	3	0	0
225 Diendou	Sankarella	10°47'11°32'	30/09/86	0	0	0	1	0	0	0
226 Téné	Conf. Dankola	11°01'11°58'	22/11/86	0	0	0	15	8	0	0
227 Téné		11°01'11°53'	09/03/88	0	0	0	1	0	0	0
228 Téné		11°01'11°49'	13/02/86	0	0	0	5	1	2	0
229 Téné		11°01'11°49'	22/11/86	0	0	0	13	4	1	0
230 Téné	Conf. Bafing	11°07'11°36'	30/09/86	0	0	0	5	0	0	0
231 Téné	Conf. Bafing	11°07'11°35'	22/11/86	0	0	0	9	2	0	0
232 Kourai	Téné	11°01'11°53'	09/03/88	0	0	0	4	0	0	0

233	Koukatamba		11°13'11°19'	30/09/86	0	0	0	15	0	20	0
234	Koukatamba		11°13'11°19'	22/11/86	0	0	0	15	3	0	0
Gambie											
235	Gambie	Ley Dima	11°40'11°55'	27/09/87	0	0	0	5	24	36	0
Tomine											
236	Koumba	Kokou	11°42'12°54'	11/11/86	0	0	0	4	14	12	0
237	Koumba	Sidipo	11°43'12°56'	11/11/86	0	0	0	1	12	6	0
Konkoure											
238	Kakrima	Soulouje	10°42'12°56'	13/02/86	0	0	0	1	1	1	0
239	Kakrima	Bougoula	10°34'12°58'	12/11/86	0	0	0	8	3	9	0
240	Kakrima	Segoghi	10°33'12°58'	03/04/87	0	0	0	13	5	5	0
241	Kakrima	Conf. Kaffima	10°50'12°57'	12/11/86	0	0	0	16	5	9	0
242	Konkouré	Conf. Kanhan	10°28'12°24'	29/09/86	0	0	0	49	4	12	0
243	Konkouré	Conf. Kanhan	10°28'12°24'	2/11/86	0	0	0	8	2	5	0
244	Konkouré	Niguisima	10°36'12°30'	12/11/86	0	0	0	3	0	1	0
245	Konkouré	Malangare	10°35'12°35'	13/02/86	0	0	0	1	0	3	0
246	Konkouré	Baléré	10°31'12°47'	12/11/86	0	0	0	12	0	4	4
247	Konkouré	Conf. Mayonkure	10°27'12°59'	13/02/86	0	0	0	11	0	6	0
248	Konkouré	Konkouré	10°27'13°00'	22/03/87	0	0	0	8	0	7	0
249	Konkouré	Tagbé/Soukia	10°25'13°12'	12/11/86	0	0	0	19	1	8	0
250	Konkouré	Konkouré	10°25'13°15'	04/04/87	0	0	0	7	0	13	0
Kolente/Great Scarcies											
251	Kolenté	Kolenté	10°04'12°38'	13/11/86	0	14	0	2	1	0	0
252	Kolenté	Malea	10°04'12°36'	13/02/86	0	7	0	2	0	1	IL.Comp
Kaba/Little Scarcies											
253	Kaba	Mange	08°56'12°51'	12/04/88	0	35	0	0	0	0	0
254	Mongo	Conf. Kaba	09°33'12°09'	21/10/86	0	3	0	3	0	0	0
255	Mabole		09°16'12°01	25/10/86	0	10	0	0	0	0	0
Rokel/Seli											
256	Rokel	Masa		21/10/86	0	5	0	3	0	0	0
257	Rokel	Bumbuna	09°04'11°43'	13/04/88	0	2	0	23	0	0	0
258	Rokel	Bumbuna	09°04'11°43'	22/05/88	0	0	0	4	0	0	0
259	Rokel	Bumbuna	09°03'11°46'	22/05/88	0	0	0	1	0	0	0
260	Rokel	Tala	08°52'11°53'	22/05/88	0	23	0	0	0	0	0
261	Rokel	Makpankaw	08°45'11°55'	12/05/88	0	26	0	0	0	0	0
262	Rokel	Magburaka	08°44'11°57'	22/10/86	0	13	0	0	0	0	0
263	Rokel	Katik	08°39'12°31	12/04/88	0	14	0	0	0	0	0
264	Rokel	Katik	08°39'12°31'	22/05/88	0	59	0	0	0	0	1xIL-A/st
Taia											
265	Pampana	Makwi	08°40'11°54'	09/05/88	0	1	0	1	0	0	0
266	Pampana	Makwi	08°40'11°54'	15/05/88	0	5	0	0	0	0	0
267	Pampana	Makwi	08°40'11°54'	18/05/88	0	2	0	0	0	0	0
268	Pampana	Makwi	08°40'11°55'	13/04/88	0	15	0	0	0	0	0
269	Teye	Gbangema	08°16'11°39'	22/05/88	0	1	0	0	0	0	0
270	Teye	Mongeri	08°19'11°44'	07/05/88	0	47	0	0	0	0	0
271	Teye	Mongeri	08°19'11°44'	14/05/88	0	3	0	0	0	0	0
272	Taia	Mogbamu	08°00'12°08'	11/05/88	0	23	0	0	0	0	0
273	Taia	Mogbamu	08°00'12°08'	12/06/88	0	10	0	0	0	0	0
Sewa											
274	Sewa	Yifin	09°07'11°16'	13/04/88	0	0	0	8	0	0	0
275	Sewa	Njaima	08°33'11°16'	22/10/86	0	0	0	13	0	0	0
MOA											
276	Meli	Sangema	08°19'10°42'	23/10/86	0	0	0	6	0	0	0
277	Moa	Gofor	07°47'11°10'	11/06/88	0	3	0	0	0	0	0
Saint Paul											
278	St. Paul	Pump Station	06°54'10°23'	10/10/86	0	0	0	10	0	0	6xD.7/st
279	St. Paul	St. Paul Falls	06°56'10°16'	13/10/86	0	0	0	8	0	0	0
Upper Sassandra											
280	Boa	Dessebadougou		28/10/84	0	0	0	0	0	0	1xA.7/A
281	Boa	Morifadougou	08°45'08°02	06/11/85	0	0	1	0	0	0	0
282	Boa	Conf. Kekouo	08°54'07°54'	30/08/84	3	0	0	0	0	0	0
283	Dilamba		08°36'08°16'	12/09/84	0	0	0	0	0	0	2x7.D/D
284	Bagbé	Kaniangoro	08°32'08°24	03/01/84	0	0	1	0	0	0	0
285	Bagbé	Kaniangoro	08°32'08°24'	12/09/84	0	0	0	1	0	0	0
286	Bagbé	Kaniangoro	08°32'08°24'	26/10/84	1	0	0	0	0	0	0
287	Bagbé		08°03'08°09'	20/02/85	0	0	2	0	0	0	0

Simulium sanctipauli subcomplex

7 The *S. sanctipauli* subcomplex was previously distinguished, cytologically, from the rest of the *S. damnosum* complex by the fixed inversions IL-P.Q, IIL-4.6 and IIIL-4.17.2 (reviewed by Post, 1986). However in the present study we have found inversions IIL-6 and IIIL-2 to be polymorphie in samples from the Fouta Djallon mountains (table 1), and a careful reanalysis of the breakpoints of the widespread inversions IIIL-C_1-C_2 (previously reported by Post (1986) from S. *sanctipauli, S. soubrense* and S. *soubrense* 'B') has shown that they are identical to IIIL-4.17. Hence chromosome sequences reported by Post (1986) as IIIL-4.17.2.C_1.C_2 can no longer be considered diagnostic. Furthermore, it is now clear that the IL is more complex than either Vajime & Dunbar (1975) or Post (1986) considered. During the course of this study it was noticed that the sequence IL-P.Q. B.C illustrated by Post (1986) was identical to the sequence IL-1 in *S. damnosum sensu stricto,* and that a hybrid between *S. sanctipauli* and *S. sirbanum* Vajime & Dunbar (Boakye & Mosha, 1988) appeared to be doubly, not triply, heterozygous in IL. Careful reexamination of the breakpoints of speciesdiagnostic IL sequences showed that a single fixed inversion normally occurred in the *S. sanctipauli* subcomplex, and not IL-P.Q as described by Post (1986). However, the single inversion had different breakpoints to those illustrated by Vajime & Dunbar (1975) for IL-6, and were apparently identical to those illustrated by Post (1986) for IL-B. Post's (1986) inversion IL-C was found to be identical to IL-1, which was previously believed to be fixed in *S. damnosum sensu stricto* and *S. sirbanum.* In summary, the *S. sanctipauli* subcomplex-is normally distinguished from the standard IL chromosome sequence (as seen in *S. squamosum* (Enderlein)) by a single inversion IL-B, and not IL-6 or IL-P.Q, which were apparently mistaken for IL-B by Vajime & Dunbar (1975) and Post (1986), respectively. However, IL-B is not fixed, but polymorphic within

the *S. sanctipauli* subcomplex, with IL-1, which Post (1986) mistook for a new inversion, IL-C.

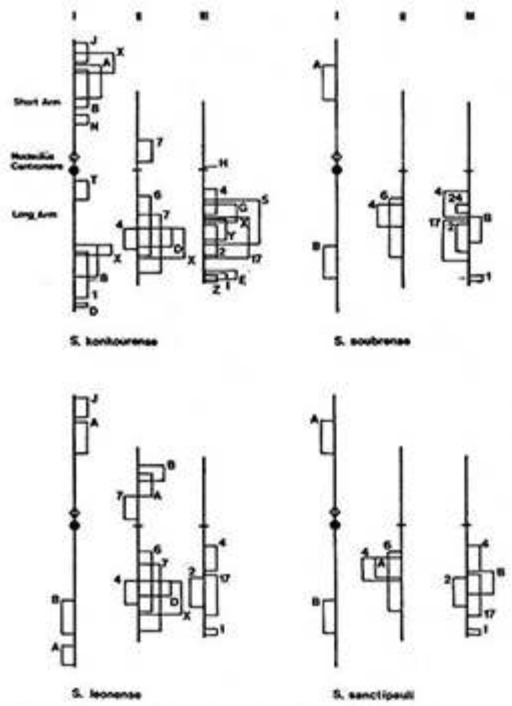

Fig. 2. Idiogrammatic representation of the chromosomal inversions found in members of the *Simulium sanctipauli* subcomplex in Guinea and the surrounding countries. Inversions plotted to the left were found fixed, and those plotted to the right were floating. The inversion breakpoints have been plotted relative to standard *S. squamosum.*

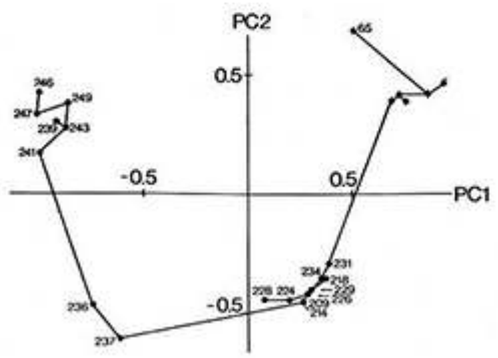

Fig. 3. Principal component plot of samples of *Simulium konkourense* sp. n. based on the correlation matrix of inversion frequencies, with minimum spanning tree superimposed. The sample numbers are as in figure 4.

8 In the present study identification of the *S. sanctipauli* subcomplex was based on homozygosity for the fixed inversion IIL-4. Members of the subcomplex were found in samples from Guinea, Mali, Sierra Leone and Liberia, but not Senegal (see table 1).

Cytological key for the identification of larvae of the *Simulium sanctipauli* subcomplex from Guinea, Mali and Sierra Leone

9 1 Inversion IIL-4 homozygous *S. sanctipauli* subcomplex 2

10 - Inversion IIL-4 absent other species of *S. damnosum* complex

11 2 Inversion IIL-A homozygous *S. sanctipauli*

12 - Inversion IIL-A absent 3

13 3 Inversion IL-A homozygous *S. leonense* sp. n.

14 - Inversion IL-A absent 4

15 4 Inversion IIIL-24 absent *S. konkourense* sp. n.

16 - Inversion IIIL-24 homozygous.*S. soubrense* 'Chutes Milo' form.

Simulium (Edwardsellum) sanctipauli *Vajime* & *Dunbar* sensu stricto

17 Inversion IIL-A (described as diagnostic of *S. sanctipauli sensu stricto* by Post (1986) was never found heterozygously in this study, even in six samples where both homozygotes occurred together (table 1), thus confirming its diagnostic value and the distinct specific status of *S. sanctipauli.*

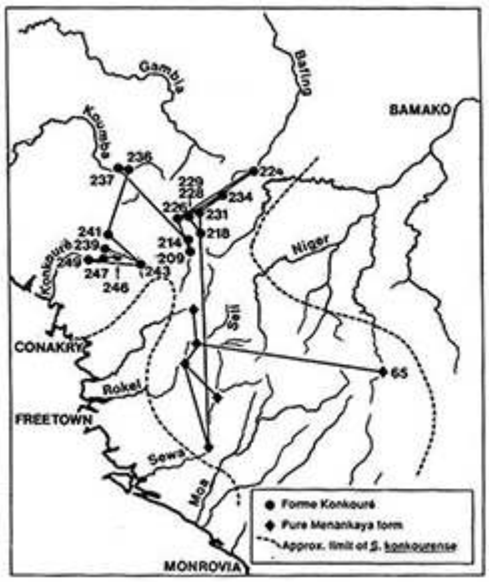

Fig. 4. Distribution map of *Simulium konkourense* sp. n. showing the genetic relatedness between samples according to the minimum spanning tree derived from the inversion frequency similarity matrix. The numbered samples are as in table 1, and the others have been taken from data published by Post (1986).

18 *Simulium sanctipauli* was only found in the eastern part of the study area, in the upper Sassandra, Sankarani, Baoulé and Bagoe river basins (table 1, fig. 1). However, it is already known from a few sites in south-eastern Sierra Leone (Post & Crosskey, 1985) and north-westem Liberia (Guzelhan & Garms, 1987).

19 All specimens karyotyped were found to be homozygous for inversions IL-B, IS-A, IIL-4.6.A and IIIL-2 relative to *S. squamosum* standards. However, as a resuit of the small sample sizes obtained for *S. sanctipauli,* it was only possible to obtain a reliable estimate of the polymorphie inversion frequencies for some samples, from the Baoulé river basin (samples 295, 296, 298 and 292). No s.ex linkage was observed among the polymorphie inversions, which are indicated on the idiogram (fig. 2), and unweighted means of sample frequencies were 0.967 for IIIL-4.17.B and 0.017 for IIIL-I.

Simulium (Edwardsellum) leonense *Boakye, Post & Mosha* sp. n.

20 *Simulium soubrense* 'B' of Post, 1986 (204–205), and subsequent citations.

21 *Diagnostic features.* Recognized on the basis of larval silk gland polytene chromosomes. Sibling species of the *Simulium sanctipauli* subcomplex (defined above by inversion IIL-4 homozygous) and distinguished from other members of the subcomplex by inversion IL-A present homozygously.

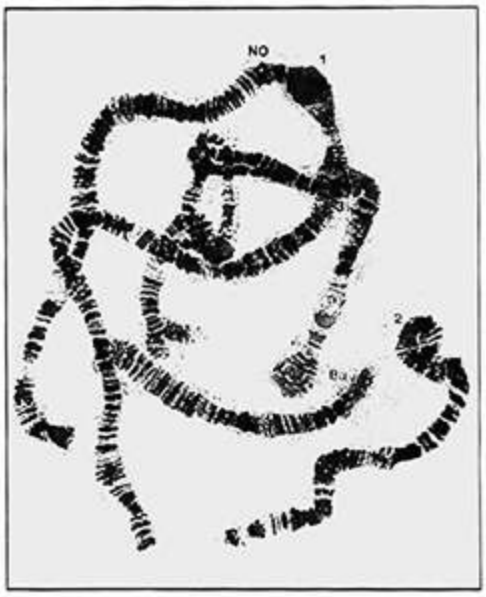

Fig. 5. Full karyotype of *Simulium leonense* sp. n. from R. Tabe near Baiima, collected 8 December 1980. IL-B.A/B.A, IS-A/A, IIL-4.6.7.D/4.6.7.D, IIS-7/7, IIIL-4/4.17.2, and IIIL-st/st. NO= Organiser. Ba=ring of Balbiani. The chromosomes are numbered at their centromeres.

Table 2. ***Simulium leonense*** **summary inversion frequencies by river basin.**

Inversion	River Basin			
	Kolenté	Kaba	Seli	Taia
IS-A	0.944	0	0	0
IS-J	0.042	0	0	0
IL-complex	0.068	0	0	0
IIS-A	0	0	0.005	0
IIS-B	0	0.009	0	0
IIL-6.7.D	0.654	0.813	0.895	0.858
IIL-X	0.346	0.187	0.105	0.142
IIIL-4.17	0.262	0.375	0.281	0.288
IIIL-I	0	0	0.009	0

Notes: Frequencies are unweighted means of sample frequencies from samples, 251,252,253,254,260, 262, 263, 264, 269 and 273. Sample sizes are usually slightly smaller than indicated in table 1 because it was often not possible to score all inversions in all larvae.

Table 3. Sex linkage in *Simulium soubrense* chutes milo form.

IIIL-B karyotype		B/B	B/st	st/st
Sankarani	males	0	29	1
	females	4	1	13
Milo	males	1	41	3
	females	2	5	37
Niandan	males	1	21	2
	females	1	3	28
Mafou	males	0	1	0
	females	0	0	1
Niger	males	0	2	0
	females	0	0	1
Sassandra	males	0	2	0
	females	0	0	0

Notes: Data compiled from samples 34, 44, 48, 59, 60, 63, 72, 73, 81, 87, 90, 91, 94, 95, 96, 111, 114, 121, 123, 125, 127, 130, 139, 142, 145, 146, 151,173, 183, 187, 189, 199, 207, 281 and 284.

22 *Material examined.* Holotype larval polytene chromosome preparation number 0712811–4 with associated larval body, SIERRA LEONE: River Gbangbaia, 1.5 km downstream of Mokasi (07°59, N/12°25'W), 07.xii.1981, collected by RJ Post and deposited in The Natural History Museum, London (NHM). Other material (excluded from the type series): larval bodies with associated chromosome preparations, collected simultaneously with holotype (NHM). Some pùpae collected simultaneously with the larval material, and some pinned adult females collected on human bait at Mokasi, are probably *S. leonense* sp. n. but cannot be unequivocally associated. They are in NHM.

Table 4. *Simulium konkourense* summary inversion frequencies by river system.

Inversion	River system					
	Milo	Niandan	Bafing	Tomine	Konkouré	Sewa
IS-A	0.334	0.300	0.068	0.386	0.045	0.000
IS-J	0.000	0.000	0.009	0.000	0.012	0.000
IS-B	0.000	0.000	0.037	0.300	0.046	0.000
IS-N	0.000	0.000	0.000	0.014	0.000	0.000
IL-D	0.000	0.000	0.000	0.000	0.000	0.000
IL-B	0.588	0.400	0.999	1.000	1.000	1.000
IL-I	0.422	0.600	0.001	0.000	0.000	0.000
IL-X	0.000	0.000	0.003	0.000	0.000	0.000
IL-T	0.000	0,050	0.000	0.000	0.000	0.000
IIS-7	0.276	0.125	0.000	0.000	0.915	0.375
IIL-6	1.000	1.000	0.834	0.289	0.862	1.000
IIL-D	0.998	1.000	0.834	0.289	0.862	1.000
IIL-7	0.973	0.950	0.834	0.289	0.862	1.000
IIL-X	0.000	0.000	0.166	0.711	0.138	0.000
IIIC-H	0.000	0.000	0.000	0.000	0.000	0.389
IIIL-5	0.425	0.000	0.000	0.000	0.000	0.389
IIIL-4.17	0.703	0.583	0.247	0.260	0.201	1.000
IIIL-X	0.000	0.000	0.000	0.654	0.761	0.000
IIIL-Y	0.000	0.000	0.017	0.000	0.000	0.000
IIIL-Z	0.000	0.000	0.037	0.000	0.000	0.000
IIIL-E	0.000	0.000	0.000	0.000	0.000	0.000
IIIL-I	0.000	0.000	0.000	0.000	0.022	0.000
IIIL-G	0.000	0.000	0.000	0.000	0.000	0.000
IIIL-2	1.000	1.000	1.000	0.199	0.205	1.000

Notes: Frequencies are unweighted means of sample frequencies from samples 70, 72, 73, 75, 123, 209, 214, 216, 218, 224, 226, 228, 229, 231, 234, 239, 241, 243, 246, 247, 249, 275, 236 and 237. Sample sizes are usually slightly smaller than indicated in table 1 because it was not always possible to score all inversions in all larvae.

23 *Distribution.* Widespread in major rivers throughout Sierra Leone. Known also from Farmington river in Liberia (Guzelhan & Garms, 1987), and the Kolenté river headwater in south-western Guinea (table 1, fig. 1).

24 *Discussion.* Inversion IL-A (described as diagnostic of S. *soubrense* 'B' by Post, 1986) was found heterozygously in only one larva although 324 were found to be homozygous (table 1), thus confirming its diagnostic value and the distinct specific status of *S. soubrense* 'B'. This species was originally described by Post (1986), but not formally named because of its unknown cytotaxonomic relationship with forme 'Konkouré' (Quillévéré *et al.,* 1982). It now seems clear that forme 'Konkouré' belongs to a newly recognized species (*S. konkourense* sp. n.-see below) which does not interbreed with *S. soubrense* 'B', even in six samples where they occurred together (table 1). Thus we name as *S. leonense* sp. n. the species originally described under the provisional name *S. soubrense* 'B' by Post (1986).

25 The pattern of polymorphic inversions was found to be largely similar to that already described, with sex linkage of IIIL-4.17, (Post (1986) as *S. soubrense* 'B' and IIIL-C_1.C_2). However it has now been possible to determine the sequence of the inversion IIL-complex2 listed by Post (1986). Homozygotes have not been seen before, but it has now been determined that heterozygotes have the karyotype IIL-4.X/4.6.7.D. Polymorphie inversions are indicated on the idiogram (fig. 2) and frequencies are summarized by river basin in table 2. Besides IL-A specimens karyotyped for this study were homozygous IL-B, IIS-7, IIL-4 and IIIL-2, relative to *S. squamosum* standards.

Simulium (Edwardsellum) soubrense Vajime & Dunbar 'Chutes Milo' form

26 Larvae of the *S. sanctipauli* subcomplex not possessing either inversion IL-A or IIL-A were defined as *S. soubrense* by Post (1986). Amongst such specimens examined in the present study inversions IIL-7.D were polymorphie but very rarely found heterozygously even in samples from the rivers Balé, Milo, Niandan and Kouya where both homozygotes (IIL-4.6/4.6 and IIL-4.6.7.D/4.6.7.D) occurred together (table 1). This observation clearly indicates a high degree of reproductive isolation between two homozygous cytotypes (informally referred to as 'Chutes Milo' and 'Menankaya' forms after the localities on the river Milo where they were first recognized), and this conclusion is supported by a consideration of the other unlinked inversions, particularly IS-A, IIIL-24 and IIIL-B.

Table 5. Sex linkage in *Simulium konkouré* forme konkouré

IIIL karyotype		2/2	2/X	X/X	X/4.17.2	2/4.17.2
Bafing	males	1	0	0	0	60
	females	44	1	0	0	8
Koumba	males	3	1	1	20	0
	females	0	0	17	6	0
Konkouré	males	0	0	6	23	0
	females	1	0	62	23	0

Notes: Data compiled from samples 236, 237, 239, 241, 243, 246, 247, 249, 209, 214, 218, 224, 226, 228, 229, 231 and 234.

Fig. 6. Long arm of chromosome I of *Simulium konkourense* sp. n. (IL-B/1) from R. Mongo near Musaia collected on 17 December 1981.

27 Inversion IIIL-24 was never found heterozygously, and homozygotes (IIIL-24/24) were nearly always found associated with IIL-4.6/4.6 ('Chutes Milo' form) and never with IIL-4.6.7.D/4.6.7.D ('Menankaya' form). The single known exception was a larva homozygous IIIL-24/24 and heterozygous IIL-4.6/4.6.7.D. However, this specimen could not have been a hybrid between the two cytotypes or it would have been doubly heterozygous. Similarly inversion IS-A was always found homozygously (IS-A/A) with IIL-4.6/4.6, but IS-A was polymorphie at lower frequencies and usually absent from larvae homozygous IIL-4.6.7.D/4.6.7.D (table 4). Inversion IIILB was never found associated with homozygotes IIL-4.6.7.D/4.6.7.D, but it was sex-linked in homozygotes IIL-4.6/4.6 such that most males were found heterozygous IIIL-B/st and most females did not possess the inversion (table 3).

28 The virtual absence of heterozygotes (table 1) and the strong gametic-phase imbalance observed between unlinked inversions was found consistently between samples over a wide geographic area and time period. This can only be the resuit of 'Chutes Milo' and ' Menankaya' forms representing two distinct sibling species.

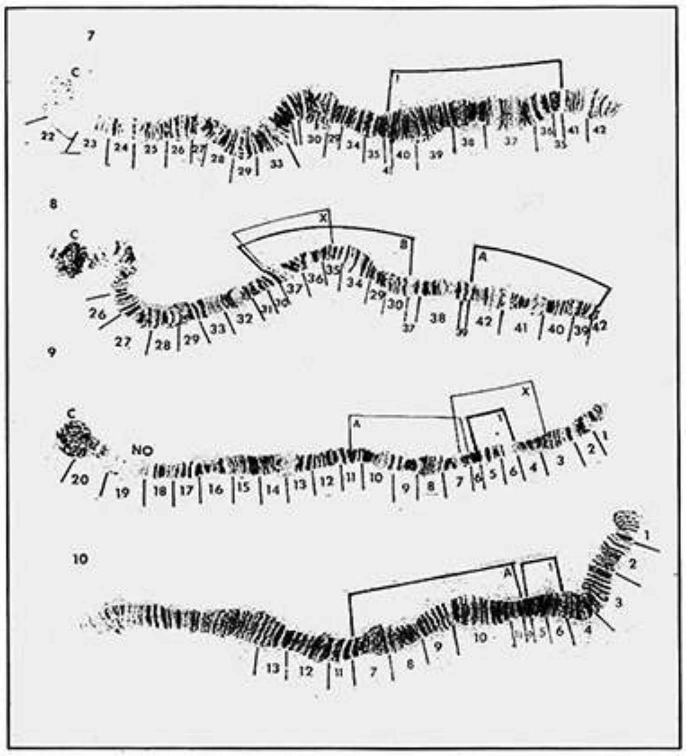

Fig. 7–10. Chromosome I: 7, long arm of *Simulium konkourense* (IL-1/1) from R. Mongo near Musaia collected on 17 December 1981; 8, long arm of *S. leonense* sp. n. (IL-B.A/B.A) from R. Taia at Mogbamu collected on 25 November 1980; 9, short arm of *S. konkourense* (IS-st/st) from R. Bagbe at Yifin collected on 18 December 1981; 10, short arm of *S. sanctipauli* (IS-A/A) from R. Sassandra at Soubre collected on 26 October 1984.

29 Populations of *S. soubrense* further east in Côte d'ivoire and Ghana are most like 'Chutes Milo'. form, with IS-A usually fixed, IL-7.D usually absent and IIIL-24 present, although polymorphic at variable frequencies, rather than fixed (unpublished data). This is also true of Vajime & Dunbar's original type specimens from the river Leraba (see Post, 1986). The exceptions to this trend are mostly found amongst samples from Togo and Nigeria, which show a somewhat lower frequency of ISA and higher frequency of IIL-7.D. However, this genetic divergence is not surprising because these are populations of the 'Beffa' form of *S. soubrense* which is already described as a distinct cytotype (Meredith *et al,* 1983; Post, 1986). Hence 'Chutes Milo' form seems to be typical *S. soubrense,* almost identical to the type material, and found throughout West Africa from Guinea eastwards where it merges into 'Beffa' form. However, the sex linkage of IIIL-B indicates that 'Chutes Milo' populations are, by definition, genetically differentiated from other *S. soubrense* populations, and therefore it seems that 'Chutes Milo' should be considered to be a geographic race within *S. soubrense.* The spectrum of fixed and polymorphie inversions observed within *S. soubrense* 'Chutes Milo' form is illustrated on the idiogram (fig. 2). All larvae were homozygous for inversions IS-A, IL-B, IIL-4.6 and IIL-4.17.2.24. Only two polymorphie inversions were recorded from *S. soubrense* 'Chutes Milo' form from Guinea. Inversion IIIL-B which is sex-linked (table 3) and IIIL-I which was only recorded from Chutes Milo at an average frequency of 0.033.

30 In spite of a few larvae found to be heterozygous for IIL-7.D (table 1) there is no evidence that these larvae are actually hybrids. Hybrids would normally be expected to be heterozygous for IIIL-24 and often IS-A as well as IIL-7.D, but in those spedmens

heterozygous for IIL-7.D, which could be fully karyotyped, chromosome arms IIIL and IS were not heterozygous. Hence no hybrids were found amongst 210 larvae fully karyotyped from sympatric samples, and therefore the rate of hybridization is less than 0.005.

Table 6. Inversion frequencies in *Simulium konkourense*

Sample[1]	River/site	Larvae[2]	Inversion frequencies IL B	I	D	C[3]	X	IS A	J	B	X	N	2L 7	D	X	2S 7	3L 5	E	I	C	Y	X	2	4.17
M 65	Milo/Tiekouradougou	8	0.312	0.750				0.063					0.938	0.938		0.625	0.313						1.000	0.750
M P1	Sewa/Njaima-Sewafe	13	0.962	0.038									1.000	1.000		0.154	0.731	0.038	0.038	0.077			1.000	0.962
M P2	Sewa/Babawahun	8	1.000		0.063			0.188					1.000	1.000		0.250	0.625	0.063					1.000	0.937
M P3	Seli/Badala	7	0.786	0.214				0.357					0.857	1.000		0.071	0.714						1.000	1.000
M P4	Bokel/Bumbuna	11	0.955	0.045				0.182					0.864	1.000		0.227	0.818						1.000	1.000
M P5	Mongo/Musaia	25	0.460	0.540		0.020		1.000					0.220	1.000		0.020	0.720						1.000	0.940
K 209	Bafing/Socotoro	21	1.000							0.105			0.762	0.762	0.238								1.000	0.286
K 218	Bafing/Lago	12	1.000					0.417					0.833	0.833	0.167								1.000	0.333
K 214	Bafing/Nduria	11	0.950	0.050				0.100	0.050				0.636	0.636	0.364						0.111		1.000	0.389
K 224	Bafing/Yegui	10	1.000					0.050		0.050			0.850	0.850	0.150							0.050	0.950	0.100
K 228	Téné/Bridge	8	1.000										0.750	0.750	0.250							0.313	1.000	0.188
K 226	Téné/Dunkolo	23	1.000							0.095			0.826	0.826	0.174								1.000	0.275
K 229	Téné Chutes	24	1.000				0.023	0.026	0.006	0.132			0.833	0.833	0.167								1.000	0.310
K 231	Téné/Bafing conf.	11	1.000							0.056			0.909	0.909	0.091								1.000	0.450
K 234	Koukatamba	19	1.000							0.033			0.895	0.895	0.105								1.000	0.313
K 247	Konkouré/Ganiya	17	1.000					0.036	0.071				0.794	0.794	0.206	0.912						0.813	0.125	0.125
K 249	Konkouré/Soulia	29	1.000					0.019					0.821	0.821	0.179	0.931			0.069			0.714	0.258	0.258
K 246	Konkouré/Bakéré	17	1.000					0.033		0.033			0.875	0.875	0.125	1.000			0.029			0.765	0.118	0.118
K 243	Konkouré/Kanhan	16	1.000					0.125					0.700	0.700	0.300	0.875			0.033			0.733	0.267	0.267
K 239	Kakrima/Bougoula	20	1.000							0.118	0.059		0.650	0.650	0.350	1.000						0.700	0.300	0.300
K 241	Kakrima/Kaflima	31	1.000					0.054		0.125	0.036		0.683	0.683	0.317	0.774						0.839	0.161	0.143
K 237	Koumba/Sidipo	23	1.000					0.306		0.306		0.028	0.250	0.250	0.750							0.636	0.364	0.227
K 236	Koumba/Kokou	32	1.000					0.466		0.293			0.328	0.328	0.672							0.672	0.034	0.293

[1]Sample numbering corresponds to table 1, except P1–5 which are from Post (1986); M= 'Menankaya' form, and K-Forme 'Konkoure'; [2]sample sizes do not always correspond exactly with table 1 because it was not always possible to observe all chromosome arms in all specimens; [3]see post (1986).

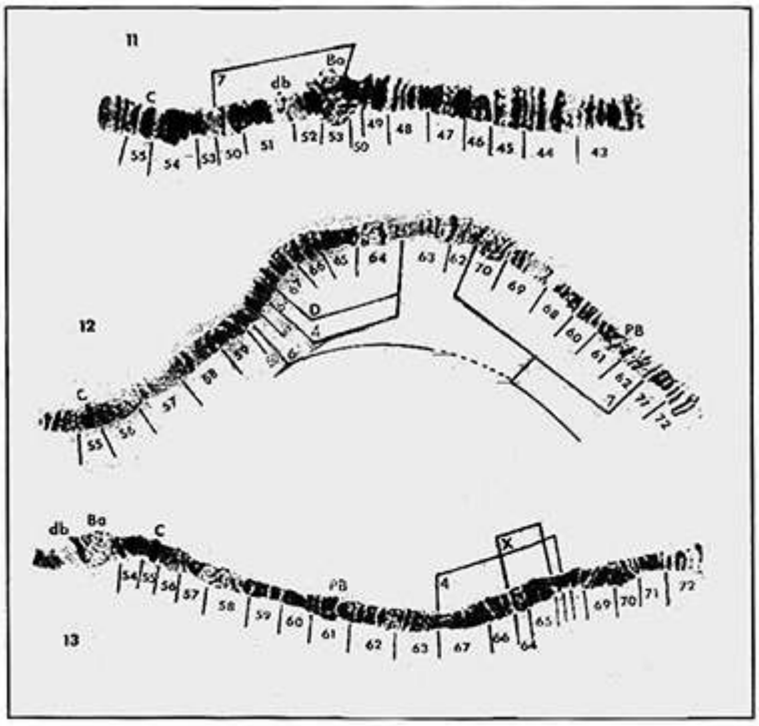

Fig. 11–13. Chromosome II: 11, short arm of *Simulium leonense* (IIS-7/7) from R. Taia at Mogbamu collected on 9 December 1980; 12, long arm of *S. leonense* sp. n. (IIL-4.6.7.D/4.6.7.D) from R. Tabe at Baiima collected on 8 December 1980; 13, long arm of *S. konkourense* sp. n. (IIL-4.X/4.X) from R. Koumba at Sidipo collected on 11 November 1986.

Fig. 14. Long arm of chromosome II from *Simulium konkourense* sp. n. (IIL-4.6.7.D/4.X) collected from R. Koumba at Sidipo on 11 November 1986.

31 If 'Chutes Milo' form is a geographic race within S. *soubrense,* it follows that 'Menankaya' form must be part of a new species, which is described below and named as *S. konkourense* sp. n.

32 In Liberia Kashan & Garms (1987), and Guzelhan & Garms (1987) described-two distinct cytotypes within *S. soubrense* (*sensu* Post, 1986). These two forms, which show restricted interbreeding, are known as the 'Farmington ' form and the 'St Paul' form, but it is not clear how they are related, taxonomically, to S. *soubrense* 'Chutes Milo' form and *S. konkourense* sp. n. If inversion 2L-7 is considered on its own, then the 'St Paul' form appears most similar to *S. konkourense* sp. n. and the 'Farmington' form appears similar to *S. soubrense* 'Chutes Milo' form. However, other inversions do not fit this pattern, and a clear description of their relationship requires a wider geographic spread of samples to determine their cytogenetic interactions in the contact zones between forms.

Simulium (Edwardsellum) konkourense Boakye, Post, Mosha & Quillévéré *sp. n.*

33 Forme 'Konkouré' of Quillévéré *et al.* 1982 (306–307), and subsequent citations.

34 *Diagnostic features.* Recognized on the basis of larval silk gland polytene chromosomes. Sibling species of the *S. sanctipauli* subcomplex (defined above by inversion IIL-4 homozygous), and distinguished from other sibling species by the absence of inversions IIL-A, IL-A and IIIL-24.

35 *Material examined.* Holotype larval polytene chromosome preparation number 2911813–3 with associated larval body, SIERRA LEONE: River Rokel at Bumbuna (09°03'N/11°44'W), 29.xi.1981, collected by RJ Post and deposited in The Natural History Museum, London. Other material (excluded from the type series): larval bodies with associated chromosome preparations, collected simultaneously with holotype (NHM). Some pupae collected simultaneously with the larval material, and some pinned adults reared from the pupae, probably include *S. konkourense* but cannot be unequivocally associated. They are in The National History Museum.

36 *Distribution.* Widely distributed in major rivers in the highlands, from the Fouta Djallon mountains in Guinea south-eastwards along the Niger basin watershed between Guinea and Sierra Leone and Liberia (table 1, fig. 1).

37 *Discussion.* Quillévéré (Quillévéré *et al.,* 1982) was the first to notice cytotaxonomic heterogeneity within thé S. *sanctipauli* subcomplex from Guinea, and the recognition of *S. konkourense* sp. n. has been developed from his original criteria for the identification of forme 'Konkouré'. Whilst the name of his form-is not available in formai taxonomie nomenclature we feel that it is appropriate to use it as the stem for the name of our-new species, and in view of this Dr D. Quillévéré has agreed to be coauthor of the species name *S. konkourense.*

38 *Simulium konkourense* sp. n. is characterized by the absence of inversions IL-A, IIS-A and IIIL-24, and inversion IS A is polymorphie at low frequency. These features distinguish *S. konkourense* sp. n. from *S. sanctipauli, S. soubrense* 'Chutes Milo' form and S. *leonense* sp. n. The fixed and. polymorphie inversions are shown diagrammatically on the idiogram (fig. 2) and frequencies are summarized by river basin in table 4.

39 However, the western Guinea populations of *S. konkourense* sp. n. do show some unique chromosomal features (table 6) and have already been described as a distinct geographic variant named forme 'Konkouré' (Quillévéré *et al.,* 1982). The geographic pattern of variation of the individual inversions (table 4) is not uniform, but multivariate analysis of inversion sample frequencies (table 6) by principal components analysis and minimum spanning tree (figs 3 and 4) suggests that the overall pattern of variation follows a stepped cline. The groups of populations correspond to, firstly the extreme westerly samples from the Koumba and Konkouré river basins, secondly the Bafing river basin and thirdly the samples from south-eastern Guinea and Sierra Leone. These three groups are geographically separated and examination of table 6 shows that there are no fixed inversion differences or other evidence of separate specific status. Indeed the overall pattern of variation seems to be typical of an intraspecific stepped cline (Endler, 1980). The two ends of this cline are genetieally substantially different, and are best considered as separate geographic races ('Menankaya' form and forme 'Konkouré') within *S. konkourense.*

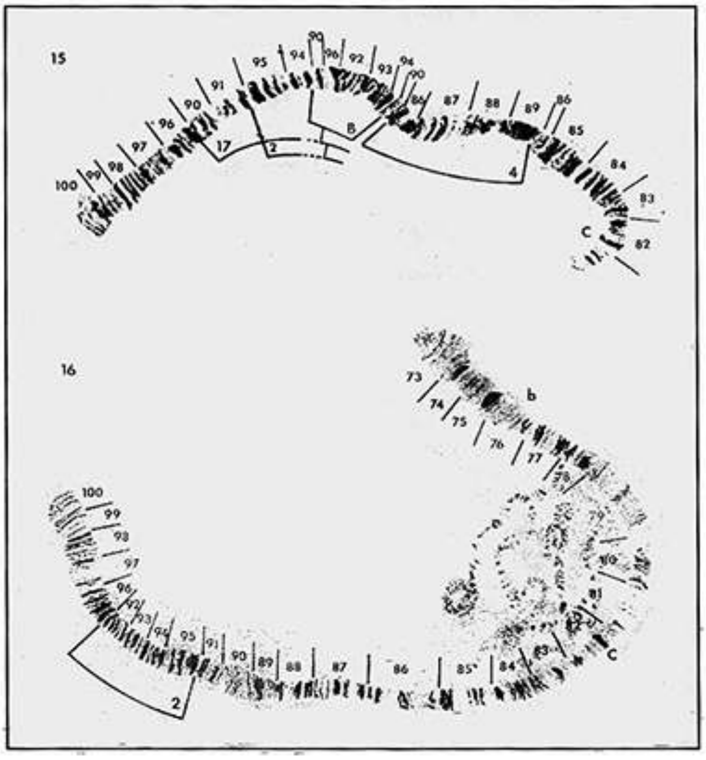

Fig. 15–16. Chromosome III: 15 *Simulium sanctipauli* sp. n. (IIIL-4.17.2.B/4.17.2.B) from R. Comoe at Mbaso on 29 July 1980; 16, *S. leonense* sp. n. (IIIS-st/st, IIIL-2/2) from R. Tabe at Baiima on 8 December 1980.

40 Within *S. konkourense* sp. n. the most characteristic feature of forme 'Konkouré' is the complex rearrangement on chromosome arm IIL already described as diagnostic by Quillévéré *et al.* (1982). Exact banding pattern analysis has shown that the complex heterozygotes consist of two sequences IIL-4.6.7.D/4.X of which the inversion IIL-X is unique, and within the whole complex rearrangement, can on its own be considered to distinguish forme 'Konkouré' from 'Menankaya' form. The use of IIL-X for the identification of forme 'Konkouré' within *S. konkourense* sp. n. is based on Quillévéré's original criteria (Quillévéré *et al.,* 1982). The inversion IIL-X was found only in the chromosome sequence I1L-4.X, which was polymorphic with IIL-4.6.7.D. Thus populations in which IIL-X occurs (either as a homozygote IIL-4.X/4.X, or heterozygote IIIL-4.X/4.6.7.D) are identified as forme 'Konkouré' whilst other populations (which are fixed IIL-4.6.7.D/4.6.7.D) are defined as 'Menankaya' form. These criteria for identification of forme 'Konkouré' and 'Menankaya' form are based on the cytogenetic characteristics of the population, as are the criteria for the identification of other geographic races within the *S. sanctipauli* subcomplex, such as 'Beffa' form (Meredith *et al.,* 1983) and 'Djodji' form (Surtees *et al.,* 1988).

41 Amongst the floating inversions there was found to be geographic variation in the sex chromosome System of *S. konkourense* sp. n., following the multivariate cytogenetic stepped cline described above. In the 'Menankaya' form of *S. konkourense* sp. n. from Sierra Leone and southeastern Guinea no sex-linked inversions were observed, whereas further west (*S. konkourense* sp. n. forme 'Konkouré') IIIL sequences were sex linked. In the Bafing river basin the most common X and Y chromosomes were IIIL-2 and IIIL-4.17.2, respectively (table 5), which is identical to the sex chromosome System in *S. leonense* sp. n.

(Post, 1986, as *S. soubrense* 'B'). In the Konkouré and Koumba river basins the most common Y chromosome is still IIIL-4.17.2., but the most common X chromosome sequence is IIIL-X (table 5), which is a new inversion (fig. 7) unknown outside the Fouta Djallon area.

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ABSTRACTS

The *Simulium sanctipauli* Vajime & Dunbar subcomplex of the West African *S. damnosum* Theobald complex is cytotaxonomically revised for the western part of the Onchocerciasis Control Programme area. The subcomplex is defined and a chromosomal key provided for the identification of the sibling species and forms recognized. Two sibling species are newly described, *S. leonense* Boakye, Post & Mosha (Sierra Leone) and *S. konkourense* Boakye, Post, Mosha & Quillévéré (Guinea and Sierra Leone). Detailed chromosomal data are provided as warranty for the conclusions about the specific or infraspecific status of the taxa recognized.

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L’onchocercose humaine en Afrique

Human onchocerciasis in Africa

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1 L’onchocercose humaine est une parasitose causée par *Onchocerca volvulus,* filaire transmise par les femelles de diptères du genre *Simulium.* Le nombre de personnes infectées par *Onchocerca volvulus* est estimé à environ 18 millions, dont plus de 99 % vivent en Afrique inter-tropicale. Les principales complications de la maladie sont oculaires et dermatologiques. On considère que 270 000 à 360 000 personnes sont aveugles du fait de l’onchocercose, ce qui fait de cette maladie la quatrième cause de cécité dans le monde, après la cataracte (16 millions), le trachome (5,9 millions) et les différentes formes de glaucome (5,1 millions). De plus, environ 500 000 autres personnes souffriraient d’une incapacité visuelle grave liée à l’onchocercose.

2 Jusque dans les années 1980, la seule méthode de lutte contre l’onchocercose était l’éradication des populations de simulies. Un programme à très large échelle basé sur cette stratégie, le Programme de Lutte contre l’Onchocercose en Afrique de l’ouest (*Onchocerciasis Control Programme* ou OCP), fut lancé en 1974 sous l’égide de quatre agences parrainantes : l’OMS, la Banque Mondiale, le PNUD et la FAO. Ce programme doit se poursuivre jusqu’en 2002 mais les bénéfices acquis jusqu’à présent, aussi bien sanitaires qu’économiques, font qu’OCP est d’ores et déjà considéré comme un succès majeur. Une nouvelle étape fut franchie dans les années 1980 grâce à la découverte de l’ivermectine, médicament microfilaricide efficace en prise orale unique et pouvant être distribué à large échelle. En 1987, la décision des Laboratoires Merck & Co Inc. de donner l’ivermectine pour le traitement de l’onchocercose à tous les états et organisations qui en font la demande, et ce pour aussi longtemps que nécessaire, a suscité la mise en place de programmes nationaux de lutte contre l’onchocercose dans la plupart des pays concernés par la maladie. Cette dynamique a conduit la Banque Mondiale à lancer, en 1995, le Programme Africain de Lutte contre l’Onchocercose (*African Programme for Onchocerciasis Control* ou APOC) dont l’objectif est d’éliminer dans les 12 prochaines années l’onchocercose en tant que problème de santé publique en Afrique grâce à la mise en place de distributions d’ivermectine gérées par les communautés elles-mêmes.

3 La lutte contre l'onchocercose suscite donc à nouveau une importante mobilisation au niveau international. De nombreuses recherches à visée opérationnelle sont entreprises pour que les opérations de lutte aient une efficacité optimale. La présente revue a pour objectif de présenter les aspects parasitologiques, cliniques, diagnostiques et épidémiologiques de l'onchocercose, aspects qui ont tous fait l'objet récemment d'avancées importantes. Un deuxième article concernera les méthodes et les stratégies de lutte contre la maladie. Sauf exception, nous n'aborderons ici que les problèmes liés à l'onchocercose africaine.

LE PARASITE ET SON CYCLE

4 On considère que l'homme est pratiquement le seul hôte définitif naturel d'*Onchocerca volvulus,* même si un ver adulte de cette espèce a été trouvé chez un gorille (1). Les seuls animaux chez lesquels l'inoculation de larves infectantes d'*Onchocerca volvulus* a permis le développement du parasite jusqu'au stade de ver adulte mature sont le chimpanzé (2) et le singe mangabey (3).

5 Les vers adultes, ou macrofilaires, vivent dans des nodules sous-cutanés ou profonds. Le mécanisme de formation des nodules est mal connu (4). Pour Albiez et Coll. (5), *les rapports faisant état de la présence de femelles adultes vivant libres dans les tissus doivent être considérés avec scepticisme ; cependant, il est certain que les vers mâles peuvent migrer d'un nodule à l'autre.* Cette migration des mâles permet à chacun d'entre eux d'inséminer plusieurs femelles. A l'exception d'un spécimen observé dans la paroi de l'aorte, aucun ver adulte n'a jamais été trouvé dans les cavités ou les organes internes (5). La longueur des femelles varie de 50 à 70 centimètres, alors que celle des mâles n'excède pas 5 centimètres. En moyenne, 80 % des nodules contiennent 1 ou 2 vers mâles et 2 ou 3 femelles.

6 Les femelles sont vivipares et produisent des embryons, les microfilaires, qui constituent le stade pathogène du parasite. Si la longévité des femelles est estimée à 15 ans, leur période de fécondité n'est en moyenne que de 9 à 11 ans et 95 % d'entre elles ne sont plus productives à l'âge de 13-14 ans (6). On estime qu'une femelle produit de 500 000 à un million de microfilaires par an (7, 8).

7 Les microfilaires, dépourvues de gaine, mesurent 250 à 330 µm de long sur 5 à 8 µm de diamètre. Leur longévité moyenne est de 12-15 mois mais peut atteindre 30 mois (8). Les microfilaires quittent activement le vagin de la femelle, pénètrent dans les lymphatiques de la capsule du nodule puis migrent vers les vaisseaux lymphatiques du derme superficiel où elles n'induisent aucun processus inflammatoire. Leur passage, spontané ou provoqué par certains médicaments, dans le tissu conjonctif du derme induit à ce niveau un processus inflammatoire aigu puis chronique, non spécifique, qui conduit à leur destruction (9). Ces phénomènes induisent l'apparition de lésions cutanées. Ils sont également à l'origine de la réaction aiguë, dite de Mazzotti, qui survient à la suite d'un traitement par la diéthylcarbamazine (DEC). La présence des microfilaires dans le circuit lymphatique fait également suggérer qu'elles pénètrent dans l'œil par les capillaires lymphatiques de l'angle irido-cornéen (10). Toutefois, il existe probablement d'autres voies de pénétration des microfilaires dans l'œil (11). Le fait que les systèmes lymphatiques et sanguins communiquent entre eux explique que les microfilaires d' *Onchocerca volvulus* sont parfois retrouvées dans le sang et les urines des sujets infectés, notamment après la prise d'un médicament microfilaricide.

8 Le cycle ne peut se poursuivre qu'après ingestion des microfilaires par les simulies. Cette ingestion se fait lors d'un repas sanguin qui fournit aux simulies femelles les protéines nécessaires à la maturation de leurs œufs. Lors de la piqûre, les pièces buccales de l'insecte dilacèrent les tissus dermiques et le micro-hématome qui se forme alors contient les microfilaires issues des lymphatiques. Le nombre de parasites ingérés est étroitement corrélé avec la densité microfilarienne dermique (12). L'ingestion du sang provoque la formation, par les cellules digestives de la simulie, d'une membrane péritrophique qui sépare rapidement la masse sanguine de la paroi de l'estomac. La plupart des microfilaires ingérées sont emprisonnées dans cette membrane, mais certaines d'entre elles, traversant assez précocément la paroi stomacale, pourront poursuivre le cycle parasitaire. L'épaisseur et la rapidité de formation de cette membrane, variables selon les espèces vectrices, influe sur la proportion de microfilaires réussissant à passer dans l'hémocèle. Chez les espèces savanicoles *Simulium damnosum* s.s. et *Simulium sirbanum,* cette proportion est d'autant plus faible que le nombre de microfilaires ingérées est plus élevé ; ce phénomène est appelé phénomène de limitation (13, 14). Chez les vecteurs de forêt, la proportion de microfilaires passant dans Phémocèle semble constante jusqu'à un seuil situé aux alentours de 10 microfilaires ingérées ; au delà, il existe comme chez les simulies de savane un phénomène de limitation (15). Après passage dans Phémocèle, les microfilaires migrent dans les muscles indirects du vol, où elles se raccourcissent et s'épaississent pour aboutir à une forme appelée forme saucisse ou premier stade larvaire (L1). Deux mues successives produisent un deuxième (L2) puis un troisième stade larvaire (L3). Ce dernier s'allonge pour prendre l'aspect définitif de larve infectante, très mobile et longue en moyenne de 650 µm. La durée totale du cycle parasitaire chez le vecteur, qui dépend de la température extérieure, est de 6 à 8 jours. Cette durée étant supérieure à l'intervalle entre deux repas sanguins (4 à 6 jours), la simulie ne peut habituellement transmettre des larves infectantes à un nouvel hôte qu'au cours de son troisième repas de sang.

9 Lors d'un nouveau repas sanguin, les larves infectantes présentes à la base des pièces buccales de la simulie s'échappent par effraction, probablement en rompant la membrane labio-hypopharyngienne. Elles sont alors déposées sur la peau de l'hôte et certaines d'entre elles pénètrent activement dans le derme par la plaie de piqûre. Dans les 3 à 7 jours suivants, les L3 subissent une nouvelle mue, probablement près de leur point d'entrée dans l'organisme (16). Il en résulte une larve de quatrième stade (L4) dont on sait peu de choses mais qui est probablement très mobile. On estime que la dernière mue, qui transforme les L4 en adultes, a lieu un à trois mois après la piqûre infectante. Les vers adultes immatures, ou juvéniles, sont de très petite taille : les femelles mesurent souvent moins d'1 centimètre. Le processus aboutissant au développement des juvéniles jusqu'au stade mature est très mal connu. La période de latence, intervalle entre la piqûre infectante et la détection de microfilaires dans le derme, est en moyenne de 7 à 12 mois mais peut atteindre trois ans (17).

LES ASPECTS CLINIQUES

10 L'augmentation progressive, du fait des infestations répétées, de la charge microfilarienne chez les sujets vivant en zone d'endémie entraîne l'apparition des complications de la maladie : l'onchocercose est une maladie par accumulation. Parmi les complications, les lésions oculaires cécitantes ont fait l'objet de la plus grande attention ;

cependant, l'impact des lésions cutanées est actuellement considéré comme également très important.

Les nodules onchocerquiens ou onchocercomes

11 Les nodules, isolés ou groupés, mesurent le plus souvent de 0,4 à 2 centimètres de diamètre. Généralement indolores, ils peuvent parfois provoquer une gêne assez importante, notamment lorsqu'ils sont situés près d'un tronc nerveux. Ils peuvent, très rarement, être calcifiés. Les nodules sous-cutanés sont principalement retrouvés en regard des plans osseux (Fig. 1), notamment au niveau de la ceinture pelvienne, des membres et de la tête. En Afrique, les nodules crâniens sont particulièrement fréquents chez les enfants. Les nodules superficiels sont le plus souvent mobiles, mais peuvent être fixés à la peau, aux aponévroses, au périoste ou aux articulations. Les nodules fixés à la peau peuvent se perforer spontanément et laisser alors échapper un ou plusieurs vers adultes. Les nodules profonds, qui représentent les deux tiers de la totalité des nodules présents dans l'organisme (8), sont principalement situés près des articulations, notamment de la hanche, et dans les aponévroses.

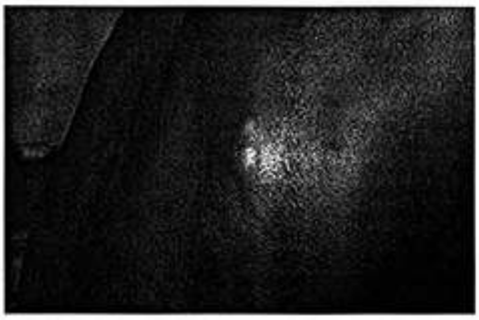

Figure 1 - *Nodules onchocerquiens au niveau du gril costal*

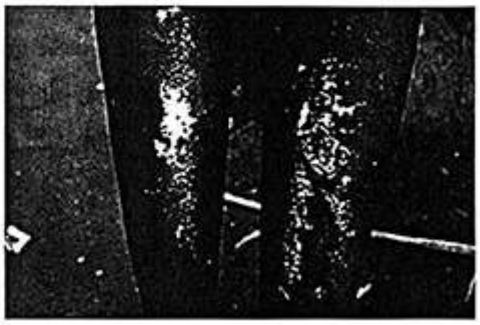

Figure 2 - *Dépigmentation cutanée au niveau des crêtes tibiales.*

Les lésions cutanées ou onchodermatite

12 La présence des microfilaires dans le derme provoque, de manière inconstante, un prurit parfois sévère pouvant se compliquer de lésions de grattage étendues et surinfectées. Sa présence et son intensité ne sont pas corrélées avec la charge microfilarienne dermique.

13 Les lésions cutanées onchocerquiennes ont fait l'objet de descriptions histologiques détaillées (18). Une classification basée sur des critères cliniques précis a été récemment proposée (19). On distingue ainsi l'onchodermatite papulaire aiguë, l'onchodermatite

papulaire chronique, l'onchodermatite lichénifiée, l'atrophie cutanée et la dépigmentation. L'onchodermatite papulaire aiguë correspond à la lésion appelée précédemment gale filarienne ou *craw craw* ; elle se présente sous forme de petites papules prurigineuses, de 1 à 3 millimètres de diamètre, largement disséminées, pouvant évoluer vers des vésicules et des pustules. L'onchodermatite papulaire chronique consiste en papules disséminées, plates, de taille variable (3 à 9 millimètres de diamètre), plus ou moins élevées et souvent associées à une hyper-pigmentation ; elle est particulièrement fréquente au niveau des fesses. L'onchodermatite lichénifiée s'observe surtout, mais non exclusivement, au Soudan et au Yémen ; touchant préférentiellement les adolescents et les adultes jeunes, elle concerne habituellement les membres inférieurs ; quand un seul membre est atteint, ce qui est fréquent, le tableau correspond à celui décrit sous le nom de *sowda* ; le tableau associe un prurit intense, une hyperpigmentation et des plaques hyper-kératosiques qui peuvent devenir confluentes. L'atrophie cutanée due à l'onchocercose diffère peu de l'atrophie sénile si ce n'est qu'elle touche préférentiellement les fesses et les membres et qu'elle survient plus précocément dans la vie. Au niveau inguinal, la perte d'élasticité de la peau peut conduire à une manifestation rare mais caractéristique de la maladie appelée aine pendante. La dépigmentation cutanée onchocerquienne est caractéristique : on observe des zones de peau complètement dépigmentées avec, à l'intérieur, des îlots de peau normalement pigmentée centrés sur les follicules pileux ; ces lésions, qui touchent surtout les sujets âgés, sont décrites sous le nom de peau de léopard ; elles sont habituellement localisées au niveau des crêtes tibiales (Fig. 2) ; leur pathogénie est controversée : pour certains, la dépigmentation résulterait de lésions de grattage dues au prurit causé par les piqûres des simulies (20) ; pour d'autres, les lésions seraient la conséquence d'une inflammation chronique causée par les microfilaires (21) ; le rôle de toxines émises par les parasites ou présentes dans la salive des simulies a également été évoqué.

14 Chez les expatriés, ou plus généralement chez les personnes provenant de zones non endémiques et exposées pour la première fois à l'infection à l'âge adulte, l'onchocercose peut produire un tableau particulier. Ces sujets présentent des papules rouges, très prurigineuses, souvent localisées à un membre, et parfois accompagnées au même niveau de myalgies profondes. On peut observer une lymphadénopathie qui, lorsqu'elle est importante, réalise le tableau connu sous le nom de gros bras camerounais (22).

Les lésions lymphatiques

15 Les sujets infectés par *Onchocerca volvulus* présentent souvent des adénopathies non douloureuses, notamment au niveau inguinal. Les ganglions contiennent des microfilaires intactes ou en voie de destruction du fait d'une réaction inflammatoire. L'existence de cas d'éléphantiasis dans des régions où la filariose lymphatique n'existe pas a conduit certains auteurs à s'interroger sur le rôle éventuel d''Onchocerca *volvulus* dans la genèse de ces lésions. Ce point est encore controversé.

Les lésions oculaires

16 Classiquement, on distingue les lésions du segment antérieur de l'œil, qui touchent la cornée et l'iris, et celles du segment postérieur, qui concernent la choriorétine et le nerf optique. Les préparations histologiques de matériel nécropsique ont permis de trouver

des microfilaires dans tous les tissus de l'œil. Cependant, hors cas exceptionnels, c'est au niveau de la cornée et surtout de la chambre antérieure que les parasites sont habituellement observés. Dans cette dernière localisation, les microfilaires sont le plus souvent vivantes et peuvent être vues facilement dans le faisceau de la lampe à fente, notamment si le patient a gardé la tête penchée en avant pendant plus de 2 minutes. L'existence de microfilaires dans la chambre antérieure est en relation avec la charge microfilarienne dermique et constitue un facteur de risque de survenue de lésions oculaires graves.

17 Dans la cornée, les microfilaires sont surtout localisées en périphérie. Bien tolérées tant qu'elles sont vivantes, elles induisent, lorsqu'elles meurent, une réaction inflammatoire mettant en jeu des réponses immunitaires locales et générales (23). Histologiquement, il se constitue autour du parasite un infiltrat de lymphocytes et d'éosinophiles associé à un œdème. Ce processus aboutit à la formation de lésions arrondies, cotonneuses, mesurant environ 0,5 millimètres de diamètre et appelées kératites ponctuées. Dans les premières semaines, la microfilaire en voie de destruction peut être vue au centre de la lésion. On a pu observer jusqu'à 50 lésions pour un seul œil. La kératite ponctuée, souvent retrouvée chez les sujets jeunes, n'induit qu'une gêne fonctionelle mineure et disparaît spontanément en quelques mois. Chez les sujets fortement parasités, la présence massive et prolongée de microfilaires dans la cornée peut entraîner une réaction inflammatoire intense conduisant à un processus de cicatrisation avec formation de néo-vaisseaux. Cliniquement, il en résulte une lésion grave : la kératite sclérosante. L'hypothèse selon laquelle cette dernière résulterait de la progression des kératites ponctuées n'est pas démontrée (23). L'aspect clinique et le mode d'évolution de la kératite sclérosante sont caractéristiques (24). La phase initiale, marquée par l'apparition d'un voile au niveau du limbe, est suivie par un envahissement cornéen superficiel par un infiltrat de cellules inflammatoires. Les lésions, d'abord localisées en nasal et en temporal, progressent rapidement vers le bas, au niveau de la cornée inférieure, pour devenir confluentes et prendre une disposition dite semi-lunaire. Progressivement, la lésion devient de plus en plus opaque et on note l'apparition de néo-vaisseaux et de taches de pigment. Le pannus ainsi constitué progresse en périphérie et vers le haut. La lésion devient cécitante quand elle affecte l'axe visuel.

18 L'atteinte de l'iris se manifeste au début par une diminution du réflexe pupillaire à la lumière. Puis se développe un iritis torpide, indolore, associé à un effet Tyndall. Au stade ultime, des synéchies apparaissent qui peuvent entraîner une déformation de la pupille. Un glaucome secondaire ou une cataracte peuvent compliquer ces lésions.

19 Les lésions choriorétiniennes ont fait l'objet de descriptions cliniques détaillées (18, 24, 25). L'atteinte débute par un remaniement de l'épithélium pigmentaire de la rétine, qui devient irrégulier. Elle touche habituellement la région située en temporal de la macula. A la phase de début, l'atrophie n'est pas complète et l'altération de l'épithélium, marquée par une disparition du pigment, ne concerne que des zones limitées. Cliniquement, les lésions ont une couleur jaunâtre ou grisâtre et des limites floues donnant un aspect en mottes ou pommelé. Puis on observe une confluence des zones altérées et une disparition complète de l'épithélium pigmentaire qui laisse apparaître le réseau vasculaire choroïdien sous-jacent. A ce stade, les zones d'atrophie de l'épithélium présentent des limites nettes et on observe fréquemment à ce niveau des mottes de pigment noires ou brunes (Fig. 3). Les vaisseaux choroïdiens sous-jacents gardent encore une couleur et un diamètre normaux et le tableau réalise alors l'aspect dit de choriorétinite tigroïde. Celle-

ci peut ne concerner qu'une zone assez limitée et se présenter alors sous la forme d'une placode. Au dernier stade, l'atteinte associe une atrophie complète de l'épithélium pigmentaire de la rétine et une sclérose des vaisseaux choroïdiens qui, normalement de couleur orange, prennent l'aspect de cordons blancs tranchant sur le fond noir de la choroïde. Les lésions concernent alors habituellement de très larges plages, voire la totalité du fond d'œil. Elles réalisent l'aspect dit en boue séchée. Les travaux concernant les mécanismes pathogéniques à l'origine de l'atteinte choriorétinienne ont fait l'objet d'une synthèse récente (26). Le processus serait initié par une réaction inflammatoire locale causée par la mort et la destruction de microfilaires dans la rétine et la choroïde ; ceci expliquerait la survenue de lésions choriorétiniennes à la suite d'un traitement par DEC. Les éosinophiles présents en abondance dans la rétine et la choroïde du fait de l'inflammation libèreraient des molécules toxiques qui entraîneraient une altération de l'épithélium pigmentaire. La poursuite du processus ne nécessiterait plus la présence des microfilaires et mettrait en jeu des phénomènes d'auto-immunité. Ces phénomènes seraient dus à une similitude entre un antigène parasitaire, appelé Ov39, et des antigènes de la rétine. Des anticorps spécifiquement dirigés contre l'Ov39 provoqueraient directement des lésions au niveau de l'épithélium pigmentaire.

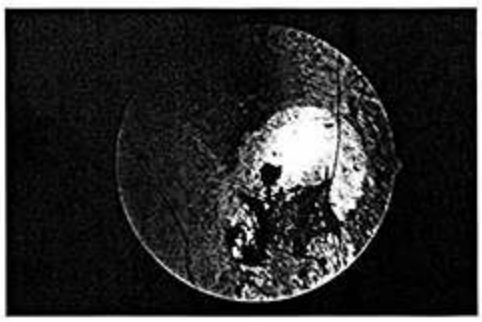

Figure 3 - *Lésions choriorétiniennes avancées.*

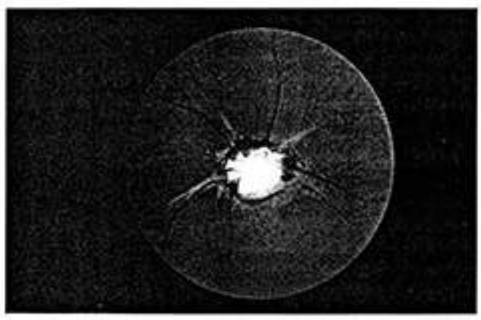

Figure 4 - *Atrophie optique avec amas de pigments et engainement des vaisseaux rétiniens.*

20 A côté des lésions choriorétiniennes typiques de l'onchocercose, qui forment un continuum, on observe chez certains sujets vivant en zone d'endémie des lésions ponctuées arrondies dont la pathogénie est mal documentée. Semba et Coll. (27) en ont décrit deux types : d'une part les dépôts intra-rétiniens blancs, de 10 à 100 µm de diamètre, susceptibles d'apparaître et de disparaître en quelques mois, plus nombreux chez les sujets présentant des lésions choriorétiniennes sévères et qui ressemblent aux lésions décrites chez des sujets traités par DEC (28) ; d'autre part les dépôts intra-rétiniens brillants, plus petits, à limites nettes, et qui ne semblent pas plus nombreux

chez les individus présentant une choriorétinite avancée. La relation entre l'onchocercose et ce dernier type de lésions mériterait d'être précisée.

21 L'atteinte du nerf optique se présente d'abord comme une pâleur papillaire sans caractère particulier. L'évolution conduit à la constitution d'une atrophie optique souvent associée à des dépôts pigmentaires en bordure de la papille et à un engainement des vaisseaux rétiniens (Fig. 4). L'association de lésions avancées de la choriorétine est un argument en faveur de l'origine onchocerquienne de l'atteinte papillaire.

22 Les lésions oculaires peuvent avoir des répercussions fonctionnelles variables pouvant aller jusqu'à la cécité. Les sujets présentant des microfilaires dans la chambre antérieure de l'œil indiquent qu'ils voient parfois les parasites bouger dans le champ visuel. L'héméralopie, fréquente, peut être liée soit à la rigidité pupillaire qui gêne la mydriase crépusculaire soit à l'atteinte de l'épithélium pigmentaire de la rétine. Les répercussions les plus graves sont la baisse de l'acuité visuelle et le rétrécissement du champ visuel. La première peut être due à une kératite sclérosante, à une iridocyclite avancée, à des lésions choriorétiniennes touchant la macula ou à une atrophie optique. La macula n'étant touchée que très tardivement, une choriorétinite isolée peut, pendant longtemps, n'entraîner que des troubles du champ visuel.

Les autres manifestations

23 Les microfilaires d'*Onchocerca volvulus,* habituellement localisées dans le système lymphatique, ont également été observées dans les organes profonds (foie, rate, reins, poumons) ainsi que dans le sang, les urines, le liquide céphalo-rachidien, les sécrétions vaginales et les expectorations (18). Cette dissémination du parasite dans l'organisme pourrait être à l'origine de manifestations qui sont classiquement regroupées sous le terme d'onchocercose systémique.

24 Une infestation importante par *Onchocerca volvulus* peut entraîner des troubles du développement staturopondéral. A cet égard, deux tableaux cliniques doivent être distingués. Le premier consiste en une diminution modérée, mais significative, du poids des sujets infectés par rapport à celui des sujets indemnes. Le second tableau, décrit pour la première fois en Ouganda où il est appelé *Nakalanga* (29), consiste en un nanisme harmonieux avec infantilisme sexuel, souvent associé à une épilepsie et à un retard mental. Ce tableau a également été observé au Burundi (30) et au Cameroun (M. Boussinesq, données non publiées). Kipp et Coll. (31) ont récemment avancé un argument en faveur de l'existence d'une association entre *Nakalanga* et onchocercose : ces auteurs indiquent que les cas de *Nakalanga,* fréquents avant les années 1960 dans la vallée du Nil Victoria, n'ont plus été observés depuis la mise en place dans cette région d'une campagne de lutte antisimulidienne ayant conduit à l'interruption totale de la transmission de l'onchocercose. Toutefois, la pathogénie de ce syndrome est à ce jour inconnue.

25 Une relation entre onchocercose et épilepsie a été signalée dans de nombreux pays. C'est à nouveau en Afrique de l'est que des études récentes ont apporté des arguments forts en faveur de cette association. Dans l'ouest de l'Ouganda, il existe une corrélation entre le niveau d'endémie onchocerquienne et la prévalence des épilepsies (32). Une relation identique a été notée dans la vallée du Mbam, au Cameroun, où la prévalence de l'épilepsie atteint dans certains villages 8 % de la population (M. Boussinesq, données non publiées). De plus, en Ouganda et au Burundi, l'infestation par *Onchocerca volvulus* est plus

fréquente chez les sujets épileptiques que dans la population générale (30, 33). Enfin, une diminution de la fréquence des crises d'épilepsie a été signalée après traitement par ivermectine dans des foyers d'onchocercose en Ouganda (34) et au Tchad (I. Bertocci, communication personnelle). Une telle association n'a cependant pas été observée dans d'autres régions (35). Les mécanismes pouvant expliquer l'association entre onchocercose et épilepsie sont inconnus. Roblès a observé au Guatemala plusieurs cas de nodules ayant perforé la voûte du crâne, dont un associé à une épilepsie (36). Des microfilaires d' *Onchocerca volvulus* ont été trouvées dans le liquide céphalo-rachidien après une prise de DEC (37, 38) et en l'absence de traitement (39) mais n'ont jamais été observées au niveau cérébral. Les conséquences sociales de l'épilepsie et la surmortalité qui frappe les épileptiques en Afrique devraient encourager la poursuite des travaux sur l'association entre onchocercose et épilepsie.

26 Enfin, il est possible que l'onchocercose ait des répercussions sur la fertilité et qu'elle provoque ou favorise la survenue d'avortements, de troubles des règles et de la lactation. Un traitement par ivermectine pourrait améliorer certains de ces troubles (40, 41).

LES ASPECTS DIAGNOSTIQUES

Le diagnostic clinique

27 Certains signes cliniques sont pathognomoniques ou très évocateurs de l'onchocercose. La consistance et la localisation particulières des nodules font que le diagnostic différentiel est en général aisé. Dans moins de 5 % des cas, cependant, on peut confondre un onchocercome avec un ganglion lymphatique ou un lipome (5). L'échographie peut permettre de faire le diagnostic différentiel (42, 43) : un nodule typique est caractérisé par un noyau central échogène, relativement homogène et contenant des particules échodenses. La localisation particulière de l'onchodermatite papulaire aiguë est également évocatrice. L'aspect et la localisation typiques de la dépigmentation cutanée d'origine onchocerquienne font que l'on peut considérer ce signe comme pathognomonique de la maladie. Certains auteurs ont cependant indiqué, qu'en Equateur, le pian pouvait provoquer une dépigmentation d'aspect similaire (44).

28 La présence de microfilaires d'*Onchocerca volvulus* dans la cornée ou dans la chambre antérieure de l'œil est évidemment pathognomonique. L'aspect des lésions rétiniennes débutantes n'est pas typique de l'onchocercose ; cependant, leur association avec d'autres lésions et leur localisation fréquente en temporal de la macula sont évocatrices de l'étiologie onchocerquienne. La choriorétinite onchocerquienne avancée présente un aspect caractéristique mais, dans certains cas, peut faire éventuellement discuter une toxoplasmose, une syphilis, voire une histoplasmose.

Le diagnostic parasitologique

29 Le diagnostic repose en fait sur la mise en évidence des microfilaires dans la peau. Celles-ci peuvent être recherchées dans le suc dermique obtenu après scarification (45). Cependant, la méthode de référence consiste à prélever avec une pince à sclérectomie une biopsie cutanée de poids calibré et à rechercher et compter au microscope les microfilaires issues de ce prélèvement après une certaine durée d'incubation. Le site de prélèvement, le type de pince, le milieu et la durée d'incubation peuvent varier, mais

l'OMS recommande de prélever deux biopsies (une à chaque crête iliaque) avec une pince de type Holth 2mm (le poids moyen de la biopsie est alors de 2,84 milligrammes) et de laisser incuber les biopsies à température extérieure pendant 24 heures dans du sérum physiologique (46). Après cette période, environ 80 % des microfilaires initialement présentes dans la biopsie se trouvent dans le liquide d'incubation (47).

Les tests à la diéthylcarbamazine

30 L'administration de 50 milligrammes de DEC chez des sujets infectés par *Onchocerca volvulus* provoque, 15 à 30 minutes après la prise, une réaction cutanée appelée réaction de Mazzotti (18). Le test diagnostique basé sur ce principe ne doit être utilisé que dans le cas où il existe une forte suspicion d'onchocercose malgré l'absence de parasite au niveau de la peau ou de l'œil (48). On a pu observer, chez les sujets fortement infectés, une toux, une tachypnée, voire un choc à la suite de la prise. Bien que ce test soit assez sensible et assez spécifique, on peut observer des faux négatifs et des faux positifs, notamment en cas d'infestation par *Mansonella streptocerca.*

31 Un test basé sur le même principe a été mis au point dans le cadre d'OCP en vue de dépister une reprise ou une recrudescence de l'infestation dans des zones où la transmission de l'onchocercose a été interrompue. Dans ce cas, la DEC n'est plus administrée par voie orale, mais est appliquée sur la peau sous forme de pommade (49). Les sujets infectés présentent, 48 heures après l'application, une réaction papuleuse caractéristique. Ce test au pansement à la DEC donne d'excellents résultats, notamment chez les enfants. Sa sensibilité semble supérieure à celle de la méthode parasitologique classique décrite plus haut Sa spécificité est en cours d'évaluation dans des zones où l'onchocercose coexiste avec d'autres filarioses.

Le diagnostic sérologique

32 De nombreux travaux ont été menés en vue de mettre au point un test sérologique permettant d'identifier non seulement les sujets porteurs de microfilaires d'*Oncho cerca volvulus* dans la peau, mais aussi les individus en phase pré-patente. La mise au point d'un tel test permettrait de détecter précocément une éventuelle reprise de la transmission, notamment dans les zones d'OCP où les opérations de lutte anti-vectorielle ont été interrompues. Ceci a des implications importantes en terme logistique car si la reprise de la transmission est détectée suffisamment tôt, on peut penser que des opérations de lutte sur une échelle assez limitée suffiront à contrôler le phénomène.

33 Les problèmes posés par les méthodes immuno-diagnostiques classiques, notamment leur manque de spécificité et de reproductibilité, ont suscité la mise au point de tests utilisant des antigènes purifiés d'*Onchocerca volvulus.* Par la suite, le développement des techniques de production d'antigènes recombinants a permis de résoudre les problèmes liés aux difficultés d'obtenir du matériel parasitaire. En 1990, l'OMS a lancé une étude multicentrique visant à évaluer la sensibilité et la spécificité de tests sérologiques utilisant 34 antigènes recombinants d'*Onchocerca volvulus* (50). La spécificité de ces tests variait de 75 à 100 % et leur sensibilité de 11 à 96 %. Par ailleurs, il est apparu que l'association de plusieurs antigènes permettait d'augmenter la sensibilité du test (51). Dans un second temps, dix antigènes sélectionnés pour leur bonne spécificité furent testés soit isolément, soit en association, pour déterminer leur capacité à détecter les

anticorps présents chez des sujets en phase de pré-patence ou d'infection débutante. Ceci a abouti à la mise au point de quatre tests utilisant soit un seul, soit un cocktail de deux ou trois antigènes recombinants complémentaires. Dans tous les cas, les anticorps dirigés contre ces antigènes sont détectés par ELISA. Les tests se font à partir d'une goutte de sang recueillie sur papier filtre. Ils permettent de dépister une infection à la phase pré-patente. Au niveau communautaire, il existe une corrélation entre le taux de séropositivité et la prévalence de la microfilarodermie à *Onchocerca volvulus.* Cependant, ces tests ne sont pas assez sensibles pour être utilisés pour le diagnostic individuel de l'infection. Il faut également noter que la séropositivité peut refléter aussi bien une infection passée qu'une infection en cours. De ce fait, dans le cadre de la surveillance d'une reprise de la transmission dans des zones où elle avait été interrompue, le test ne peut être utilisé que chez les enfants nés après l'interruption de la transmission.

Le test diagnostique basé sur l'utilisation de sondes d'ADN

34 Ce test consiste à rechercher dans la peau des individus infectés, grâce à des sondes d'ADN, la présence d'ADN parasitaire et plus précisément de séquences répétées spécifiques d'*Onchocerca volvulus* (52). Les séquences mises en évidence par le test appartiennent à une famille de séquences étroitement apparentées appelée 0-150 car sa longueur unitaire est d'environ 150 paires de bases. Au sein de cette famille, spécifique du genre *Onchocerca,* il existe des variantes permettant de distinguer clairement les différentes espèces d'onchocerques et, au sein d'*Onchocerca volvulus,* plusieurs souches de pathogénicité différente. Après amplification des séquences par PCR, l'application de sondes d'ADN spécifiques d'*Onchocerca volvulus* ou de certaines souches de parasites permettent de faire le diagnostic (53). Le test réalisé à partir d'une biopsie cutanée est beaucoup plus sensible que la méthode parasitologique classique. Du fait de la méthode utilisée (PCR), il ne donne cependant pas d'indication sur la densité microfilarienne. Il peut être réalisé par méthode ELISA (54). Son coût a été estimé à 0,8 US$ par échantillon. La durée pendant laquelle l'ADN parasitaire reste présent dans la peau est encore à déterminer mais on estime que le test devrait permettre de distinguer une infection passée et une infection en cours. La sensibilité d'un test réalisé selon le même principe mais à partir de fragments de peau obtenus par simple grattage avec un vaccinostyle est en cours d'évaluation.

LES VECTEURS D'*ONCHOCERCA VOLVULUS*

Identification et répartition

35 En Afrique, l'onchocercose est transmise par *Simulium damnosum, Simulium neavei* et *Simulium albivirgulatum.* A partir de 1966, l'étude des chromosomes géants des glandes séricigènes des larves de simulies a montré que *Simulium damnosum* était un complexe d'espèces jumelles très proches du point de vue morphologique mais pouvant être distinguées par la disposition des bandes chromosomiques (55). L'étude cytotaxonomique de *Simulium damnosum sensu lato* (s.l.), menée d'abord dans l'aire d'OCP (56, 57) et qui se poursuit dans toutes les zones d'endémie, a permis de décrire plus de 40 formes cytologiques différentes, dont la moitié ont reçu un nom latin classique (48). Schématiquement, le complexe *Simulium damnosum* s.l. rassemble *(a)* le sous-complexe

Simulium damnosum qui regroupe plusieurs espèces de savane, notamment *Simulium damnosum sensu stricto* (s.s.) et *Simulium sirbanum* dont la répartition s'étend de l'Afrique de l'ouest jusqu'au Soudan, *(b)* le sous-complexe *Simulium sanctipauli* qui regroupe des espèces, notamment *Simulium sanctipauli* s.s. et *Simulium soubrense,* vivant en zones humides d'Afrique de l'ouest, *(c)* le sous-complexe *Simulium squamosum* qui regroupe des espèces, notamment *Simulium squamosum* s.s. et *Simulium yahense,* vivant en forêt mais aussi sur les hautes terres d'Afrique de l'ouest, *(d)* l'espèce *Simulium mengense* présente notamment au Cameroun et, *(e)* l'espèce *Simulium kilibanum vecteur* local dans les zones de montagne d'Afrique de l'est (48, 58). Les caractéristiques bio-écologiques des plus répandues de ces espèces (longévité, capacité de dispersion, préférences trophiques, caractéristiques des gîtes de reproduction) sont actuellement bien connues, notamment en Afrique de l'ouest La capacité vectrice varie également entre les différentes espèces : le rendement parasitaire (rapport entre le nombre de larves infectantes et le nombre de microfilaires ingérées) est beaucoup plus important pour les membres du sous-complexe *Simulium sanctipauli* que pour ceux du sous-complexe *Simulium damnosum* (56). Ceci est dû au fait qu'il existe, chez ces derniers, un phénomène de limitation marqué, même si le nombre de microfilaires ingérées est faible (voir plus haut).

36 Dans les zones où plusieurs membres du complexe *Simulium damnosum* s.l. coexistent, il peut être important de distinguer non plus les larves, mais les femelles adultes des différentes espèces. Bien que ces dernières soient proches morphologiquement, un nombre limité de caractères (couleur ou taille de certains éléments de l'insecte) permet de construire des clés d'identification (56). La variabilité de chacun des caractères au sein d'une même espèce et le fait que certains d'entre eux peuvent être communes à plusieurs espèces rendent nécessaire l'utilisation combinée de plusieurs caractères. L'analyse statistique multivariée permet de prendre en compte l'ensemble des caractères observés et d'obtenir des résultats fiables (59). Les autres techniques utilisées pour identifier au niveau spécifique les femelles adultes du complexe *Simulium damnosum* (analyse des hydrocarbones cuticulaires, sondes d'ADN, cytotaxonomie, analyse des isoenzymes) n'ont pas donné de résultat satisfaisant ou ne sont pas applicables dans les conditions de terrain (48).

37 *Simulium neavei,* vecteur de l'onchocercose en Afrique de l'est (Ouganda, Tanzanie, Ethiopie, est de la République Démocratique du Congo (RDC)), est aussi un complexe d'espèces dont les principales sont *Simulium neavei* s.s., *Simulium woodi* et *Simulium ethiopense.* Quant à *Simulium albivirgulatum,* il s'agit d'un vecteur dont la répartition est limitée à la région de la cuvette centrale de la RDC.

Biologie

38 Les femelles de *Simulium damnosum* s.l. pondent leurs œufs dans des cours d'eau rapides (70 à 200 cm/seconde). La répartition géographique de chacune des espèces du complexe dépend notamment du pH de l'eau au niveau des gîtes potentiels. Ainsi, en Afrique de l'ouest, on retrouve *Simulium squamosum* et *Simulium yahense* au niveau des petites rivières de forêt, où le pH est en moyenne de 6,6, tandis que *Simulium soubrense* et *Simulium sanctipauli* peuplent les grandes rivières de forêt, où le pH est en moyenne de 7,8 (56). Les œufs, fixés sur des supports rocheux ou végétaux immergés, éclosent en 24 à 48 heures. Les larves, qui restent fixées sur les supports immergés et se nourrissent de particules en suspension, subissent six mues successives en l'espace de 5 à 12 jours. Cette durée est

d'autant plus courte que la température est plus élevée. La larve de 7e stade mue pour donner une nymphe, également aquatique, qui ne se nourrit pas.

39 L'émergence de l'adulte se produit un à trois jours après. *Simulium neavei* s.l. pond dans les petits cours d'eau très ombragés. Après les premières mues, les larves se fixent sur la carapace de crabes du genre *Potamonautes.* Leur développement jusqu'au stade de nymphe se fait en 26 à 76 jours.

40 Les mâles se nourrissent uniquement de sucs végétaux. Les femelles sont hématophages et absorbent environ 1 mm^3 de sang lors de chaque repas qui dure en moyenne 3 à 4 minutes. Ce sang est indispensable à la maturation des œufs. Peu après l'émergence, les femelles sont inséminées par les mâles et les spermatozoïdes définitivement stockés dans un réservoir, la spermathèque. Les œufs sont fécondés pendant la ponte, lors de leur passage dans l'oviducte. L'intervalle de temps séparant deux pontes, et donc deux repas sanguins, est appelé cycle gonotrophique. En Afrique de l'ouest, sa durée varie de 4 à 6 jours en fonction de la température et de l'âge des femelles (15). L'intervalle entre la ponte et le repas de sang suivant est inférieur à 24 heures. Il est impossible de déterminer le nombre de cycles gonotrophiques accomplis par une femelle de *Simulium damnosum,* mais l'examen des reliques folliculaires permet de distinguer les femelles pares (ayant pondu au moins une fois) et nullipares. Le taux de parturité, ou proportion de femelles pares, permet d'apprécier l'âge moyen des populations de simulies.

41 La dispersion des femelles de *Simulium damnosum* s.l. est essentiellement influencée par le couvert végétal, la nébulosité et l'hygrométrie (60). En zone de savane, pendant la saison sèche, le déplacement des simulies est limité aux galeries forestières longeant les cours d'eau (dispersion linéaire). En revanche, quand le couvert végétal ou la nébulosité offrent une protection aux femelles, celles-ci se déplacent dans toutes les directions à partir des cours d'eau (dispersion radiaire). Quand les conditions sont favorables, la dispersion linéaire peut atteindre 80 kilomètres et la dispersion radiaire plus de 20 kilomètres. En zone de savane, la dispersion radiaire des simulies nullipares est plus importante que celle des simulies pares (61). Les capacités de dispersion de *Simulium neavei* s.l. sont beaucoup plus faibles que celles de *Simulium damnosum* s.l.

42 Outre la dispersion active, les simulies peuvent migrer passivement sur plusieurs centaines de kilomètres en se laissant porter par les vents (62). C'est, notamment le cas en Afrique de l'ouest pendant la première moitié de la saison des pluies : la migration des simulies se fait alors du sud-ouest vers le nord-est grâce au front inter-tropical. Ce phénomène a des implications considérables sur la stratégie de lutte d'OCP.

43 Les simulies ne piquent que le jour et à l'extérieur des habitations. L'activité de piqûre est très réduite quand la température excède 30°C. Ceci se traduit par une courbe journalière d'agressivité montrant habituellement deux pics, l'un en matinée et l'autre en fin d'après-midi. Les membres du complexe *Simulium damnosum* ne se nourrissent pas exclusivement sur l'homme. Leur degré de zoophilie dépend de la disponibilité des hôtes et de l'espèce ; de plus, au sein d'une même espèce, il peut varier en fonction des zones géographiques. Ainsi, *Simulium sirbanum* et *Simulium soubrense* manifestent une très nette tendance à la zoophilie dans la partie nord de leurs aires de répartition. En zone de forêt, *Simulium soubrense* et *Simulium sanctipauli* semblent beaucoup plus zoophiles que *Simulium squamosum* et *Simulium yahense.* Certaines espèces zoo-anthropophiles du complexe *Simulium damnosum* peuvent être vectrices non seulement d'*Onchocerca volvulus* mais aussi d'autres filaires notamment *Onchocerca ochengi,* parasite du bétail. Les larves infectantes

de ces deux espèces étant indifférentiables morphologiquement, il est nécessaire, pour apprécier le taux d'infestation des simulies par *Onchocerca volvulus,* de recourir à des sondes d'ADN spécifiques de cette espèce (voir plus loin).

44 La longévité des simulies n'excède pas un mois. Elle est plus élevée en savane qu'en forêt. En Afrique de l'ouest, la proportion de femelles encore vivantes 9 jours après leur premier repas de sang est de 9 % en zone de forêt et de 47 % en savane.

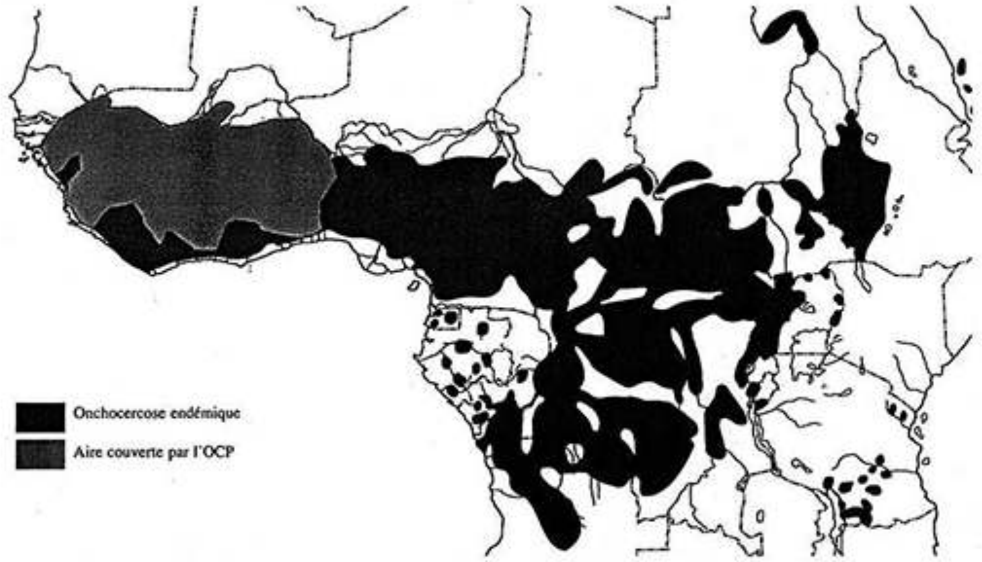

Figure 5 - *Répartition géographique de l'onchocercose en Afrique et dans la Péninsule arabique (selon (48)).*

LES ASPECTS EPIDEMIOLOGIQUES

La répartition géographique

45 L'onchocercose est endémique dans quelques foyers d'Amérique latine et du Yémen, mais 99 % des sujets onchocerquiens vivent en Afrique inter-tropicale (Fig. 5). Le nombre de personnes infectées dépasse un million dans quatre pays : RDC, Nigeria, Cameroun et Ouganda (48). Ces estimations sont en cours d'actualisation grâce à la technique de la cartographie épidémiologique rapide de l'onchocercose (*rapid epidemiological mapping of onchocerciasis* ou REMO) basée sur la mesure de la prévalence des onchocercomes (63, 64). A l'échelle d'une région, la répartition géographique de l'onchocercose est déterminée par la biologie des simulies, notamment l'existence et l'étendue des gîtes larvaires et les capacités de dispersion des adultes.

Le contact homme-vecteur

46 Schématiquement, la densité de la population simulidienne et donc l'intensité du contact entre les vecteurs et la population humaine décroissent au fur et à mesure que l'on s'éloigne des gîtes larvaires. Toutefois, les simulies peuvent parcourir de longues distances en remontant les affluents des cours d'eau où se trouvent les gîtes. Ainsi, si d'une manière générale le niveau d'endémie onchocerquienne tend à diminuer quand on s'éloigne des gîtes, on constate parfois que certains villages situés à distance des gîtes sont plus fortement infectés que des communautés qui en sont plus proches. Par ailleurs,

le schéma général doit prendre en compte la position des villages les uns par rapport aux autres : les villages situés en première ligne par rapport aux gîtes constituent un écran pour les simulies, qui auront moins tendance à se disperser vers les localités situées plus loin. A distance égale par rapport à un gîte, un village de première ligne sera plus fortement infecté qu'une localité située en deuxième ligne (65).

47 Au niveau individuel, un sujet vivant dans l'aire de dispersion d'une population de simulies est d'autant plus piqué que la densité humaine par espace utilisé est plus faible. Une augmentation de cette densité entraînera une dilution des piqûres et on a pu montrer qu'en zone de savane d'Afrique de l'ouest le taux de cécité était inférieur à 5 % lorsque la densité était supérieure à 50 habitants par km^2 (66). Dans les régions où les simulies sont zooanthropophiles, la présence de troupeaux peut diminuer le contact homme-vecteur. Les sujets exerçant une activité près des cours d'eau (pêcheurs, etc.) sont bien entendu plus piqués que les autres. La différence de niveau d'infestation souvent observée entre les deux sexes est probalement due au fait que le contact avec les populations vectrices peut varier entre les deux sexes, à cause de la division du travail (48). Cependant, les études menées sur des modèles animaux montrent que, pour certaines filarioses, le sexe est un facteur pouvant influer sur la densité microfilarienne (O. Bain et G. Petit, communication personnelle).

Les faciès épidémiologiques

48 A partir des années 1950, plusieurs auteurs ont signalé la variabilité des tableaux cliniques de l'onchocercose en fonction des zones géographiques. L'aspect le plus frappant de cette hétérogénéité était la prévalence supérieure des complications oculaires dans les régions de savane d'Afrique de l'ouest par rapport à celle observée dans les zones de forêt De nombreux travaux ont été menés pour confirmer ces différences et tenter de les expliquer (67). Elles furent d'abord attribuées à des différences d'intensité de l'infestation dans les communautés. Le fait qu'en zone de savane les simulies ont une longévité plus importante et se dispersent peu à partir de leurs gîtes de reproduction explique en effet que, dans ces régions, l'intensité de transmission est très élevée dans les villages situés à proximité des cours d'eau (60). Un pas supplémentaire a été effectué quand les enquêtes parasitologiques furent réalisées en utilisant les pinces à sclérectomie permettant d'obtenir des biopsies cutanées de poids calibré. Cette méthode permet de mesurer de façon reproductible la charge microfilarienne moyenne dans une communauté. On put alors démontrer que, pour une charge microfilarienne dermique moyenne donnée, la densité microfilarienne moyenne au niveau de l'œil pouvait varier en fonction des régions et que, pour une charge microfilarienne oculaire moyenne donnée, la prévalence des lésions oculaires pouvait être plus ou moins élevée (68, 69, 70, 71, 72). Les résultats obtenus en Sierra Leone montrent également que l'onchocercose est cécitante dans certaines zones de forêt (72). Ces observations indiquent que les microfilaires peuvent, selon les régions, être d'une part plus ou moins invasives et d'autre part plus ou moins pathogènes pour l'œil.

49 Ceci confirme les observations faites dès 1966 qui révélaient l'existence de plusieurs souches parasitaires. Il apparaissait en effet que, d'une manière générale, le rendement parasitaire chez les simulies d'une région donnée était plus important quand les microfilaires provenaient de sujets originaires de la même zone bio-géographique (73). Ces résultats ont conduit à la notion de complexes *Onchocerca-Simulium.* Par la suite, de

nombreux travaux mirent en évidence des différences morphologiques, iso-enzymatiques et génétiques, ainsi que des variations portant sur la répartition de l'activité phosphatase acide, la localisation des microfilaires dans le derme, le développement des parasites chez le chimpanzé et la sensibilité aux médicaments (67, 74). Mais, la seule étude mettant en évidence une différence de pathogénicité entre différentes souches parasitaires est celle qui a consisté à comparer les lésions oculaires apparues chez le lapin après injection sous-conjonctivale ou intra-oculaire de microfilaires d'origines différentes. Les microfilaires de savane camerounaise se sont révélées être plus invasives et plus pathogènes au niveau de la cornée que celles provenant d'une région de forêt (75,76). En revanche, très peu de différences ont été notées dans le développement des lésions du segment postérieur (77, 78).

50 Le développement des techniques de biologie moléculaire a permis de mettre au point deux sondes d'ADN, appelées pFS-1 et pSS-1BT, spécifiques respectivement des souches de forêt et de savane d'Afrique de l'ouest (79). Ces sondes correspondent à la séquence O-150 évoquée plus haut Par la suite, il a été démontré que la fréquence des complications oculaires en Afrique de l'ouest est en relation avec la présence de l'une ou l'autre des deux souches identifiées par ces sondes (80). Ces résultats confirment que les souches d' *Onchocerca volvulus* peuvent être plus ou moins pathogènes pour l'œil. Les deux sondes décrites plus haut sont actuellement utilisées en routine dans le cadre d'OCP pour identifier, à partir de broyats de simulies, les souches d'*Onchocerca volvulus* transmises à un moment donné dans une zone donnée. Ceci permet de suivre les éventuelles modifications dans la répartition géographique des souches les plus pathogènes dans l'aire d'OCP et de définir la stratégie de lutte optimale à mettre en place (81). La caractérisation de souches parasitaires dans d'autres régions endémiques montre que la classification utilisée à OCP ne peut être appliquée à la totalité de l'Afrique (82).

IMPACT DEMOGRAPHIQUE, SOCIAL ET ECONOMIQUE

L'impact sur la mortalité et la fécondité

51 Une étude menée en savane d'Afrique de l'ouest a montré que l'espérance de vie des aveugles était diminuée d'environ 13 ans par rapport à celle des non-aveugles (83). L'impact de l'infestation elle-même sur la longévité est controversé ; cependant, on a pu observer dans certaines zones une surmortalité chez les hommes fortement infectés et ne présentant pas de baisse marquée de l'acuité visuelle (84). Enfin, si la relation avec l'épilepsie était confirmée, la surmortalité liée à cette pathologie serait à mettre au compte de l'onchocercose.

52 L'impact de l'onchocercose sur la fécondité est mal connu. En Afrique de l'ouest, le rapport du nombre d'enfants âgés de 0 à 4 ans sur le nombre de femmes en âge de procréer a été calculé dans des zones hypo-, méso et hyper-endémiques (83). Bien que ce rapport soit légèrement diminué dans certaines zones hyperendémiques, les auteurs concluent que l'onchocercose n'a pas d'influence directe sur la fécondité. En revanche, il semble qu'en Equateur il existe une relation entre le niveau d'endémie et le taux d'incidence des avortements spontanés (41).

Les mouvements de population

53 Dans les années 1950, plusieurs auteurs émirent l'hypothèse que le phénomène de dépeuplement observé dans certaines régions de savane d'Afrique de l'ouest était dû principalement à l'onchocercose. Outre la morbidité liée à la maladie, l'appauvrissement des communautés du fait de l'existence d'une forte proportion d'aveugles peu productifs aurait poussé les adultes jeunes à quitter des vallées pourtant fertiles. Des études plus fines montrèrent que le dépeuplement avait été provoqué dans un premier temps par des épidémies, notamment de trypanosomiase, et par les contraintes imposées aux populations pendant la période coloniale (travail obligatoire, impôts,...). La chute de la densité de population humaine a entraîné une augmentation du nombre de piqûres de simulies par individu et, par suite, un accroissement de l'intensité de l'infestation par *Onchocerca volvulus* et de la fréquence des complications oculaires dans les communautés. Ce phénomène, avec les conséquences économiques qu'il entraîne, aurait provoqué de nouveaux mouvements de population à distance des cours d'eau (85).

L'impact social

54 Une étude menée en Guinée a montré que la cécité et la baisse d'acuité visuelle ont des répercussions importantes sur la mobilité, l'activité professionnelle et le taux de mariage des individus (86). Par ailleurs, dans une zone de forêt du Nigeria, on a constaté que les femmes présentant une onchodermatite sévère se mariaient plus tard et que la période d'allaitement au sein était plus courte chez les femmes infectées par *Onchocerca volvulus* (87). Une étude multicentrique a montré d'une part que le prurit entraîne une insomnie et une fatigue générale et d'autre part que les personnes présentant une dermatite papulaire ou lichénifiée se sentent dévalorisées, qu'elles ont des difficultés à s'imposer à des postes de responsabilité dans la communauté et, plus généralement, qu'elles sont frappées d'un certain ostracisme (88, 89). Enfin, il semble que les enfants dont les parents souffrent d'onchodermatite sévère ont deux fois plus de chances que les autres d'interrompre leur scolarité prématurément (90).

L'impact économique

55 Plusieurs composantes doivent être prises en compte pour évaluer l'impact économique de l'onchocercose. On distingue les coûts directs liés aux dépenses des individus pour leur santé et les coûts indirects correspondant au temps de travail perdu du fait de la maladie. Ainsi, une étude menée dans une plantation de café en Ethiopie a montré que les personnes souffrant d'onchodermatite sévère avaient une productivité diminuée d'environ 15 % (91). De plus, une étude effectuée au Nigeria, au Soudan et en Ethiopie a permis d'évaluer les coûts indirects liés à l'onchocercose et a montré que les personnes souffrant d'onchodermatite dépensent en moyenne chaque année 100 FF de plus pour leur santé que les personnes non atteintes, cette différence pouvant représenter jusqu'à 15 % du salaire annuel (90).

56 Murray (92) a récemment présenté une nouvelle méthode de mesure de l'impact des pathologies basée sur le calcul du nombre d'années de vie ajustées sur l'incapacité (*disability-adjusted life years* ou DALYs) perdues du fait de la maladie. Cette mesure tient

compte du nombre d'années perdues du fait de la surmortalité et du nombre d'années vécues avec un handicap lié à la maladie. A chaque type de handicap est affecté, en fonction de sa sévérité, un poids compris entre 0 (vie en bonne santé) et 1 (décès prématuré). Les poids attribués au prurit, à la baisse d'acuité visuelle et à la cécité, par exemple, sont respectivement de 0,068, 0,064 et 0,6. La Banque Mondiale a estimé que l'impact de l'onchocercose en Afrique est de 884 000 DALYs perdues chaque année, la contribution respective de la cécité, de la baisse d'acuité visuelle, du prurit et de la surmortalité liés à la maladie étant respectivement de 290 400, 34 380,446 760 et 112 460 DALYs (90). Ces chiffres indiquent que, selon cette méthode de calcul, le prurit contribue pour moitié au poids global de l'onchocercose. C'est pourquoi les opérations de lutte contre cette maladie ne se limitent plus aux régions où l'onchocercose a des répercussions oculaires.

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RÉSUMÉS

Avant les années 1980, la seule méthode de lutte contre l'onchocercose était l'élimination des populations de simulies vectrices. Cette stratégie a été utilisée avec un succès considérable dans le cadre du Programme de Lutte contre l'Onchocercose en Afrique de l'ouest (OCP). La découverte de l'ivermectine, premier médicament efficace contre *Onchocerca volvulus* et pouvant être distribué à large échelle, suscite depuis 10 ans une nouvelle mobilisation internationale tant au niveau de la recherche fondamentale que de l'élaboration de nouvelles stratégies de lutte applicables en dehors de l'aire d'OCP. Cet article résume l'état des connaissances sur les aspects parasitologiques, cliniques, diagnostiques et épidémiologiques de l'onchocercose. Si le mode de développement des premiers stades parasitaires chez l'homme est encore très mal connu, des progrès importants ont été accomplis dans la connaissance de la dynamique de population d' *Onchocerca volvulus.* L'étude de la pathogénie des lésions dermatologiques et oculaires a fait l'objet de nombreux travaux. Des études épidémiologiques sont menées en vue d'apprécier l'importance des manifestations systémiques de l'onchocercose. De nouvelles méthodes diagnostiques, plus sensibles que la technique parasitologique classique, ont été mises au point. La répartition de l'onchocercose a été précisée grâce à l'utilisation systématique d'une méthode d'évaluation rapide des niveaux d'endémie. Les techniques de biologie moléculaire, utilisées en routine en Afrique de l'ouest, permettent de différencier les différentes espèces du genre *Onchocerca* ainsi que plusieurs souches d'*Onchocerca volvulus,* de pathogénicité différente. Enfin, de nouvelles méthodes de quantification des handicaps ont permis de préciser l'impact socio-économique des complications oculaires et cutanées de l'onchocercose.

Before the 1980s, the only available method for control of onchocerciasis was elimination of blackfly vector populations. This strategy was used with considerable success in the Onchocerciasis Control Programme in West Africa (OCP). The discovery of ivermectin, the first effective drug suitable for mass treatment of onchocerciasis, has revived international interest not only in fundamental research but also in development of new strategies to control onchocerciasis in the countries outside the OCP area. This report gives an overview of current parasitological, clinical, epidemiological and diagnostic data about onchocerciasis. Although little is known about the early development of *Onchocerca volvulus* in the human host, significant insight has been gained into the population dynamics of the parasite. The pathogenesis of cutaneous and ocular manifestations in onchocerciasis is now better understood. Epidemiological studies are under way to evaluate the extent of systemic manifestations. Recently developed diagnostic methods are more sensitive than conventional parasitological techniques. A new method for rapid assessment of endemic level has provided a detailed picture of the distribution of onchocerciasis. Species-and strain-specific DNA probes have been developed for identification of parasites in West Africa. New methods for quantifying disability allow evaluation of the socio-economic impact of the cutaneous and ocular complications of onchocerciasis.

INDEX

Mots-clés : Onchocercose, Onchocerca volvulus, Simulie, Afrique noire

Keywords : Onchocerciasis, Onchocerca volvulus, Simulium, Black Africa.

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Article sollicité.

Analysis of the effects of rotational larviciding on aquatic fauna of two Guinean rivers: the case of permethrin

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1. Introduction

2 In 1974 WHO launched the Onchocerciasis Control Programme for the elimination of an illness of public importance in west Africa, river blindness. The programme was based on the control of the blackfly *Simulium* damnosum, vector of the filariasis caused by the parasitic worm *Onchocerca volvulus* (WHO, 1997).

3 The strategy of larviciding was designed to interrupt the transmission of the parasite for longer thin the longevity of their adult worms in the human host tes rimated to be about 14 years) by destroying larval stages of the vector through the aerial application of insecticides at breeding sites in the rivers. The developnent oc the aquatic stage from egg to pupae is around one week hence, insecticide was applied weekly. At the peak of larviciding activities about 50.000 km of river: were treated weekly in 11 countries in West Africa The biological insecticide *Bacillus thuringiensis* stotype H-14 (a biologicai control agent) and temepres. and organophosphorus degradable molecule were, until 1979, the only insecticides applied. Following the development of resistance in vector populations, the strategy was to alternate other insecticides such as chlorphoxim and

pyraclophos (organophosphorus compounds), permetrin (pyrethroid), carbosulfan (carbamate) and etofenprox (pseudopyrethroid) (Hougard et al., 1997).

4 Because of the extent of the aquatic environment exposed to regular larviciding and the consequent possible effects on non-target organisms, larvicides applications, both in terms of compounds and frequency of spraying, were planned and carefully monitored from the beginning of the programme, taking into account the criteria and advice of an independent ecological group. A comprehensive review of the environmental assessment of larvicide use in the programme has been recently published (Calamari et al., 1998).

5 Permethrin was selected in 1984 for potential operational use following the increase in resistance of target populations to applied larvicides. Preliminary assessments of its effects on aquatic fauna were made through acute toxicity on fish (Yaméogo et al., 1991) and by means of semi-field experiments in mini-gutters (Yaméogo et al., 1992). Following the results of these studies, a pilot scale field trial was conducted on the Sassandra river in Cote d'Ivoire to evaluate its impact in operational conditions (Yaméogo et al., 1993).

6 The resuit of this study led to the conclusion that the conditions of fish and fisheries before and after the experiment period were significantly unchanged, and thus, operational use of permethrin by the programme would not be expected to have permanent adverse effects on non-target fauna (Lévêque et al., 1998)

7 However, this research demonstrated that permethrin has a stronger impact on invertebrates than other insecticides used in the programme (Yaméogo et al., 1992). It has been therefore decided to pay particular attention to a few rivers where the Chemical larvicides permethrin and organophosphates were applied. Organophosphates on several occasions had limited impact on aquatic fauna when used only in combination with the biological insecticide *B.t.* H-4 (Crosa et al., 1998).

8 Detailed, long-term series of biological data were available to make a more specific assessment for permethrin. This paper presents and comments on the results of a comprehensive analysis of the biological data collected during the monitoring programme in two Guinean rivers.

9 The objective of this paper is the detailed evaluation of the short-, medium-and long-term biological variations related to larvicide applications with particular attention to permethrin.

10 To account for biological variations due to the strong dependence of aquatic organisms on the seasonal fluctuation of river discharges, the results of the data analyses are evaluated and commented on with reference to the hydrological condition of the river.

2. Treatments, biological data, rivers hydrology and analysis strategy

11 The analysis regards the invertebrate and the fish sampled in the rivers Niandan (Sansambaya, field station) and Milo (Boussolé, field station), respectively. (Fig. 1). The rivers are located in Guinea and were selected from among other Guinean rivers because of their dctaiied series of biological and hydrological data, as well as their type of treatments.

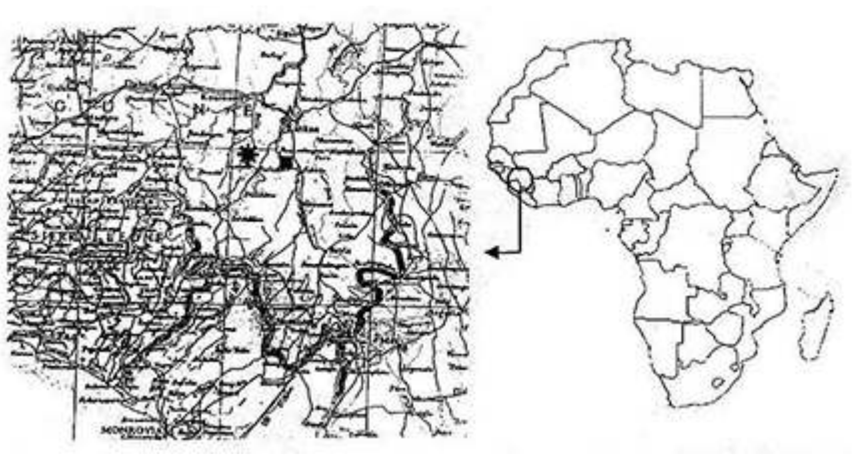

Fig. 1. Fish (black square) and invertebrate (black star) sampling sites location, respectively, on Milo and Niandan rivers.

2.1. Treatments

12 The rivers were treated with *B.t.* HI4, the organophosphates, themephos and pyraclophos, and the pyrethroid permethrin.

13 The length of time, the number of treatments and the available discharge data as well as the number of invertebrate and fish samples for each monitored year are shown in Fig. 2. Invertebrates and fish monitoring started 3 and 2 years, respectively, before the beginning of the treatments.

2.2. Biological data

14 Invertebrates were collected in shallow riffles over rock substrate using a modified 25 x 25 cm Surber sampler (Dejoux et al., 1979). For each site five samples were normally replicated and the mean number of individuals as well as 95% confidence limits were calculated.

15 All sampled individuals were classified according to their family level and their trophic role: predators, shredders, scrapers and filtering or gathering collectors. This second classification method is based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories (Cummins, 1973).

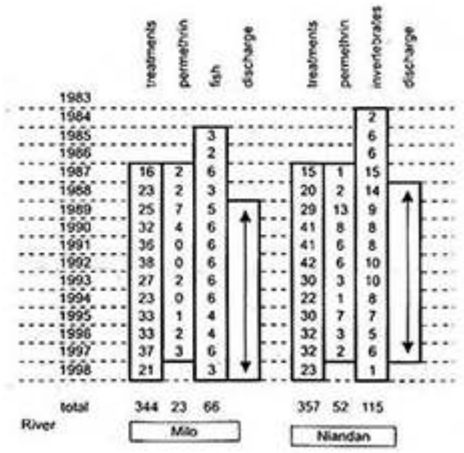

Fig. 2. Schematic representation of the periods of the data analysed. The numbers of the weekly larviciding plus permethrin and the numbers of invertebrates and fish samples arc dctaiied for each year.

16 The variables used for the analyses are the total invertebrate individuals collected in each Surber sample (individuals/625 cm$^{2)}$ and the percentage of each trophic guild. Principal component analyses (PCA) were applied to the relative log-transformed invertebrate abundance to detect any change in taxonomie composition or relative abundance of each systematic unit.

17 The fish were sampled since 1980 with standard batteries of five mesh size gill nets: 15, 20, 25, 30 and 40 mm. The number and total weight of individuals for each species caught by the different mesh size were calculated, all the results were standardised as catch per unit effort (CPUE) which is the number, or the weight, of fish caught in 100^{m2} of net per night (Abban et al., 1997).

18 The variables used for the analyses are the total catch per unit of effort expressed as number and weight of specimens calculated for each of the five mesh sizes and specific richness. For the principal component analysis the relative abundance of each species was used.

2.3. Hydrological data

19 The water regime of the studied rivers is characterised by a well-defined seasonal pattern with high water periods from June-July to December followed by low water periods from January to May–June.

20 The hydrographs show a time-related increase of the water volumes flowing yearly through the Milo measure section Figs. 8–10. For the Niandan discharges, two low water periods were recorded during 1992–1993 and no long-term trend is evident (Figs. 3 and 7).

2.4. Data analysis

21 The numerical analysis strategy was selected in order to assess community structure variations as well as invertebrate and fish reductions in terms of total number of individuals or species richness.

22 For assessing community structure changes, principal component analysis (SAS, 1989) was applied to the logtransformed abundance of the invertebrate and fish taxa collected.

The results of this ordination analysis are illustrated by means of functional graphs, which consist in representing the sample co-ordinates along with the sampling time.

23 PCA is one of the multivariate techniques that arrange samples along axes (principal component) on the basis of data pertaining to species composition: presence and relative abundance. The aim of this technique is to ordinate the samples so that samples with similar axis scores correspond to sites that are similar in species composition, and samples that are far apart correspond to sites that are dissimilar in species composition

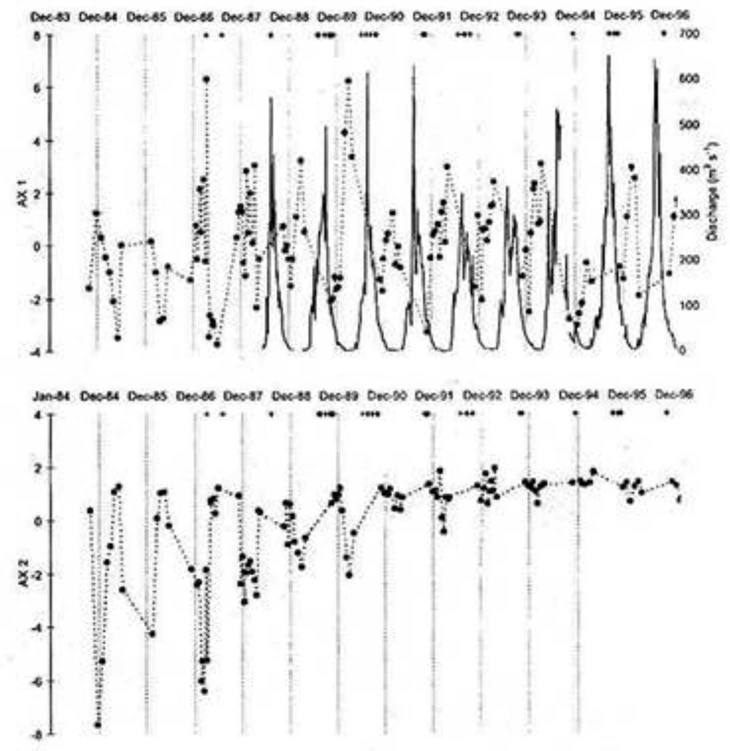

Fig. 3. Niandan hydrograph and invertebrate samples ordination scores (dotted line), percentage of total data variability 21% AXI. 10% AX2. In the upper part of the graphs permethrin applications are marked with black crosses.

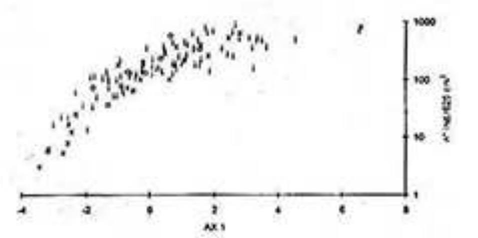

Fig. 4. Relationship between the invertebrate samples ordination scores (1st axis) and the invertebrate density; each sample is marked as follows: o = pre-treatment period, x = first year of larvicides application, i = 2nd to last year of larvicides application.

24 Due to the strong and seasonal relationship expected between the river discharges and the aquatic community structures, the biological variations measured were related to hydrological conditions in order to assess natural biological changes with respect to larvicide applications.

3. Results

3.1. Invertebrates

3.1.1. Taxonomic classification

25 The results of the ordination analysis of Niandan invertebrate communities and the trend in discharge for the period 1988–1996 are illustrated by means of functional graphs in Fig. 3. In the upper part of the graphs, black crosses mark the periods of permethrin.

26 The ordination axes account for 31% of total invertebrates variance: 21% first axis, 10% second axis. Considering the high variability of aquatic communities. such percentages can be considered adequate to analyse the major specific sources of variation.

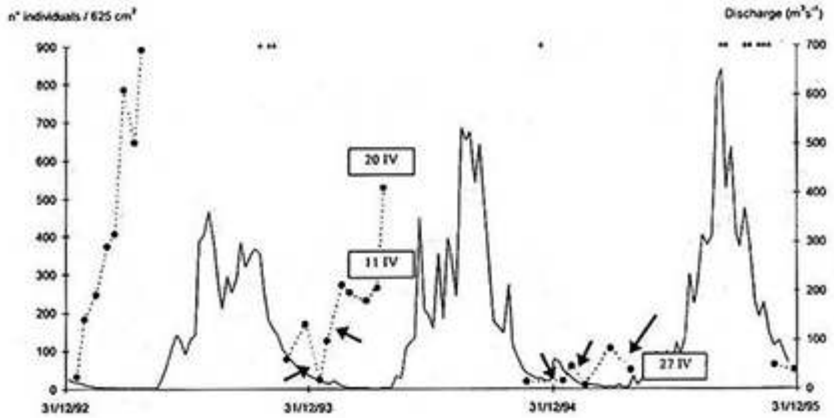

Fig. 5. Detailed representation of discharge and invertebrate density trends during 1993–1995 for Niandan river. Dotted line marks invertebrate densities, the crosses in the upper part of the graph mark permethrin and the arrows mark pyraclofos applications.

27 The sample co-ordinates of the first axis (Fig. 3 upper) show no long-term trend, in that the communities sampled during the treatment period have ranges and patterns of variation similar to those occurring during the pre-treatment periods. This pattern results from the cyclic variation of the sample co-ordinates throughout the investigated period with low values at the end of wet seasons and an increasing trend during dry seasons.

28 With respect to the second axis, a clear, long-term trend of sample co-ordinates is evident from the graph (Fig. 3 lower). Although maximum values during the pre-treatment period still occurred throughout the research, the variation ranges of sample scores were reduced soon after the beginning of the treatments.

29 Biological fluctuation mainly reflects the seasonal variation of invertebrate densities as demonstrated by the significant correlation between the first axis sample co-ordinates and the mean invertebrate density ($r = 0.8$, $P < 0.001$). This relationship is shown in Fig. 4 where pre-treatment samples, the samples collected during the first year of treatment and the remaining ones are diflerently marked. This graph clearly demonstrates that the sample scores present high, intermediate or low values without any reference to the three different operational phases of the programme (pre-treatment, first year of larviciding, succeeding years of larviciding).

30 Excluding the data collected during 1995 (for which a detailed discussion is provided in the following) no significant changes of this seasonal pattern occur during the years of larvicide application.

31 To explain invertebrate community changes previously outlined during 1995 by the first co-ordinate scores, and for a better evaluation of the short-term effects of permethrin and pyraclofos applications, invertebrate data collected during 1993–1995 have been analysed in detail. For this, invertebrate densities and discharge values are plotted on the same graph with the indication of permethrin and pyraclofos application as described in Fig. 5.

32 The graph indicates low invertebrate density values occurring during the first months of 1995. Because the hydrograph of the previous wet season (1994) is not significantly different from those of other years, the outlined low density values might be partially explained by larvicide applications. A specific effect of permethrin cannot be reasonably assumed because more permethrin application occurred during the 1993 wet season compared to the 1994 wet season and no such reduction of invertebrate densities can be shown for the following dry season.

33 On the contrary, the low density values measured during the first months of 1995 can be partially explained considering that, in this period, invertebrate sampling took place shortly after the weekly applications of pyraclofos. For example, during April 1995 Surber samples were collected 17 days after pyraclofos spraying, while during April 1994 the invertebrates were sampled twice, 70 and 79 days after pyraclofos treatments, a sufficient lag time for invertebrate communities to recover.

34 Considering that the long-term trend of the invertebrate structure illustrated by the second axis scores in Fig. 4 is not related to river hydrology, a detailed evaluation of taxonomic units showing significant and timecorrelated trends is illustrated in Fig. 6.

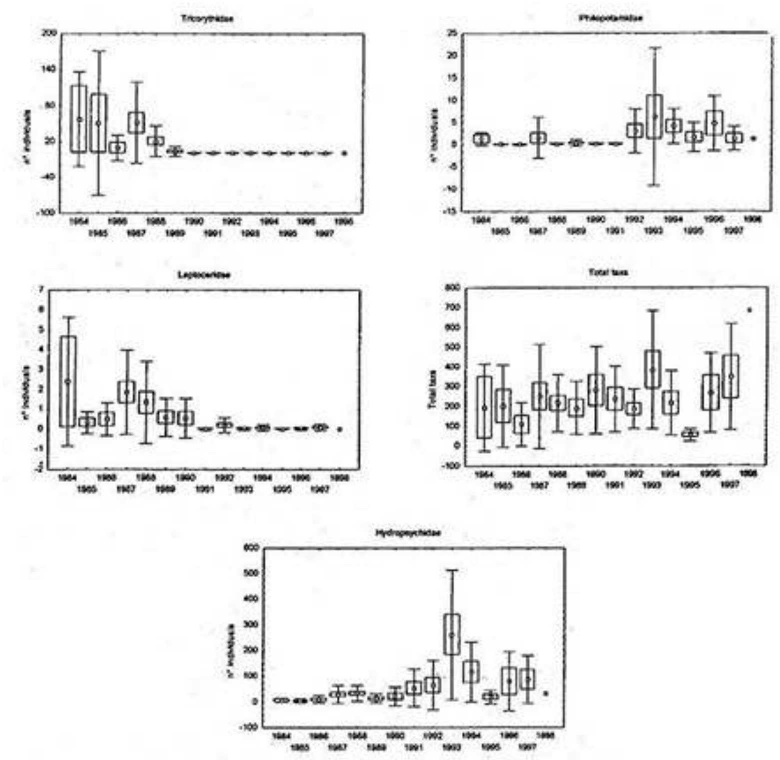

Fig. 6. Niandan average invertebrate densities (individuals/625 cm^{3}). Inner box = standard error, whisker = standard deviation.

35 Tricorythidae and Leptoceridae decrease in abundance as treatments take place and become increasingly rare from 1990–1991 until the final year of monitoring. On the contrary, the low, pre-treatment densities of both Hydropsychidae and Philopotamidae increase from 1991–1992.

36 As a result of this relative shift of taxa abundance, total invertebrate density was not significantly modified within the studied period. The only remarkable invertebrate density reduction occurred during 1995 and was due to the short lag period between treatments and sampling which did not allow time for recovery. It should be noted that this biological alteration did not last long and was completely reversed after the 1995 wet season.

3.1.2. Functional classification

37 The two most abundant invertebrate trophic guilds are the gathering and the filtering collectors that, on an average, contain more than 95% of total invertebrates.

38 The relative percentage variation of these two guilds during the study period is represented in Fig. 7 superimposed on the river discharge. In the saine figure, permethrin applications are marked with black crosses.

39 The percentage values show a cyclic pattern characterised by the dominance of gathering colleetors at the beginning of the dry season shifting to the dominance of the filtering collector group in the months before the beginning of the wet season.

40 With reference to pre-treatment years, both trophic guilds present a wider seasonal pattern in their relative abundance for all the monitored years after the beginning of larviciding. This “amplification” of a seasonal and natural biological variation can thus be

attributed to larvicide applications. It should be considered, however, that, although the invertebrate communities show a marked reduction in relative abundance of gathering collectors at the end of the dry season (and a corresponding increase of the filtering collectors), after each wet season the trophic compositions of the communities arc similar to those in pre-treatment sampling.

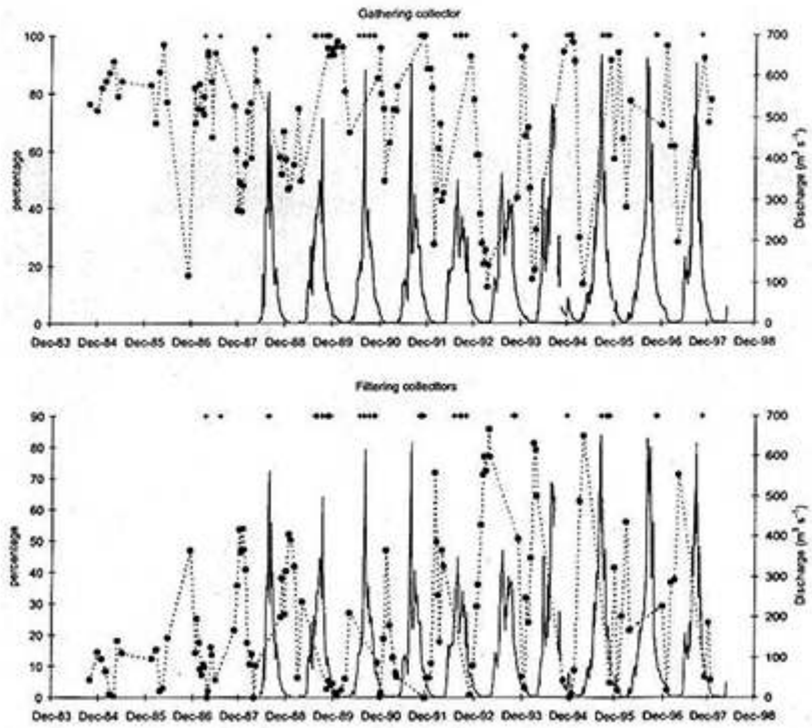

Fig. 7. Niandan flow rates and relative percentages of the two most abundant functional groups of the invertebrate communities (dotted line). In the upper part of the graphs permethrin applications are marked with black crosses.

41 In Fig. 7, the periods of permethrin applications do not show corresponding eflects on variation in invertebrate trophic structure commented above.

3.2. Fish

3.2.1. Species structure and fish abundance

42 Taxonomie structure changes of fish communities sampled in the Milo river during 1985–1998 are shown in Fig. 8 by means of the first two axes scores plotted along with the sampling time. In the same graphs the river discharges are available from 1988.

43 The first two axes account for 25% of the total variance of the sampled communities (15% firs axis, 10% second axis). As previously noted, for invertebrates the percentage of variation can be considered adequate to identify major sources of variation in the fish communities.

44 The biological changes described by the first axis scores show two variation patterns with respect to time. The first is related to the increase in sample scores during the dry seasons of 1995–1996 and 1998, the second one is the seasonal, flow-related, variation of the sample scores showing low values during the high flow periods and high values

during the dry seasons. No short-term eflects of these patterns are demonstrable after permethrin applications.

45 The first axis sample scores are significantly related to the CPUEs ($r = 0.63$, $P > 0.001$) showing high scores in concomitance of high catch values recorded during the low water periods lasting from November until January (Fig. 9).

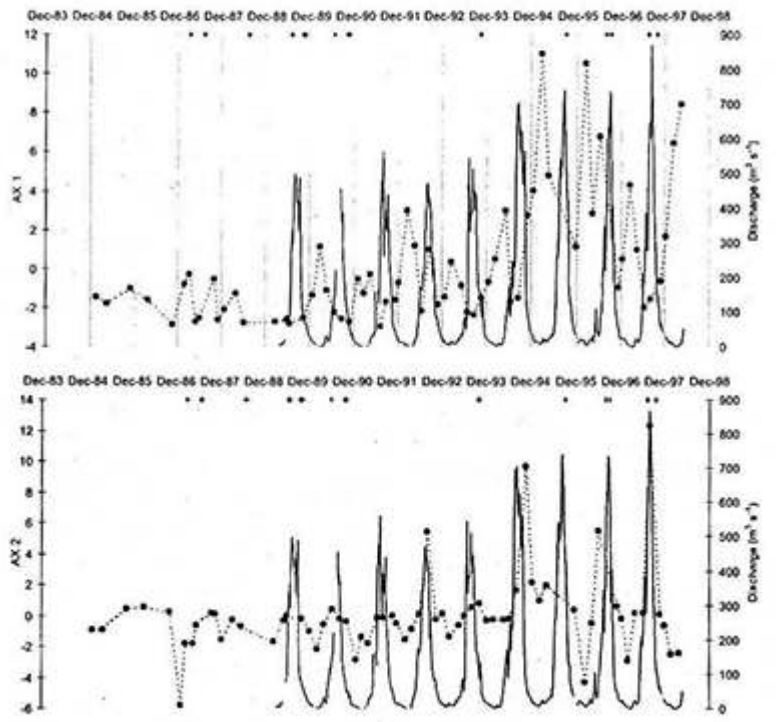

Fig. 8. Milo hydrograph and fish samples ordination scores (dotted line), percentage of total data variability 15% AX1.10% AX2. In the upper part of the graphs permethrin applications are marked with black crosses.

46 Visual inspection of long-term variations in CPUEs shows a reduction of the mean yearly catches during the period 1988–1993. Although this reduction can partially be the resuit of the initial phase of the treatments, it can be better explained by the low discharge values of those years. In fact, as the hydrologic regime shows an increase in volume of flowing water (1994–1997), the CPUEs increase in spite of larvicide applications.

3.2.2. Fish species richness

47 The number of fish species caught during the monitoring period shows seasonal fluctuation with maximum values at low water periods and a slight increment during the last 5 years (Fig. 10). Both these variations can be related to the number of fish caught, in particular the seasonal fluctuation can partly be explained by the maximum efficiency of the gill nets during the dry season. The long-term trend of species richness corresponds almost exactly to CPUE values whose variation can be ascribed to long-term flow variation. No reduction in fish species occurs during the final years of larvicide application.

4. Conclusion

48 Data analysis of fish and invertebrate monitoring in two Guinean rivers treated with *B.t.* temephos, pyraclophos and permethrin allows for the first time a comprehensive field assessment of the impact of permethrin on aquatic life when used in rotation with other Chemicals already evaluated as having limited or no impact.

49 The two biological components investigated for detecting adverse environmental responses after larvicide applications, in particular permethrin, produced a body of experimental evidence on the biological variation occurring in the rivers during the monitoring period. In general, most of this variation is strongly, long-or shortterm related to the hydrological river conditions. Evidence of this finding is provided, for example, by the Milo river, where the long-term trend in fish catches follows the increase in discharges or, more in general, for the invertebrates for which short-term seasonal variation, both in terms of total density and trophic structure, are significantly related to the river discharge.

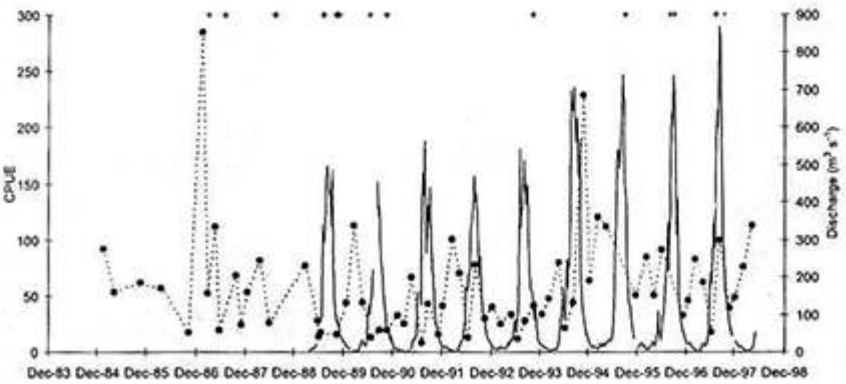

Fig. 9. Milo hydrograph and trend in fish caiches per 100 m^2 of gill nets (dotted line). CPUE in number of individuals. Black crosses mark permethrin applications.

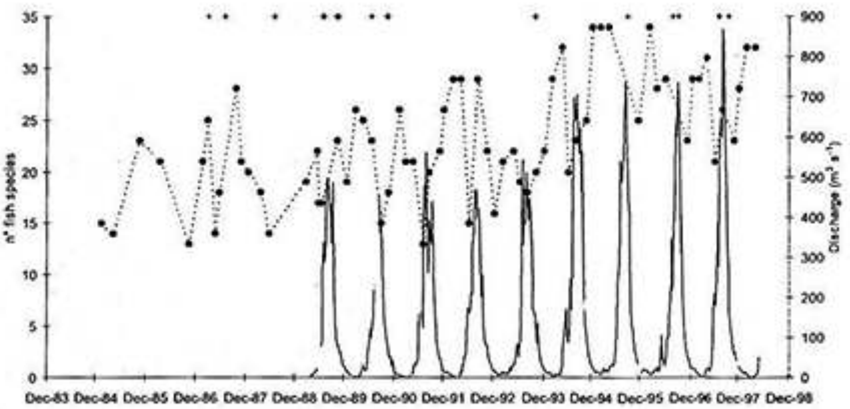

Fig. 10. Milo hydrograph and trend in number of fish species per sample during the monitoring period (dotted line). In the upper part of the graph permethrin applications are marked with black crosses.

50 Flow and treatment-related aspects of biological response differ for invertebrates and fish according to the different durations of their life cycles. Invertebrates, in fact, with their short life cycles are better indicators of short-term Chemical shock and seasonal flow

changes than fish, for which the seasonal (short-term), variations are mainly related to the high efficiency of the gill nets during low water periods.

51 Results on fish communities do not suggest any reduction in CPUEs or species richness that can be attributed to larvicide applications; observed variations are mainly flow-related.

52 The invertebrates collected have been classifiai according to their taxonomic levels as well as to their functional feeding group (trophic role). Analysis of biological variation focused on both these structural and functional attributes. Whereas the first aspect concerns, besides the faunistic interest over loss of global biodiversity, the quality of biomass available for the upper trophic levels. the second is related to the stability of energy flows. On account of the invertebrate position in the first levels of the river food webs, changes here can signal greater detrimental effects on the ecological characteristics of the whole river System. In this context most invertebrate variations are seasonal and flowrelated and the results do not support any evidence of specific effects of permethrin application on the biological targets monitored.

53 Larvicide applications have influenced the community structures by putting pressure on some taxonomical groups, causing for example the rarefaction of Tricorythidae and Leptoceridae in Niandan river since 1990. In spite of the above results, the scarcity of some systematic invertebrate units does not resuit in a significant reduction of total invertebrate density because of the corresponding increase in other systematic units as Hydropsychidae and Philopotamidae.

54 Only occasionally, for example, during 1995 in the Niandan river, can the reduction of total invertebrate density be related to larvicide applications; however, the experimental evidence previously discussed reveals that this adverse biological response is a particular, shortlasting case: invertebrate density completely recovered in 3–8 weeks.

55 Invertebrate trophic guilds with an increase in their seasonal variation during the treatment period completely recovered at the end of each high water period.

56 Considering the short-time recovery capabilities of invertebrate communities, it can be assumed that sampling sites will be recolonised by taxa shortly after the conclusion of the programme.

57 The biological variations discussed above are to be considered ecologically acceptable, according to the rationale for evaluating adverse effects of larvicide applications on aquatic fauna indicated by the ecological group (Calamari, 1998): “temporary and seasonal variation in invertebrate populations other than Simulium could be accepted” and considering that in nature aquatic communities would rarely be in equilibrium because of frequent natural stresses, like drought and spate events.

58 In conclusion the acceptability of the discussed results is in agreement with the two fundamental principles for which the monitoring programme was organised (Lévêque et al., 1998):

1. repeated long-term treatments could change the reproductive cycle of fish, either by affecting their physiology or by acting directly on eggs or offspring. If so, there could be changes in fish recruitment and, on a long-term basis, a decrease in fish abundance. This would apply to the fish community as a whole, or to particular species, which could be more sensitive to insecticides.
2. Insecticides could affect the food chain leading to a serious reduction in diet.

59 *Note:* A complete set of data is available upon request.

Acknowledgements

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ABSTRACTS

Within the Onchocerciasis Control Programme about 50,000 km of west African rivers have been regularly sprayed with larvicides to control the vector of dermal filariasis caused by *Onchocerca volvulus.* Since the beginning of the programme invertebrates and fish data were collected to monitor adverse effects on non-target organisms. The regular series of biological and hydrological data collected in two Guinean rivers were analysed to evaluate the effects of rotational larviciding with particular attention to permethrin, as preliminary acute toxicology tests and semi-field experiments suggest it has stronger effects on non-target fauna in respect to other larvicides. Invertebrates ard fish variations in biomass and species richness are seasonal and flow-related and the results presented here do not support any evidence of specific effects of permethrin application on the biological targets monitored. Larvicide applications influence community structures, putting pressure on some taxonomic groups, causing. for example, the rarefacion of some taxa. In spite of the above results, the scarcity of some invertebrate systematic units does not resuit in a significant reduction of total invertebrate density because of the corresponding increase in other systematic units. In nature the studied aquatic communities would rarely be in equilibrium because of frequent natural stresses, such as drougst and spate events, the biologicai variations discussed are to be considered ecologically acceptable.

INDEX

Keywords: Permethrin, Onchocerciasis, Simulium damnosum, Aquatic invertebrates

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Effects of larvicide treatment on invertebrate communities of Guinean rivers, West Africa

Giuseppe Crosa, Laurent Yaméogo, Davide Calamari, Fafondé Kondé and Kélétigui Nabé

Introduction

1 From 1974 to 1998, up to 50 000 km of West African rivers located in an area of approximately 1 300 000 km^2 were treated with larvicides to eliminate the aquatic stage of the blackfly *Simulium damnosum,* vector of the human parasite *Onchocerca volvulus* (Zimmerman et al., 1992). The control agents were aerial sprayed weekly, on the basis that the development of the aquatic stage of the vector, from egg to pupae, is around 1 week.

2 At the beginning of the programme, the biological control agent. *Bacillus thuringiensis* serotype H-14, and temephos, an organophosphorus compound, were the only insecticides applied. From 1979, the appearance of certain forest cytotypes of the vector resistant to temephos (Guillet et al., 1980) enforced a search for new compounds and the implementation of a renewed strategy based on the rotational application of the larvicides, with intervening periods during which application was suspended. The new compounds applied were: chlorphoxim and pyraclophos (organophosphorus compounds), permethrin (pyrethroid), carbosulfan (carbamate) and etofenprox (pseudopyrethroid) (Hougard et al., 1997). A comprehensive review on the environmental assessment of the applied larvicides has been published (Calamari et al., 1998) and the environmental behaviour and mode of action of the various substances applied are discussed in Dejoux et al. (1985) and Yaméogo et al. (1991).

3 The strategy for larvicide application within the programme (Onchocerciasis Control Programme – OCP), both in term of compounds and frequency of spraying, has been carefully planned and, to control the possible effects on the non-target fauna, monitoring

has been undertaken applying the criteria and the advice of an independent Ecological Group. An aquatic environmental monitoring programme was established and operationally applied before the application of larvicides to monitor their possible side-effects on aquatic communities. Two biological components were identified and regularly sampled during all periods of larvicides spraying: benthic invertebrates and fish. Among the principles that define the acceptability of new insecticides is one relating to evaluation of induced modifications in aquatic communities. This is indicated within the mandate of the Ecological Group: "temporary and seasonal variation in invertebrate populations, beside the Simulium, could be accepted".

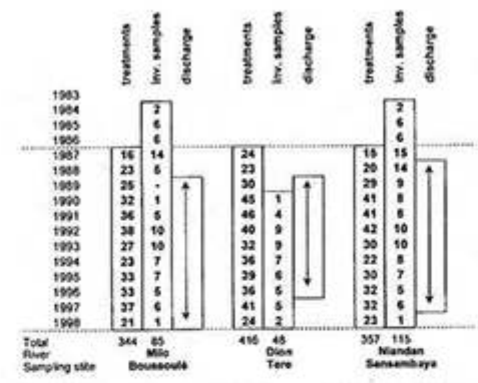

Figure 1. Schematic representation of the analysed data. The number of the weekly applications of larvicides plus the number of invertebrates samples are detailed for each year.

4 A number of studies were published to evaluate the possible side-effects on the fish and non-target insect populations in various areas and during different periods (i.e. Lévêque et al., 1988; Yaméogo et al., 1988).

5 This paper presents and discusses the taxonomie and trophic structure variation of benthic invertebrate communities collected from 1984 to 1998 in three Guinean rivers. The objective of the study was to verify whether any short-, medium-or long-term biological variations can be related to the larvicides application.

6 The time periods to which these relate are: (1) short-term: any change in the biological structures recovering within a season (wet or dry season); (2) medium term: any change in the biological structures recovering within one complete hydrological cycle (wet and dry season); (3) long-term: any permanent change in the biological structures.

7 The rationale we adopted to analysis the data collected in rivers located within a limited spatial range (one country) was intended to avoid possible bias due to the ecological differences that characterise water courses flowing in dissimilar regions. Moreover, the rivers were selected according to the types of treatment and, among the other Guinean rivers, because of their detailed series of biological and hydrological data.

Materials and methods

8 Since 1987. the studied rivers were treated with the biological control agent *B.t.* H14, the organophosphates temephos and pyraclophos, and the pyrethroid permethrin. The time extern of the treatments, the available discharge data and the number of invertebrate samples for each of the monitored year are shown in Figure 1. Invertebrate sampling started 3 years before the beginning of larvicide treatment in the Milo and Niandan

rivers. No pre-treatment samples are available for the Dion river for which invertebrate collection started in 1990, 3 years after the first larvicide application.

9 The invertebrates were collected in shallow riffles over rock substrate using a modified 25x25 Surber sampler (Dejoux et al., 1979), the sampling stations were chosen because, being the breeding sites of *S. damnosum,* these areas were subject of direct application of the larvicide compounds. For each site, 5 samples were replicated and the mean number of individuals were calculated.

10 All the sampled invertebrates were classified at family level and to their trophic role: predators, shredders, scrapers and filtering or gathering collectors. This second classification method is based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories (Cummins, 1973).

11 Since the invertebrates collected were classified with respect to their taxonomie level as well as to their functional feeding group (trophic role), the analysis of the biological variation focused on both structural and functional attributes. Whereas the first aspect of concern, beside the faunistic interest over loss of global biodiversity. is related to the quality of the biomass available for the upper trophic levels, the second one is related to the quantity and stability of energetic flows. On account of the position of invertebrates in the first levels of river food webs, changes in energy flows can be an alarm signal about greater detrimental effects upon the ecological characteristics of the whole river system.

12 The variables used for the analyses were the total number of invertebrates collected in each Surber sample (individuals/625 $cm^{-2)}$ and the percentage of each trophic guild.

13 For detecting any change in the taxonomie composition or in the relative abundance of each taxonomie unit. Principal Component Analysis (PCA) was applied to the relative log-transformed invertebrate abundance expressed in term of the number of individuals for each taxa. The resulting first two axes were then opportunely rotated (45°) to facilitate the description of variation in the scores for each axis.

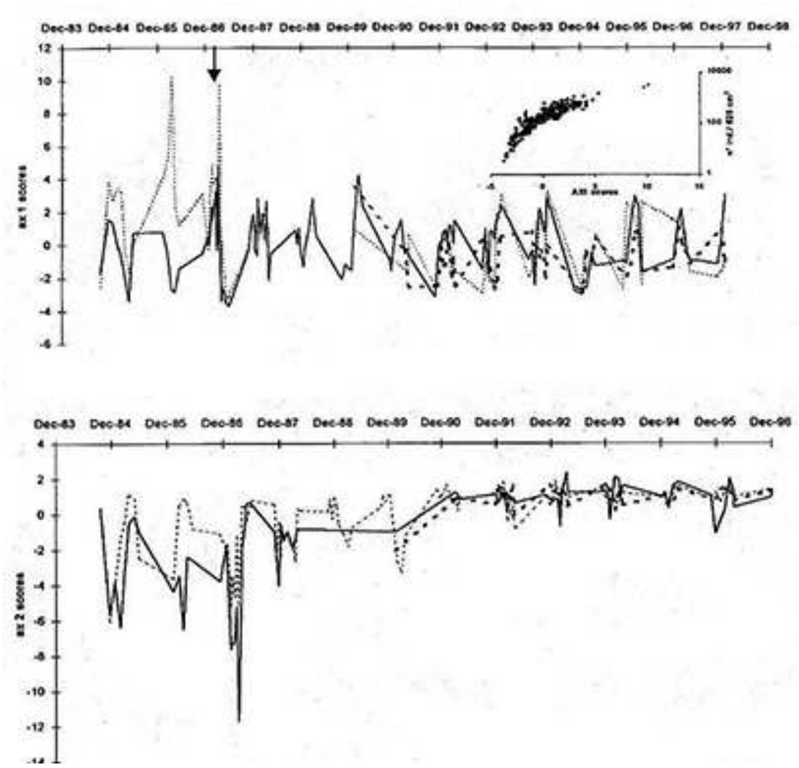

Figure 2. Samples ordination scores resulting from the Principal Component Analysis of invertebrate data collected in the three Guinean rivers studied: dotted line = Milo; unbroken line = Niandan; bold dotted line = Dion. In the upper graph, the relationship between the first axes ordination scores and the densities of the total classified invertebrates are shown and the arrow marks the beginning of the larvicides application.

14 An invertebrate data matrix was created by assembling all the samples classified according sampling date and river location.

15 Sample ordination scores of the first two components resulting from the analysis are separately illustrated along with sampling time in order to outline any time-related biological variation.

16 The water regime of the selected rivers shows a defined seasonal pattern with high discharges from June-July to December followed by low discharges during the dry season (January to May–une); details of hydrological and Chemical characteristics of the rivers and of the flowing waters are reported in Iltis & Lévêque (1982) and in Moniod et al. (1977).

Results

17 Ordination scores of the log-transformed invertebrates data on the first two axes are shown in Figure 2. In the upper part of the graphs, the arrow marks the beginning of the larvicide treatments, and each point in the figure represent a samples ordination score plotted in correspondence of the sampling day (x-axis).

18 The ordination diagrams account for 35% of the total invertebrates variance: 28% 1st axis, 7% 2nd axis. As discussed in the following, the ordination scores represent mean invertebrate densities (1st axis) and the relative abundance changes of the sampled taxonomic groups (2nd axis).

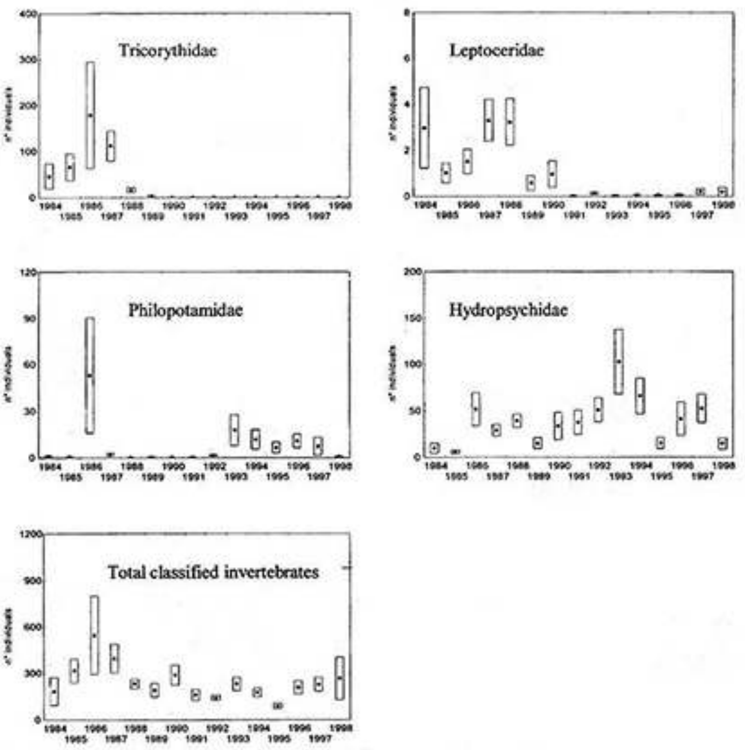

Figure 3. Long-term lime related variation of the average invertebrates densities (individuals 625 cm^{-2}) sampled in the studied Guinean rivers. Boxes represenl the standard errors of the calculated means values.

19 The two axes' ordination scores present different time-related patterns. The first one results from the seasonal variation of the sample ordination scores and shows no evidence of any long-term trend in invertebrate community structures. Medium-term variation is evident for all the three sampled rivers, and reflects seasonal changes in invertebrate densities as demonstrated by the significant correlation between the first axis samples ordination scores and mean invertebrate densities (r=0.8, *P<0.001)* shown within the upper graph of Figure 2.

20 With respect the 2nd ordination axis, a long-term trend in ordination scores for the sample is evident from the corresponding graph. Although the maximum values resulting during the pre-treatment period still occur throughout the sampling period, soon after the beginning of the treatments the samples scores for the 2nd axis reduce their variation range. Considering that this trend can not be reasonably related to natural factors presenting such a continuous change as the river hydrology, a detailed analysis of the taxonomic units showing significant time related trends are provided in Figure 3.

21 Tricorythidae and Leptoceridae show extremely low abundance since, respectively, the first and the fifth year from the beginning of the larvicides applications; Tricorythidae in particular were absent from 1990 and for the remainder of the sampling period (until 1998). Conversely, Hydropsychidae and Philopotamidae show slightly higher abundance values during the treated years compared to the densities measured during the pre-treatment period. As a result of the density increase of the above taxonomic units, total invertebrate abundance does not show a significant reduction during the final years of the monitoring period or any other related long-term trend.

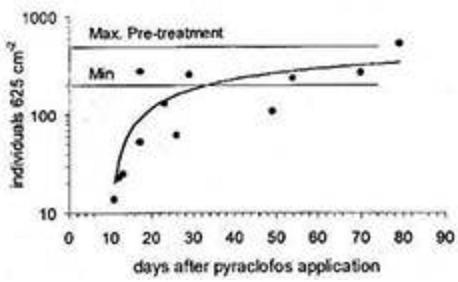

Figure 4. Total invertebrates densities after pyraclofos applications measured in Niandan river during 1993–1995.

22 To analyse of the short-term effects of larvicide applications on invertebrate communities, and to provide a description of their resistance and resilience, we selected the data sampled in the Niandan river during the years 1993–1995. This period was chosen because of the low invertebrate density measured during 1995, and also due to the fact that, during these years, invertebrate communities in the Niandan river were sampled after pyraclophos applications with different time lags; from a minimum of 11 days after pyraclofos treatments up to 79, with intermediate lags. In addition, previous studies provided experimental evidence of the severity of this Chemical in relation on the nontarget aquatic fauna. Finally, the hydrological cycles within the selected years do not present in Niandan river any significant difference.

23 The invertebrate density measured during the above selected period is shown in Figure 4. In the graph, each abundance value is plotted with respect to the number of days elapsed since pyraclofos treatment and the maximum and mean invertebrate densities measured during the pre-treatment period are marked as reference values.

24 Although the invertebrates sampled within a short period of time after pyraclofos application (11–13 days) show low densities, in all probability induced by the Chemical, as the time elapsed since treatment increases, the communities recover their original densities. After a period of 3–8 weeks, invertebrate abundance recovers to within the range of variation measured during the pre-treatment years.

25 To address the last question of this study, regarding the possible side-effects of larvicide applications on invertebrate trophic structure, the time-related variation of the relative abundance of the principal functional groups sampled in Milo river are presented as representatives of the situation typical response of the studied Guinean rivets (Fig. 5). To facilitate the interpretation of the seasonal biological variation, the hydrograph is plotted on the same graph of analysed trophic guilds and the missing flow values of the period 1984–98 are replaced by the average monthly discharges calculated using the available data.

26 The most abundant trophic guilds are the gathering and the filtering collectors that show seasonal and, consequently, flow-related variations similar to those of the other two sampled rivers: a dominance of the gathering collectors at the end of the wet season that decreases during the dry season.

27 Although the data preceding the commencement of applications of larvicide are themselves poorly correlated with seasonality, this cyclic pattern is described, with narrower variation ranges, by the communities sampled during the pre-treatment period

28 The graphs indicate that the larvicide applications affect the trophic structure of the invertebrate communities, reducing the gathering collectors abundance during the dry

season. However, after each wet season, the two trophic guilds show percentages similar to those calculated for the pre-treatment samples. For example, the low proportion of gathering collectors during March 1995 fell to 10%, a value very different from the percentages measured for the pre-treatment samples although, by November of the same year, the gathering collectors abundance was more than 90%. This finding suggests that the larvicides effect on the relative abundance of the trophic guilds of the invertebrate communities is not permanent: no more than 7–8 months after the first sign of alteration.

Discussion

29 The data collected provide experimental evidence of the variation in invertebrate communities during the Onchocerciasis Control Programme in Guinea. These allow an evaluation of the biological response to larvicide applications, with reference to short-, medium- and long-term trends addressed in the present study.

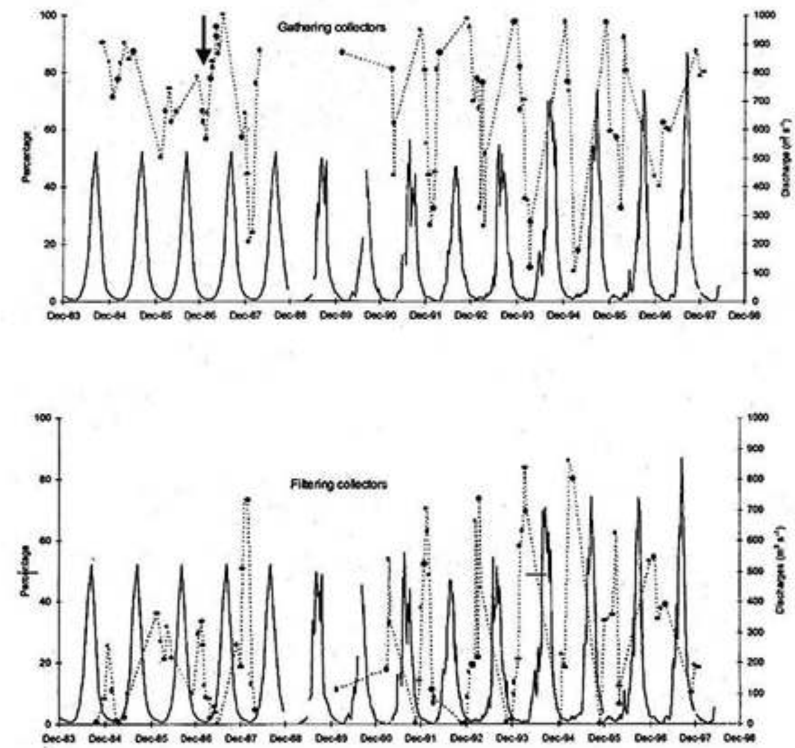

Figure 5. Milo flow rates (solid lines) and relative percentages of the two mos abundant invertebrate functional groups (dotted lines). The missing flow values of the period 1984–98 are replaced by the average monthly discharges calculated using the available data. In the graphs, the first year of treatment is marked by an arrow.

30 Most of the biological variation measured during the monitored period in the three studied Guinean rivers is flow and medium-term related. The variability of the total invertebrate density, showing higher values during the dry season, results mainly regulated by the natural hydrological cycle and does not shows any significant trend within the monitored period.

31 Also, trophic structure appears not to be permanently affected by larvicide applications; unfortunately, the 1988 year-class is missing and this make more difficult the interpretation of recovery following treatment.

32 Although the amplification of the variation in invertebrates trophic guilds occurring during the treatment period may be induced by the applied larvicides, the trophic structures show a complete recovery at the end of each high water period. It has to be noted that the recovery of population may be due to in-section resilience as well to recolonisation, from unaffected reaches upstream and in tributary, between wet cycles.

33 Both of these two aspects of the invertebrate communities, total density and trophic structure, suggest they are highly resilient with respect to natural driving forces (hydrology) or to induced stresses (larvicide application).

34 Examining the present data in the context of published papers on the same subject, the results of the analysis of the invertebrates sampled during the Onchocerciasis Control Programme show that, with reference to the pre-treatment observations, and during *B.t.* applications, the relative abundance of the gathering collectors decrease. Conversely, during the application of Chemicals such as permethrin and phoxim, the filtering collectors are strongly affected (Crosa et al., 1988). This differing response between invertebrate trophic structures can be partially justified by the larvicide properties and the feeding habits of the invertebrates; for example the gathering collectors feed on surface deposit and thus can be more affected *by B.t.* because to its rapid adsorption to soil particles (Tuosignant et al., 1993). Since the biological control agent was applied only during the low water periods (up to 1 $m^3 s^{-1}$), the above consideration can partly explain the density reduction of the gathering collectors measured during the low water periods, and suggests a direct effect of *B.t.* application on invertebrate trophic structures.

35 As for short-term biological variation induced by larvicide application, the data collected in the Niandan river during the period 1993–95 suggest that the Chemical shock, although affecting the invertebrate communities and reducing their total density, are short-lasting and in the quoted example result completely overcome after a period of 3–8 weeks.

36 Analysis of long-term trends in changes in invertebrate taxa demonstrates that the rarefaction of Tricorythidae and Leptoceridae in the studied rivers may be induced by the application of larvicides. Considering the above discussed short-term recovery capabilities of the invertebrate communities, recolonization of river habitats by the above quoted taxa will probably be accomplished within a short period after the conclusion of the programme.

Conclusion

37 In evaluating biological variations, we adopted the concept of “significant ecological change” used by Onchocerciasis Control Programme in assessing the ecological impact of larvicide application (Lévêque et al., 1977). Within the mandate of the Ecological Group, two criteria were indicated: “the vector control activities should not reduce the number of invertebrate species, nor cause a marked shift in the relative abundance of species”; and “temporary and seasonal variations in invertebrate populations other then *Simulium* could be accepted.” With reference to these criteria, the biological variation we have measured in the regional contest of Guinea can be considered ecologically acceptable.

Acknowledgements

38 We thank the anonymous reviewers for helpful comments and suggestions on improving the manuscript.

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ABSTRACTS

Biological and hydrological data collected from 1984 to 1998 in three Guinean rivers were analysed to evaluate adverse effects of biological and Chemical larvicides applied for the control of blackfly *Simulium damnosum,* vector of the parasitic digenean worm *Onchocerca volvulus.* Although most of the variation in invertebrate populations were flow-related, larvicide applications affect community structure reducing the abundance of the most sensible taxa. In spite of these results, in the long term the rarefaction of some invertebrate taxa (i.e. Tricorythidae) does not cause a significant reduction of total invertebrate densities because of the corresponding increase of other taxa (i.e. Hydropsychidae and Philopotamidae). The functional structure of the communities is also not affected.

INDEX

Keywords: Onchocerciasis, Simulium damnosum, aquatic invertebrates, larvicides

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Onchocerciasis control by large-scale ivermectin treatment

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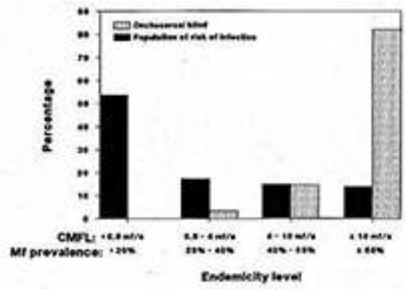
1 SIR,—Since ivermectin was introduced as a treatment for onchocerciasis[1] it has been shown to be applicable for community-based treatment[2] and annual application has been found to be effective for the control of onchocercal ocular disease.[3] These developments have offered an alternative to vector control in onchocerciasis. However, the effect of annual ivermectin mass treatment in the control of transmission of onchocerciasis is limited[4] and large-scale ivermectin treatment, even for "morbidity control", cannot be achieved quickly.[5]

2 The Onchocerciasis Control Programme in West Africa has shown that onchocerciasis can be severely blinding, savanna type[6] or mildly blinding, forest type.[7] In the savanna, the prevalence of onchocercal blindness increases with increasing community microfilarial load (CMFL) (an index of intensity of infection), and onchocercal blindness starts becoming a public health problem from a CMFL of 10 microfilariae per skinsnip (mf/s). Severely blinding onchocerciasis merits and is receiving control. Further analysis of the programme's data has revealed that 80% of the onchocercal blind are in communities with a CMFL of 10 mf/s or more or a prevalence of 55% and over, among 15% of the population living in a savanna onchocerciasis-endemic area at high risk of onchocerciasis. Screening communities with a CMFL of 4-10 mf/s or a prevalence of 40-55% increases the pick-up rate to 97% but doubles the population covered to 30% (figure). Onchocercal blindness does not occur in communities with a CMFL below 0,5 mf/s or a prevalence of 20% or less.



Distribution of onchocercal blind and population at risk of infection by endemicity level in savanna areas of Senegal. Guinea, and Mali.

3 These findings underline the importance of epidemiological mapping in onchocerciasis endemic areas,[8] before starting any ivermectin mass treatment to control onchocerciasis as a public health problem. Such maps identify the areas and communities that need to have a sustained annual treatment, and also identifies communities that willingly accept treatment. Although ivermectin is supplied free of charge, the cost of its delivery cannot be overlooked even with simplified logistics. An efficient delivery strategy is therefore essential to keep costs down to enhance sustainability. These analyses indicate that before ivermectin mass treatment to control onchocerciasis as a public health problem, it should be clear that the activity will have to be long term. Targeting of the treatment is essential to achieve the most cost-effective and sustainable strategy.

4 Onchocerciasis Control Programme in West Africa. BP549. Ouagadougou. Burkina Faso

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Toxicité pour la faune aquatique non cible de quelques larvicides antisimulidiens

II — L'Actellic^{R} M20 / II. — Actellic^{R} M20

Toxicyty for aquatic fauna of some black-flies larvicides

Claude Dejoux et Jean-Jacques Troubat

INTRODUCTION

1 Les travaux[1] (réalisés sur cet insecticide font partie d'un ensemble de tests effectués sur le terrain afin de rechercher la toxicité de produits de remplacement du téméphos Abate^{R}, organophosphoré utilisé pour le contrôle des larves de *Simulium damnosum* s.l.

1. CARACTÉRISTIQUE DU PRODUIT UTILISÉ

2 L'Actellic^{R} M20 est un produit ICI/Plant Protection. Il est constitué par une suspension de microcapsules d'un diamètre moyen de 2 µm et d'une densité de 1,05 qui contiennent 200 g de matière active par litre (Pirimiphos-methyl).

3 Les microcapsules appartiennent d'une manière générale à deux types bien différents :

- le type Dow Chemical, théoriquement imperméable lorsqu'il est dans le milieu aquatique et qui se dissout dans l'intestin. Dans ce cas, on utilise les propriétés physico-chimiques de l'intestin moyen (pH, érosion par le bol alimentaire ambiant, sécrétions de l'intestin) de la cible pour entraîner sa mort ou tout au moins son décrochement ;
- le type ICI (c'est le cas pour l'Actellic), poreux quel que soit le milieu (aquatique ou intestin). Dans ce cas, on joue sur la durée du transit intestinal, l'effet toxique étant d'autant plus fort que ce transit est lent.

2. ZONE D’ÉTUDE ET MODALITÉS DES TRAITEMENTS

4 Les premiers essais d’une formulation microencapsulée d’Actellic^{R} M20 ont été effectués sur le Baoulé au Mali en octobre 1978. Les épandages étaient réalisés par hélicoptère à la concentration 0,075 ppm/10 minutes, le débit du cours d’eau à cette époque était de 22 m^3 /s et la température de l’eau de l’ordre de 26 à 28°C.

5 La stabilité du produit soumis aux essais s’est révélée médiocre, provoquant une libération prématurée de la matière active dans le milieu aquatique, réduisant ainsi fortement l’avantage théorique de ce type de formulation.

6 Devant la relative inefficacité de cette formulation sur le groupe cible, il fut alors décidé de la modifier. En 1981, une nouvelle formulation a donc été testée en Côte d’Ivoire, sur la Férédougouba (Bagbé), affluent du Sassandra. Dans ce second essai, la température était de 28° à 8 heures, la transparence de 1 mètre au disque de Secchi, le débit du cours d’eau de l’ordre de 2,5 m^3 et la concentration utilisée de 0,2 ppm/10 mn. L’épandage a également été effectué par hélicoptère.

3. MÉTHODES ET TECHNIQUES

7 Deux méthodes ont essentiellement été utilisées, de manière classique (DEJOUX, 1980). Un profil de dérive *in situ* était établi en récoltant la dérive naturelle, puis la dérive provoquée, au sein du cours d’eau traité, durant 24 heures, à l’aide d’un jeu de deux petits filets à entrée circulaire de 113,1 cm^2 et de vide de maille de l’ordre de 200 µ.

8 Les filets étaient laissés en place 1 minute pour chaque estimation de l’indice de dérive.

9 La seconde méthode mettait en œuvre le système de gouttière mis au point par DEJOUX et TROUBAT, permettant un bilan exact, *in situ,* de l’impact immédiat d’un pesticide.

4. RÉSULTATS

4.1. Première série d’expérimentations sur le Baoulé

10 Sans vouloir insister sur ces résultats déjà partiellement publiés par ailleurs (DEJOUX et GUILLET, 1980) il est toutefois nécessaire de rappeler quelques faits marquants :

- l’augmentation maximale instantanée de la dérive survenue environ 1 heure après l’épandage était de 132 fois la dérive normale, ce qui dénote d’un impact important ;
- les groupes les plus touchés, *in situ,* ont été les Baetidae, les Tricorythidae et les Chironomidae d’une manière générale ;
- l’impact sur la dérive fut de longue durée et était encore sensible le lendemain du traitement ;
- à 6 km en aval du point d’épandage, aucune augmentation significative de la dérive n’a été décelée ;
- en gouttière, les observations n’ont pu être conduites que durant les 12 heures suivant le traitement. Durant cette période, 21,3 % de la faune testée (5 176 individus) ont décroché de leur support, ce qui peut être considéré comme un taux élevé.

11 Avec cette méthode, les Baetidae apparaissent à nouveau comme très sensibles avec 95,2 % de décrochement pour 355 individus testés. D’autres groupes ont également été très touchés :

Tricorythidae	93,1 %	(pour 29 testés)
Chironomini	63,8 %	(152)
Tanytarsini	35,9 %	(39)
Chimarra petri (Trichoptera)	21,9 %	(160)
Hydrophilidae	100 %	(10)

12 Les résultats obtenus sur les Simuliidae : 17,6 % (527) pour *S. damnosum* et 11,1 % (3 289) pour les autres espèces, étaient peu encourageants et nous conduisirent à rejeter cette formulation.

4.2. Deuxième série d'expérimentations sur la Férédougouba

13 Un profil de dérive *in situ* a été établi sur 46 heures, centré sur le traitement qui a eu lieu à 10 h 30, par hélicoptère, à environ 200 mètres en amont du point d'observation. Les premières réactions des invertébrés ne se font sentir que deux heures après le traitement. L'effet est alors violent et très rapide, le maximum de décrochement étant atteint après 15 minutes (soit 2 h 15 mn après le traitement) avec une valeur de l'indice de dérive (ID) de 2 980,8, ce qui est considérable[2].

14 Le coefficient instantané d'augmentation maximal de la dérive, qui est le rapport de la valeur maximale atteinte après le passage de l'insecticide à la valeur de l'indice de dérive immédiatement avant traitement, est de 1 461. En comparaison avec celles ordinairement obtenues avec le téméphos qui varient entre 75 et 100 pour une concentration de 0,5 m/1/10 mn, cette valeur est extrêmement forte.

15 Le coefficient d'augmentation pondérée de la dérive, qui est le rapport entre la valeur moyenne de ID, calculée durant 1 heure et centrée sur l'acrophase du décrochement, et la valeur moyenne de ID durant l'heure précédant le traitement, est de 222,8. C'est également une valeur élevée (elle est ordinairement de l'ordre de 50 avec le téméphos), qui traduit un effet prolongé dans le temps de la toxicité de l'Actellic[R] M20.

TABLEAU **1. Compositions comparées de la dérive (en nombre d'organismes récoltés en 60 secondes et %)**

	Avant traitement (11 h)		Au maximum d'intensité de dérive (12 h 45)		2 heures après les premiers effets (14 h)	
	$\bar{N}$	%	$\bar{N}$	%	$\bar{N}$	%
Baetidae	0	0	990	45,3	259	51,2
Caenidae	0	0	27	1,2	4	0,8
Leptophlebiidae	0	0	36	1,6	11	2,2
Heptageniidae	0	0	216	9,9	12	2,3
Tricorythidae	0	0	36	1,6	3	0,6
Ecnomidae	0	0	3	0,1	2	0,4
Hydropsychidae	0	0	186	8,5	16	3,1
Hydroptilidae	0	0	6	0,3	5	1
Leptoceridae	0	0	12	0,5	2	0,4
Philopotamidae	0	0	24	1,1	4	0,8
Polycentropodidae	0	0	0	0	2	0,4
Simuliidae	0,5	14,3	30	1,4	2	0,4
Chironomini	1,5	42,8	159	7,3	58	11,4
Tanytarsini	0	0	240	10,1	51	10
Orthocladiinae	1	28,6	195	8,9	37	7,3
Tanypodinae	0	0	3	0,1	4	0,8
Dytiscidae	0,5	14,3	0	0	6	1,2
Elmidae	0	0	9	0,4	5	1
Pyralidae	0	0	12	0,5	23	4,5
TOTAL	3,5		2184		506	

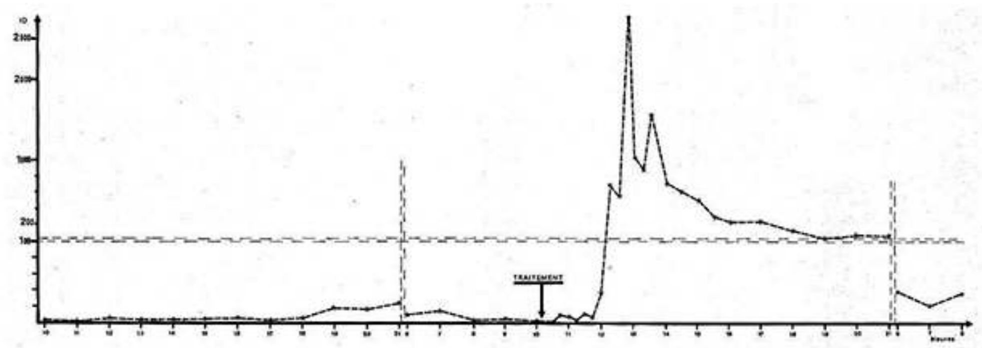

FIG. 1. – Profil de dérive des invertébrés établi *in situ* durant 46 heures, centré sur le traitement à l'Actellic[R] M20

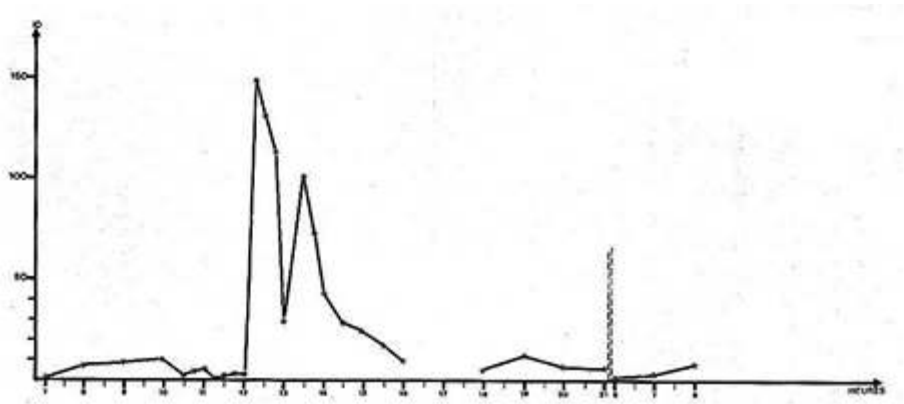

FIG. 1. – Cinétique de la dérive des invertébrés obtenue en gouttière, *in situ* et durant 24 heures

TABLEAU II. Bilan de l'expérimentation en gouttière (dérive des organismes durant 24 heures)

	Faune dérivée en 24 h	Faune restante après 24 h	Total testé	% de décrochement en 24 h

Baetidae	142	2	144	98,61
Caenidae	21	3	24	87,50
Leptophlebiidae	6	4	10	60
Heptageniidae	9	0	9	100
Tricorythidae	386	18	404	95,54
Ephemeridae	1	0	1	100
Neoperla	17	144	161	10,56
Libellulidae	—	2	2	0
Zigoptera	1	0	1	100
Ecnomidae	1	3	4	25
Hydropsychidae	62	21	83	74,70
Hydroptilidae	9	23	32	28,12
Leptoceridae	11	15	26	42,31
Philopotamidae	80	281	361	22,16
Polycentropodidae...	0	2	2	0
Trichoptera autres.	1	1	2	50
Hemiptera	1	0	1	100
Ceratopogonidae	2	8	10	20
Simuliidae	207	86	293	70,65
Chironomini	157	51	208	75,48
Tanytarsini	70	41	111	63,06
Orthocladiinae	63	42	105	60
Tanypodinae	2	5	7	27,57
Dytiscidae	26	11	37	70,27
Elmidae	47	129	176	26,70
Pyralidae	13	6	19	68,42
Hydracarina	5	3	8	62,79

TOTAL	1340	901	2241	59,79

16 Cet effet toxique, schématisé fig. 1, est fortement étalé dans le temps et se fait sentir durant toute l'après-midi et la soirée, l'indice de dérive restant supérieur à 100 jusqu'à 21 heures. Le lendemain du traitement, à 8 heures soit 21 h 30 après l'épandage, la dérive est encore 20 fois supérieure à ce qu'elle était la veille du traitement, à la même heure.

17 Une comparaison de la composition des dérives avant et après traitement montre la grande sensibilité des Ephéméroptères Baetidae, qui constituent pratiquement la moitié des organismes dérivés. Cinq autres groupes taxinomiques dérivent ensuite avec une intensité du même ordre de grandeur (7 à 10 % du total). Ce sont les Heptageniidae, les Hydropsychidae, les Chironomini, Tanytarsini et Orthocladiinae (tabl. I). Les Simuliidae par contre sont durant les premières heures suivant l'épandage assez peu affectés par l'insecticide.

18 Cette image représente l'impact sur le bief étudié mais reflète mal la sensibilité exacte de chaque groupe d'organismes présent car il est impossible de connaître leurs densités initiales à l'échelle de quelques centaines de mètres carrés.

19 L'utilisation de la technique des gouttières *in situ* permet à nouveau une estimation quantitative plus précise de l'impact de cet essai. Les résultats obtenus sont consignés dans le tableau II. Sur 2 241 organismes testés, appartenant à 27 groupes taxinomiques, près de 60 % dérivèrent en 24 heures dont 57 % par effets du traitement. Cette valeur est élevée si on la compare toujours au téméphos pour lequel, dans un même cas, nous aurions obtenu un décrochement de l'ordre de 25 à 30 % seulement.

20 L'examen des pourcentages de décrochement met à nouveau en évidence l'hypersensibilité des Baetidae mais aussi celle des Tricorythidae, à moindre titre celle des Caenidae. Par contre, l'espèce visée, *S. damnosum,* ne subit que 70,65 % de décrochement, ce qui permet de conclure qu'à cette concentration et sous cette formulation, l'Actellic n'est pas un insecticide utilisable pour la lutte contre ce Diptère, le but impératif étant de détruire 100 % de la population larvaire.

21 Avec 75 % de décrochement, les Hydropsychidae sont très affectés ainsi que les Chironomidae d'une manière générale. Seuls les Plécoptères, pourtant abondants dans l'expérimentation, sont peu sensibles au traitement.

22 La cinétique du décrochement en gouttière a été schématisée fig. 2. Elle est très proche du profil de dérive obtenu au sein même du cours d'eau montrant à nouveau l'intérêt de cette technique. On retrouve particulièrement le second pic de 13 h 15 (soit 3 h après le traitement), essentiellement dû à une recrudescence de la dérive des Baetidae et au commencement de décrochement des Simuliidae.

5. CONCLUSION

23 D'une manière générale, les insecticides utilisés pour détruire les populations larvaires de *S. damnosum* s.l. sont introduits dans le milieu aquatique sous forme de concentré liquide émulsifiable. Étant donné la technique d'épandage par voie aérienne, leur passage dans un gîte proche du point de traitement — ce qui est le cas des essais étudiés — est nécessairement bref, même si l'adsorbsion d'une partie de l'insecticide sur la matière en

suspension dans l'eau ou couvrant les substrats du gîte (matière organique, limon, periphyton, etc.) retarde quelque peu le passage.

24 Ordinairement 24 heures après le passage de la vague insecticide, l'effet toxique n'est pratiquement plus décelable, sauf sur le groupe cible où des décrochements tardifs peuvent encore survenir.

25 L'idée de l'emploi de produits microencapsulés est bonne car elle vise à rendre leur action plus sélective. Dans le cas de microcapsules digestibles par exemple, on peut penser que seuls les invertébrés microphages et détritivores risquent d'être atteints au même titre que les Simuliidae, les carnivores ne l'étant, éventuellement et avec une moindre intensité que par le biais de la chaîne alimentaire.

26 Dans le cas de microcapsules de type poreux qui peuvent se dissoudre lentement dans l'eau, les risques sont par contre plus grands et liés à leur temps de présence sur les gîtes. S'il est long, l'effet sur le groupe cible est amélioré mais l'impact sur les groupes non cible a tendance à s'accentuer.

27 La formulation soumise aux essais était par ailleurs très instable les microcapsules s'étant brisées soit durant le transport, le stockage à haute température ou bien les manipulations d'épandage. Il en résulte un double effet toxique, l'un immédiat dû au pirimiphos-méthyl prématurément libéré des capsules, l'autre, plus lent et retardé, dû à la diffusion progressive de la matière active au travers des capsules restées intactes.

28 L'emploi d'une formulation microencapsulée aussi instable que celle que nous avons testée n'est donc pas recommandable en campagne de lutte contre *S. damnosum,* d'autant plus que son efficacité contre le groupe cible n'a pas été totale à la concentration utilisée.

29 *Manuscrit reçu au Service des Éditions de l'O.R.S.T.O.M. le 7 décembre 1981.*

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NOTES

1. Travaux réalisés dans le cadre d'une convention O.R.S.T.O.M.-O.M.S. Lutte contre l'Onchocercose ; Surveillance de l'environnement aquatique.

2. (1) Rappelons que l'indice de dérive (ID) est le nombre théorique d'organismes dérivant chaque seconde dans un mètre cube d'eau d'une rivière. Il est calculé à partir de récoltes à l'aide de filets à mailles fines par la formule $ID = \frac{\overline{N}}{v.s.t.}$, où $\overline{N}$ est le nombre moyen d'organismes récoltés dans un filet, v est la vitesse du courant mesurée à l'entrée du filet (en cm/s) ; s est la surface d'entrée de ce même filet (en cm^2) et t le temps de mise en œuvre (en s).

RÉSUMÉS

Des traitements aériens à l'Actellic^R M20 (Pirimiphos methyl) en formulation microencapsulée ont été réalisés sur différentes rivières du Mali et de Côte d'Ivoire, en vue de ta destruction des larves de Simulium damnosum s.l., vecteur de l'Onchocercose.

A la concentration de 0,2 mg/l/10 mn, aucune mortalité de poissons n'a été observée. Par contre, les évaluations de l'intensité de dérive témoignent d'une forte toxicité des traitements vis-à-vis des invertébrés benthiques. Cet effet est maximum environ deux heures après les épandages et atteint un niveau vingt fois supérieur à celui provoqué par l'utilisation du téméphos (Abale^R) dans les conditions normales de campagne.

Baetidae, Tricorythidae et Chironomidae sont parmi les taxons les plus sensibles et un niveau global de décrochement pour l'ensemble des invertébrés de l'ordre de 60 % a été obtenu sur une période de 24 heures en utilisant la méthode des « gouttières in situ ».

Un tel niveau d'impact sur la faune des invertébrés n'est pas sans risque pour l'environnement aquatique et l'utilisation de l'Actellic^R M20 microencapsulé sur une grande échelle n'est pas recommandable, sous la forme de ta formulation testée.

Aerial applications of Actellic^R M20 (Pirimiphos methyl), in a micro-encapsulated formulation were tried as a larvicide against Simulium damnosum s.l. in different rivers in Mali and Ivory Coast. Al 0.2 mg/l/10 mn concentration, no fish mortality was observed. However, drift rate measurements indicate a strong effect on the invertebrate fauna, about two hours after application with a maximum level of 20 times in comparison to a normal temephos (Abale^R) treatment.

Baetidae, Tricorythidae and Chironomidae are the most sensitive taxa and a total detachment of 60% have been recorded during a 24 hours period, using the in situ *gutter technique.*

Such an impact on the invertebrale fauna is not without any risk for the aquatic environment and the use *of Actellic R M20 micro-encapsuled on a large scale is not recommended, as far as the tested formulation is concerned.*

INDEX

Mots-clés : Pesticides, Toxicité, Invertébrés, Eaux courantes, Afrique de l'Ouest
Keywords : Pesticides, Toxicity, Invertebrates, Running waters, Western Africa

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Nouvelle technique pour tester *in situ* l'impact de pesticides sur la faune aquatique non cible

Claude Dejoux

1 La mise en œuvre à grande échelle de pesticides destinés à l'éradication des vecteurs de grandes endémies est toujours précédée d'une phase expérimentale destinée à connaître de la manière la plus exacte possible la toxicité des produits utilisés. Outre leur toxicité spécifique pour les espèces que l'on désire contrôler, il est indispensable d'estimer leur impact sur les différentes composantes biologiques du milieu naturel. En ce qui concerne le milieu aquatique, 2 grands types d'études sont généralement utilisés : les expériences de laboratoire en « milieu confiné » et les expériences *in situ* en grandeur naturelle.

2 Notre propos n'est pas de faire la critique de ces 2 techniques qui apportent l'une et l'autre des renseignements intéressants et souvent indispensables mais plutôt d'en proposer une troisième qui évite en partie les inconvénients des 2 premières tout en conservant leurs avantages.

3 L'expérimentation de laboratoire donne généralement des résultats précis, chaque test pouvant être répété dans des conditions standardisées de manière à fournir des résultats statistiquement exploitables. Les organismes testés étant placés dans de petits volumes d'eau, leurs réactions sont aisément visibles et peuvent être minutieusement suivies. Par contre, les conditions expérimentales s'éloignent de celles du milieu aussi sophistiquées que soient les techniques employées. Il en résulte donc une incertitude pour l'extrapolation des résultats aux conditions naturelles.

4 L'expérimentation en vraie grandeur, si elle élimine d'emblée cet inconvénient, a par contre le désavantage d'être beaucoup moins précise. En effet, seuls les résultats « extrêmes » sont nettement visibles (absence totale d'action ou au contraire toxicité entraînant une mortalité spectaculaire). Les effets intermédiaires sont difficilement quantifiables à moins que n'ait été réalisée une étude faunistique quantitative très précise, ce qui est rarement le cas étant donné le volume de travail nécessaire. Par ailleurs, elle nécessite de grandes quantités de pesticides qui, outre un prix de revient

élevé, risquent de provoquer des « accidents » sur un plan écologique étant donné l'incertitude liée à l'expérimentation. Cette méthode a par contre l'avantage d'être appliquée sur des organismes en place avec des conditions écologiques de milieu naturelles. Les résultats obtenus, malgré leur imprécision relative en comparaison des méthodes de laboratoire, seront extrapolables à grande échelle avec beaucoup plus de rigueur.

5 L'idée directrice nous ayant amené à tester un troisième type d'expérimentation a été de conserver les avantages des 2 méthodes habituellement utilisées tout en éliminant leurs inconvénients. Dans les lignes suivantes nous décrirons brièvement l'appareil utilisé et la technique expérimentale en soulignant les avantages acquis. Nous proposerons enfin, après une courte analyse des résultats, quelques améliorations résultant de nos essais préliminaires.

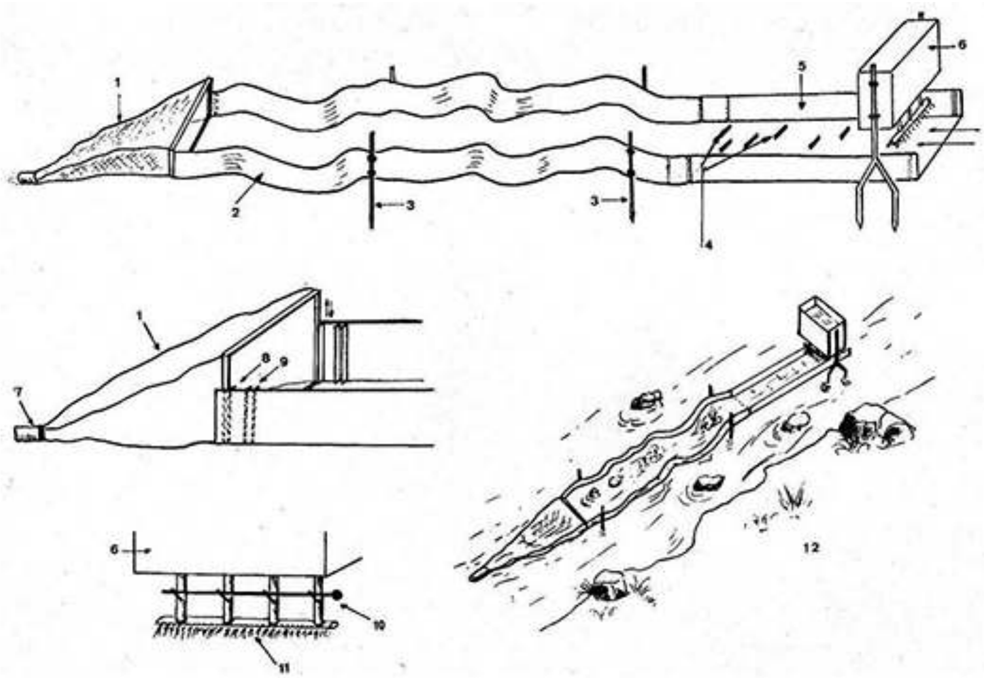


FIG. 1. — 1. Filet de récolte des organismes dérivants. — 2. Partie « expérimentale » de l'appareil. Sinueuse sur le prototype, elle mesure 2 mètres de long pour une largeur de 40 centimètres et une hauteur de 25 centimètres. — 3. Système de positionnement de l'appareil permettant un réglage en hauteur. — 3. Petites palettes métalliques soudées sur le fond et assurant une meilleure répartition du pesticide pendant l'écoulement. — 5. Partie rectiligne de 1 mètre de longueur où il est possible de mesurer facilement le débit de l'appareil. — 6. Réservoir contenant le pesticide à tester. — 7. Collecteur. — 8. Glissière dans laquelle est introduit le filet de récolte de la dérive. — 9. Glissière servant à la mise en place d'une plaque filtrante pendant le changement du filet de récolte de la dérive. — 10. Système de tringle permettant de laisser écouler le pesticide. — 11. Rampe assurant un épandage en pluie. — 12. Schéma de l'appareil en place dans un cours d'eau.

1. Description de l'appareil utilisé

6 Destiné à être utilisé en milieu tropical et réalisé localement, nous avons recherché un type d'appareil qui soit simple à construire, robuste et transportable. Le « prototype » expérimenté, schématisé figure 1 et présenté en action sur la photo 1 est constitué par une gouttière de tôle galvanisée, mise en forme de manière à simuler un modèle réduit de cours d'eau. Réglable en hauteur et en inclinaison, il est formé de 2 parties amovibles, l'une de 1 mètre et rectiligne, l'autre de 2 mètres et sinueuse. Mis en place dans les cours

d'eau, parallèlement au sens du courant, la partie amont de l'appareil comporte une glissière destinée à fixer une fermeture filtrante. Cette partie est surmontée d'un réservoir qui renferme le pesticide à tester.

7 La partie aval comporte 2 glissières contiguës, la plus en amont destinée à recevoir une fermeture filtrante identique à la précédente et la plus en aval servant à la mise en place d'un filet de récolte de la dérive.

2. Technique de l'expérimentation

8 — L'appareil est tout d'abord ancré dans un endroit peu profond d'un cours d'eau. Éventuellement et pour plus de précision dans les résultats, il peut être placé dans une zone destinée à être ultérieurement traitée avec le pesticide testé. Dirigé dans le sens du courant, partie rectiligne vers l'amont, il est semi-immergé de manière à ce qu'une dizaine de centimètres restent hors de l'eau.

9 — Le fond de la partie sinueuse est alors garni à l'aide d'une certaine quantité de substrat naturel du cours d'eau. On prendra par exemple des cailloux du fond, des graviers, du sable, des morceaux de branches immergées... Le but recherché est de reconstituer dans cette partie de l'appareil une sorte de « réplique » du cours d'eau et de ses biotopes. Ces différents substrats seront mis en place avec précaution de manière à ce que la faune les peuplant soit ainsi transplantée dans l'appareil. Avec une certaine habitude et selon la richesse du lieu d'expérimentation, on jugera de la quantité suffisante de substrat transplantée.

10 — On laisse ensuite l'appareil *in situ,* ouvert aux 2 extrémités, pendant au moins 6 heures de manière à ce que les organismes qui n'ont pas été emportés par le courant au moment de la transplantation des substrats, se redistribuent dans le modèle et retrouvent leur biotope habituel.

11 — Cette période préparatoire terminée, on peut passer à l'expérimentation proprement dite. Les opérations devront se dérouler de la manière suivante et dans l'ordre.

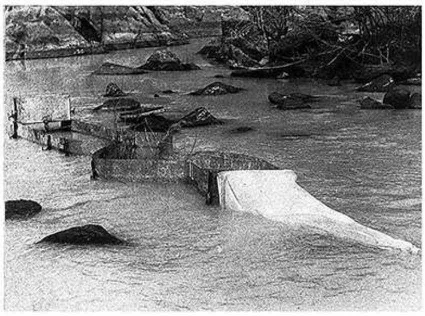
- Fermer par une plaque filtrante, dont les mailles ont 300 µ de large par exemple, la partie amont.
- Introduire le filet de récolte de la dérive dans la glissière la plus en aval.
- Mesurer dans ces conditions le débit de l'appareil. La vitesse pourra être mesurée à l'aide d'un moulinet ou plus simplement avec un objet flottant parcourant toute la longueur de l'appareil.
- Connaissant le débit, calculer la quantité de pesticide à déverser, sa concentration et sa durée d'écoulement en fonction du type d'expérience que l'on veut faire.
- Relever le filet de récolte de la dérive et le nettoyer.
- Mettre en place le pesticide à tester dans le réservoir.
- Replacer le filet de récolte de la dérive.
- Noter le temps t_0 puis faire déverser l'insecticide pendant la durée calculée.
- Au temps t_0 + 30 minutes, changer le filet de récolte de la dérive en ayant soin pendant l'opération d'obturer la partie avale de l'appareil par une plaque filtrante introduite dans la gouttière destinée à cette fin. Cette opération doit être très rapide.
- Le contenu du filet prélevé est récolté, dûment étiqueté et fixé pour une étude ultérieure. Le filet est ensuite correctement lavé pour être à nouveau utilisé.

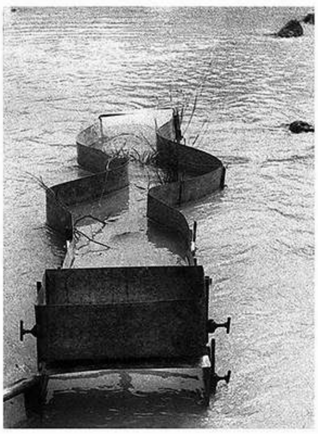
- Au temps t_0 4-1 heure, la même opération sera faite et ainsi de suite aux temps t_0 + 2h ;t_0+4h ; t_0+6h ; t_0 + 8 h et t_0 + 24 h.
- Après la dernière récolte de la dérive (t_0 + 24 h), les 2 ouvertures de l'appareil seront fermées à l'aide des plaques filtrantes puis *la totalité des substrats* contenus à l'intérieur sera récupérée dans des bassines d'eau formolée. Les blocs seront brossés soigneusement, le reste tamisé et trié de manière à récolter *toute la faune encore présente dans l'appareil.* Cette faune est ensuite fixée et triée à part car elle représente la fraction des organismes non affectés par le passage du pesticide.

3. Exploitation des résultats

12 Elle peut se faire de différentes manières, toutefois les données de base seront les suivantes :

1. Pour chaque temps, 30', 1 h, 2 h..., 24 h, on connaîtra la quantité totale de faune décrochée, déterminée au niveau de l'espèce, du genre, de la famille suivant les groupes. Une analyse comparée indiquera les groupes les plus sensibles et, en fonction du temps d'action du pesticide, le moment où se manifeste l'effet toxique du composé testé.
2. En faisant la somme des quantités d'organismes dérivés et de la quantité recueillie vivante dans l'appareil en fin d'expérience, qui représente comme nous l'avons vu la fraction de faune non affectée par le pesticide, on connaîtra pour chaque espèce ou genre le nombre total d'individus testés. Il sera alors aisé de déduire pour les différentes unités taxonomiques, les pourcentages de décrochement en fonction du temps.





4. Résultats d'une expérience préliminaire

13 Afin de tester le fonctionnement de l'appareil que nous venons de décrire, une expérience a été réalisée à Marabadiasa en Côte d'ivoire, dans le Bandama (*). Le produit testé était un insecticide organophosphoré destiné à être employé pour l'éradication de *Simulium damnosum.* L'écoulement dura 2'15", correspondant à une action de 2 ppm d'insecticide.

TABLEAU 1. — Résultats de l'expérience préliminaire.

Groupes décrochés		*Pourcentages décrochés*	
Plécoptères (*Neoperla spio*)		74,1	(27)
Éphéméroptères	*Cloeon* sp.	81,6	(703)
	Baetis sp.	88,2	(328)
	Ephemera sp.	95,5	(9)
Lépidoptères Pyralidae		0	(1)
Trichoptères		74,9	(227)
Coléoptères	Elmidae	84,1	(69)
	Hydrophilidae	84,7	(13)
Odonates	Libellulidae	100	(8)
	Paragomphus sp.	100	(7)
Hémiptères	*Naboandelus* sp.	100	(1)
	Micronecta sp.	100	(5)
	Plea sp.	100	(2)
Diptères :			
— Ceratopogonidae		55,6	(9)
— Simulidae		83,4	(12)
— Chironomidae	• Tanypodinae	51,8	(29)
	• Chironominae	52,7	(76)
	Tanytarsini	33,4	(36)
	• Orthocladiinae	68,6	(35)
Nématodes		0	(1)
Oligochètes		16,7	(6)
Hydracariens		50	(4)
Alevins		100	(10)

14 Dans le tableau 1, nous avons exprimé les pourcentages de décrochage du support pour les différents organismes présents dans l'appareil, 19 h après le passage de l'insecticide. Le nombre total d'organismes testés est donné pour chaque groupe entre parenthèses.

15 Ces quelques résultats montrent nettement que les groupes réagissent très différemment au toxique, certains comme les Éphéméroptères et les Hémiptères étant particulièrement sensibles alors que d'autres comme les Chironomidae ou les Oligochètes le sont beaucoup moins. Pour les groupes dont les effectifs testés sont suffisants, il est possible de dresser une courbe de sensibilité en notant l'intensité du décrochage en fonction du temps. Il apparaît par exemple que 2 genres voisins d'un même ordre d'insectes aquatiques (*Cloeon* et *Baetis*) peuvent avoir une sensibilité différente (fig. 2), l'un réagissant immédiatement au passage du toxique, l'autre plus lentement.

16 Bien que les effectifs expérimentés soient faibles, il est probable que la sensibilité des Chironomides Tanytarsini soit encore d'un autre type car leur réaction au passage de l'insecticide est très lente.

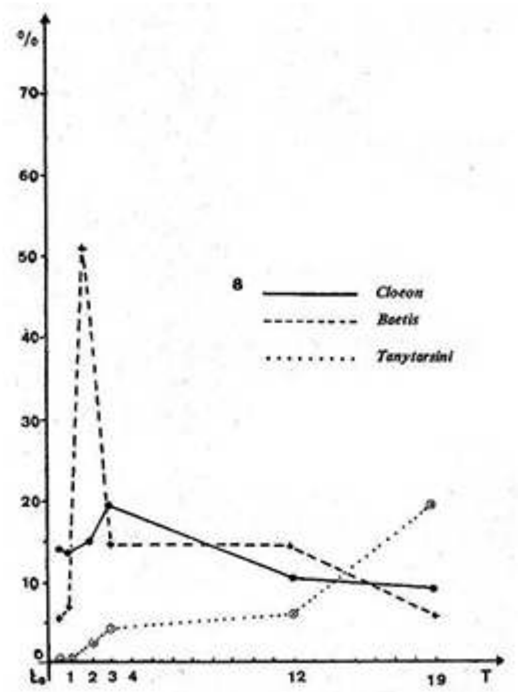


FIG. 2. — Pourcentages d’organismes décrochés, en fonction du temps, par rapport au nombre total testé. Les récoltes ont été faites aux temps t_0 et 30’ ; t_0 + 1 h ; t_0 + 2 h ; t_0 + 3 h ; t_0 + 12 et enfin t_0 + 19 h.

17 (*) Nous remercions vivement MM. TROUBAT et LÉVÊQUE, respectivement technicien hydrobiologiste et malacologue de l’O.R.S.T.O.M. qui, outre la réalisation matérielle de notre appareil, ont bien voulu effectuer le premier essai *in situ.*

18 Ces résultats préliminaires demandent bien entendu à être confirmés ; ils montrent toutefois les avantages de la méthode employée.

19 — L’expérimentation se déroulant *in situ,* les « qualités » de l’eau, tant physiques que chimiques sont identiques à celles des milieux qui subiront les traitements en vraie grandeur.

20 — La faune observée est celle des biotopes naturels et *les rapports numériques entre les organismes sont conservés* si le transfert des substrats est fait avec précaution.

21 — *Les organismes reprennent* pendant la phase préparatoire, donc avant le passage du pesticide, *leur position respective* dans le biotope.

22 — Le nombre d’organismes expérimentés est généralement *élevé* ce qui permet une interprétation statistique des résultats obtenus.

23 — Plusieurs appareils peuvent être mis en action « en parallèle » ce qui permet soit la duplication d’un même test, soit la réalisation de tests avec plusieurs concentrations ou avec des pesticides différents.

24 — Les pesticides employés sont testés en *faible quantité,* ce qui ne porte pas atteinte au milieu naturel et laisse le coût de l’expérimentation à un niveau très bas.

25 Quelques inconvénients enfin sont apparus au cours du test préliminaire mais peuvent toutefois être partiellement éliminés. D’une part la réalisation locale de la partie sinueuse est difficile à effectuer ; par ailleurs la fermeture amont de l’appareil par une plaque filtrante diminue la vitesse du courant à l’intérieur. En conséquence, nous préconisons d’employer un appareil plus simple, formé d’une gouttière en U, rectiligne et en 2

parties ; la plaque filtrante amont sent supprimée. L'estimation de la quantité d'organismes entrant dans l'appareil et provenant de la dérive naturelle sera estimée en disposant de chaque côté de l'ouverture 2 filets de récolte de la dérive et en admettant que la moyenne de leurs récoltes pour chaque période expérimentée équivaut sensiblement à la quantité d'organismes entrée dans l'appareil pendant le même temps.

26 *Manuscrit reçu au S.C.D. de l'O.R.S.T.O.M., le 20 janvier 1975.*

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RÉSUMÉS

Après avoir réalisé plusieurs séries d'expérimentations, en laboratoire et dans le milieu naturel, afin de tester l'impact de nouveaux insecticides sur la faune aquatique non cible, nous avons pu juger les avantages et les inconvénients liés à ces 2 techniques. Afin d'améliorer la représentativité des résultats

obtenus, nous proposons une technique nouvelle d'expérimentation qui tout en gardant les avantages des méthodes classiques, en supprime les inconvénients. Cette méthode utilise un modèle réduit de cours d'eau, installé in situ *et dont l'utilisation permet la mise en évidence de la sensibilité des différents organismes testés vis à vis du pesticide expérimenté.*
Un premier essai de l'appareil a donné des résultats qui confirment ceux obtenus par d'autres méthodes.

Lethal effects of new pesticides on non-target organisms are often estimated on the basis of laboratory bioassays. The relevance of such experimental data to natural conditions is questionable. In other respects, field trials mode on a large scale, can have unexpected and noxious effects on the aquatic environment.
Our purpose was to carry out a new in situ *method having the same accuracy as laboratory tests. The environmental conditions of the treated area are simulated.*
The method is based on the utilization of one apparatus which has been specially designed and which represents a reduced model of a river bed. It is made of sheet-iron, and ils lower part is closed by removable drift-net.
The pesticide formulation to be tested is kept in a storage tank, set up above the upper part of the apparatus and can flow regularly into the System. This device is anchored into shallow and running water beds. Different types of substrates (sand, gravels, stones, etc.), containing fauna, are transferred into the U-shaped part of the apparatus, and this one is kept open at both ends during 12 h for recovering. After this preparation phase, the lower part is closed with a drift-net and the pesticide solution is allowed to flow down, during a given time.
Samples are taken successively, after 30 minutes, I, 2, 4, 8 and 24 h by removing the drift net collector. Finally, the substrates are taken off and the remaining fauna, not killed by the pesticide, is sorted by washing and sieving the sediment, after fixation into formalin. The total amount of experimented fauna is equal to the sum of killed fauna present in collectors plus living fauna which remains at the end of the bio-assay.
The degree of susceptibility of experimented species can be derived from quantitative analysis of the different samples and curves depicting specific drift-rates versus time can be drawn. By that method sufficient numbers of organisms are tested and therefore, specific reactions related to different pesticides can be assessed statistically.

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Toxicité pour la faune aquatique de quelques nouveaux insecticides

III - La deltaméthrine / III. The deltamethrine

Toxicity of some new insecticides towards the aquatic fauna

Claude Dejoux

1 L'emploi des pyréthrinoïdes naturels ou de synthèse comme insecticides n'est pas récent, cependant, ce n'est qu'à partir de 1978 qu'un nouveau groupe fut découvert, présentant à la fois un grand spectre d'action et une forte stabilité à l'air et à la lumière.

2 La deltaméthrine (OMS 1998) ou Kothrine®, ou décaméthrine, ou NRDC 161, fait partie de ces nouveaux composés stables dernièrement mis au point, dont l'emploi diversifié couvre un champ varié. Dans le présent travail, et après un bref rappel concernant la toxicologie générale de ce produit, nous envisagerons son impact sur la faune aquatique tropicale, dans le cadre de son emploi éventuel comme insecticide antisimulidien, ainsi qu'en lutte adulticide contre les glossines. Nous dirons enfin quelques mots de son utilisation en riziculture.

1. TOXICOLOGIE GÉNÉRALE

3 La deltaméthrine présente une toxicité orale aiguë pour le rat et le chien de respectivement 130 à 140 et 300 mg kg^{1} (DL 50). On peut la considérer comme modérée. Les oiseaux paraissent également peu sensibles et la DL 50 par voie orale chez le poulet est d'environ 1 g kg^{-1} et 4,5 g kg^{1} chez le canard (Anon. 1979).

4 Chez l'homme, la manipulation de ce produit et son inhalation peuvent provoquer des irritations faciales, nasales et oculaires. Il y a donc lieu, comme pour de nombreux pesticides, de le manipuler avec précaution. La toxicité à long terme pour les mammifères est faible, principalement en raison de la grande vitesse de métabolisation de ce composé. Cette métabolisation intervient d'ailleurs rapidement et d'une manière générale, dans tout milieu biologiquement actif. Dans le sol, la deltaméthrine pénètre peu en profondeur et est adsorbée par les particules inorganiques fines.

5 Enfin, il faut signaler que le fabricant lui-même insiste sur la toxicité de ce produit pour la faune aquatique en général et invite à prendre des précautions en cas d'utilisation dans un tel milieu. Nos expérimentations, ainsi qu'une analyse de certains résultats obtenus dans le cadre de la lutte antiglossines, nous permettent de donner dans le présent travail quelques précisions quant à la toxicité de ce pyréthrinoïde en milieu tropical.

2. ACTION DE LA DELTAMÉTHRINE SUR LA FAUNE LOTIQUE

6 L'action insecticide de la deltaméthrine étant largement démontrée (MULLA, 1978; BALDRY *et al.*, 1978; RUSCOE, 1977...), son utilisation sans risques écologiques dans le domaine de la santé publique permettrait d'adjoindre un élément de choix à l'arsenal des moyens de lutte contre certains vecteurs de grandes endémies.

7 Afin de rechercher sur le terrain l'impact de ce produit en milieu tropical, nous avons réalisé quelques expérimentations particulières ayant donné les résultats suivants.

2.1. Effets toxiques d'une application à la concentration 0,003 mg/1/10 minutes, en lutte antisimulidienne

8 Ce premier essai a été conduit sur la rivière Baoulé, dans l'ouest du Mali. La deltaméthrine utilisée était sous la forme d'un concentré émulsifiable à 2,5% de matière active (Décis® de Procida, ref. OMS 1998). Elle contenait un synergisant, le pipéronylbutoxide, à la concentration de 125 gl^{-1}. L'épandage a été réalisé par hélicoptère, à environ 200 mètres en amont de notre point d'observation. Les eaux étaient relativement turbides (environ 50 cm de transparence au disque de Secchi), et chaudes (30 °C). Les observations consistèrent essentiellement en une analyse de la cinétique de dérive des invertébrés, à l'aide de filets jumelés de 60 cm de longueur, 12 cm de diamètre d'entrée et environ 250 p. de vide de maille. Chaque récolte durait exactement 30 secondes.

9 L'épandage eut lieu tôt le matin et les premiers effets se manifestèrent 40 minutes plus tard, le maximum d'intensité de dérive étant mis en évidence une heure après le traitement. Quelques valeurs rassemblées dans le tableau I ainsi que la schématisation de la cinétique de dérive (fig. 1) donnent une bonne indication sur l'impact du traitement qui est très violent, mais de courte durée. Cet aspect, largement différent de ce que nous connaissons avec l'utilisation d'insecticides organophosphorés par exemple, semble constant et caractéristique du mode d'action des pyréthrinoïdes qui ont un effet de choc sur les organismes.

10 Le rapport d'augmentation instantanée maximale de l'indice de dérive est de 87,9 et celui d'augmentation pondérée, de 21,2[1]. Ce sont des valeurs du même ordre de grandeur que celles obtenues par action sur un milieu vierge d'une formulation de téméphos 200 CE à la concentration 0,1 $mgl^{-1}10\ mn^{-1}$. De même, les Baetidae présentent le maximum de sensibilité et les Hydracariens paraissent très affectés par le traitement.

TABLEAU I. Variations de l'indice de dérive moyen ($\overline{ID}$) de quelques taxocènes (Invertébrés), en fonction de l'utilisation de différentes concentrations de décaméthrine. Rappelons que l'indice de dérive ID est donné par la relation $ID = \frac{N}{V}$ N est le nombre d'organismes récolté dans un filet à dérive durant un temps donné et V le volume d'eau ayant été filtré pendant ce même temps, exprimé en mètres cubes

	Concentration 0,003 mg l^{-1} 10 mn^{-1}		Concentration 0,007 mg l^{-1} 10 mn^{-1}	
Principaux taxocènes	$\overline{ID}$ avant traitement	$\overline{ID}$ après traitement *	$\overline{ID}$ avant traitement	$\overline{ID}$ après traitement *
Baetidae	5,6	677,8	8,8	7982,7
Caenidae	0	27,8	0	442,5
Leptophlebiidae	0	5,6	0	182,9
Hydropsychidae	16,7	22,2	0	265,5
Philopotamidae	5,6	27,8	0	94,4
Leptoceridae	0	16,7	0	70,8
S. damnosum	0	5,7	0	23,6
Simuliidae	2,2	5,7	0	64,9
Orthocladiinae	0	5,7	8,3	590
Chironomini	11,1	5,7	2,4	88,5
Tanypodinae	5,7	15,8	0	106,2
Tanytarsini	-	-	5,9	82,6
Elmidae	5,7	5,7	0	35,4
Dytiscidae	0	16,7	0	5,9
Micronecta sp.	0	11,1	0	64,9
Anisops sp.	0	11,1	-	-
Hydracariens	0	105,5	0	106,2

• Calculé au maximum d'impact décelé par des prélèvements faits toutes les 10 minutes.

11 Une toxicité de ce niveau pourrait être acceptable, mais à cette concentration l'efficacité sur le groupe cible n'est que partielle (DEJOUX et GUILLET, 1980). Il n'est donc pas possible de préconiser un tel dosage en campagne de lutte antisimulidienne.

2.2. Effets d'une application à la concentration 0,005 mg1^{-1} 10 mn^{-1}

12 Dans le but de rechercher dans un même test l'action de très faibles concentrations de deltaméthrine et celle de concentrations moyennes, nous avons réalisé l'expérimentation en deux temps, utilisant la méthode des gouttières «in situ»[2]. Dans une première phase d'une durée d'une heure, nous ayons recherché l'intensité du décrochement produit par l'action d'une concentration de 0,0005 mg l^{-1} 10 mn^{-1} sur une faune stabilisée durant une semaine. Dans une seconde phase, nous avons observé le décrochement induit par l'introduction de deltaméthrine à la concentration de 0,005 mg H 10mn$^{-1,}$ sur la faune demeurée en place à l'issue de la première heure. L'expérience a été conduite sur la rivière Maraoué, en Côte d'Ivoire.

13 Sur les 1 608 organismes soumis au traitement, 701 (43,6%) ont décroché dans la première heure suivant le passage de l'insecticide (0,0005 mg l^{-1} 10 mn^{-1}), puis 643 (40%) durant les 23 heures suivant la deuxième application (0,005 mg l^{-1} 10mn^{-1}).

14 On notera l'importance des effets du faible dosage qui entraîna un décrochement immédiat des invertébrés, l'indice de dérive atteignant 220 dans les 10 premières minutes. Après la seconde application, le maximum d'intensité atteint par la dérive fut plus faible (ID max = 103), en raison de l'extrême réduction numérique de la faune survenue dans la première heure. Le bilan de l'expérimentation est présenté dans le tableau II.

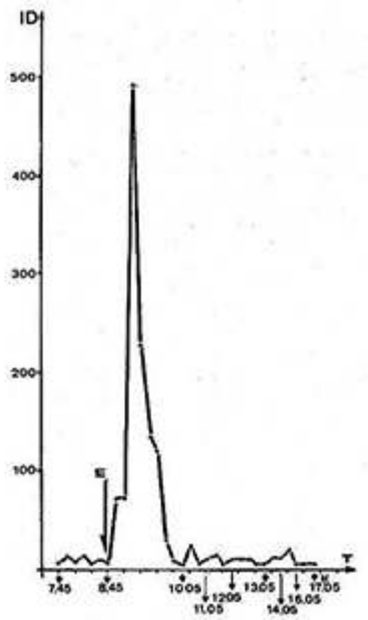


FIG. 1. — Variation de l’indice de dérive des invertébrés lotiques dans une rivière du Mali, après épandage d’une formulation de deltaméthrine à la concentration 0,003 ppm/10 minutes. ID = indice de dérive = nombre d’organismes dérivants chaque seconde dans 1 mètre cube d’eau de la rivière. $ID = \frac{10^4 . \bar{N}}{v.s.t.}$ où N est le nombre moyen d’organismes récolté dans un filet à dérive; v la vitesse du courant en cm/s mesurée à l’entrée du filet; s la surface d’entrée d’un filet (en cm^2) et t le temps de récolte en secondes

15 Tous les taxocènes présents eurent une forte dérive dans la première heure, malgré le faible dosage utilisé. Les Éphéméroptères présentèrent cependant le maximum de réaction avec des pourcentages de décrochement en 24 heures variant entre 76,5% (Oligoneuridae) et 100% (Heptageniidae). Parmi les Trichoptères, *Chimarra petri,* avec 82,3% de décrochement, apparaît également comme très sensible.

16 Il semble qu’à l’effet de choc soit associé un effet de fuite instantanée, faisant entrer volontairement les invertébrés dans la dérive. Nous retrouverons ce phénomène encore plus marqué avec l’ichtyofaune.

2.3. Effets d’une application à la concentration 0,007 mg l^{-1} 10 mn^{-1}

17 Cet essai a été effectué sur le Badinn Ko, au Mali, sur un gîte à simulies formé par de violents rapides situés à l’entrée d’un cañon étroit et encaissé d’environ 10 km. Notre point d’observation était situé à une dizaine de mètres à l’intérieur de la zone de rapides, au seul endroit accessible. La dérive, régulièrement récoltée selon la méthodologie déjà utilisée au cours des essais précédents, provenait donc dans le cas présent, en partie de la zone calme située entre le point d’épandage et le début des rapides (environ 300 mètres), mais surtout de la première dizaine de mètres de ceux-ci.

18 Si l’on en juge par l’extrême abondance de la faune Théophile que nous avons récoltée, tout laisse à penser que le décrochement global, à l’échelle du bief traité, fut catastrophique.

19 Le pesticide atteignit notre point d’observation en 30 minutes environ. Immédiatement après s’ensuivait une dérive spectaculaire atteignant son maximum en 10 minutes (fig. 2), avec une valeur de ID supérieure à 9 000. Le rapport d’augmentation instantanée

maximale de cet indice est de 510,9, soit près de 6 fois plus élevé que dans le cas du traitement à 0,003 mg l^{-1} mn-1. En raison de l'énorme quantité de faune présente dans la dérive, le rapport d'augmentation pondérée est lui-même très élevé (148,5).

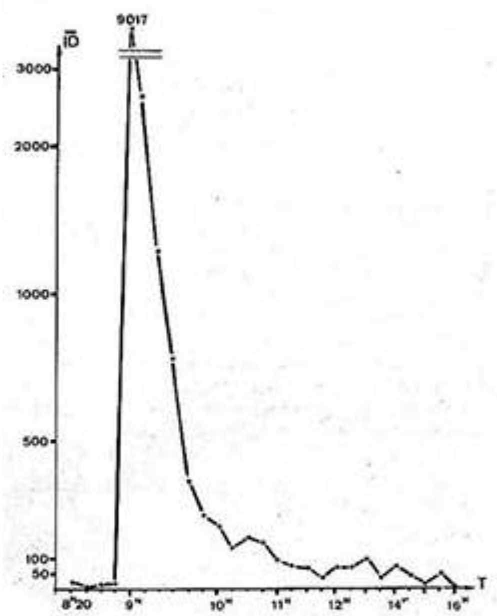


FIG. 2. — Variation de l'indice de dérive des invertébrés lotiques dans une rivière du Mali, après épandage d'une formulation de deltaméthrine à la concentration de 0,005 mg 10 mn^{-1}

TABLEAU II. Bilan de l'expérimentation en gouttière de la deltaméthrine à la concentration 0,0005 mg l^{-1} puis 0,005 mg l^{-1} 10 mn^{-1} Correspondance des codes pour les Trichoptères: T1 = *Cheumatopsyche falcifera*, T2 = *Amphipsyche* sp., T10 = *Cheumatopsyche digitata*, T32 = *Aethaloptera dispar*, T14 = *Orthotrichia straeleni*, T16 = *Chimarra petri*, T20 = *Oecetis* sp.

TAXONS	Faune dérivée	Faune restante	Faune totale testée	% de décrochement
Bastidae	213	12	225	94,7
Caenidae	175	9	184	95,1
Leptophlebiidae	62	4	66	93,9
Heptageniidae	59	0	59	100
Trycorythidae	309	11	320	96,6
Oligoneuriidae	13	4	17	76,5
Povilla sp.	1	0	1	100
Neoperla sp.	2	0	2	100
Libellulidae	2	□	2	100
Agrionidae	1	6	7	14,3
T1	58	47	105	55,2

T2	4	0	4	100
T10	7	1	8	87,5
T32	7	0	7	100
T14	5	2	7	
T16	130	28	158	82,3
T20	2	0	2	100
Veliidae	0	1	1	0
Ceratopegonidae	0	4	4	0
Simulidae	136	79	215	63,2
Chironomini	14	4	18	77,8
Tanytarsini	1	0	1	100
Orthocladiinae	69	23	92	75,0
Tanypodines	57	18	75	76,0
Tipulidae	1	0	1	100
Rhagionidae	0	3	3	0
Hydrophilidae	0	3	3	0
Dytiscidae	3	0	3	100
Elmidae	7	4	11	63,6
Hydracariens	6	0	6	100
Bissanodonta sp.	0	1	1	0
TOTAL	1344	264	1608	% 83,58

20 Il est pratiquement certain qu'en raison de la configuration du gîte, l'impact de ce traitement expérimental se fit sentir sur une grande distance et fut très meurtrier. Dans de telles conditions et malgré une efficacité totale vis-à-vis du groupe cible, l'emploi d'une telle concentration en campagne de lutte antisimulidienne ne peut être envisagée sans risque d'entraîner de graves déséquilibres écologiques dans les milieux traités.

2.4. Tests de toxicité *in situ*

21 Nous avons vu que la deltaméthrine a un effet de choc très violent sur l'ensemble des organismes aquatiques, même à de très faibles concentrations. Afin de rechercher si cet

effet entraînait la mort des organismes, nous avons réalisé, sur certains invertébrés et quelques espèces de poissons, une série d'expérimentations particulières sur la Maraoué en Côte d'Ivoire.

Dispositif destiné à l'observation *in situ* de la réaction des invertébrés après contamination dans des bains de deltaméthrine de concentrations différentes :
A. Tuyau d'amenée de l'eau du cours d'eau.
B. Rampe de distribution.
C. Alimentation individuelle des boites d'observation.
D. Boites d'observation avec grillage circulaire retenant les organismes et laissant s'écouler l'eau par débordement.
E. Plaque de support des boites, réglable en hauteur pour une semi-immersion des bacs d'observation nécessaire à la régulation thermique du système.

2.4.1. ACTION SUR LES INVERTÉBRÉS

22 Les individus testés étaient prélevés dans le milieu naturel le plus délicatement possible, plongés à l'aide de petits paniers grillagés dans les solutions de deltaméthrine aux concentrations voulues, durant un temps précis, puis replacés dans le cours d'eau après rinçage rapide, à l'intérieur de petites boîtes de matière plastique parcourues par un courant d'eau continu (cf. photo). Leur comportement était ensuite régulièrement observé.

23 Le détail des résultats obtenus est présenté dans le tableau III. Il apparaît que dans la majorité des cas la mortalité survient rapidement après un effet de choc immédiat. Ce n'est qu'avec des concentrations inférieures au 1/100 de mg l^{-1} que les chances de survie existent, bien que souvent des séquelles de la contamination semblent affecter le comportement des individus (déplacements ralentis, «prostration», abandon des tubes chez les Trichoptères...) phénomènes jamais observés dans le cas des individus témoins.

2.4.2. ACTION SUR L'ICHTYOFAUNE

24 Les tests auxquels ont été soumis les poissons sont disparates en raison des difficultés d'obtention rapide et en grande quantité de poissons vivants en bon état. Cependant, ils donnent des indications intéressantes et inédites sur la réaction de ces organismes à la deltaméthrine. Les individus testés ont été pêchés de nuit à l'aide d'un épervier puis mis en observation in situ, dans des cages, durant au moins 12 heures avant d'être contaminés. Cette contamination se faisait alors en plongeant les individus durant un temps précis dans des solutions de deltaméthrine de concentrations connues et variées. Ils étaient ensuite remis immédiatement dans des grandes cages (50 cm de côté), disposées dans des endroits particuliers de la rivière, choisis pour être représentatifs de leur biotope habituel. Leur comportement était alors observé durant 24 heures.

TABLEAU III. **Effets de la deltaméthrine sur quelques invertébrés aquatiques**

TAXONS	Nbre d'ind. testés	Concentrations et temps de contamination	Observations
Amphipsyche sp.	6	1mg l^{-1}5mn^{-1}	K.O immédiat. 3 meurent après 2 heures 30, 1après 5 heures 30, les deux derniers sont trouvés morts le lendemain du traitement et sont certainement morts depuis longtemps (observation nocturne *in situ* impossible).
	5	0,1mg l^{-1}10mn^{-1}	K.O immédiat. 3 meurent après 2h 30. Les deux derniers meurent dans la nuit.
	8	0,05mg l^{-1}10mn^{-1}	K.O immédiat. 5 meurent après 2 h 30, les 3 derniers meurent durant la nuit.
	5	0,003mg l^{-1}2mn^{-1}	Légère traumatisation immédiate caractérisée par une agitation intense. Les 5 sont encore vivants après 24 heures mais n'ont pas reconstruit leur abri.
	5	Témoins	Activité normale. Reconstruisent rapidement un abri avec le sable et les graviers mis à leur disposition. 1 est trouvé mort après 24 heures.
Aeptageneidae *Afronurus* sp.	6	0,01mg l^{-1}1mn^{-1}	4 traumatisés avec perte d'équilibre pendant la contamination. 1 mort après 2 heures. 5 vivants après 24 heures avec un comportement normal.
	7	0,05mg l^{-1}mn^{-1}	Traumatisation de 2 individus durant la contamination. 2 morts après 2 heures, 1 mort après 24 heures. Le reste a un comportement normal.
	11	0,005mg l^{-1}10mn^{-1}	Traumatisation de tous les individus en cours de contamination. 3 morts après 2 heures. 8 vivants après 24 heures.
	13	Témoins	1 mort après 24 heures. Tous les autres ont un comportement normal.
Tricorythidae *Tricorythus* sp.	25	0,003mg l^{-1}1mn^{-1}	Forte agitation pendant la contamination. Observation après 2 heures : tous vivants. Après 15 heures : 9 morts, 1 mourant et 15 vivants à comportement normal.
	2	0,1mg l^{-1}1mn^{-1}	1 mort et 1 vivant après 20 heures.
	6	Témoins	6 vivants après 18 heures.
Baetidae	14	0,003mg l^{-1}1mn^{-1}	Traumatisation immédiate. 14 morts après 1 heure.
	7	Témoins	5 morts après 18 heures. 2 vivants.
Leptophlebiidae *Adenophlebiodes* sp.	3	0,1mg l^{-1}1mn^{-1}	Meurent en moins d'une heure.
Macronema	9	0,003mg l^{-1}1mn^{-1}	8 morts et 1 vivant après 18 heures. (traumatisé).
Polymorphomisus	1	0,05mg l^{-1}10mn^{-1}	Meurt au bout d'une heure. Très taumatisé durant le traitement.
Odonates *Phyllomacromia* sp.	4	0,05mg l^{-1}10mn^{-1}	Perte d'équilibre durant le traitement - masque inerte se détache de la tête et pend ! Traumatisation durant plus de deux heures puis mort d'un individu. Les 3 autres reprennent leur activité normale après 18 heures.
	1	Témoin	Vivant après 24 heures.
Cleopatra sp. (Mollusque)	10	0,05mg l^{-1}10mn^{-1}	Rentrent tous dans leur coquille pendant le traitement. Reprennent leur déplacement après 2 ou 3 heures. Deux demeurent "sur le dos" après 18 heures mais ne sont pas morts.
	10	Témoins	Vivants après 24 heures, se déplacent régulièrement.

TABLEAU IV. Bilan des tests *in situ* de contamination par la deltaméthrine de quelques espèces de poissons de Côte d'Ivoire

Espèces testées	Nombre	Concentration et temps de contamination	% de mortalité	Durée en heures
Tilapia zillii	1		0	24
Alestes maroe	6		0	24
Barilius senegalensis	2	*Témoins*	0	24
Labeo parvus	1		0	24
Alestes rutilus	2		50	24
Barbus sp.	1		0	24
T. zillii	1	$100 mg\ l^{-1} 20\ s^{-1}$	0	24
T. zillii	1		0	24
A. Maroe	1		0	24
L. parvus	1	$100 mg\ l^{-1} 10 s^{-1}$	100	2,30
A. rutilus	1		100	4
B. sp.	1		100	15
A. maroe	2	$100 mg\ l^{-1} 5 s^{-1}$	0	24
A. maroe	1	$100 mg\ l^{-1} 2 s^{-1}$	0	24
B. senegalensis	1		0	17
L. parvus	1		0	17
T. zillii	1	$50 mg\ l^{-1} 50 s^{-1}$	100	4
B. senegalensis	1	$50 mg\ l^{-1} 40 s^{-1}$	100	2
Hemichromis bimaculatus	1		100	1
A. maroe	1	$50 mg\ l^{-1} 20 s^{-1}$	100	0,10
B. senegalensis			100	2
B. sp	1	$50 mg\ l^{-1} 10 s^{-1}$	0	17
A. maroe	2	$50 mg\ l^{-1} 5 s^{-1}$	50	15
L. parvus	1	$10 mg\ l^{-1} 2 mn^{-1}$	0	24
T. zillii	2	$10 mg\ l^{-1} 100$	0	24
A. Maroe	1	$10 mg\ l^{-1} 50$	100	6,30
A. maroe	1	$1 mg\ l^{-1} 10 mn^{-1}$	100	24
B. sp.	1		100	6
A. rutilus	7		86	0,05 - 2
Alestes longipinnis	1	$1 mg\ l^{-1} 2 mn^{-1}$	0	18
A. longipinnis	3	$1 mg\ l^{-1} 1 mn^{-1}$	33	16
A. maroe	11		63	2-15
A. rutilus	2	$1 mg\ l^{-1} 20 s^{-1}$	100	0,25

25 Bien qu'il n'ait pas été possible de tester de séries complètes de concentrations, ce qui eût apporté une meilleure validité statistique aux résultats obtenus, nos observations concernèrent 62 poissons appartenant à 8 espèces. 13 furent conservés comme témoins dont 1 fut trouvé mort après 24 heures; sur les 49 ayant été contaminés, 30, soit 61%, moururent durant ces mêmes 24 heures.

26 Les résultats obtenus figurent dans le tableau IV.

27 D'une manière générale, les concentrations utilisées sont élevées et correspondent à des conditions que l'on ne rencontre en campagne qu'aux points d'épandage ou dans les environs immédiats. Il faut cependant noter que l'effet de choc sur certaines espèces est pratiquement instantané ce qui, in situ, se traduit par l'impossibilité de fuite et le maintien des individus dans la zone contaminée durant un temps suffisamment long pour entraîner la mort.

28 La première réaction des poissons, dès qu'ils sont en contact avec la deltaméthrine est la fuite, au sein de l'eau, ou en surface avec bonds hors de l'eau. Ce phénomène est probablement dû à un effet très irritant du produit, mais il est difficile de savoir s'il est le fait de la matière active ou bien s'il provient de l'action de l'un des constituants de la formulation. Une telle réaction avait également été observée lors d'un survol à basse altitude du Badinn Ko au Mali, traité à la concentration 0,003 mg h^{1} mm^{1}. Si la concentration est trop forte (100 mg l^{-1}) ou le temps d'exposition trop long (5 à 10 minutes), l'effet de choc plus ou moins rapide se traduit par un déséquilibre avec immobilisation des individus. Ce phénomène apparaît plus ou moins rapidement selon les espèces et celles ayant un métabolisme rapide (poissons de pleine eau à nage continue), sont choquées plus vite que les espèces à métabolisme lent *(Tilapia, Labeo...)*.

29 Les *Alestes* par exemple, soumis à de fortes concentrations durant un temps court, perdent leur équilibre en quelques secondes et tombent au fond. La durée de survie en état d'immobilisation est variable selon les individus et l'intensité du choc et il peut y avoir reprise d'activité normale après quelques minutes si les poissons sont placés dans une eau non contaminée et bien oxygénée.

30 Dans le cas de concentrations plus faibles, mais avec une longue exposition (10 minutes par exemple), après une vive réaction de fuite, il y a ralentissement de l'activité, puis nage hésitante avec recherche apparente d'oxygène en surface. Généralement, le déséquilibre survient avant la fin de l'exposition, suivi par un rétablissement quand les conditions écologiques sont à nouveau normales.

31 Il semble donc qu'en conditions de campagne, les risques d'entraîner une mortalité directe des poissons soient localisés et fonction de la topographie des biefs traités. Ils demeurent cependant toujours présents.

3. OBSERVATION CONCERNANT L'IMPACT SUR LE MILIEU AQUATIQUE D'UN EMPLOI EXPÉRIMENTAL DE LA DELTAMÉTHRINE EN RIZICULTURE

32 La deltaméthrine ayant montré une bonne efficacité contre les insectes «borers» du riz, l'emploi de ce pyréthrinoïde est susceptible de se développer en régions tropicales. Nous avons donc jugé intéressant d'étudier succinctement les effets possibles de cet insecticide sur le milieu aquatique particulier que représente une rizière (DEJOUX *el al.,* 1977).

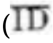
33 Plusieurs modes d'application de la deltaméthrine peuvent être envisagés selon la période végétative du riz et selon la superficie à traiter. Il peut ainsi y avoir un apport par les canaux d'amenée de l'eau dans les différents casiers (cas le moins fréquent), ou bien traitement par pulvérisation, soit depuis le sol (dos d'homme, mécanisé...), soit par voie aérienne.

3.1. Traitement d'un canal d'irrigation

34 La deltaméthrine (formulation en concentré émulsifiable à 20 g de matière active par litre, sans synergisant) a été introduite dans une vasque située au niveau du déversoir d'un petit lac de barrage, d'où partait le canal d'irrigation d'une rizière expérimentale située près de Bouaké, en Côte d'Ivoire.

35 Sachant que la concentration normalement employée pour lutter contre les «borers» du riz est de 750 ml de formulation par hectare, nous avons traité la vasque à une concentration 10 fois supérieure afin d'obtenir, compte tenu des pertes et du coefficient de renouvellement de l'eau, une concentration dans les casiers situés en aval qui soit proche du dosage actif.

36 Le canal d'irrigation des casiers faisant suite à la vasque traitée présentait un riche peuplement d'algues filamenteuses (*Spirogyra* sp.) et d'invertébrés, ainsi qu'une ichtyofaune relativement abondante.

Le passage de la deltaméthrine dans ce canal d'irrigation entraîna une dérive considérable et immédiate des invertébrés, Baetidae et Ceratopogonidae étant les plus affectés. L'indice de dérive calculé avant et 30 minutes après traitement, présenta un coefficient d'augmentation de 27 ( passant de 29,3 à 796,6).

37 Les poissons réagirent également aussitôt après le passage du produit et il y eut une dévalaison complète des individus présents dans le canal, 89 poissons appartenant à 7 espèces ayant été récoltés dans la poche d'un filet situé à 40 mètres en aval du point de traitement. Il faut toutefois signaler que 68 poissons ainsi capturés furent placés en observation dans un casier à riz de 24 m^2, vierge d'insecticide et qu'aucune mortalité n'a été constatée en 48 heures.

3.2. Traitements sur casiers

38 La seconde expérimentation consista en un épandage direct de deltaméthrine, en milieu stagnant et par pulvérisation depuis le sol, à la concentration de 750 ml.ha^{-1} de formulation.

3.2.1. IMPACT SUR LES POISSONS

39 Trois casiers de 24 m^2 chacun, contenant respectivement 5,5; 2,8 et 3,4 m^3 d'eau ont été traités après avoir été empoissonnés deux jours avant le test.

40 Les différentes charges ont été faites à partir des milieux aquatiques environnants, après pêche électrique. La faune présente dans chaque casier est détaillée dans le tableau V.

TABLEAU V. **Distribution des différentes espèces de poissons testés, dans les trois casiers expérimentaux et concentration effective de deltaméthrine, en fonction du volume de chaque casier**

Taxons	Casier 1	Casier 2	Ca3ier 3
Tilapia zillii	5	2	1
Clarias sp.	2	4	-
Heterobranchus sp.	1	10	-
Epiplatys sp.	8	6	2
Barbus spurelli	14	1	8
Ctenopoma kineleyae	1	3	-
Neolebias unifasciatus	3	9	20
Hemichromis bimaculatus	6	5	9
Total	40	40	40
Matière active par litre	0.0065mg	0.013mg	0,011mg

41 Cette expérimentation nous plaçait dans le cas d'un impact maximal sur le milieu stagnant. Les quelques graminées présentes dans chaque casier pouvaient être assimilées

à de jeunes pousses de riz et la deltaméthrine risquait peu d'être décomposée par la matière organique, comme c'est le cas au moment où le riz est dans une phase végétative avancée.

42 La formulation a été pulvérisée directement à la surface de l'eau. En raison de la forte turbidité, il ne fut pas possible d'observer un effet de choc entraînant le déséquilibre de certains individus comme nous l'avions vu en rivière, mais l'application du produit provoqua une fuite en tous sens de quelques-uns d'entre eux, décelable aux remous produits au sein de l'eau.

43 Les différents casiers ont été vidangés 48 heures après le traitement et seul un *Neolebias unifasciatus* était trouvé mort, sans qu'il soit possible de mettre l'insecticide en cause avec certitude.

3.2.2. ACTION SUR LES INVERTÉBRÉS

44 Au moment du traitement, chaque casier était peuplé d'une abondante faune d'insectes aquatiques où dominaient les Coléoptères, les Hémiptères et les Diptères Chironomides.

45 Moins d'une heure après l'épandage, les premiers individus morts venaient flotter à la surface. Les organismes les plus touchés étaient ceux à respiration aérienne ou semi-aérienne, parmi lesquels il faut citer :

- Les Gyrinidae du genre *Dineulus.*
- Les Hydrophilidae Hydrobiinae.
- Les Dytiscidae des genres *Hydrocanthus, Canthydrus* et *Hydalicus.*
- Les Hémiptères des genres *Ranatra, Laccotrephes, Hydrocyrius Anisops, Micronecta, Enithares* et *Diplonychus.*

46 Ensuite moururent les Diptères Chironomides (Chironomini, Tanytarsini et Tanypodinae).

47 Le lendemain de l'expérimentation, pratiquement toute l'entomofaune avait succombé.

48 Ces premières observations concernant l'effet de la deltaméthrine sur la faune aquatique des rizières devraient être répétées. Elles permettent toutefois d'envisager les hypothèses suivantes:

- Si la concentration utile est bien respectée, les mortalités directes de poissons sont peu probables, tout au moins celles de grands individus. Par contre, l'entomofaune souvent riche de ce type de milieu sera régulièrement décimée à chaque traitement.
- Si les épandages sont suffisamment espacés dans le temps (tous les deux à trois mois par exemple), une reconstitution rapide des peuplements aura lieu par des individus provenant des plans d'eau voisins non traités, l'impact indirect sur l'ichthyofaune étant faible et ne risquant d'affecter que les espèces strictement insectivores.

4. RISQUES LIÉS A L'UTILISATION DE LA DELTAMÉTHRINE DANS LA LUTTE CONTRE LES GLOSSINES RIVERAINES

49 Parmi les insecticides pouvant être utilisés pour le contrôle des populations adultes de *Glossina palpalis* et *Glossina tachinoïdes,* se trouvent des produits très toxiques pour l'environnement aquatique, comme par exemple l'endosulfan et la deltaméthrine. Les effets marginaux de ce dernier insecticide sur la faune non cible ont été plusieurs fois

étudiés lors de traitements adulticides expérimentaux (ÉLOUARD *et ai,* 1979; BALDRY *et al.,* 1978, 1981; TAKKEN *el al.,* 1978; SMIES *el al.,* 1980...).

50 La lutte contre les glossines est réalisée par épandages d'insecticides dans les galeries forestières, de manière séquentielle (aérosols) ou résiduelle (traitement direct de la végétation constituant les lieux de repos des adultes). Au cours de ces traitements réalisés très près des cours d'eau, une partie de l'insecticide peut être épandue, accidentellement ou par négligence, directement dans l'eau. Il est cependant très difficile de connaître la quantité de produit atteignant réellement le milieu aquatique. Sur certains biefs et en fonction de facteurs divers (conditions météorologiques, densité du couvert végétal, système d'épandage, habileté du pilote ou de l'épandeur au sol...), la majeure partie de l'insecticide peut atteindre la rivière, alors que sur d'autres les retombées seront pratiquement nulles.

51 Quelle que soit cette fraction, l'impact sur le milieu peut être dans la majorité des cas décelable à l'échelle macroscopique et nous signalerons ci-dessous quelques résultats obtenus en Afrique de l'ouest et centrale.

52 Des épandages expérimentaux de deltaméthrine ont été effectués en Côte d'Ivoire contre les glossines de galeries forestières (EVERTS, 1979). Les traitements étaient réalisés par hélicoptère, au-dessus de la rivière Maraoué et dirigés vers l'intérieur de la galerie. Ils étaient de type résiduel, à 12,5 g de matière active par hectare.

53 Les hydrobiologistes de l'équipe responsable de ces traitements ont étudié les effets sur la faune non cible; ils ont obtenu les résultats suivants:

- Les Baetidae disparaissent rapidement après traitement et recolonisent vite.
- Les Tricorythidae subissent également de lourdes pertes mais recolonisent très lentement. Il en est de même pour les Heptageniidae, les Caenidae et Polymitarcyidae.
- Les Trichoptères sont très affectés mais recolonisent rapidement entre deux traitements.
- Les Chironomidae présentent un développement anormalement élevé de leur peuplement.
- Les Simuliidae sont décimés à chaque traitement mais repeuplent en moins de deux semaines.
- Une dérive très importante de Gerridae, Veliidae et Hydrometridae est constatée, de même que celle d'Elmidae (Coléoptère) et Pyralidae (Lépidoptère).
- Les crustacés *Caridina africana* sont exterminés mais les populations de *Macrobrachium vollenhouenii* récupèrent après un effet de choc paralysant d'assez courte durée (10 heures).

54 Nous avons mis à profit notre présence sur les lieux pour confirmer leurs résultats.

55 — Les effets immédiats se manifestèrent par une dérive des invertébrés de type catastrophique, concernant l'ensemble des espèces présentes, à tous leurs stades de développement. Une estimation de l'indice de dérive effectuée 3 heures après le traitement, donc très certainement en dehors du maximum d'impact, donna une valeur de 3 284 ind. m^{-3} alors que l'ordre de grandeur normal pour la Maraoué, à ce niveau, est en moyenne annuelle de l'ordre de 3 ind. m^3 !

56 — Des estimations de densité des invertébrés, réalisées à l'échantillonneur de Surber 5 jours plus tard, sur les rochers situés dans le courant et recouverts d'un petit phanérogame *(Tristicha trifaria),* ont été comparées à celles présentes sur une autre station de la même rivière, à une vingtaine de kilomètres en amont de la zone traitée. Les résultats, schématisés figure 3 témoignent du violent impact du traitement. En effet, malgré une recolonisation par dérive en provenance de l'amont non traité et par reproduction des adultes ailés, les peuplements d'insectes aquatiques sont extrêmement

faibles. Les Éphéméroptères, qui généralement dominent, ont totalement disparu et les Hydropsychidae (autre groupe abondant) sont peu nombreux et représentés en majeure partie par de très jeunes stades, certainement éclos depuis le jour du traitement. Seuls les Chironomini semblent avoir peu souffert, peut-être en raison d'une moindre sensibilité (comme les Pyralidae) ou plus sûrement par repeuplement à partir de pontes et en profitant de la disparition de leurs prédateurs (ELOUARD *el al.*, 1979).

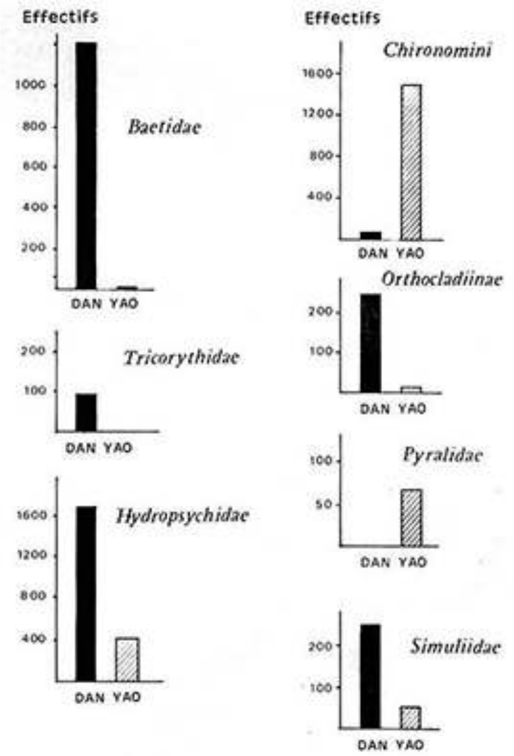


FIG. 3. — Densités comparées des invertébrés à la surface des rochers dans le courant, pour deux stations. La première située sur la Maraoué près de Danangoro (DAN) est non traitée, la seconde située près de Yaokro (YAO) se trouve en zone de traitement anti-glossines, à la deltaméthrine. Effectifs moyens pour une surface de 625 cm^2

57 Ce type de résultat n'est pas spécifique à la Maraoué. Ainsi, à l'issue de traitements expérimentaux réalisés en Haute-Volta sur la Comoé, il a été constaté également un fort impact sur la faune aquatique d'épandages à la concentration de 12,5 g de m.a. ha^{-1}.

58 Si aucune mortalité de poisson n'a été décelée, l'ensemble des peuplements de macro-crustacés (essentiellement *Caridina africana* et *Macrobrachium raridens*) ont été décimés. Seule la première espèce était à nouveau présente sur les lieux un an après traitement, la seconde demeurant introuvable (BALDRY *et al.*, 1981).

59 Une dérive considérable des invertébrés est également indirectement signalée, les mêmes auteurs indiquant leur présence en grande abondance dans les estomacs de poissons, après traitement.

60 Les peuplements ichtyologiques étudiés sur un plan qualitatif, avant et après traitement, montrent de profonds changements caractérisés par une disparition rapide de nombreuses espèces de la zone traitée, ce qui laisse à penser à une fuite importante de ces taxons.

61 Des résultats identiques avaient déjà été obtenus au Nigeria par SMIES *et al.* (1979) et confirment donc le type d'action de ce pyréthrinoïde. Les effets y étaient d'autant plus marqués que les traitements étaient effectués à 30 g de m.a. ha^{-1}

4. CONCLUSION

62 L'ensemble des résultats obtenus sur le terrain, en milieu tropical, met en évidence la forte toxicité de la deltaméthrine pour la faune aquatique et sa faible sélectivité. Ces résultats sont confirmés par la littérature et l'impact souvent catastrophique sur les invertébrés lotiques de traitements anti-glossines réalisés avec ce pyréthrinoïde, a maintes fois été mis en évidence.

63 Si l'on ne prend en considération que les traitements antisimulidiens, le fait le plus marquant est la rapidité d'action de la deltaméthrine sur la faune aquatique non cible. Cet effet de choc, même s'il n'entraîne pas systématiquement la mort des individus, peut très souvent être la cause d'une mortalité indirecte: prédation, transport dans des biefs écologiquement non viables. Enfin, la toxicité de ce pyréthrinoïde croît rapidement avec la concentration utilisée, même à des doses très faibles. Si l'impact sur les invertébrés peut par exemple être considéré comme acceptable à 0,003 mg l^{-1} 10 mn^{-1}, il devient catastrophique à 0,007 $mg1^{-1}$. On conçoit alors que l'utilisation de ce produit dans les campagnes de lutte anti-simulies, si tant est qu'il eût été efficace sur le groupe cible à 0,003 mg l^{-1}, présenterait un risque permanent pour la faune non cible dans la mesure où, à une aussi grande échelle de temps et d'espace, un respect très strict d'une concentration aussi précise est pratiquement irréalisable.

64 En conséquence, il est impossible du point de vue écologique, de recommander l'emploi de la deltaméthrine en campagne de traitements antisimulidiens sans s'exposer à des risques certains et graves pour la faune aquatique lotique.

65 Dans d'autres domaines d'emploi, comme par exemple la lutte contre les glossines ou la lutte contre les insectes « borers » du riz, une utilisation plus ponctuelle et plus limitée dans le temps de cet insecticide peut être envisagée en cas de grande nécessité et en cas d'inefficacité d'autres produits moins toxiques. Il sera toutefois nécessaire de s'assurer que les applications de deltaméthrine soient faites avec le maximum de précautions, à la dose minimale utile et avec un espacement dans le temps permettant la recolonisation des zones traitées à partir des biotopes voisins non traités.

66 *Manuscrit reçu au Service des Éditions de l'O.R.S.T.O.M. le 18 mai 1983*

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NOTES

1. Le rapport d'augmentation instantanée maximale est le rapport de la valeur de l'indice de dérive immédiatement avant traitement à sa valeur au maximum mesuré de l'impact. Le rapport d'augmentation pondérée est celui de la valeur moyenne de l'indice de dérive dans l'heure précédant le traitement (généralement 4 mesures) à sa valeur moyenne calculée sur une heure (généralement 6 mesures), centrée sur l'acrophase de décrochement.
2. Pour toute explication concernant la technique des tests en gouttières *in situ*, cf. DEJOUX 1975 et 1980.

RÉSUMÉS

La deltaméthrine est un insecticide pyréthrinoïde de synthèse très actif et ayant un large spectre d'action. Son utilisation dans le domaine de la santé publique et animale, et surtout en agriculture, semble être largement envisagée. De nombreuses observations de terrain effectuées en pays tropical dans un cadre varié d'utilisation (lutte antisimulidienne, anti-glossines, contre les insectes «borers» du riz...) montrent cependant la forte toxicité de ce produit pour l'environnement aquatique, même à des doses extrêmement faibles.

Si l'ichtyofaune ne semble pas être directement menacée par l'utilisation de deltaméthrine aux doses efficaces contre la plupart des groupes cibles, il n'en est pas de même pour les invertébrés dont certains, comme les Éphéméroptères et surtout les macro-crustacés, sont extrêmement sensibles. Par ailleurs, la sensibilité des différents invertébrés aquatiques augmente très vite avec la concentration d'emploi de ce pyréthrinoïde, ce qui rend d'autant plus dangereuse son utilisation en campagne de traitements de grande envergure, dans le temps et l'espace, comme c'est le cas par exemple pour la lutte antisimulidienne.

Compte tenu de ces résultats, l'emploi de cet insecticide à grande échelle, dans ou près des milieux aquatiques, n'est pas recommandé et doit être réalisé avec de très grandes précautions s'il s'avère indispensable.

*Deltaméthrine is a synthetic pyrethroid insecticide, very efficient and with a large spectrum of action. Its use for public health as well as agriculture purposes seems to be largely considered. However, numerous field experiments, carried out in tropical countries and related to various types of use (*Simulium *or TseTse fly control, fight against rice borer insects...) have pointed out the high toxicity of that compound for aquatic environment, even at very low dosages.*

The fishfauna seems to be directly unaffected by use of dellaméthrine al the normal dosages utilised against the main target groups of insects. It is not the case for invertebrates, and some of them such as Ephemeroptera or chiefly macro-crustacea are extremly sensitive. In other respects, the sensitiveness of invertebrates rise up rapidly in relation to a slight increase of concentration of that pyrethroid. That makes the use of such a compound, in large campaigns and for a long time as it is the case for Simulium damnosum *control for example, very unsafe.*

According to these results, the use of deltaméthrine on a large scale, into or near aquatic habitats, is not recommended or, if necessary, have to be done with great precautions.

INDEX

Mots-clés : Insecticide, Pyréthrinoïde, Impact, Faune non cible, Afrique, Invertébrés, Poissons
Keywords : Insecticide, Pyrethroid, Impact, Non target fauna, Africa, Invertebrates, Fish

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Toxicité pour la faune non-cible de quelques insecticides nouveaux utilisés en milieu aquatique tropical

IV. Le Bacillus thuringiensis var. israelensis H-14 / IV — Bacillus thuringiensis VAR. israelensis H-14

Toxicity for the non-target fauna of some new insecticides used under tropical aquatic conditions

C. Dejoux, F. M. Gibon et L. Yaméogo

1 Essentiellement conduite à l'origine avec un insecticide organophosphoré, l'Abate® ou téméphos, la lutte antisimulidienne réalisée en Afrique de l'Ouest par l'Organisation Mondiale de la Santé (OCP)[1] s'est maintenant diversifiée. L'apparition en 1981 d'une résistance au téméphos de certains cytotypes du complexe *Simulium damnosum* a en effet nécessité la mise en œuvre sur le terrain (essentiellement en Côte d'Ivoire) de produits de remplacement. Afin de prendre toutes les garanties nécessaires pour leur emploi à grande échelle, sans risques trop importants pour l'environnement aquatique, ces produits ont fait l'objet, avant utilisation, de tests de toxicité *in situ.* Dans le présent travail, nous avons essayé de synthétiser les différents résultats obtenus pour l'un deux : le *Bacillus thuringiensis israelensis* sérotype H-14 (de BARJAC, 1978)[2].

1. CARACTÉRISTIQUES DU PESTICIDE

2 Le *Bacillus thuringiensis* Berliner 1915 est un insecticide d'origine biologique puisqu'il s'agit d'une bactérie de la famille des Bacillaceae dont les spores ont la propriété de produire une protéine toxique (delta-endotoxine). On en distingue actuellement plusieurs sérotypes ayant une grande spécificité d'action. D'une manière générale, le cristal protéinique entraîne après ingestion une paralysie des pièces buccales, ainsi qu'une lyse des tissus épithéliaux du système digestif (WHO/VBC/79-750).

3 Utilisé depuis plusieurs années en agriculture, principalement pour lutter contre les chenilles de Lépidoptères, le *Bacillus thuringiensis* est considéré comme un insecticide non dangereux pour l'environnement. Le sérotype H-14 de la variété *israelensis,* dont l'action entomopathogène vis-à-vis des larves de moustiques a été mise en évidence par de BARJAC (1978 a, b et c), fut reconnu comme efficace contre les larves de Simuliidae, en Côte d'Ivoire (GUILLET, 1979 ; GUILLET-ESCAFFRE, 1979 a), et très peu toxique à court terme pour les invertébrés lotiques (DEJOUX, 1979). Il devenait ainsi potentiellement utilisable en campagne de lutte contre l'onchocercose.

2. PRODUITS TESTÉS ET MÉTHODOLOGIE

2.1. Recherche de la toxicité à court terme

4 Le *B.t.i.* a été essentiellement testé sous deux formes différentes. La première était une poudre primaire du sérotype H-14, référencée R-153-78, produite par le laboratoire Roger Bellon Biochem, d'une teneur en matière active supérieure à 1 000 unités internationales de *Bacillus thuringiensis israelensis,* par mg. Elle est peu miscible à l'eau et devait mécaniquement être mise en suspension dans l'eau des milieux à traiter, avant épandage.

5 La seconde forme testée était une formulation fournie par le laboratoire Sandoz Ldt (Agrochemical Dpt., Bâle, Suisse), le Tecknar®, référencée Sandoz 402 I.W.DC. Elle se présentait sous forme d'une suspension liquide épaisse, de couleur brun clair (densité spécifique 1,1 mg/ml à 180 °C), obtenue à partir du sérotype H-14.

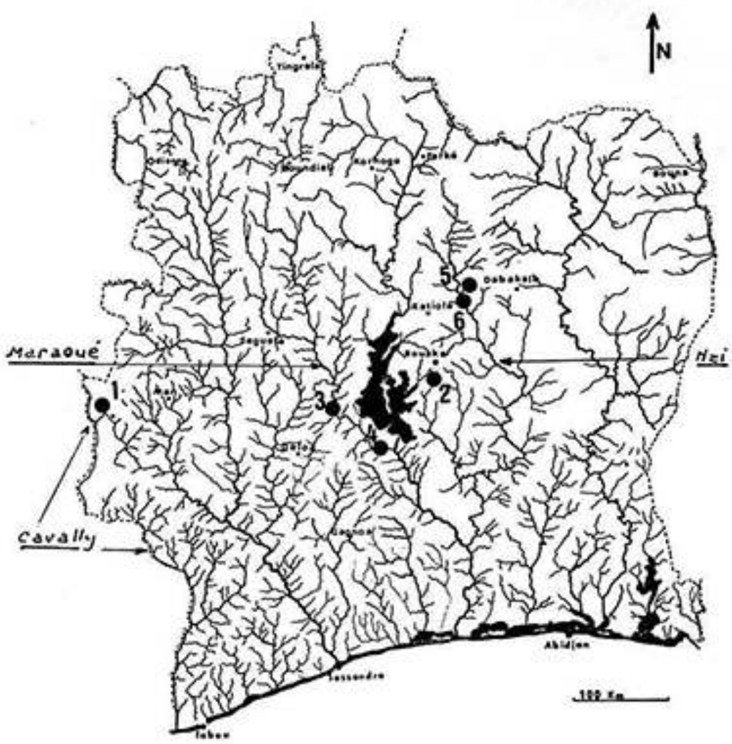

FIG. 1. — Carte de situation des différentes études. 1. Site de Wâ sur le Goué, affluent du haut Cavally ; 2. Site du Kan (affluent du N'zi), près de Bouaké ; 3. Site de Danangoro sur la Maraoué, au Nord-Ouest de Bouaflé ; 4. Site d'Entomokro sur la Maraoué. au Sud-Est de Bouaflé ; 5. Site de l'affluent du N'zi en amont de la route Katiola-Dabakala ; 6. Nzi au pont de Timbé

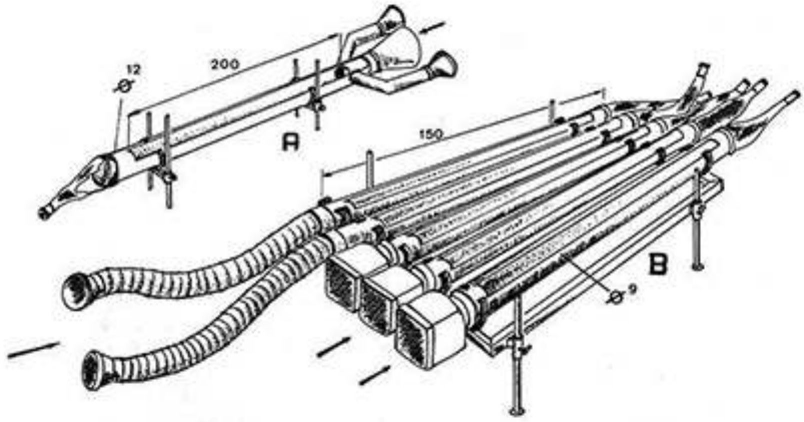

FIG. 2. — Schéma des deux systèmes de gouttières (simple A et multiples B), utilisés *in situ* pour étudier le décrochement des invertébrés, après action du *B.t.i.*

TABLEAU I. Récapitulatif des différentes observations réalisées concernant la toxicité du *Bacillus thuringiensis israelensis* H-14, sous forme de poudre primaire ou de formulation

	Produit utilisé	Localisation des expériences	Méthodes d'observation - Caractéristiques diverses
	A - Observationsen milieu lotique		
Effets à court terme	Poudre primaire	Goué, affluent du Cavally (site 1)	Tests en gouttières simples durant 24 heures* (traitement dans la gouttière) - Traitement d'un bief entier et étude de la dérive in situ.
	-Id-	Bouaké, affluent du Kan (site 2)	Tests en gouttières multiples durant 24 heures.
	Tecknar[R] Formulation Sandoz 402 I.W.DC.	Maraoué à Danangoro (site 3)	Conditions hydrologiques de fin de crue, débit élevé. Epandage par bateau, en hélicoptère, à 5 km en amont du bief d'étude. Observation de la dérive in situ, durant 48 h. centrées sur le traitement. Filets de 225 cm² d'ouverture et de 250 µ de vide de maille.
	-Id-	Maraoué à Entomokro (site 4)	Conditions d'étiage. Epandage par hélicoptère à 300 m en amont du gîte étudié. Etude de la dérive in situ durant 48 h. - Etude de la densité d'organismes sur dalles rocheuses (Surber de 225 cm²) : 10 prélèvements 24 h. avant épandage, 10 autres après. Etude de substrats artificiels benthiques en place depuis 3 mois (blocs de ciment de 4 x 7 x 7 cm), 10 prélevés avant épandage, 10 après.
	-Id-	Affluent du Nzi (site 5)	Conditions d'étiage, épandage par hélicoptère à 100 m en amont du bief étudié. Etude de la cinétique de dérive in situ durant 6 h. et de la faune benthique, avant et après traitement.
Effets à moyen terme	Tecknar[R] Formulation Sandoz 402 I.W.DC.	Bouaké, affluent du Kan (site 2)	Traitements manuels hebdomadaires. Etude de la dérive in situ sur 48 h. centrées sur le 1er épandage Récolte de la faune benthique au Surber, chaque semaine durant 2 mois sur un bief aval traité et un bief amont non traité (5 à 8 échantillons de 33 cm² pris 24 h. avant et 24 h. après chaque épandage). Même étude sur fonds meubles avec un carottier de 3,3 cm de Ø. Prélèvements hebdomadaires de substrats artificiels formés de 4 à 5 blocs de latérite dans un filet grillagé.
	Tecknar[R] Formulation Sandoz 402 ISC	Site 3, site 4 et site 6 (Nzi au pont de Timbé)	Traitements réguliers hebdomadaires par hélicoptère (OMS). Faune benthique analysée dans 5 prélèvements au Surber de 225 cm². Recherche de l'intensité de dérive diurne et nocturne.
	B - Observations en eau stagnante		
Effets à court terme	Tecknar[R] Formulation Sandoz	Bouaké	Tests en laboratoire. 10 séries de 2 béchers de 500 ml contenant chacun 5 larves d'Aedes aegypti et 5 larves de Baetidae (Centroptilum sp.). Une série est gardée comme témoin, les 9 autres reçoivent des concentrations allant de 85 à 780 ppm. Tests extérieurs en bacs plastiques pour des concentrations de 25 et 100 ppm.

* Les méthodes signalées dans ce tableau sont décrites en détail dans Dejoux, 1980.

6 Dans les tests en milieux lotiques, la concentration de 1,6 mg/1 pour un temps de passage de 10 minutes a toujours été utilisée. En milieu stagnant, des concentrations variant entre 25 et 780 ppm ont été testées, les mortalités d'organismes étant notées après 24 heures d'action.

2.2. Recherche de la toxicité à moyen terme

7 Deux séries d'observations ont été effectuées. La première concernait le traitement expérimental, à l'aide de la formulation Sandoz 402 I.W.DC. (1,6 mg/1/10 minutes), d'un petit affluent du Kan situé près de Bouaké en Côte d'Ivoire (site 2)[3]. Des épandages hebdomadaires y ont été réalisés durant deux mois.

8 La seconde série concerna les effets de traitements réguliers de certaines portions de cours d'eau ivoiriens dans le cadre d'une surveillance de routine liée à la campagne OCP. Dans ce cas, la formulation utilisée était référencée Sandoz 402 I.S.C. et le dosage était identique. Trois sites ont été régulièrement échantillonnés (sites 3-4 et 6). Les sites 3 et 4 ont été traités de février à fin avril 1982 au Tecknar®, après avoir été auparavant régulièrement traités a l'Abate® et au Chlorphoxim®. Le site 6 a été traité au Teknar® à partir du mois d'avril 1982 date de reprise de l'écoulement. Au préalable ce site avait également été traité à l'Abate® et au Chlorphoxim®.

9 Les renseignements concernant la localisation des sites et les méthodes utilisées pour la recherche de la toxicité des différentes présentations du *B.t.i.* H-14, sont reportés dans le tableau I. Un schéma des deux dispositifs de gouttières utilisés dans plusieurs tests, est présenté figure 2.

3. RÉSULTATS

3.1. Action à court terme de la poudre primaire

10 Les résultats obtenus après épandage de poudre primaire sur un bief du Goué sont schématisés figure 3. L'intensité de dérive calculée pour plusieurs périodes de la journée, avant et après traitement, y est reportée.

11 Il apparaît nettement une grande différence de cinétique de dérive entre la faune non-cible (14 taxons) et les Simuliidae. Les premiers présentent une faible augmentation de leur intensité de décroche ment après traitement (1,3 fois au maximum décelé), alors que les Simuliidae, très nombreux dans le biotope traité, dérivent intensément (augmentation maximale de 81,2 fois !). De nombreuses larves de Simulies meurent par ailleurs, sans quitter leur support.

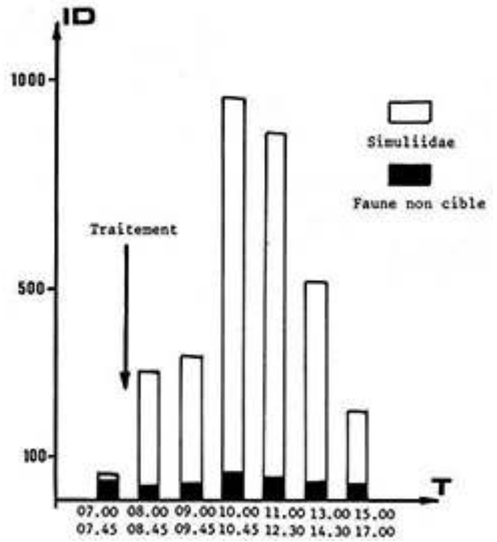

FIG. 3. — Variation de l'indice de dérive (ID) à la suite d'un traitement au *B.t.i.* du Goué (dosage : 0,2 mg/l de poudre primaire/ 10 minutes)

TABLEAU II. Cinétique de dérive *in situ* pour différents groupes d'invertébrés du Goué, traité à 0,2 mg/1 de poudre primaire R-153,78 de *B. t. israelensis* pendant 10 minutes (en % du nombre moyen dérivé, par prélèvement)

Taxocènes	Avant traitement (4 prélèvements)	Après traitement (25 prélèvements)
Orthocladiinae	33,3	45,9
Chironomini	14,2	1,6
Tanytarsini	4,8	7,4
Tanypodinae	0	8,1
Ceratopogonidae	9,5	1,6
Rhagionidae	0	0,8
Baetidae	19,0	15,4
Caenidae	4,8	1,6
Libellulidae	0	0,8
Elmidae	0	4,0
Pyralidae	4,8	6,4
Hydropsychidae	4,8	3,2
O1igochètes	4,8	1,6
Hydracariens	0	1,6

TABLEAU III. Bilan de la dérive en gouttière témoin

TAXONS	Faune totale testée	Faune totale dérivée en 24 heures	Pourcentage d'individus ayant dérivé par taxon
DIPTERES			
Simuliidae	3010	680	22,6
Ceratopogonidae	6	5	83,3
Rhagionidae	10	4	40,0
Orthocladiinae	667	119	17,8
Chironomini	155	36	23,2
Tanytarsini	428	48	11,2
Tanypodinae	55	9	16,4
Tipulidae	2	0	0
EPHEMEROPTERES			
Caenidae	36	5	13,9
Baetidae	284	56	19,7
Leptophlebiidae	9	9	100,0
Tricorythidae	28	1	3,6
TRICHOPTERES			
Cheumatopsyche sp	68	22	32,4
Chimarra sp.	304	27	8,9
Polymorphanysus sp	1	1	100,0
Ecnomus sp	1	1	100,0
Oecetis sp	11	3	27,3
Orthotrichia sp	12	3	25,0
Amphipsyche sp	6	1	16,7
Catoxyethira sp	20	0	0
COLEOPTERES			
Elmidae	290	48	16,6
LEPIDOPTERES			
Pyralidae	74	25	33,8
ODONATES			
Libellulidae	155	6	3,9
Agrionidae	4	1	25,0
HYDRACARIENS	7	5	71,4
COELENTERES	1	1	100,0
PLANIPENNES	7	7	100,0
OLIGOCHETES	16	5	31,3
NEMATODES	2	1	50,0
MOLLUSQUES			
Ancylidae	214	6	2,8
Biomphalaria sp.	1	0	0
Anisus sp.	1	0	0
HIRUDINEES	57	0	0
TOTAUX	5941	1134	Pourcentage global de dérive : 19,08
Faune non-cible seule	3031	454	" 14,98

TABLEAU IV. Bilan d'un traitement à la poudre primaire de *B.t.i.,* en gouttière, à la concentration de 0,2 mg/l (pour un écoulement de 10 minutes)

TAXONS	Faune totale testée	Faune totale dérivée en 24 heures	Pourcentage d'individus ayant dérivé par taxon
DIPTERES			
Simuliidae	6513	6060	93
Rhagionidae	4	2	50
Orthocladiinae	521	60	11,5
Chironomini	91	35	38,5
Tanytarsini	300	46	15,3
Tanypodinae	32	4	12,5
Ceratopogonidae	3	3	100
EPHEMEROPTERES			
Baetidae	279	51	18,3
Tricorythidae	12	0	0
Caenidae	58	6	10,3
COLEOPTERES			
Elmidae	7	5	71,4
Hydrophilidae	2	1	50
ODONATES			
Libellulidae	135	12	8,9
Agrionidae	1	1	100
TRICHOPTERES			
Hydropsychidae			
- Sp T 1	42	0	0
- Sp T 10	8	5	62,5
Leptoceridae sp.			
Oecetis sp	7	5	71,4
Ceraclea sp	1	0	0
Philopotamidae			
Chimarra sp.	344	22	6,4
Hydroptilidae			
Orthotrichia sp.	11	9	81,8
Catoxyethira sp.	24	0	0
PLECOPTERES			
Neoperla sp.	1	0	0
LEPIDOPTERES			
Pyralidae	55	9	16,4
MOLLUSQUES			
Ancylidae	43	10	23,3
Biomphanaria sp.	6	4	66,7
Anisus sp.	3	3	100
HIRUDINEES	38	1	2,6
OLIGOCHETES	11	7	63,6
NEMATODES	2	1	50
HYDRACARIENS	5	4	80
Faune totale	8763	6397	Pourcentage global de dérive : 76,42
Faune non-cible (= sans Simuliidae)	2250	337	" " 14,97

12 Un examen des pourcentages moyens de décrochement pour les différents constituants de la faune non-cible, avant et après épandage, permet de déceler une légère augmentation pour les chironomides autres que les Chironomini, mais dans l'ensemble, les effets du traitement que l'on peut considérer comme négligeables, à court terme, n'ont pas perturbé la structure de la dérive normale de la faune non-cible (tabl. II).

13 Le test réalisé en gouttière sur le même site, avec une concentration identique, permet une analyse plus précise de l'impact. Le bilan de cette expérimentation est dressé dans les tableaux III et IV. Durant les 24 heures d'observation le total du décrochement dans la gouttière témoin correspond à une dérive d'environ 19 % de la faune présente dont 15 % sont relatifs à la faune non-cible. Cette valeur doit être regardée comme normale si l'on considère que le système expérimental n'était en place que depuis 48 heures avant le test, temps relativement trop court pour que les perturbations crées par la transplantation de la faune, du lit du cours d'eau aux gouttières, soient totalement estompées.

14 Après traitement, le bilan dans la gouttière traitée fait état d'une dérive globale de 76,4 % de l'ensemble de la faune, mais de seulement 15 % si l'on considère que la faune non-cible. Il y a donc une même intensité générale de dérive des espèces non-cible, avec et sans traitement (l'identité parfaite étant certainement fortuite).

15 Si l'on prend en considération séparément les taxocènes dont les effectifs testés étaient supérieurs à 50 individus et que l'on effectue une comparaison statistique des pourcentages (comparaison de leur différence Sd_q à la variance standard de cette même différence, Sd_q) il apparaît qu'elle est hautement significative pour les Simuliidae, mais également, au seuil P = 0,01, pour les Chironomini, les Trichoptères du genre *Orthotrichia* et les mollusques Ancylidae, ces deux derniers groupes accusant respectivement des augmentations de leur intensité de dérive de 56 et 20 %.

16 Il semble donc finalement que quelques groupes non-cible aient été légèrement traumatisés par le traitement alors que les autres présentent des différences d'intensité de dérive non significatives. Signalons aussi qu'à l'observation directe *in situ,* à la loupe binoculaire, il n'a pas été possible de déceler d'organismes morts « en place », autres que des Simuliidae.

3.2. Toxicité comparée de la poudre primaire et de la formulation

17 Cette expérimentation, réalisée à l'aide du système à gouttières multiples, porta sur une faune préalablement établie dans l'appareil depuis 4 jours, donc théoriquement stable. Le bilan en est présenté dans le tableau V.

18 Les Simuliidae accusent la plus forte réaction, bien que la poudre primaire n'entraîne que 33,3 % de décrochement des espèces présentes contre 69,8 % pour la formulation. Cette différence est significative au seuil P = 0.01 (d_q = 0,487 pour une valeur seuil de P = 0,01 égale à 0,049). Il se peut toutefois que la formulation entraîne un décrochement supérieur à celui provoqué par la poudre primaire pour une mortalité du même ordre, les individus morts décrochant dans ce cas plus facilement de leur support.

19 Les différences constatées pour le reste de la faune ne sont par contre pas significatives et les pourcentages varient peu d'une gouttière à l'autre. C'est le cas notamment pour les Chironomini qui, dans les premières expériences (cf. 3.1), accusaient une certaine sensibilité à la poudre primaire.

20 Il apparaît donc dans ce test que la formulation de *B.t.i.* n'est, à court terme, pas plus toxique pour la faune non-cible que la poudre primaire, mais qu'elle est par contre plus active vis-à-vis des Simuliidae.

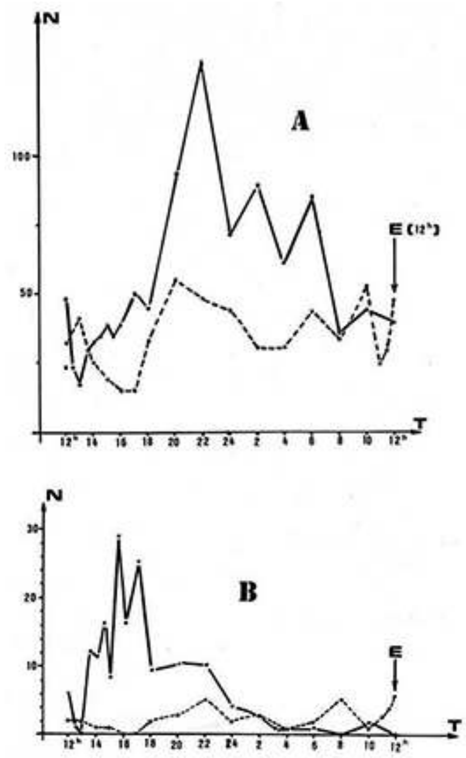

FIG. 4. — Cinétique de dérive de l'ensemble de la faune non cible (A) et des Simuliidae seuls (B), lors d'un épandage expérimental de *B.t.i.,* en formulation et durant la crue (dosage 1,6 mg/1/10 minutes). N = nombre moyen d'organismes récoltés dans la dérive ; T = temps ; ----- avant traitement, ——— après traitement ; E = épandage

TABLEAU V. **Bilans comparés des impacts du *B.t.i.,* sous forme de poudre primaire et sous forme de formulation. Expérimentation en « gouttières *in situ* »**

Taxocènes	Gouttière témoin faune testée	Gouttière témoin reste	Gouttière témoin % dérivé	Traitement avec poudre primaire faune testée	Traitement avec poudre primaire reste	Traitement avec poudre primaire % dérivé	Traitement avec formulation faune testée	Traitement avec formulation reste	Traitement avec formulation % dérivé
DIPTERES									
Simuliidae									
S. adersi	552	427	22,6	845	654	22,6	672	86	87,2
S. hargreavesi	115	99	14,6	97	59	60,8	100	9	91,0
S. ruficorne	498	466	0,0	434	204	53,0	550	304	44,6
Chironomidae									
Chironomini	397	349	12,1	499	432	13,4	872	828	5,0
Orthocladiinae	66	48	26,1	54	40	25,9	291	271	6,9
Tanytarsini	33	28	15,1	61	54	11,5	43	36	16,3
Tanypodinae	223	221	0,9	119	111	6,7	149	143	4,0
Tipulidae	1	1	0,0	-	-	-	-	-	-
TRICHOPTERES									
Hydropsychidae	658	613	5,3	1384	1323	4,4	983	961	2,3
Polycentropodidae	4	4	0,0	-	-	-	-	-	-
Hydroptilidae	19	13	31,6	11	9	18,2	18	16	11,1
Philopotamidae	6	5	16,7	8	7	12,5	4	4	0,0
EPHEMEROPTERES									
Baetidae	76	75	1,3	3	1	33.3	-	-	-
Caenidae	4	4	0,0	-	-	-	-	-	-
ODONATES									
Libellulidae	2	2	0,0	-	-	-	1	1	0,0
Zygoptères	-	-	-	1	1	0,0	-	-	-
PLECOPTERES	-	-	-	1	1	0,0	-	-	-
Pyralidae	1	0	100,0	2	1	50,0	-	-	-
HYDRES	2	0	100,0	9	1	88,9	9	2	77,8
NEMATODES	6	6	0,0	3	3	0,0	2	2	0,0
Totaux	2662	2361	$\bar{x}$ = 11,3	3631	2901	$\bar{x}$ = 17,8	3694	2663	$\bar{x}$ = 27,9
Simuliidae seuls	1165	992	$\bar{x}$ = 14,8	1376	917	$\bar{x}$ = 33,3	1322	399	$\bar{x}$ = 69,8
Faune non-cible	1497	1369	$\bar{x}$ = 8,5	2155	1984	$\bar{x}$ = 7,9	2372	2264	$\bar{x}$ = 4,5

3.3. Action à court terme d'une formulation de *B.t.i.* employée durant la crue. Modification du rythme nycthéméral de dérive

21 Schématisée figure 4, l'augmentation de l'intensité de dérive après traitement se fait sentir faiblement sur la faune non-cible, quatre heures après épandage. Tout en étant significative (environ du simple au double), cette augmentation doit être considérée comme peu importante, d'autant que nous nous trouvons en période de hautes eaux et que la stabilité écologique du milieu est faible. Il est par contre intéressant de noter un certain effet prolongé du traitement qui se traduit par une intensité globale de dérive, durant la nuit suivant l'épandage, encore environ deux fois supérieure à sa valeur de la nuit le précédant. Les Simuliidae accusent une dérive nettement plus élevée.

22 Il existe une légère différence dans la réaction au traitement des différents taxocènes. En calculant l'augmentation de l'intensité de dérive moyenne pour deux périodes de 22 heures correspondantes, l'une avant traitement et l'autre après, nous trouvons les pourcentages d'augmentation suivants :

Simuliidae	+70 %
Chironomidae	+ 46,2 %
Ephéméroptères	+39 %
Trichoptères	+ 27,6 %

Reste de la faune	+ 52 %

23 La plus forte augmentation est donc obtenue pour les Simuliidae, mais à nouveau il faut noter que de très nombreux individus moururent sans décrocher de leur substrat ; cette valeur est donc sous estimée.

24 Mis à part le « reste de la faune » qui correspond à un ensemble de taxons variés, aux faibles effectifs, ce sont les chironomides qui réagissent le plus fortement au traitement, résultat à rapprocher de ce que nous avions déjà trouvé sur le Goué (3.1.) et qui est également signalé par d'autres auteurs (SINÈGRE *et al.,* 1979 b ; YAMÉOGO, 1980). A l'opposé, les Trichoptères accusent une très faible réaction à ce traitement qui a d'une manière générale un impact sur la faune lotique non-cible peu important et étalé dans le temps.

25 A titre de comparaison, il faut enfin signaler que le rapport d'augmentation maximale[4] de l'intensité de dérive était dans cette expérience d'environ 3,5 alors que dans des conditions semblables il est de l'ordre de 40 à 80 fois dans le cas d'un traitement au téméphos (0,5 mg/1/10') et de 100 à 200 avec le Chlorphoxim®, autre organophosphoré (0,025 mg/1/10').

3.4. Action à court terme d'une formulation de *B.t.i.* employée à l'étiage

26 Agissant sur une faune concentrée dans les zones de rapides bien oxygénées, les traitements effectués durant l'étiage ont un impact particulièrement important qui concerne une faune Théophile largement à l'origine des repeuplements de l'ensemble des cours à la reprise des écoulements.

3.4.a. IMPACT D'UN TRAITEMENT EXPÉRIMENTAL DE LA MAROUÉ

Modification du cycle nycthéméral de dérive

27 La partie du cycle relatif à la seule faune non-cible, établi 24 heures avant traitement (fig. 5), présente un profil classique.

28 L'introduction de l'insecticide provoque une augmentation rapide de la dérive des Simuliidae durant 3 à 4 heures. Les valeurs trouvées restent cependant faibles car de nombreux individus morts ne décrochent pas de leur support.

29 La réaction de la faune non-cible est plus lente, mais l'intensité de dérive présente cependant un pic bien individualisé durant la journée après traitement. L'amplitude en demeure toutefois faible (2,5 fois au maximum de décrochement décelé), ce qui doit être considéré comme une action toxique extrêmement minime comparée à celle d'un organophosphoré, dans les mêmes conditions hydrologiques.

30 Le pic de dérive de nuit se situe ensuite au même moment que la veille du traitement, témoignant de l'absence de perturbation profonde du rythme de dérive. A partir de minuit et jusqu'au lendemain matin, on note à nouveau une augmentation sensible de l'intensité de dérive qui est environ 2 fois plus élevée que la nuit précédant le traitement. Cette observation rejoint celle faite en saison des pluies et on peut en conclure que la faune non-cible a subit une certaine traumatisation et que les individus, quand ils reprennent leur activité nocturne, sont légèrement affaiblis et résistent un peu moins à

l'action du courant. D'une manière générale, le traitement a eu une très faible incidence sur l'intensité du décrochement de la faune non-cible.

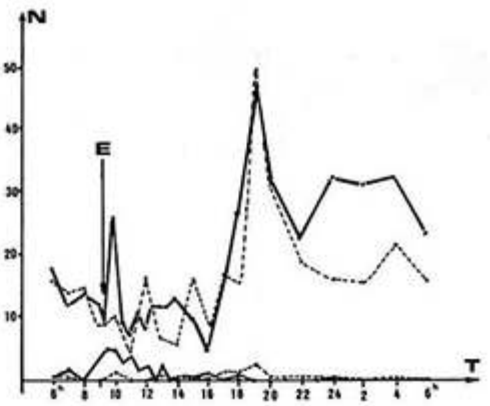

FIG. 5. — Cinétique de dérive de l'ensemble de la faune non cible (courbes supérieures) et des Simuliidae (courbes inférieures) lors d'un épandage expérimental de *B.t.i.*, en formulation, durant l'étiage (dosage 1,6 mg/1/10'). N = nombre moyen d'organismes récoltés dans la dérive ; T = temps ; ---- avant traitement, — après traitement ; E = épandage

Variation des densités de faune benthique en place

31 Les résultats obtenus par l'étude de la faune des rochers dans le courant du site traité, sont consignés dans le tableau VI. L'hypothèse de départ est qu'une forte action toxique du *B.t.i.* va entraîner une importante baisse de densité des organismes en place, due à leur décrochement et à leur passage dans la dérive. Cette hypothèse est vérifiée pour les Simuliidae (test de Wilcoxon positif au seuil P = 0,05), mais pas pour les groupes non-cible suffisamment bien représentés. Pour ces derniers, certains effectifs sont même plus élevés après traitement qu'avant. Globalement, la série récoltée après traitement présente une densité plus élevée de 7,7 % si l'on considère tous les organismes et de 19,7 % si l'on ne prend en compte que la faune non-cible.

TABLEAU VI. **Estimation des densités d'organismes benthiques sur dalles rocheuses, avant et après un traitement avec une formulation de *B.t.i.* (concentration 1,6 mg/1/10')**

TAXONS	AVANT TRAITEMENT m (10 ECH.)	%	Ecart type	24 h. APRES TRAITEMENT m (10 ECH.)	%	Ecart type	% de variation Avant/après
Oligochètes	0,9	0,06	-	0,6	0,04	-	- 33,3
Baetidae	5,6	0,41	5,79	4,80	0,32	4,10	- 14,3
Caenidae	2,4	0,17	4,57	1,80	0,12	3,91	- 25,0
Hydropsychidae	579,1	42,15	697,78	860,40	57,75	549,56	+ 32,7
Leptoceridae	-	-	-	0,10	0,007	-	-
Philopotamidae	-	-	-	0,30	0,02	-	-
Ceratopogonidae	-	-	-	1,40	0,09	-	-
Simulium adersi	180,0	13,10	323,56	22,30	1,50	33,75	- 87,6
S. tridens	4,10	0,30	-	0,80	0,05	-	- 80,5
S. damnosum	-	-	-	0,10	0,007	-	-
Chironomini	470,0	34,21	274,11	454,90	30,53	137,63	- 3,2
Tanytarsini	54,6	3,97	69,28	64,10	4,30	47,54	+ 14,8
Orthocladiinae	34,3	2,50	36,72	45,40	3,05	58,34	+ 24,4
Tanypodiinae	10,3	0,75	16,78	11,10	0,75	14,50	+ 7,2
Tipulidae	8,3	0,60	9,74	5,10	0,34	6,28	- 38,6
Pyralidae	17,0	1,24	15,03	14,5	0,97	19,17	- 14,7
Hémiptères	-	-	-	0,10	0,007	-	-
Hydracariens	1,5	0,11	-	2,00	0,13	-	+ 26,0
Mollusques	5,9	0,43	-	-	-	-	-
Totaux	1374,0	100	-	1489,80	99,98		+ 7,7 %

32 Cette augmentation des densités s'explique mal. Elle peut être due à une distribution très hétérogène des taxons dans les biotopes échantillonnés, mais on ne peut écarter l'hypothèse d'un apport d'organismes par la dérive, qui seraient alors retenus par la végétation ténue que constitue les *Tristicha trifaria* recouvrant les rochers. Cet apport serait alors dans certains cas supérieur à l'intensité du décrochement.

33 Quoi qu'il en soit, il n'est pas possible de conclure à un effet marqué du traitement, décelable au niveau des densités d'organismes peuplant les rochers dans le courant.

Variation des densités de peuplement des substrats artificiels

34 Les résultats regroupés dans le tableau VII conduisent à une conclusion similaire. La variation globale des densités est faible mais encore positive (+ 10,3 % en considérant toute la faune et + 14,5 % en ne considérant que la faune non-cible). Les variations des densités moyennes des différents taxocènes ne sont significatives que pour les Simuliidae, toutefois on constate une augmentation notable des peuplements chironomidiens.

35 A nouveau, faut-il considérer que les substrats jouent le rôle de pièges à dérive comme cela a été montré par quelques auteurs, en l'absence de pollution (CELLOT, 1982 ; BOURNAUD *et al.*, 1973) ? Ils auraient ainsi « capté » les larves de chironomides dont on sait que l'intensité de dérive a tendance à augmenter sous l'effet du *B.t.i.* L'absence de végétation sur les substrats ne permettant plus d'avancer l'hypothèse d'une filtration mécanique ; il faudrait alors admettre qu'il y a eu simple accumulation en raison d'une dérive plus intense de ce groupe. S'il en est ainsi, nous pouvons en conclure que les individus dérivant après un traitement sont aptes à se raccrocher en aval, ce qui n'est pas toujours le cas après action d'autres insecticides (DEJOUX, 1983), donc que les effets toxiques du traitement ne traumatisent que faiblement ces organismes.

TABLEAU VII. **Densité avant et après traitement sur les substrats artificiels. Concentration de *B.t.i.*, égale à 1,6 mg/l/10 minutes**

TAXONS	Avant traitement		Après traitement		% de variation avant/après
	m	%	m	%	
Oligochètes	1,5	0,28	1,2	0,20	- 20
Baetidae	0,7	0,13	0,1	0,02	- 85,7
Caenidae	3,0	0,55	0,8	0,13	- 73,3
Leptophlobiidae	0,3	0,05	0,8	0,13	+ 62,5
Ecnomidae	-	-	2,5	0,41	-
Hydropsychidae	364,1	67,13	368,1	60,85	+ 1,1
Hydroptilidae	-	-	1,7	0,30	-
Leptoceridae	-	-	1,8	0,30	-
Philopotamidae	-	-	1,2	0,20	-
S. damnosum	-	-	0,1	0,02	-
Simulies	27,8	5,12	1,3	0,20	- 95,3
Chironomini	76,9	14,18	100,4	16,60	+ 23,4
Tanytarsini	9,3	1,71	29,5	4,88	+ 68,5
Orthocladiinae	52,2	9,62	84,5	13,97	+ 38,2
Tanypodiinae	1,9	0,35	1,6	0,26	- 15,8
Diptères autres	0,4	0,07	2,0	0,33	+ 80,0
Elmidae	0,5	0,09	0,3	0,05	- 40,0
Pyralidae	2,3	0,42	4,6	0,76	+ 50,0
Sisyridae	0,1	0,02	-	-	-
Hydracariens	0,8	0,15	1,4	0,23	
Gastéropodes	0,6	0,11	-	-	-
Bivalves	-	-	1,0	0,16	- 42,9
Totaux	542,4	99,98	604,9	100	+ 10,3 %

3.4.b. IMPACT D'UN TRAITEMENT EXPÉRIMENTAL D'UN AFFLUENT DU N'ZI

36 La réalisation, sur un petit affluent du N'zi très riche en invertébrés, d'un traitement expérimental similaire à celui effectué sur la Maraoué, nous permet de préciser certains résultats précédemment obtenus.

37 A nouveau nous ne constatons pas d'action importante sur l'intensité de dérive, dans les 5 heures qui suivirent l'épandage (fig. 6). Cette dernière augmente légèrement un quart d'heure après le passage de l'insecticide, atteignant, compte tenu des Simuliidae, une valeur environ 4 fois supérieure à son niveau prétraitement. Ceci doit être considéré comme faible, d'autant que si l'on exclue le groupe cible, le rapport d'augmentation pour le reste des invertébrés n'est plus que de 2 environ, valeur déjà rencontrée dans les expériences précédentes qui correspond essentiellement à une dérive légèrement accrue des chironomides Orthocladiinae.

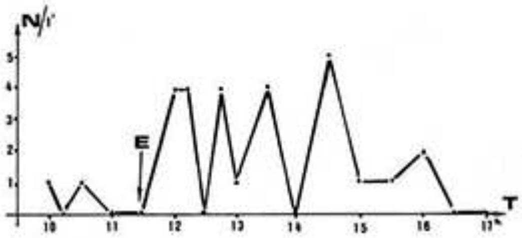

FIG. 6. — Cinétique de dérive à court terme observée au cours d'un traitement expérimental d'un affluent du N'zi, à l'aide d'une formulation de *B.t.i.*

TABLEAU VIII. Variation des densités d'organismes benthiques récoltés dans deux séries de 5 échantillons (Surber), avant et 5 heures après un traitement avec une formulation de *B.t.i.* (nombre moyen d'individus pour 225 cm^2)

TAXONS	Densité avant traitement	Densité après traitement	% de variation
Hydropsychidae	86,2	193,6	+ 55,5
Hydroptilidae	9,4	31,6	+ 70,3
Philopotamidae	11,0	14,2	+ 22,5
Leptoceridae	1,0	1,4	+ 28,6
Simuliidae	0,6	1,6	+ 62,5
Orthocladiinae	240,8	325,8	+ 26,1
Tanypodinae	0,6	0,4	-33,3
Chironcomini	7,0	9,4	+ 25,5
Tanytarsini	24,2	32,4	+ 25,3
Tipulidae	0	0,4	-
Raghionidae	0,4	6,4	+ 93,8
Ceratopogonidae	1,6	1,0	-37,5
Baetidae	65,0	91,6	+ 29,0
Caenidae	16,2	20,8	+ 22,1
O1igoneuridae	0,6	0	-
Pyralidae	49,0	43,0	-12,2
Dytiscidae	0	0,6	-
Libellulidae	0	0,8	-
Plécoptères	0,2	0,2	0,0
Hydracariens	0,2	0	-
Total	514,0	775,2	% 33,7

38 Dans le tableau VIII ont été regroupés les résultats d'analyse de deux séries de prélèvements à l'échantillonneur de Surber. Nous retrouvons une fois encore des densités globalement plus élevées dans la 2e série d'échantillons (environ 34 %), que la première.

Pour les taxocènes ayant des effectifs suffisants (plus de 10 individus en moyenne par prélèvement), ces variations sont significatives.

39 Le biotope échantillonné est ici très particulier. Il s'agit d'une dalle d'une dizaine de mètres de long, en forme de déversoir incliné, recouverte de *Tristicha triforia* sur une épaisseur de 6 à 7 cm. Elle est parcourue par une lame d'eau d'environ 10 cm, s'écroulant à environ 60 cm par seconde. Les échantillons ont été récoltés tête-bêche dans la partie avale de cette dalle.

40 Nous nous trouvons donc dans un cas mécaniquement plus simple que les précédents et il est ici nettement plus concevable que les organismes ayant décroché de l'amont de ce biotope aient été transportés vers l'aval où ils se sont relativement concentrés en se raccrochant dans la végétation, volontairement ou par simple effet mécanique.

41 Il y aurait donc un phénomène identique à celui des cas précédents, le piège à dérive fonctionnant simplement avec une plus forte intensité en raison de la configuration morphologique du milieu.

42 Notre hypothèse émise en III.4.a se trouve ainsi quelque peu confirmée, mais nous retiendrons surtout et une fois de plus, le faible impact à court terme induit par le *B.t.i.*

3.5. Effets à moyen terme d'une formulation de *B.t.i.*

3.5.a. ACTION DU PREMIER TRAITEMENT

43 L'impact du premier traitement du bief choisi risquant d'être plus marqué que celui des suivants, comme c'est généralement le cas lors de l'emploi d'autres pesticides (DEJOUX, 1973), un certain nombre d'observations ont été réalisées à cette occasion.

- La transparence de l'eau a permis de noter une dévalaison temporaire de l'ichtyofaune présente, immédiatement après le traitement (*Tilapia* sp.). De jeunes alevins d'*Alestes* sp. ont ensuite été régulièrement capturés dans les filets de récolte de la dérive, durant plus de 12 heures, alors qu'ils étaient très rares la veille du traitement. On peut supposer que la gène était occasionnée par les constituants de la formulation (solvant, émulsifiant...) mais qu'elle n'était que passagère, puisque des poissons ont par la suite régulièrement été observés sur le bief traité.
- Aucune action du même type n'a été observée pour les larves de Batraciens (*Bufo regularis* essentiellement) qui étaient pourtant bien représentés dans la zone traitée.
- Les invertébrés réagirent de manière identique à celle déjà signalée dans les expériences précédentes, le traitement induisant une augmentation globale de l'intensité de dérive dans la demie heure suivant son application (environ 5 fois sa valeur prétraitement), les Simuliidae et Chironomidae en étant en majeure partie responsables.

44 Après un rapide retour à la normale, l'intensité de dérive nocturne est à nouveau plus forte (environ 2 fois) durant la première partie de la nuit suivant le traitement, que la nuit précédente.

45 Si l'on compare cependant les indices de dérive ID (indices qui, rappelons-le, correspondent au nombre théorique d'organismes « en dérive », chaque seconde, dans un mètre cube d'eau), entre la zone traitée et la zone non traitée située immédiatement en amont, on élimine ainsi les variations générales pouvant avoir lieu entre une nuit et la suivante. On intègre d'autre part les grandes « pulsions » intrinsèques de la dérive (commencement d'émergence d'une espèce par exemple...) qui se produisent alors

simultanément aux deux points de mesure distants de quelques dizaines de mètres. Les différences constatées entre les deux courbes de variation des valeurs de ID sont alors en majeure partie dues à la perturbation produite par l'insecticide (fig. 7).

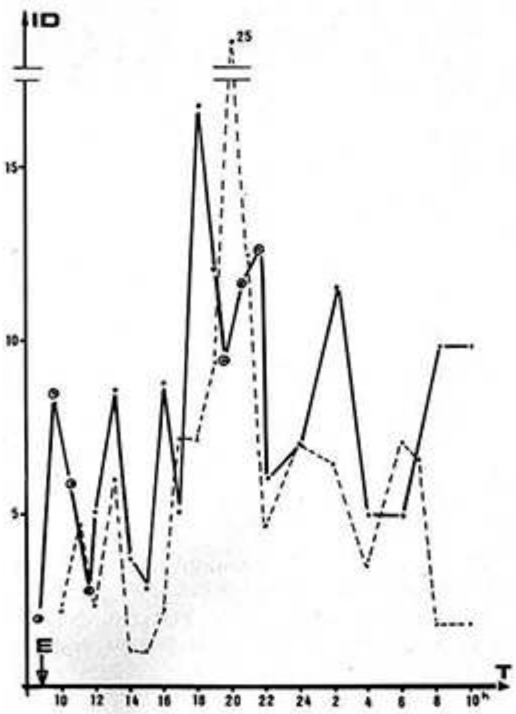

FIG. 7. — Cinétique comparée de la dérive des invertébrés durant 24 heures, dans la zone traitée (—) et dans la zone témoin non traitée (-------). ID = indice de dérive ; E = épandage ; ◉ = valeurs moyennes de plusieurs mesures faites dans l'heure ; ● = valeur de la seule mesure faite à l'heure indiquée

46 Dans le cas présent, apparaît nettement une augmentation de la valeur de l'indice de dérive après traitement, sans qu'il y ait perturbation des rythmes internes de dérive qui demeurent semblables dans les deux zones. Par ailleurs, la valeur supérieure de ID en zone traitée se maintient le lendemain de l'épandage, témoignant d'un effet léger mais étalé dans le temps, de la formulation de *B.t.i.*

3.5.b. MODIFICATIONS APPORTÉES AUX PEUPLEMENTS PAR 9 SEMAINES DE TRAITEMENT

Peuplements des rochers en eau courante

47 Nous avons procédé à une analyse comparée des densités, dans la zone traitée et dans la zone témoin. Dans chacune d'elles, les prélèvements ont été réalisés 24 heures avant (E — 24) et 24 heures après l'épandage (E + 24). Nous avons recherché dans un premier temps si une différence existait en zone témoin dans l'évolution des densités, entre les deux séries de mesures (fig. 8).

48 Il faut tout d'abord remarquer que les résultats obtenus au cours de l'échantillonnage réalisé la 9^{e} semaine apparaissent comme aberrants et tout laisse à penser qu'ils résultent d'une perturbation importante du milieu, survenue entre la 8^{e} et la 9^{e} série du prélèvement. Nous n'en avons pas tenu compte dans le calcul des droites de régression.

49 D'une manière générale, on constate dans la zone témoin une augmentation de la densité moyenne de l'ensemble du benthon tout au long de l'étude. Cette augmentation est pratiquement synchrone entre les deux périodes E — 24 et E + 24, durant les 5 premières

semaines, puis s'accentue différemment par la suite. La pente de la droite de régression pour la période E + 24 est nettement plus forte et le coefficient de corrélation (r = 0,73) est supérieur au seuil significatif pour P = 0,05 (seuil = 0,706).

50 Parmi les groupes taxonomiques principaux, on retrouve une évolution semblable tout au long de l'étude pour les Hydropsychidae, avec une différence cependant moins marquée entre les deux périodes d'échantillonnage (E — 24 et E + 24). Les Chironomidae quant à eux ne présentent aucune tendance à l'augmentation de densité durant le même temps, une légère baisse étant même constatée pour la période E — 24 (r non significatif).

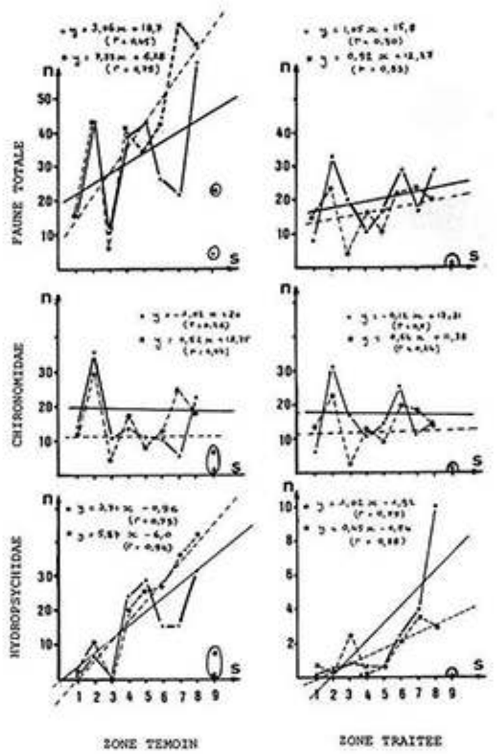

FIG. 8. — Évolution au cours des 9 semaines de traitement des effectifs moyens d'invertébrés récoltés en zone témoin et en zone-traitée 24 heures avant épandage (—) et 24 heures après (------). Faune des rochers dans le courant

51 Pour tous les groupes taxonomiques, une assez grande dispersion des points de part et d'autre des droites témoigne d'une forte distribution agrégative des espèces.

52 Un examen de la série de droites de régression correspondant à la zone traitée permet plusieurs conclusions intéressantes sur l'impact des épandages.

53 — Quand on considère l'ensemble de la faune, nous retrouvons comme en zone témoin une tendance à l'augmentation des densités entre le début et la fin de l'étude, mais elle est peu marquée (a = 1,05 et 0,92) et les coefficients de corrélation r sont largement inférieurs au seuil de signification. La différence qui par ailleurs existe entre les deux périodes E — 24 et E + 24 représente un écart d'environ 20 % (tabl. IX), dont Hydropsychidae et Tipulidae sont les principaux responsables.

TABLEAU IX. **Densités comparées des organismes peuplant les rochers en eau courante, entre zone témoin et zone traitée (9 semaines de traitement) Densités moyennes par mètre carré**

	Densités moyennes (T - 24 h.)	Peuplement en % moyen (T - 24 h.)	Densités moyennes (T + 24 h.)	Peuplement en % moyen (T. + 24 h.)	Différence de densités moyennes en %
(A) ZONE TEMOIN					
Chironomidae	4 222	47,5	4 582	42,2	+ 7,8
Simuliidae	111	1,3	77	0,7	- 30,6
Ceratopogonidae	34	0,4	13	0,1	- 61,8
Tipulidae	178	2	391	3,6	+ 54,5
Hydropsychidae	4 279	48,2	5 777	53,2	+ 25,9
Baatidae	57	0,6	20	0,2	- 64,9
	Total 8 881	100	10 860	100	$\bar{D}$ = + 18,2 %
(B) ZONE TRAITEE					
Chironomidae	4 563	81,6	3 848	87,0	- 15,7
Simuliidae	61	1,1	18	0,4	- 7
Ceratopogonidae	73	1,3	67	1,5	- 8,3
Tipulidae	167	3,0	88	2,0	- 47,3
Hydropsychidae	721	12,9	403	9,1	- 44,1
Baatidae	4	0,1	0	0	-
	Total 5 589	100	4 424	100	$\bar{D}$ = - 20,8 %

54 On peut également supposer, *a priori,* que les densités d'organismes auraient, en absence de traitements, évolué de façon semblable entre zone témoin et zone traitée. En conséquence, les densités dans la zone traitée auraient été plus élevées en fin d'étude au temps E-)-24 qu'au temps E — 24 et la différence constatée en présence de traitement n'est plus 20 % mais de l'ordre de 40 % (20,8 + 18,2).

55 D'autre part, si l'on ne tient pas compte des périodes d'échantillonnage et que l'on regroupe dans chaque zone les effectifs récoltés aux temps E — 24 et E + 24, on obtient un bilan présenté dans le tableau X où l'on retrouve une nette différence de densité moyenne entre bief témoin et bief traité (environ 50 % en moins en zone de traitement). Les densités de chironomides sont pratiquement identiques, mais les autres groupes taxonomiques sont nettement moins denses, à l'exception des Ceratopogonidae.

- Les droites de régression concernant les Chironomides varient peu entre le bief traité et le bief témoin, ce qui suppose une stabilité des peuplements, pris dans leur ensemble. En pourcentage relatif (tabl. X), ils apparaissent toutefois plus importants dans le bief traité, bénéficiant du peu de densité des autres taxons.
- Les Hydropsychidae, qui ont largement augmenté leurs effectifs en zone témoin durant 9 semaines (r supérieur au seuil de 0,834 pour P = 0,01, au temps E + 24), sont demeurés rares en zone traitée. Ils accusent d'ailleurs, avec les Tipulidae, le plus fort écart de densité entre les périodes E — 24 et E + 24 (tabl. IX), ce qui suppose une sensibilité certaine, à moyen terme, à la formulation de *B.t.i.* Le coefficient de corrélation r = 0,75 correspondant à la droite de régression pour les périodes E — 24 est significatif au seuil P = 0,05.

TABLEAU X. Bilan comparé des peuplements d'invertébrés sur fond rocheux en eau courante, entre zone témoin et zone traitée à 1,6 mg/l/10' d'une formulation de *B.t.i.*

TAXOCENES	Zone témoin $\bar{N}/m^2$	Zone témoin %	Zone traitée $\bar{N}/m^2$	Zone traitée %	Différence de densité moyenne
Chironomidae	4402	45,06	4205,5	84,0	- 4,5 %
Simuliidae	94	0,96	39,5	0,79	- 58,0 %
Ceratopogonidae	23,5	0,24	70,0	1,40	+ 66,4 %
Tipulidae	284,5	2,92	127,5	2,55	- 55,2 %
Hydropsychidae	5028	51,46	562	11,23	- 88,8 %
Baetidae	38,5	0,39	2	0,04	- 94,8 %
	9770,5		5006,5		- 48,8 %

Peuplement des fonds meubles en eau peu courante

56 Les résultats obtenus sont résumés dans le tableau XI. Ils concernent des groupes taxonomiques très différents de ceux vivant sur les dalles rocheuses et sont tributaires de variations physiques de la nature de leur biotope, nettement plus importantes.

57 Bien que significative au seuil P = 0,05, la différence globale de densité constatée entre les deux zones peut difficilement être attribuée au seul traitement. Cette remarque s'applique *a fortiori* pour les chironomides qui accusent une différence encore plus faible.

Modifications constatées au niveau des peuplements des substrats artificiels

58 En raison des différences de forme qui existent nécessairement entre chacun des substrat employés, il n'est possible que de comparer la composition relative de leurs peuplements, au cours de l'étude. Mis en place une semaine avant les premiers traitements, les premières récoltes faites immédiatement avant épandage prouvèrent que la colorisation de ces milieux était grossièrement semblable dans les deux biefs étudiés, avec toutefois une légère différence pour les Hydropsychidae, moins denses dans la future zone traitée que dans la zone témoin. Rappelons qu'une telle différence avait également été notée sur les dalles rocheuses.

TABLEAU XI. Densités moyennes des organismes peuplant les fonds de sable, en zone traitée et en zone non traitée (moyennes établies sur 9 semaines)

Taxocènes	Zone non traitée $\bar{N}/dm^2$	Zone non traitée %		Zone traitée $\bar{N}/dm^2$		Zone traitée %	Différences entre les densités moyennes
Chironomini	51	46,4		32,9		39,0	
Tanytarsini	47,8	43,5	93,4	8,8	82,1	10,4	- 12,1 %
Tanypodinae	2,2	2,0		8,3		9,8	
Orthocladiinae	1,7	1,5		19,3		22,9	
Ceratopogonidae	0,5	0,5		2,2		2,6	+ 77,3 %
Caenidae	1,4	1,3		0		0	-
Potadoma sp.	5,4	4,9		9,4		11,1	+ 42,6 %
Hydropsychidae	0	0		3,5		4,1	-
Total	110			84,4			- 23,3 %

59 Après 9 semaines de traitements, la structure moyenne des peuplements entre les deux biefs présente peu de différences, sauf pour deux familles : les Orthocladiinae et les Hydropsychidae. Les premiers ont vu leurs effectifs augmenter, à l'inverse des seconds

qui accusent une baisse sensible de leur pourcentage. Ce résultat est à rapprocher de celui obtenu par l'échantillonnage des dalles rocheuses et confirme un impact moyen des traitements sur ces organismes.

TABLEAU XII. **Proportions moyennes des différents taxocènes ayant peuplé les substrats artificiels durant 9 semaines, en zone témoin et en zone traitée**

	% en zone témoin 7 échantillons	% en zone traitée 7 échantillons	Différence
Chironomini	18,4	18,3	-0,1
Tanytarsani	8,2	12,3	+ 4,1
Tanypodinae	1,7	2,5	+ 0,8
Orthocladiinae	8,4	35,3	+ 26,9
S. adersi	4,5	1,5	-2,5
S. hargreavesi	0,2	0.5	+ 0,3
S. ruficorne	1,2	0	-1,2
Baetidae	3,2	8,4	+ 5,2
Caenidae	0,3	1,7	+ 1,4
Hydropsychidae	51,8	14,7	-37,1
Hydroptilidae	1,0	1,1	+ 0,1
Philopotamidae	0,4	0,9	+ 0,5
Libellulidae	0,1	0	-0,1
Zygoptères	0,03	0,2	+ 0,17
Potadoma sp.	0,3	1,5	+ 1,2
Oligochètes	0,27	1,0	+ 0,73

3.6. Premiers résultats fournis par le protocole de surveillance des milieux aquatiques lors de traitements de moyenne durée au *Bacillus thuringiensis,* sérotype H-14

60 Nous avons pris en considération les sites 3 et 4 de la Maraoué, qui ont été régulièrement traités aux organophosphorés depuis mi-1977, puis ensuite et durant 3 mois à l'aide de Teknar® (février à avril 1982), et les densités moyennes d'insectes benthiques présents sur ces sites durant des périodes calendaires identiques. On remarquera tout d'abord la très forte variabilité des peuplements, aussi bien d'un mois au suivant que d'une année à

l'autre. De telles variations, dues en partie à des conditions hydrologiques différentes, empêchent une analyse détaillée de l'effet de traitements de courte durée (tabl. XIII).

61 Le protocole de surveillance est en effet conçu pour un suivi à long terme et se prête mal à des comparaisons partielles. Nous pouvons toutefois faire quelques observations.

62 — Seuls les chironomides demeurent nettement plus abondants durant les périodes traitées que durant les périodes non traitées. Ils bénéficient d'une situation créée par l'application régulière d'organophosphorés qui les favorisent par rapport aux autres taxons ayant des cycles de développement plus longs (ELOUARD et JESTIN, 1982). Il apparaît toutefois une certaine tendance à la baisse de leurs effectifs, compensée par une augmentation des densités d'Hydropsychidae, au cours des 3 mois d'application du Teknar®. Elle peut correspondre à un retour à une situation plus proche de celle prévalant avant tout traitement, auquel cas on peut conclure à un effet du *B.t.i.* inférieur à celui de l'Abate®.

TABLEAU **XIII. Peuplements des dalles rocheuses aux sites de Danangoro et d'Entomokro, sur la Maraoué. Nombre moyen d'individus par prélèvements (5 échantillons au Surber de 225 cm^2 récoltés chaque mois). 1976 et 1977 : non traité. Traitement hebdomadaires au Teknar® en 1982**

Années	Danangoro 1976				Danangoro 1977				Danangoro 1982				Entomokro 1982			
Mois	Fév.	Mars	Avril	$\bar{N}$	Fév.	Mars	Avril	$\bar{N}$	Fév.	Mars	Avril	$\bar{N}$	Fév.	Mars	Avril	$\bar{N}$
Baetidae	194,2	13,4	79,6	95,7	176,0	489,0	2,4	222,5	43,2	362,0	10,4	138,5	58,8	12,8	4,8	25,5
Caenidae	52,4	2,6	0	18,3	0	1,0	0	0,3	20,4	0	0	6,8	19,4	17,6	1,2	12,7
Tricorythidae	11,8	0	0	3,9	0	0	0	0	1,2	0	0	0,4	5,8	0	0	1,9
Hydropsychidae	395,2	10,8	1610,4	672,1	328,2	85,4	186,0	199,9	369,0	282,0	485,0	378,7	101,4	902,4	688,2	564,0
Hydroptilidae	28,6	0	0	9,5	1,2	3,2	0	1,6	2,4	0,2	0	0,9	0	3,2	0	1,1
Philopotamidae	1,4	0	4,6	2,0	12,8	0	0	4,2	9,6	0	0,4	3,3	0,8	4,8	0	1,9
Simulium spp.	0	0	0	0	40,6	9,0	2,4	17,3	44,4	19,5	1,0	21,6	400,0	639,0	245,0	428,0
Chironomini	32,4	0	2,4	11,6	44,8	3,6	2,4	16,9	3027,0	60,0	49,8	1045,6	249,6	1973,0	157,8	793,5
Tanytarsini	277,2	0	0	92,4	0	0	0	0	159,0	28,0	6,6	64,5	1107,0	272,0	13,0	464,0
Orthocladiinae	9,0	31,0	132,8	57,6	61,6	58,0	80,4	66,7	1627,0	128,0	83,6	612,9	859,0	40,0	136,4	345,1
Tanypodiinae	18,4	0,2	0	6,2	29,2	28,0	1,2	19,5	90,6	6,5	0,2	32,4	29,8	59,2	1,6	30,2
Pyralidae	19,2	0	0	6,4	94,2	10,4	0,8	36,1	13,8	25,0	2,4	13,7	62,0	34,4	39,4	45,3
Total	1039,8	58,0	1829,8	975,9	788,6	687,6	275,6	583,9	5407,6	911,2	639,4	2319,4	2893,6	3958,4	1287,4	2713,1

63 Un résultat du même ordre a été trouvé sur le N'zi (site 6) traité de mai à juillet 1982 au Teknar®, après avoir subit des traitements au Chlorphoxim® et à l'Abate®. Enfin si l'on examine les résultats des analyses de dérive, aucune action drastique imputable aux traitements au *B.t.i.* n'est décelable. La dérive de jour (dérive traumatique) demeure faible, témoignant d'une traumatisation négligeable des peuplements présents. La dérive nocturne (d'activité biologique) est toutefois moyennement élevée, très certainement en raison des impacts à long terme des traitements antérieurs aux organophosphorés.

3.7. Effets d'une formulation de *B.t.i.* sur les invertébrés d'eau stagnante

64 Bien que les expériences réalisées aient été peu nombreuses et d'une portée limitée, elles ne furent pas sans intérêt dans la mesure où les informations dans ce domaine sont rares en pays tropical. Nous ne les considèrerons cependant que comme des recherches préliminaires dont les résultats ont été les suivants :

65 — En laboratoire, l'addition d'une quantité progressive allant de 1 à 9 gouttes de formulation de *B.t.i.* à des béchers contenant 400 cc d'eau et chacun 5 larves de Culicidae

et 5 larves de Baetidae, a entraîné la mort de tous les Culicidae en 24 heures ainsi que les mortalités suivantes de Baetidae :

Concentrations en mg/l	85 mg/l (1 g)	170 — (2 g)	255 — (3 g)	340 — (4 g)	425 — (5 g)	510 — (6 g)	595 — (7 g)	680 — (8 g)	765 — (9 g)
1re Série................	0	0	0	1	0	0	2	1	1
2e Série.................	0	1	0	0	1	0	1	0	2
Série témoin.............	— Aucune mortalité —								

66 Si cette expérience a peu de signification dans la pratique, dans la mesure où les concentrations normalement employées contre les larves de moustiques sont beaucoup plus faibles que celles testées (environ 1 mg/litre), il est toutefois intéressant de noter que jusqu'à 500 fois cette dose, le taux de mortalité des Baetidae n'a pas dépassé 20 % en 24 heures.

67 Dans l'expérience conduite à l'extérieur, dans des bacs comportant les éléments faunistiques normaux d'un gîte à moustiques, les résultats consignés dans le tableau XIV ont été obtenus.

TABLEAU **XIV. Mortalités obtenues après 24 heures dans les bacs d'eau stagnante peuplés d'organismes aquatiques. Bac 1 avec 25 mg/1 de formulation *de B.t.i.* (Sandoz 402 A WDC). Bac2 avec 100 mg/l. Aucune mortalité dans un bac témoin**

	Bac 1		Bac 2	
	VIVANTS	MORTS	VIVANTS	MORTS
Chaoboridae	3	0	4	0
Chironomini	26	0	11	0
Orthocladiinae	13	0	17	0
Tanypodinae	-	-	2	0
Culicidae	0	21	0	32
Microvelia sp.	3	0	-	-
Anisops sp.	16	0	5	1
Paragomphus sp.	-	-	2	0
Libellulidae	-	-	3	0
Dytiscidae	7	0	3	0
Orthotrichia sp.	3	0	6	0
Hydracariens	-	-	3	0
Oligochètes	2	0	47	0
Hydres	16	8	13	27
Biomphalaria	-	-	2	0
Ancylidae	-	-	1	0
Potadoma sp.	17	0	41	0
Ostracodes	-	-	10	2
Ciliés	très nbx	?	très nbx	?
Bufo regularis	3	0	34	0

68 A la concentration 25 mg/l qui est bien au delà de la dose léthale pour les Culicidae, seule une mortalité chez les Hydres a été observée (50 %). Tous les autres organismes ont survécu après 24 heures, hormis peut être quelques Ciliés mais il est pratiquement impossible pour ce groupe de retrouver les morts.

69 Dans le cas de la concentration 100 mg/l, la sensibilité des Hydres se manifeste à nouveau avec une mortalité de près de 70 %. De même apparaît une certaine mortalité chez les Ostracodes (16 %) et les Hémiptères *Anisops sp.* (17 %). Il est difficile de faire la part des « responsabilités » dans ces faibles taux de mortalité mais l'hypothèse d'une toxicité du solvant n'est pas à écarter.

70 Les résultats de ces quelques tests en eau stagnante, compte tenu de leur caractère préliminaire, semblent indiquer que le *B.t.i.* sous forme de formulation Sandoz, ne présente aucune toxicité à court terme pour de nombreux organismes non-cible tropicaux. Ceci rejoint les observations effectuées en climat tempéré, bien qu'il ait dans ce cas été rapporté une toxicité du *B.t.i.* en formulation, vis-à-vis des larves de Chironomidae, du même ordre de grandeur que celle obtenue pour les Culicidae (SINÈGRE *et al.*, 1979 a et b). Nous n'avons pas noté cette toxicité dans nos tests, bien qu'ayant expérimenté de très fortes concentrations.

4. CONCLUSION

71 Bien que les différents essais dont nous venons de présenter les résultats ne couvrent pas toutes les situations rencontrées en campagne de lutte contre Simulies ou moustiques, nous pouvons conclure à une innocuité marquée du *B.t.i.* vis-à-vis des invertébrés aquatiques non-cible, tout au moins à court et moyen terme.

72 — Introduit dans un milieu lotique, le *B.t.i.* en formulation n'induit qu'une faible augmentation de la dérive des invertébrés, contrairement à ce qui se passe avec plus d'une dizaine d'autres insecticides employés dans les mêmes conditions. L'augmentation du taux de dérive est seulement de 3 à 5 fois, au maximum d'intensité. Il faut toutefois noter que le phénomène se prolonge sur presque 24 heures, témoignant d'une certaine traumatisation des organismes, légère mais prolongée.

73 — Certains taxons sont plus affectés que d'autres par les traitements. C'est par exemple le cas des *Ortholrichia* sp. (Trichoptères) ou des Simuliidae (*S. adersi S. tridens* et bien entendu *S. damnosum,* espèce qui disparaît totalement des biefs traités).

74 — L'examen des premiers résultats fournis par le Programme de surveillance montre cependant que sous traitements hebdomadaires, des populations importantes de Simuliidae autres que *S. damnosum* peuvent se développer. Par ailleurs, il ne se confirme actuellement pas l'hypothèse selon laquelle le *B.t.i.* H-14 aurait maintenu, comme ce fut le cas au cours des traitements expérimentaux d'un affluent du Kan, les populations d'Hydropsychidae à un niveau très faible. Peut-être faut il chercher dans ce dernier cas d'autres explications à ce phénomène comme par exemple une hétérogénéité du milieu entre zone traitée et zone non traitée avec des différences de nature du substrat, de vitesse de courant, d'ensoleillement... Nous savons par ailleurs que des formulations différentes d'une même matière active peuvent avoir des effets eux aussi différents.

75 — Si l'on en juge par le bilan réalisé après 9 semaines de traitements expérimentaux de l'affluent du Kan, encore jamais traité auparavant, il faut malgré tout se garder de conclure en une innocuité totale des épandages et si l'on considère l'ensemble de la biocénose du bief traité, la baisse de densité des organismes benthiques constatée en fin d'étude n'est certainement pas fortuite et doit, au moins partiellement, être imputée à l'action de la formulation de *B.t.i.* Son amplitude est cependant faible et n'atteint pas celle constatée après des applications régulières d'Abate® que l'on peut considérer comme sans conséquences péjoratives et à long terme pour l'environnement aquatique.

76 — Bien que restant faible, la toxicité de la formulation est sensiblement supérieure à celle de la poudre primaire, probablement en raison d'une meilleure dispersion dans le milieu, mais on ne peut également écarter une action toxique spécifique de ses constituants (solvant, dispersant...).

77 — D’une manière générale et au moment où nous écrivons ces lignes, la durée d’emploi du *B.t.i.* dans le cadre des traitements réguliers des cours d’eau de Côte d’Ivoire doit être considérée comme trop courte pour qu’ai pu s’établir — s’il y a lieu — une amélioration de la situation hydrobiologique. En effet, ce n’est pas en quelques mois que peuvent se reconstituer les équilibres faunistiques d’avant traitement, dans des milieux qui ont subit des épandages hebdomadaires d’Abate® puis de Chlorphoxim® depuis plus de huit années. Même si les effets de ces deux produits peuvent être considérés comme non catastrophiques, il est certain que la pression insecticide qu’ils ont exercé sur les écosystèmes constitue un facteur défavorable à un rapide retour à la situation d’origine.

78 — L’ensemble des résultats obtenus concernant la toxicité du *Bacillus thuringiensis israelensis* sérotype H-14 est cependant suffisamment positif pour que cet agent biologique soit considéré comme un insecticide utilisable en campagne permanente de lutte contre *Simulium damnosum,* sans que des risques soient à craindre, sur le plan écologique, pour la faune associée à ce complexe d’espèces cibles vectrices de l’onchocercose humaine. Malgré cela, il est nécessaire que soit maintenue une surveillance attentive des milieux traités afin de prévenir d’éventuels effets à long terme.

79 — Les quelques essais réalisés en eau stagnante doivent être considérés comme très favorables dans la mesure où les doses utilisées étaient largement supérieures à celles habituellement mises en œuvre en opérations larvicides contre les moustiques, aussi bien que lors de traitements agricoles sur rizières, contre les insectes borers du riz.

80 Des essais répétés et de plus grande envergure sont naturellement nécessaires pour confirmer ce faible impact sur les hydrosystèmes tropicaux stagnants, mais ces premiers résultats sont encourageants.

81 *Manuscrit accepté par le Comité de Rédaction le 26 février 1985 et reçu au Service des Éditions le 28 février 1985*

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NOTES

1. OCP = Onchocerciasis control programme.
2. Ce travail a fait l'objet d'un accord contractuel entre l'Organisation Mondiale de la Santé et l'ORSTOM.
3. La position des sites numérotés est reportée sur la figure 1.
4. Le taux d'augmentation maximale de dérive est le rapport entre la valeur de son intensité mesurée immédiatement avant passage d'un insecticide et sa valeur maximale atteinte après son passage.

RÉSUMÉS

Le Bacillus thuringiensis *var.* israelensis *est un insecticide entomopathogène qui agit par ingestion et provoque une lyse des tissus épithéliaux de l'intestin ainsi qu'une paralysie des pièces buccales. Le sérotype H-14* (de BARJAC), *présente une très forte spécificité d'action vis-à-vis des larves de moustiques ainsi que des simulies.*
Utilisé de manière expérimentale en Côte d'Ivoire dans le cadre d'essais de contrôle des populations larvaires de Simulium damnosum *s.l., puis en campagne de lutte contre ces mêmes Diptères, il s'est avéré peu toxique pour la faune aquatique non-cible.*
Son épandage dans un milieu vierge entraîne une augmentation de l'intensité de dérive des invertébrés présents qui ne dépasse pas 4 à 5 fois sa valeur normale (contre 50 à 200 fois pour des insecticides organophosphorés comme le téméphos ou le chlorphoxim). L'action traumatisante induite par les traitements semble par contre plus longue qu'avec d'autres produits mais est sans conséquences pour le maintien des équilibres.
Une action significative sur les Trichoptères Hydropsychidae, qui s'était traduite par une baisse sensible de leur densité après neuf semaines d'un traitement expérimental de saison sèche avec une formulation de B.t.i. *H-14, n'a pas été retrouvée à la suite de traitements de campagne de plusieurs mois effectués sur de nombreuses rivières de Côte d'Ivoire, avec une autre formulation.*
En milieu stagnant, des concentrations très supérieures aux doses actives contre les larves de moustiques ont été lestées et se sont révélées très peu toxiques pour la faune non-cible.
D'une manière générale, l'utilisation de B.t.i. *H-14 comme moyen de lutte antivectorielle, avec application en milieu aquatique, ne semble pas devoir entraîner de risques écologiques, tout au moins à court et moyen terme.*

Bacillus thuringiensis *var.* israelensis *is a spore forming bacterium with a pathogenic action caused by the production of a toxic protein. The ingestion by insects of the cristal produced by the spores lead to a paralysis of the mouthparts and a rapid destruction of the gut epithelium. The H-14 serotype isolated by* de BARJAC *(1978) present a strong larvicide specificity against mosquitoes and also Simuliidae.*
Experimental use of the primary powder or formulations have been done in several rivers of the Ivory Coast,

in order to estimate their toxicity against aquatic non-target organisms in the case of Simulium *control applications.*
Some medium term experiments have also been studied as well as a dry season campaign of weekly treatments, covering the main rivers of that country.
Applications of a formulation of B.t.i. *H-14 on a virgin biotope produces an increase of the invertebrate drift intensity which rise about 4 to 5 times its normal value. Such an increase as to be considered as very low compared to the value which occures after organophosphorus insecticides treatments (respectively 50 to 200 times with temephos and chlophoxim for example...). However, the traumatisation induced on invertebrates by action of* B.t.i. *seems to be longer than in the case of application of other compounds, but is without any consequences lo the aquatic population balance.*
A significant decrease of density was found for Hydropsychidae after a 9 weeks experimental treatment of a small river with a B.t.i. *formulation. The same phenomenon was not detected after the campaign of treatments with an other formulation, covering most of the Ivorian rivers.*
A B.t.i. *H-14 Sandoz formulation experimented in stagnant biotopes in order to control mosquitoes larvae has been applied without any injury for the non target fauna, even at high dosages never used in a normal campaign.*
It can be concluded from the present results that B.t.i. *H-14 have a nearly total short term innocuity against non target fauna associated lo* Simulium damnosum *s.l., or mosquitoes larvae. There is also no evidence of a deleterious action on invertebrate populations after a regular application at medium term.*

INDEX

Mots-clés : Insecticides, Toxicité, Invertébrés aquatiques, Afrique, Côte d'Ivoire.
Keywords : Insecticides, Toxicity, Aquatic invertebrates, Africa, Ivory Coast.

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Action de l'Abate sur les invertébrés aquatiques cinétique de décrochement à court et moyen terme

Claude Dejoux et Jean-Marc Elouard

INTRODUCTION

1 Parmi un certain nombre d'insecticides organophosphorés, susceptibles d'être employés pour lutter contre les larves de *Simulium damnosum* en Afrique de l'Ouest, l'Abate a été reconnu comme l'un de ceux présentant la moindre toxicité pour la faune non-cible, tout en gardant une grande efficacité vis-à-vis des Simulies (LE BERRE et *al.,* 1971 ; LAUZANNE-DEJOUX, 1973 ; DEJOUX-TROUBAT, 1974 ; DEJOUX-TROUBAT, 1975).

2 Cependant, l'insecticide employé n'ayant pas une action spécifique, il peut détruire une partie de la faune non-cible et par là même entraîner un déséquilibre des écosystèmes lotiques. Si certaines espèces de l'entomofaune aquatique et de l'ichtyofaune sont touchées par effet toxique direct de l'insecticide, d'autres espèces prédatrices risquent de voir leurs densités diminuer par raréfaction de certains maillons de la chaîne trophique.

3 Après maintenant une année et demie d'épandages aériens, aucun effet toxique catastrophique n'a été observé sur la faune des rivières traitées en Afrique de l'Ouest. Toutefois des effets ponctuels et localisés dans le temps peuvent être mis en évidence à la suite de chaque épandage. Le recul n'est pas encore suffisant pour que nous soyons certains que ces effets ne se cumuleront pas de façon telle qu'un déséquilibre du milieu s'ensuivrait. Il existe cependant actuellement un certain traumatisme permanent des invertébrés des rivières traitées qui se traduit par un taux de dérive journalier supérieur en moyenne à celui rencontré dans les cours d'eau non traités.

4 Dans le cadre du Programme régional de lutte contre l'Onchocercose dans le bassin de la Volta, un programme de surveillance du milieu aquatique a été mis en place sous l'égide

de l'O.M.S., afin de prévenir toute action drastique des traitements insecticides sur l'environnement. C'est dans cette optique et avec l'aide d'un financement de l'O.M.S. que nous avons étudié le niveau de toxicité, à court et moyen terme, de l'Abate.

1. MÉTHODES ET TECHNIQUES UTILISÉES

1.1. Récolte de la faune dérivante

5 Elle est réalisée selon une méthode désormais classique (ELOUARD-LÉVÊQUE, 1975), basée sur l'utilisation d'un jeu de 3 filets à mailles fines, placés dans les zones de courant fort. Ces filets travaillent pendant un temps déterminé selon le but recherché, temps que nous spécifierons pour chaque expérimentation effectuée. Après avoir trié les organismes récoltés, un indice de dérive moyen (ID) est calculé, qui représente le nombre moyen théorique d'organismes dérivants chaque seconde dans un mètre cube d'eau de la rivière étudiée.

6 Dans les travaux de surveillance du programme de lutte contre *S. damnosum,* la dérive de jour est toujours récoltée une heure et demie avant le coucher du soleil et les filets travaillent durant 30 minutes.

1.2. Emploi de gouttières

7 Nous ne nous étendrons pas sur cette méthode déjà décrite par ailleurs (DEJOUX, 1975 ; DEJOUX-TROUBAT, 1976). La technique consiste à prélever dans un cours d'eau une certaine quantité de substrat présent et à le transplanter, avec la faune qu'il contient, dans une gouttière semi-immergée, placée dans la même rivière. On fait passer une concentration déterminée d'insecticide pendant un temps donné. Les organismes atteints dérivent dans la gouttière et sont recueillis au moyen d'un filet à mailles fines à l'extrémité avale, selon une chronologie préétablie.

8 Afin de mieux cerner les effets à court terme de l'Abate, nous nous sommes placés dans différentes conditions (épandages mécaniques aériens, manuels sur le terrain, expérimentaux en gouttières) et avons utilisé des concentrations différentes. Par ailleurs, nous avons comparé les effets de différentes formulations d'Abate (Procida et American cyanamid), sur la faune non-cible.

9 Les stations où ont été effectuées les observations ou les expérimentations sont localisées sur la figure 1.

2. ACTION DE L'ABATE 200 CE PROCIDA

2.1. Effets toxiques à court terme d'un épandage par hélicoptère, dans les conditions générales du programme O.M.S.[1] (observations à « Niaka » sur le Bandama

10 L'épandage étudié était le 40^{e} effectué dans cette station depuis le début des traitements hedomadaires exécutés dans le cadre du programme O.C.P.[2] (; la quantité d'Abate déversée (10 litres), devrait permettre d'obtenir une concentration de 0,05 ppm pour une durée de passage d'environ 10 minutes. Le déversement de l'insecticide a eu lieu à 11 h 55.

11 Un cycle naturel de dérive a été établi selon la méthode classique (récoltes périodiques de 3 minutes) durant les 24 heures précédant l'épandage puis pendant les 24 heures suivant l'épandage. Les résultats sont exprimés en indice de dérive et reportés dans le tableau 1.

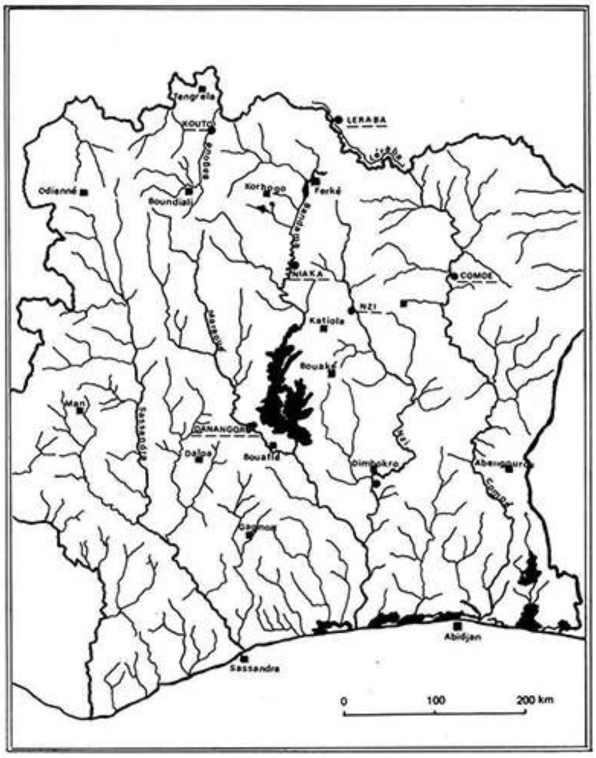

Fig. 1. — Orographie générale de la Côte d'Ivoire. Situation des stations.

12 Ils sont par ailleurs schématisés sur les figures 2 et 3. Il apparaît de façon nette, une augmentation de l'indice de dérive après épandage. Durant la période diurne, de 13 à 18 h., la valeur moyenne calculée de ID est de 1,46 alors que pendant la même période, après épandage, elle atteint 2,30 soit un facteur d'augmentation de 1,58. Durant la période nocturne, les valeurs moyennes des indices de dérive sont de 7,17 avant épandage et de 10,88 après, soit un coefficient d'accroissement de 1,5. Enfin, durant la matinée du lendemain de l'épandage, nous avons un coefficient d'accroissement de l'indice de dérive de 1,54, les indices moyens d'avant et après épandage étant de 3,0 et 4,6. L'action de l'insecticide se traduit donc par une augmentation non négligeable et relativement constante de la quantité totale d'organismes dérivants. Ce phénomène est particulièrement net pour les groupes les plus abondants : Éphémères Baetidae et Caenidae, Chironomides (fig. 3).

TABLEAU 1. **Variation des indices de dérive de quelques groupes d'organismes lors d'un épandage dans les conditions du programme, d'Abate 200 Procida à la concentration 0,05 ppm.**

TAXONS	13h	15h	17h	18h	19h	20h	22h	2h	4h	6h30	8h	9h	10h	10h30	11h	11h30	12h	12h30
Baetidae	0.39	0.36	0.71	1.36	2.49	2.04	2.81	1.90	2.16	1.42	1.30	0.65	0.36	0.36	0.41	0.50	0.12	0.71
Caenidae	0.33	0.24	0.77	1.57	1.69	4.65	2.76	2.76	2.52	2.05	1.27	0.53	0.21	0.27	0.68	0.47	[illegible]	0.21
Ephemeridae		0.03		0.09	0.06		0.09			0.04		0.03			0.03		0.06	
Euthyplociidae				0.03			0215			0.09	0.03					0.06	0.03	
Tricorythidae	0.03		0.03		0.09		0.03			0.04			0.06			0.03		0.12
Leptophlebiidae			0.03		0.09		0.06											
Oligoneuriidae		0.03		0.03	0.06		0.03	0.06			0.03						0.03	
Chaoboridae	0.36	0.30	0.36	0.44	0.39	0.12	0.95	0.86	0.83	1.19	0.56	0.30	0.27	0.15	0.39	0.18	0.39	
Simuliidae				0.03	0.12	0.03		0.09		0.06	0.06	0.06	0.03	0.06	0.03		0.33	
Chironomini		0.03	0.06	0.03	0.03	1.75	0.15	0.65	0.03				0.06				0.33	
Orthocladiinae		0.03	0.12	0.30	2.25		1.87	0.18	1.27	0.89	0.12	0.12	0.03	0.03	0.03	0.03	0.03	[illegible]
Tanypodinae			0.06	0.03	0.06		0.06	0.09						0.06				[illegible]
Ceratopogonidae	0.03	0.03	0.03				0.03				0.03	0.03		0.06				
Veliidae							0.03	0.03	0.06									
Agrionidae	0.03											0.03				0.03		
Libellulidae	0.03				0.03	0.03	0.09			[illegible]			0.03			0.03		
Gomphidae	0.				0.03						0.03			0.03		0.03		
Hydracariens	0.12	0.06	0.03	0.21	0.24	0.12	0.15	0.06	0.09	0.39	0.27	0.09	0.03	0.09	0.24	0.06		
Naididae	0.03									0.02			0.03			0.03		
Nématodes			0.03	0.03	0.09													
Sisyridae				0.21	0.18	0.12	0.18	0.03		0.04	0.06					0.03	0.06	0.36
Hydres				0.03		0.03	0.03		0.03	0.02	0.03						0.33	
Hirudinea													0.03					
Poissons	0.03			0.09	0.06				0.09	0.03			0.03					
T1. Hydropsychidae				0.06			0.06	0.12	0.03	0.03	0.03		0.03	0.06				
T11 Ecnominae			0.03	0.06		0.06	0.06	0.06		0.06			0.06			0.03	0.06	
T13 Polycentropodidae								0.03	0.03									
T14 Hydroptilidae					0.03		0.03		0.18		0.03	0.03			0.03		0.03	0.29
T33 Philopotamidae				0.03	0.03													
Biomphalaria								0.03			0.03							
Diptères autres							0.06			0.03								
Oligochètes							0.03	0.03	0.03	0.03								
ΣD	1.36	1.13	1.90	3.79	7.85	5.39	11.65	7.2	6.64	6.62	4.09	1.90	1.24	1.15	1.84	1.51	2.49	1.33

TAXONS	13h	13h30	14h	14h30	15h	15h30	16h	17h	18h	19h	20h	22h	2h	4h	6h	7h	8h	10h
Baetidae	1.39	0.36	0.71	1.71	0.68	1.48	1.24	0.56	0.68	3.17	4.06	2.07	3.82	2.04	1.19	0.71	1.57	1.63
Caenidae	0.59	0.24	0.83	0.24	0.41	0.62	1.27	0.27	0.47	2.73	5.45	2.75	5.98	3.73	1.30	0.89	2.43	1.96
Ephemeridae	0.12					0.12	0.06			0.09	0.12	0.06	0.30	0.03	0.03		0.06	0.24
Euthyplociidae							0.06			0.09	0.24	0.03	0.06	0.12				0.03
Tricorythidae	0.03	0.03					0.03				0.12	0.03	0.03	0.06			0.15	0.03
Leptophlebiidae	0.03			0.03	0.06		0.03	0.03		0.33	0.12	0.12	0.18	0.09	0.06	0.09		0.15
Oligoneuriidae				0.03				0.03		0.03	0.12		0.06	0.03				0.03
Chaoboridae	0.33		0.15	0.15	0.15	0.03	0.03	0.15	0.21	0.28	0.50	0.80	0.74	0.77	0.12	0.12	0.15	0.15
Simuliidae	0.12			0.06	0.03	0.09			0.03	0.12	0.06	0.09	0.27	0.03	0.06		0.30	0.15
Chironomini			0.03	0.03			0.03		0.03	0.66	0.59	0.03	0.09	0.06	0.03			0.06
Orthocladiinae	0.09		0.03	0.06	0.06	0.06	0.18		0.27	1.36	2.22	2.19	1.16	2.31	0.44	0.24	0.50	0.03
Tanypodinae			0.06				0.06		0.06	0.12	0.24	0.12	0.41	0.06	0.03	0.03	0.09	0.06
Ceratopogonidae							0.03			0.03			0.03					0.06
Veliidae										0.03	0.03	0.03		0.06				
Agrionidae			0.03								0.03		0.06	0.03				
Libellulidae		0.03	0.03					0.03	0.03	0.09	0.03	0.06	0.15	0.03	0.03	0.03		0.06
Gomphidae						0.03												
Hydracariens	0.15	0.12	0.24	0.03	0.15	0.15	0.03	0.12	0.12	0.21	0.18	0.12	0.30	0.27	0.18	0.18	0.33	0.15
Naididae										0.06	0.06				0.09			
Nématodes									0.03	0.03								
Sisyridae		0.03		0.03	0.03	0.15	0.15	0.06	0.09	0.62	0.30	0.39	0.74	0.30	0.09	0.12	0.09	0.24
Hydres	0.06			0.03						0.06		0.06	0.03					0.03
Hirudinea																		
Poissons	0.03	0.03				0.03	0.03						0.06	0.06	0.03	0.06	0.03	
T1. Hydropsychidae	0.43	0.03		0.12	0.03	0.06	0.33		0.03	0.15	0.12	0.06	0.30	0.09	0.03	0.03		0.03
T11 Ecnominae	0.03			0.03				0.03	0.03	0.09	0.21	0.03	0.71	0.21	0.09	0.12	0.15	0.15
T13 Polycentropodidae											0.03							
T14 Hydroptilidae			0.06				0.12	0.03		0.03	0.09		0.12	0.12	0.06	0.03	0.03	0.09
T33 Philopotamidae			0.06											0.09				
Biomphalaria																		
Diptères autres	0.03		0.03			0.03						0.03	0.12			0.06	0.03	0.09
Oligochètes	0.03	0.06	0.03				0.03				0.09	0.03		0.03			0.09	0.03
ΣD	3.11	0.92	2.34	1.66	1.84	3.17	3.55	1.48	2.04	11.61	15.14	9.18	[illegible]	10.52	3.88	2.78	6.04	5.72

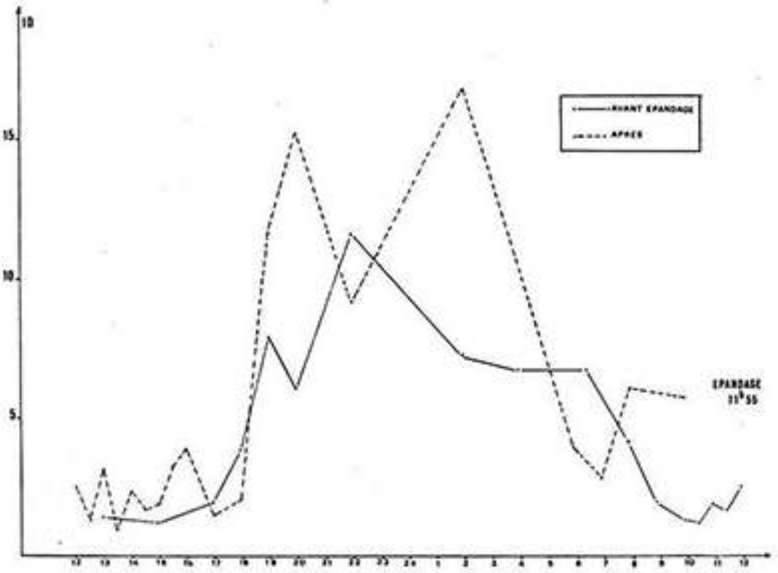

Fig. 2. — Toxicité de l'Abate 200 CE standard Procida. Cinétique de décrochement obtenue à « Niaka » pour l'ensemble des groupes, à la concentration 0,05 ppm.

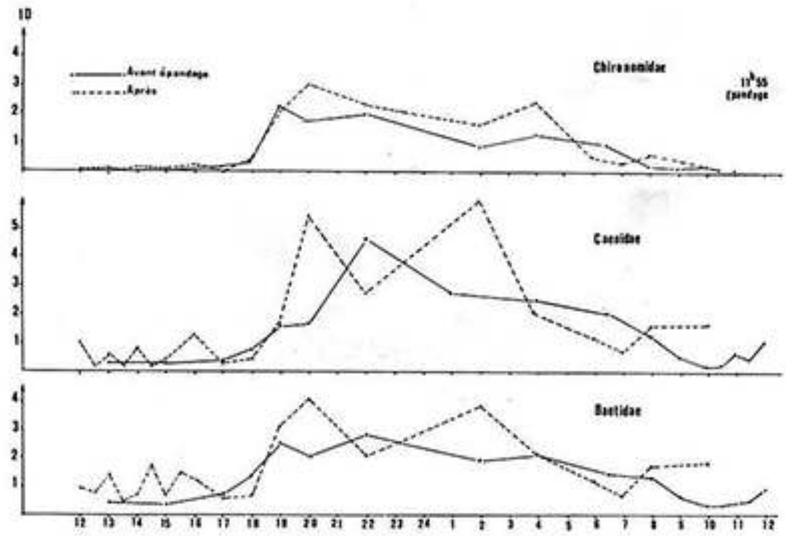

Fig. 3. — Toxicité de l'Abate 200 CE standard Procida. Cinétique de décrochement obtenue à « Niaka » pour les 3 principaux groupes taxonomiques.

13 Bien que l'augmentation du décrochement des organismes par action du traitement soit nette, nous verrons par la suite qu'elle est moins importante que celle observée après épandage d'insecticide sur un gîte d'une rivière non traitée régulièrement.

14 Tout se passe comme si la faune présente dans les rivières régulièrement traitées avait subi une certaine sélection ou bien présentait une tolérance plus grande au toxique que celle des rivières non traitées.

2.2. Effets toxiques à court terme d'un épandage expérimental fait au vide vite, à la concentration 0,1 ppm

15 L'épandage a été réalisé sur la Léraba après une période de deux mois d'arrêt des traitements réguliers à l'Abate. La population simulidienne était alors telle que tous les supports immergés étaient littéralement couverts de larves et de nymphes. Nous n'avons pris en considération, dans cette étude, que les taxons numériquement abondants dans la dérive naturelle, à savoir les Trichoptères, les Simulies, les Éphémères et les Chironomides. Les résultats sont consignés dans le tableau 2 et schématisés sur la figure 4.

TABLEAU 2. **Variation des indices de dérive de quelques groupes d'insectes aquatiques lors d'un épandage expérimental d'Abate 200 Procida à la concentration 0,1 ppm.**

Avant épandage

Temps	15 h	18 h	20 h	22 h 30	1 h	4 h	6 h	9 h
Éphémères	0,04	0,11	0,77	0,4	0,44	0,24	0,13	0,15
Chironomides N.	0,28	0,86	1,01	—	—	—	0,1	—
Chironomides L.	0,31	0,31	1,44	0,97	0,47	0,26	0,06	0,06
Trichoptères	0,02	—	0,33	0,22	0,09	0,08	—	0,04
Simulies	0,86	0,53	2,77	3,84	4,08	2,66	1,66	0,77

Après épandage

Temps	9 h 15	9 h 45	11 h	11 h 30	12 h	15 h	18 h	20 h	20 h 30	24 h	2 h
Éphémères	0,44	5,48	7,35	17,24	9,11	1,68	1,31	0,48	2,02	0,06	0,42
Chironomides N.	—	0,44	1,75	1,44	1,02	0,24	0,72	0,64	0,68	0,04	—
Chironomides L.	0,28	2,53	9,62	19,37	9,8	1,37	2,11	0,28	2,8	0,06	0,28
Trichoptères	—	0,04	2,42	4,66	6,26	2,31	3,25	0,53	3,51	0,4	0,37
Simulies	1,24	10,02	23,11	22,73	18,66	6,24	9,15	0,17	4,35	0,28	0,04

16 Une augmentation spectaculaire de l'indice de dérive a lieu une demi-heure après l'épandage de l'insecticide qui eut lieu à 9 heures. L'indice total de dérive atteint alors la valeur de 44,25 à 11 heures alors qu'il n'était que de 6,32 à sa valeur maximum durant la nuit précédant l'épandage et de 1,02 juste avant l'épandage, soit respectivement 7 et 43 fois plus ! L'acrophase de dérive se situe entre 1 h 30 et deux heures après l'épandage pour les Simulies, les Chironomides et les Éphémères, les Trichoptères présentant un décrochement plus tardif avec deux maximums. La valeur de l'indice de dérive redescend ensuite progressivement à un taux voisin de la normale.

17 Il est à noter que le taux de dérive nocturne est plus important lors de la nuit suivant l'épandage que la nuit le précédant. Ceci est dû au fait que de nombreux organismes se trouvent affaiblis par l'insecticide et présentent dès lors une résistance moins grande aux courants affrontés lors de leurs déplacements trophiques nocturnes.

2.3. Expérimentation en gouttière de la toxicité à court terme de l'Abate

18 Afin de pouvoir quantifier de manière plus précise la toxicité de l'Abate à court terme, nous avons réalisé une série d'expériences *in situ* en utilisant le système des gouttières.

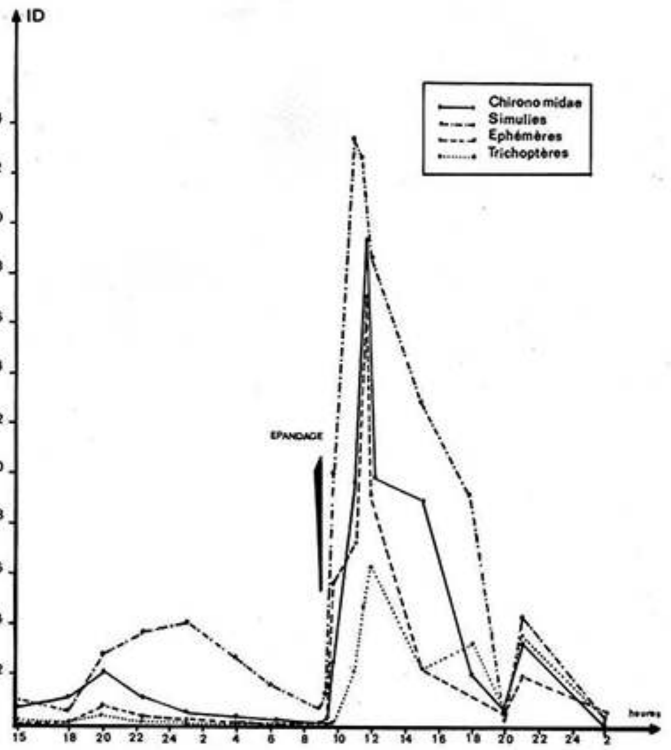

Fig. 4. — Toxicité de l'Abate 200 CE Procida. Cinétique de décrochement obtenue à Niaka pour 4 groupes taxonomiques importants, après action de la concentration 0,1 ppm.

19 Trois concentrations différentes ont été testées : 0,025 ppm, 0,05 ppm et 0,1 ppm avec un temps de passage de l'insecticide de 10 minutes, les deux premières dans la Maraoué, rivière jamais traitée, la troisième dans le Bandama, au radier de Niakaramandougou, rivière subissant un traitement hebdomadaire régulier d'Abate depuis près d'un an.

20 Dans chaque cas, l'appareil recevant l'insecticide était doublé d'un appareil témoin indemne de tout traitement, qui permettait une estimation précise des indices de dérive naturelle.

a) EXPÉRIMENTATION EN RIVIÈRE NON TRAITÉE (MARAOUÉ), À LA CONCENTRATION 0,025 PPM DURANT 10 MINUTES

21 Dans la gouttière témoin, au cours des 24 heures d'observation, les indices de dérive ont varié entre les valeurs extrêmes 0,24 et 1,60 avec une moyenne de 0,82. Le total des organismes ayant dérivé pendant cette même période a été de 270, pour une faune total expérimentée de 8 316 organismes, ce qui représente une dérive naturelle de 3,25 % de la faune présente.

22 Dans le tableau 3 sont consignés les résultats obtenus dans la gouttière ayant reçu l'insecticide. Il apparaît immédiatement que tous les groupes ne sont pas touchés de la

même manière et que certains d'entre eux sont particulièrement sensibles. Ce sont, entre autres, les Éphémères Baetidae, les Trichoptères Hydropsychidae du genre *Macronema,* les Odonates Agrionidae et les Chironomides de la tribu des Chironomini.

23 Les indices de dérive ont varié durant 24 heures entre les valeurs extrêmes 2,6 et 233,9 avec une valeur moyenne de 21,64 soit 26 fois supérieure à celle du témoin.

TABLEAU 3. **Variation des indices de dérive des organismes aquatiques lors d'un épandage en gouttière d'Abate 200 Procida aux concentrations 0,025 ppm et 100 ppm**

Taxons	Faune totale dérivée		Faune résiduelle non tuée		Faune totale expérimentée	% tué	
	0,025 ppm	100 ppm	avant 100 ppm	après 100 ppm		0,025 ppm	100 ppm
Baetidae	1.878	1.264	1.450	186	3.328	*56,4*	*87,3*
Caenidae	34	13	242	229	276	12,3	5,4
Leptophlebiidae	10	0	109	109	119	8,4	0
Ephemeridae	3	0	0	0	3	100	0
Heptageniidae	0	0	1	1	1	0	0
Tricorythidae	0	3	6	3	6	0	100
Cheumatopsyche sp.	419	1.474	3.240	1.766	3.659	11,5	*45,5*
Macronema sp.	203	97	183	86	386	*52,6*	*53,0*
Orthotrichia sp.	84	31	214	183	298	28,2	14,5
Sericostomatidae T 19	3	0	3	3	6	50,0	0
Sericostomatidae T 22	3	0	28	28	31	9,7	0
Philopotamidae	0	21	21	0	21	0	100,0
Chironomini	37	0	41	41	78	*47,4*	0
Tanytarsini	13	0	28	28	41	31,7	0
Tanypodinae	16	77	101	24	117	13,7	*76,2*
Orthocladiinae	24	335	678	343	702	3,4	*49,4*
Rhagionidae	2	8	97	89	99	2,0	8,2
Ceratopogonidae	1	0	0	0	1	100	0
Simuliidae	0	127	128	1	128	0	*99,2*
S. damnosum	22	0	0	0	22	100	0
Plécoptères	0	1	7	6	7		104,3
Agrionidae	44	3	3	0	47	*93,6*	100
Libellulidae	67	114	133	19	200	33,5	*85,7*
Pyralidae	6	4	25	21	31	19,4	16,0
Elmidae	20	10	922	912	942	2,1	1,1
Sysiridae	1	0	0	0	1	100	0
Hydracariens	0	3	6	3	6	0	50
Batraciens	4	2	2	0	6	66,7	100
Ancylidae	0	1	1	0	1	0	100
Cleopatra sp.	0	0	3	3	3	0	0
Totaux	2.894	3.528	7.672	4.084	10.566	27,4	46,8

24 La courbe représentant l'évolution des indices de dérive sur 24 heures est de type classique (fig. 5), présentant un maximum extrêmement marqué dans l'heure suivant l'épandage puis une diminution régulière qui aboutit au bout de 24 heures A une situation peu différente de la normale.

TABLEAU 4. Variation des indices de dérive des organismes aquatiques lors d'un épandage en gouttière d'Abate 200 Procida à la concentration 0,05 ppm

TAXONS	Faune dérivée 0,05 ppm	Faune résiduelle non dérivée	Faune totale expérimentée	% de mortalité
Baetidae	1.881	1.008	2.889	*65,1*
Caenidae	59	1.022	1.081	5,5
Tricorythidae	2	0	2	100
Leptophlebiidae	10	50	60	16,7
Ephemeridae	3	48	51	5,9
Cheumatopsyche sp.	334	2.341	2.675	12,5
Macronema sp.	177	287	464	*38,1*
Orthotrichia sp.	95	176	271	*35,1*
Dipseudopsis sp.	1	0	1	100
Sericostomatidae T 19	3	0	3	100
Calamoceratidae	1	0	1	100
Philopotamidae	4	13	17	23,5
Sericostomatidae T 22	20	1	21	95,2
Chironomini	110	458	568	19,4
Tanytarsini	16	308	324	4,9
Orthocladiinae	60	568	628	9,6
Tanypodinae	40	24	64	*62,5*
Rhagionidae	2	39	41	4,9
S. damnosum	15	5	20	75,0
Simuliidae	0	62	62	0
Agrionidae	118	12	130	*90,8*
Libellulidae	1	0	1	100
Pyralidae	1	103	104	1,0
Elmidae	16	333	349	4,6
Neoperla spio	2	24	26	7,7
Poissons	2	0	2	100
Hydracariens	2	0	2	100
Ancylidae	1	2	3	33,3
Oligochètes	0	12	12	0
TOTAUX	2.976	6.896	9.872	$\bar{M}$ 30,15

25 La faune totale expérimentée était de 10.566 individus et la quantité ayant dérivé en 24 heures, de 2 894 organismes ; ceci correspond à un décrochement de l'ordre de 27,4 % de la faune testée. Si l'on retranche de cette valeur celle du décrochement naturel observée dans le témoin, nous obtenons un décrochement net par effet de l'insecticide égal à environ 24 % de la faune présente.

b) EXPÉRIMENTATION EN RIVIÈRE NON TRAITÉE (MARAOUÉ), À LA CONCENTRATION 0,05 PPM DURANT 10 MINUTES

26 Les résultats obtenus pour la gouttière témoin sont les suivants :

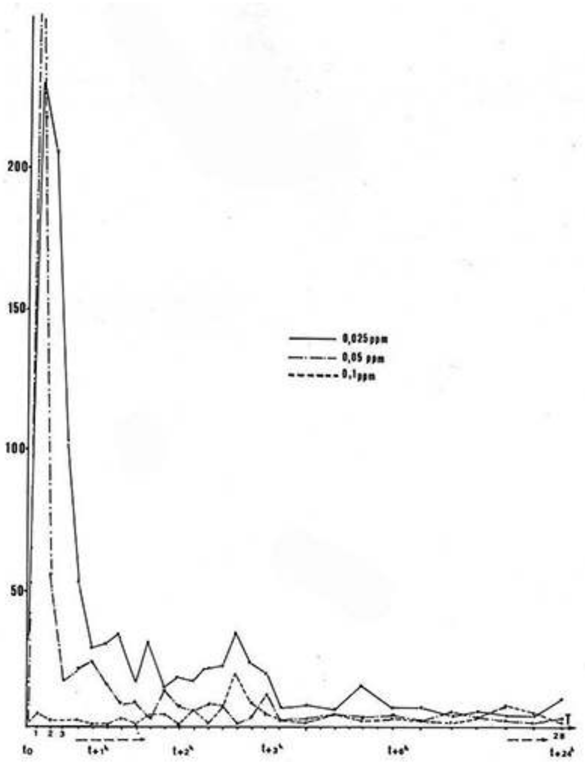

Fig. 5. — Toxicité de l'Abate 200 CE Procida. Expérimentation en gouttière.

Nombre d'individus expérimentés	216
Nombre d'individus décrochés	356
Pourcentage de décrochement	4,33 %
Valeurs extrêmes de l'indice de dérive	0,120-1,54
Valeur moyenne de l'indice de dérive	0,55

27 Dans le tableau 4 sont consignés les résultats obtenus dans la gouttière ayant reçu l'insecticide. Une faune totale de 9 872 individus a été testée. Au cours des 24 heures d'expérience 2 976 organismes dérivèrent, soit 30,15 % de la faune présente.

28 Les groupes qui apparaissent les plus sensibles (pourcentages en italique dans le tableau), peuvent être considérés comme identiques à ceux de l'expérimentation précédente malgré cependant quelques petites différences comme par exemple une moindre sensibilité des Chironomini et au contraire un plus fort décrochement des Tanypodinae.

29 La courbe de l'évolution des indices de dérive sur 24 heures est tracée sur la figure 5. Son aspect général est le même que celui de la courbe correspondant à la concentration 0,025 avec toutefois un maximum beaucoup plus marqué dans la première heure suivant l'épandage et au contraire un niveau inférieur après une heure et jusqu'à la fin de l'expérience.

30 Les valeurs extrêmes de variation de l'indice de dérive durant 24 heures ont été de 1,6 et 312 avec une moyenne de 20,5.

31 A nouveau, si l'on tient compte du pourcentage de dérive naturelle estimé grâce au témoin (4,33 % de la faune en place), nous obtenons un décrochement net par effet du toxique, de l'ordre de 26 %, soit seulement légèrement supérieur à celui obtenu par action de la concentration 0,025 ppm.

c) EXPÉRIMENTATION EN RIVIÈRE TRAITÉE (BANDAMA), À LA CONCENTRATION 0,1 PPM DURANT 10 MINUTES

32 Pour des raisons pratiques, cette expérimentation n'a pas été réalisée sur la Maraoué mais sur le Bandama, rivière déjà traitée à l'Abate depuis un an.

33 L'étude de la dérive naturelle dans un témoin nous a permis d'obtenir les éléments suivants :

Nombre d'individus testés	5 033
Nombre d'individus décrochés	230
Pourcentage de décrochement	4,57
Valeurs extrêmes de l'indice de dérive	0,35 -2,48
Valeur moyenne de l'indice de dérive	0,80

34 Les résultats obtenus dans la gouttière ayant reçu l'insecticide sont consignés dans le tableau 5 et la courbe d'évolution des indices de dérive a été tracée sur la figure 5.

35 Sur un nombre total Lesté de 4 875 individus, seulement 538 décrochèrent au cours des 24 heures, soit 11 %. Comme dans la Maraoué, les Trichoptères *Macronema* apparaissent comme sensibles, de même que les Chironomides Chironomini. Par contre, les Hydracariens semblent ici fortement touchés, avec un décrochement de près de 53 % et les Éphéméroptères Caenidae présentent une grande sensibilité. Les Tricorythidae, très abondants dans notre expérience, sont peu sensibles au traitement ; leur décrochement n'atteint pas 5 %.

36 Les valeurs extrêmes de l'indice de dérive sur 24 heures sont comprises entre 0 et 9,5 avec une moyenne de 1,74, soit à peine plus du double de la valeur obtenue dans le témoin, ce qui correspond à un pourcentage de décrochement dû à l'insecticide de l'ordre de 6,5 % de la faune expérimentée.

37 Sans préjuger du niveau de décrochement que l'on aurait obtenu sur la Maraoué avec la concentration 0,1 ppm, on peut penser qu'il aurait été supérieur à ceux obtenus avec les concentrations plus faibles.

TABLEAU 5. **Variation des indices des invertébrés aquatiques lors d'un épandage en gouttière de l'Abate 200 Procida aux concentrations 0,1 ppm et 100 ppm.**

TAXONS	Faune totale dérivée		Faune résiduelle non tuée		Faune totale expérimentée	% tué	
	0,1 ppm	100 ppm	avant 100 ppm	après 100 ppm		0,1 ppm	100 ppm
Tricorythidae..	97	20	2.028	2.008	2.125	4,6	1,0
Caenidae......	47	2	66	64	113	*46,6*	3,0
Baetidae......	60	136	727	591	787	7,6	18,7
Oligoneuriidae.	2	0	2	2	4	50,0	0
Ephemeridae..	0	0	1	1	1	0	0
Heptageniidae.	0	0	2	2	2	0	0
Cheumatopsyche sp..........	124	183	448	265	572	21,7	40,8
Macronema sp.	11	6	16	10	27	*40,7*	37,5
T14 *Orthotrichia* sp..........	7	0	3	3	10	70,0	0
Philopotamidae.	34	124	195	71	229	14,8	63,6
Chironomini...	17	0	2	2	19	*89,5*	0
Orthocladiinae.	112	17	470	453	585	19,2	3,6
Tanypodinae..	1	1	16	15	17	5,9	6,2
Tanytarsini....	0	2	303	301	303	0	0,7
Rhagionidae...	2	1	34	33	36	5,6	2,9
Plécoptères....	0	0	1	1	1	0	0
Hydracariens..	18	0	16	16	34	*52,9*	0
Elmidae.......	3	0	2	2	5	60,0	0
Nématodes....	3	1	4	3	7	42,9	25,0
Planipenne....	0	0	1	1	1	0	0
TOTAL......	538	493	4.337	3.844	4.875	11,0	11,4

38 En fait, dans le Bandama, nous avons obtenu un décrochement nettement inférieur.

39 Il apparaît donc qu'*un traitement isolé portant sur une faune ne subissant ordinairement aucun effet toxique, entraîne un décrochement important, même à une faible concentration. Par contre, si ce traitement intervient sur une faune déjà régulièrement soumise aux effets de l'Abate, la mortalité induite par un traitement isolé est plus faible.* Ceci explique que, malgré un traitement prolongé à l'Abate, nous ayons encore dans les rivières traitées une faune relativement abondante.

d) ACTION DE TRÈS FORTES CONCENTRATIONS

40 Il est bien connu que dans la zone d'épandage et pendant un court laps de temps, les concentrations d'Abate présentes dans le milieu peuvent être très élevées.

41 Dans deux des expériences précédentes, après 24 heures d'observation, nous avons fait agir une concentration de 100 ppm pendant une durée de 5 secondes environ. Nous avons ensuite étudié le décrochement sur une durée de 3 heures. Les résultats sont consignés dans les tableaux 3 et 5.

42 Dans le cas de la Maraoué, non traitée, nous avons obtenu au bout de 3 heures un décrochement de 46,8 % de la faune testée. Dans le Bandama déjà traité, le décrochement pendant le même temps n'a été que de 11,4 %. Ces résultats confirment ce que nous disions précédemment, à savoir la relativement forte toxicité de l'Abate en traitement isolé et au contraire sa plus faible toxicité instantanée dans le cas de traitements répétés, ce qui n'est toutefois pas incompatible avec l'obtention à moyen terme d'un effet cumulé atteignant le niveau de celui d'un traitement isolé.

2.4. Toxicité de l'Abate à moyen terme

a) ÉTUDE DES EFFETS DE DEUX MOIS DE TRAITEMENT

43 Durant 2 mois, un gîte à *S. damnosum* du N'zi[3] (a été traité à l'Abate 200, dans une zone encore indemne de tout traitement, à raison d'un épandage chaque semaine. Trois points étaient étudiés et échantillonnés à l'aide de filets à dérive. Le premier point (A), non traité était situé en zone de rapides, environ 50 mètres en amont du point d'épandage ; le second (B) et le troisième (C) étaient en aval du point d'épandage, respectivement à environ 100 m et 7 km.

44 Les résultats obtenus ont été consignés dans le tableau 6. Les données du 2/XII/76 représentent la situation aux trois points, la veille du premier épandage. Les échantillons ont par la suite été récoltés simultanément en A et B, 1 heure après épandage. La récolte de la dérive en C avait lieu 24 heures après épandage, ceci afin que la vague d'insecticide ait eu le temps de parcourir les 7 kilomètres. Une concentration de 0,05 ppm a été employée durant la première moitié de l'étude correspondant à des conditions hydrologiques de saison des pluies et 0,1 ppm pendant la seconde, correspondant à des conditions hydrologiques de saison sèche.

Nous pouvons considérer la situation de départ comme relativement homogène sur les 3 points avec une valeur de ID comprise entre 0,4 et 0,9. Au point A non traité, les fluctuations n'ont été durant les deux mois que de faible amplitude avec une valeur moyenne de ID de 0,14. Par contre, au point B, immédiatement après le point d'épandage, la valeur moyenne de ID est de 32,29 soit près de 80 fois celle trouvée en A et 40 fois celle de départ.

TABLEAU 6. **Variation des indices de dérive en 3 points du N'zi durant 2 mois d'épandages d'Abate 200 CE.**

Avant épandage	DATES	A	B	C
	2/XII/76	0,41	0,87	0,62
Pendant épandage	3-4/XII/75	0,18	3,35	0,79
	8-9/XII/75	0,27	0,74	1,76
	16-17/XII/75	0,32	38,47	4,78
	21-22/XII/75	0,27	0,46	2,56
	29-30/XII/75	0,87	29,96	3,70
	6-7/I/76	0,65	21,04	3,92
	13-14/I/76	0,18	86,13	0,72
	20-21/I/76	0,08	111,68	3,82
	27-28/I/76	0,12	0,20	3,56
	3-4/II/76	1,16	30,34	0,41
	Moyenne	0,41	32,29	2,60

Au point C qui ne reçoit plus qu'une faible partie de l'insecticide, la moyenne de ID est de 2,60 soit 6 fois la valeur trouvée en A et seulement 4 fois supérieure à la valeur trouvée avant traitement.

45 Il apparaît donc nettement une fois de plus que le passage de l'Abate provoque un décrochement important de la faune aquatique dans un délai très bref. Cet effet s'atténue avec la distance, en partie en raison de l'importante dilution du produit épandu.

b) ÉTUDE À MOYEN TERME DE LA DÉRIVE DE JOUR DANS 6 STATIONS DE CÔTE D'IVOIRE

46 Parmi les paramètres suivis dans le cadre du programme de surveillance de l'environnement lié au Programme de lutte contre l'Onchocercose, l'étude de la dérive de jour permet de mettre en évidence l'intensification du décrochement des organismes aquatiques par l'effet des traitements.

47 Quelle que soit l'intensité de l'activité biologique des rivières de la zone du programme, on peut considérer que la valeur de l'indice de dérive de jour, mesuré 1 h 1/2 avant le coucher du soleil, au niveau des radiers, varie entre 0,20 et 4. Tout dépassement de ces limites est presque toujours dû à l'action d'un facteur artificiel ou tout au moins inhabituel.

Dans le tableau 7 nous avons consigné les valeurs de ID calculées depuis 1975 pour 6 stations. Sur les 4 stations traitées, il apparaît nettement des groupes de valeurs excessivement fortes, qui dépassent le seuil de ID = 4, parfois dans des proportions considérables (Niaka ID = 307,2 en mars 1976 !).

TABLEAU 7. **Variation des valeurs de ID pour 6 stations de Côte d'Ivoire.**

	1975								1976										$\overline{ID}$	σ^2
	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O		
*Bagoué	—	—	0,88	—	1,30	—	0,37	—	1,31	—	1,47	—	1,08		3,19	0,60	0,81	1,28	1,23	0,59
*Maraoué	0,70	0,72	1,12	1,91	0,82	1,27	2,51	0,38	1,42	0,94	3,32	2,71	0,32	2,10	2,67	1,20	3,28	0,56	1,55	0,98
Niaka	3,52	1,02	0,18	2,98	1,04	11,25	1,47	0,53	1,79	1,88	*307,2*	0,55	0,75	0,23	1,77	0,63	0,30	3,03	18,89	5183,41
Leraba	1,40	0,20	*11,49*	1,56	1,10	1,17	1,02	0,5	*3,38*	1,44	0,09	0,57	1,70	0,15	0,46	0,92	0,98	1,14	1,63	6,63
N'zi	0,25	—	*4,03*	2,61	1,96	0,74	*10,95*	0,61	0,84	—	—	—	1,80	0,88	*5,83*	1,32	0,69	3,92	2,60	8,40
Comoe	5,79	—	3,10	1,20	0,44	0,41	1,74	2,0	0,24	0,13	0,57	*46,3*	*5,83*	*6,19*	0,60	1,70	3,04	1,74	4,77	118,61

* Ces deux stations sont non traitées.

D'une manière générale dans toutes les stations traitées, l'indice de dérive de jour moyen est supérieur à celui calculé pour les deux stations non traitées. L'examen des variances correspondantes montre que seulement dans ces deux dernières stations, la variance de ID est inférieure à sa moyenne ce qui traduit une distribution régulière et homogène des valeurs de ID. Par contre, dans les stations traitées, la variance est supérieure à la moyenne et même très supérieure dans le cas de Niaka.

La station de la Léraba ne présente pas un indice de dérive moyen très élevé. Cependant cette station est dans l'ensemble pauvre en faune et des valeurs très basses de ID s'y rencontrent (0,09-0,15-0,20...). Il est évident que dans une série aussi faible, une valeur de ID égale à 11,49 (juillet 75) traduit un fort décrochement de la faune en place.

Il faut enfin remarquer que pour des raisons logistiques, les échantillonnages d'une fois sur l'autre ne sont pas toujours faits avec un intervalle de temps identique après l'épandage. Il a été montré précédemment que la valeur de ID est d'autant plus grande que cet intervalle de temps est court. En conséquence, la différence constatée entre les valeurs moyennes de ID pour les stations traitées et pour les stations non traitées, serait encore plus grande, si l'échantillonnage pour la surveillance du milieu pouvait être fait systématiquement après l'épandage et le même jour que celui-ci.

3. ACTION A COURT TERME DE L’ABATE 200 CE AMERICAN CYANAMID

48 Nous avons déjà montré que des différences de toxicité peuvent exister selon le type de formulation de l’Abate qui est utilisée (LAUZANNE-DEJOUX, 1973 ; DEJOUX-TROUBAT, 1975) aussi avons-nous testé les effets de l’Abate 200 de l’American Cyanamid Company sur la faune non-cible afin de rechercher s’ils diffèrent de ceux de l’Abate 200 de Procida.

TABLEAU 8. **Étude de la toxicité de l’Abate 200 CE American Cyanamid. Chronologie des récoltes et résultats généraux.**

DATE et HEURE	Numéro du prélèvement	Nombre d’organismes récoltés	Indice de dérive correspondant
11/6/1975			
8 h 20	1	60	3,56
8 h 50	2	69	4,09
9 h 20	3	54	3,20
9 h 50	4	25	1,48
10 h	Épandage	—	—
10 h 10	5	22	2,17
10 h 20	6	558	55,11
10 h 30	7	766	75,65
10 h 40	8	493	48,69
10 h 50	9	305	30,02
11 h 00	10	70	6,91
11 h 10	11	38	3,75
11 h 20	12	308	30,42
11 h 30	13	118	11,65
11 h 40	14	158	15,60
11 h 50	15	124	12,25
12 h 00	16	101	9,98
13 h 00	17	106	10,47

13 h 30	18	104	10,27
14 h 00	19	114	11,26
14 h 30	20	86	8,49
15 h 00	21	65	6,42
15 h 30	22	65	6,42
16 h 00	23	26	2,57
16 h 30	24	37	3,65
17 h 00	25	8	0,79
17 h 30	26	68	6,72
18 h 00	27	39	3,85
12/12/1975			
7 h 00	28	46	2,73
7 h 30	29	58	3,44
8 h 00	30	38	2,25
8 h 30	31	46	2,73
9 h 00	32	34	2,01

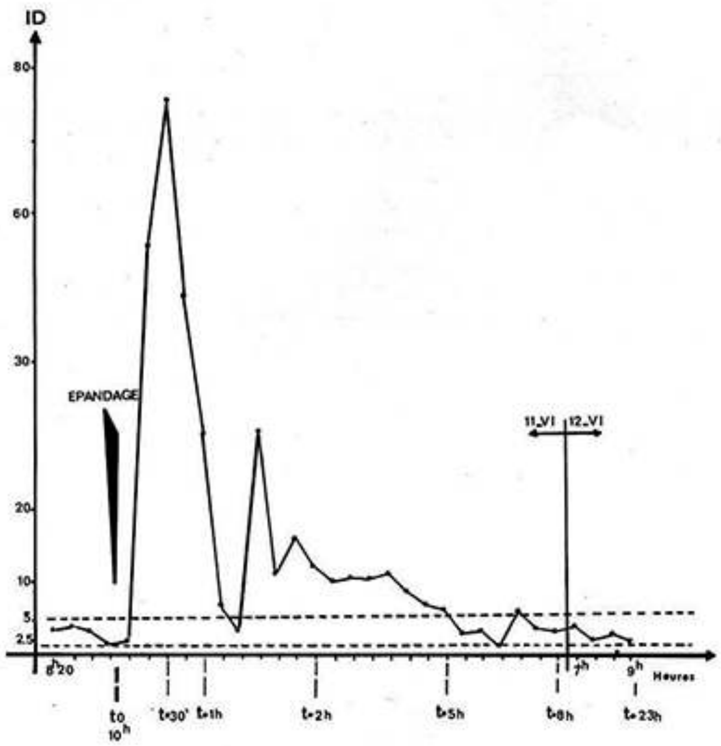

Fig. 6. — Toxicité de l'Abate 200 CE American Cyanamid. Cinétique de décrochement de l'ensemble des organismes.

49 L'épandage étudié a été réalisé au vide-vite sur la Maraoué, rivière non traitée. La concentration employée était de 0,1 ppm durant 10 minutes. Un filet de récolte de dérive était mis en place avec une périodicité définie et à chaque fois pendant une durée de 5 minutes avant épandage et de 3 minutes après épandage. Les résultats sont consignés dans le tableau 8 et schématisés sur les figures 6, 7 et 8.

50 Immédiatement après l'épandage l'indice de dérive s'accroît de façon considérable et devient rapidement (30' après) plus de 20 fois ce qu'il était avant le passage de l'insecticide. Au bout d'une heure, on note une première chute de l'indice de dérive suivit d'un deuxième pic dont l'amplitude n'atteint cependant pas la moitié du précédent. Plusieurs pics se succèdent ensuite, de plus en plus atténués et 5 heures après l'épandage nous retrouvons un indice de dérive « normal » (situé entre les lignes pointillées indiquant les limites des valeurs normales de l'indice de dérive de jour pour cette rivière et cette station).

51 Le lendemain de l'épandage, aucune augmentation notable et significative n'apparaît dans les valeurs calculées de l'indice de dérive, ce qui laisse à penser que les conditions locales sont redevenues identiques à celles existant avant le traitement.

52 Globalement, la valeur moyenne de ID est de 3,08 avant épandage. Elle passe à 16,22 le jour de l'épandage et retombe à 2,63 le lendemain. Nous avons une valeur 5,3 fois plus forte après épandage revenant ensuite à 0,85 fois la valeur initiale, ce qui traduit à la fois le retour à une situation normale mais aussi la perte momentanée en organismes sur les lieux du traitement.

53 Trois éléments faunistiques constituent l'essentiel de la dérive : deux Éphéméroptères Baetidae et un Chironomidae Orthocladiinae. A moindre titre, il faut noter une présence abondante de Caenidae et d'Hydracariens. Selon les groupes considérés, la cinétique de

décrochement peut être légèrement différente. C'est ainsi que les Caenidae ne présentent leur maximum de décrochement que deux heures environ après le passage de l'insecticide alors qu'il suit de seulement quelques minutes pour les Baetidae.

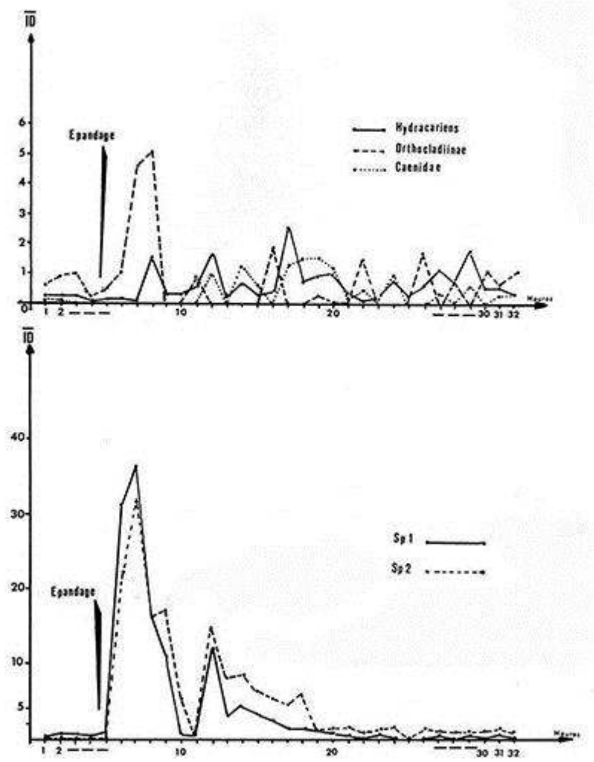

Fig. 7. — Toxicité de l'Abate 200 CE American Cyanamid. Expérimentation sur la Maraoué, cinétique de décrochement de quelques groupes d'invertébrés.
Fig. 8. — Toxicité de l'Abate 200 CE American Cyanamid. Expérimentation sur la Maraoué, cinétique de décrochement de deux espèces de Baetidae.

54 Nous retrouvons donc un schéma d'action en tout point identique à celui obtenu pour l'Abate 200 Procida. En fait, seules les intensités du décrochement peuvent varier d'une formulation à l'autre mais leurs modes d'action demeurent du même type, induisant un effet immédiat souvent très important, qui s'atténue rapidement.

4. CONCLUSION

55 Dans toutes les expériences réalisées apparaît un effet immédiat du traitement, se traduisant par une augmentation très importante et rapide du taux de dérive des organismes en place. Cet effet sera d'autant plus important que le milieu est vierge de tout traitement préalable. L'indice de dérive peut prendre des valeurs dix à cent fois supérieures à la normale, témoignant ainsi d'un fort décrochement des organismes de leur substrat. Les effets aigus d'un traitement s'atténuent toutefois rapidement et sont souvent à peine décelables 24 heures après passage de l'insecticide.

56 Si tous les groupes affectés réagissent globalement de la même manière, la cinétique de décrochement de chacun d'eux peut varier légèrement d'une espèce à l'autre, compte tenu soit d'une plus faible sensibilité au toxique, soit d'une localisation particulière dans les biotopes les exposant plus ou moins aux effets mécaniques d'arrachement par le courant.

57 Dans la majorité des cas, la composition de la faune dans la portion de rivière traitée n'est pas connue, il se peut que les éléments qui dominent dans la dérive après traitement soient aussi ceux qui dominent dans le milieu naturel. En conséquence, la plus grande abondance de ces organismes dans la dérive ne signifie pas nécessairement qu'ils présentent une plus grande sensibilité aux insecticides que d'autres groupes, numériquement moins bien représentés. Seules les expériences réalisées à l'aide des gouttières permettent une estimation précise des sensibilités relatives de chaque constituant des peuplements testés et l'on peut affirmer que les Éphéméroptères Baetidae et Caenidae, les Trichoptères *Macronema* et *Orthotrichia,* les Chironomides chironomini, constituent avec les Simulidae les organismes les plus affectés par les traitements.

Nous avons montré par ailleurs (ELOUARD-LÉVÊQUE, 1975) que la dérive de jour a un caractère traumatique. Ceci implique que l'indice de dérive dans les rivières en équilibre est normalement faible. Au contraire, une augmentation notable de cet indice dans un cours d'eau traduit l'action d'un effet traumatisant certain. Pratiquement, nous avons retrouvé durant plus d'un an d'observation sur 6 stations, des valeurs de ID nettement supérieures dans les rivières traitées que dans les rivières non traitées de Côte d'Ivoire. Ceci traduit l'existence d'une traumatisation permanente de la faune des cours d'eau traités, qui, si elle n'est pas catastrophique, n'en est pas pour autant négligeable.

58 L'effet cumulé à moyen terme, n'apparaît pas comme étant la sommation arithmétique des effets partiels de chaque traitement. Si une telle situation existait, on aboutirait rapidement à un dépeuplement complet des rivières traitées. Dans le cas de traitements répétés, chaque épandage provoque un décrochement des organismes présents les plus sensibles, entraînant ainsi une sélection individuelle et peut-être spécifique de la faune restante.

59 En saison sèche et d'une manière générale, les effets toxiques maximums se font sentir dans les quelques centaines de mètres situés immédiatement après les points d'épandage ; par contre, la faible vélocité du courant, la dilution rapide et la labilité du produit sont telles que ces effets ne sont que d'un niveau très faible quelques kilomètres en aval.

60 La modalité d'action de l'Abate American Cyanamid sur la faune non-cible est enfin très similaire à celle obtenue avec l'Abate 200 de Procida.

61 *Manuscrit reçu le 7 septembre 1977 au Service des publications de l'O.R.S.T.O.M.*

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NOTES

1. Organisation Mondiale de la Santé.
2. Onchocerciasis Control Program.
3. Situation signalée par une étoile sur la figure 1.

RÉSUMÉS

Le contrôle des populations de Simulium damnosum *dans le bassin des Voila est actuellement réalisé à l'aide d'épandages hebdomadaires d'un insecticide organophosphoré : l'Abate 200 CE. Malgré une sélectivité certaine de ce produit vis-à-vis du vecteur de l'Onchocercose, les groupes d'insectes non cibles des milieux aquatiques traités subissent un certain impact du produit utilisé, impact que nous avons cherché à mettre en évidence de manière expérimentale ou à l'aide d'observations suivies et régulières des milieux traités. Quelle que soit la concentration employée, l'épandage d'Abate dans un cours d'eau entraîne un décrochement des organismes en place qui induit une très forte augmentation des valeurs de l'indice de dérive. Cette augmentation suit toujours de très près le passage de la vague insecticide, témoignant d'une réaction extrêmement rapide de la faune d'Invertébrés. L'effet du toxique s'atténue ensuite rapidement et devient négligeable après 24 heures. Tous les groupes présentent une cinétique de décrochement de ce type avec cependant des variations en fonction de leur sensibilité intrinsèque. Parmi les plus sensibles, il faut signaler les Éphémèroptères Baetidae et Caenidae, les Trichoptères du genre* Macronema *et les Chironomides d'une manière générale. Il est très important de noter que les traitements isolés sur un milieu vierge sont le plus souvent très toxiques et peuvent entraîner, selon la concentration employée, une mortalité des Invertébrés de 50 % et plus. Par contre, quand les traitements sont répétés régulièrement, après une première série ayant des effets toxiques importants, les suivants n'induisent à chaque fois qu'un faible décrochement. Ce fait explique que, après maintenant deux années de traitements à l'Abate, la faune d'invertébrés des rivières ouest africaines soit toujours abondante.*

Population control of Simulium damnosum *in the Voila basin is presently done by weekly sprays of Abate 200 CE, an organophosphorus insecticide. In spite of the relative selectivity of Abate against the larvae of blackflies, the non-target organisms living in the aquatic biota are affected by the toxicity of the product.*

By using experimental methods and also after regular observations carried out on treated and untreated rivers, we try to point out the toxicity rate of Abate against the aquatic invertebrate fauna.

At all the concentrations tested in the rivers, Abate treatments induce detachment of invertebrates from their substrates, especially insect larvae, and an increase of the drift rate. This increase always appears very soon after the passage of the insecticide wave, giving evidence of a rapid response of the organisms to the Chemical. After this very sensitive effect, the drift rate decreases rapidly and rises to its normal level 24 hours later.

The general detachment kinetic is similar for all the invertebrate groups but some differences occur in relation to the specificity of the animals concerned. Ephemeroptera: Baetidae and Caenidae, Chironomids in general and Trichoptera of the genus Macronema *are some of the more sensitive taxa.*

It is very important to point out that isolated treatments done on virgin biotas often have a very drastic effect and could involve a mortalily of 50% and more at normal concentrations used for Simulium *control. On the other hand, if treatments are regularly done in a river, after a first series of drastic effects, the following ones each time induce a relative small detachment of organisms. This is certainly the reason why the invertebrate fauna is always present in the treated rivers at a quite satisfactory level, even after two years of traitments.*

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Impact of temephos (*Abate*) on the non-target invertebrate fauna

A — Utilization of correspondence analysis for studying surveillance data collected in the onchocerciasis control programme[1] / A — Utilisation de l'analyse des correspondances pour analyser les données de surveillance récoltées dans le programme de lutte contre l'Onchocercose dans le bassin de la Volta

Impact du téméphos (abate[R]) sur les invertébrés non-cibles

Jean Marc Elouard and Jean Marie Jestin

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1 For the past six years the World Health Organization (WHO) has undertaken the control of human Onchocerciasis in West Africa. Because of logistic and economic considerations, an organophosphorous larvicide, temephos (Abate[R]), has been used in the control programme. Weekly treatments of all potential breeding sites of *Simulium damnosum s.l.* within the control area are required because of the brievity of the larval cycle of these black fly vectors. The insecticide concentrations range from 0.05 to 0.1 ppm which are active against the vector show some effect on the other benthic invertebrate fauna.

2 Such systematic riverine pollution over several years poses a threat to the lotic ecosystem involved. In order to predict and prevent eventual ecological long term catastrophy, ecological surveillance has been conducted during the past five years.

3 From numerous past experiments both in experimental gutters and *in situ* (DEJOUX, 1977a; DEJOUX, 1977b; DEJOUX et ELOUARD, 1977; ELOUARD et FORGE, 1977; DEJOUX, 1978a; DEJOUX, 1978b) we already knew that temephos presented a certain amount of short term toxicity for the non-target insect fauna. Therefore, it was important to know if this non-catastrophic and limited effect would have serious long term repercussions. The mathematical treatment of surveillance data should, therefore, answer the following questions:

- Does temephos have a long term effect on the benthic fauna?
- Was the protocol for ecological surveillance of the rivers adopted after the beginning of the programme, adequate for the evaluation of such an effect? Because the sampling techniques employed were, in fact, chosen *a priori* at the start of the programme, and it was not evident

that these techniques were sensitive enough to demonstrate evidence of a low magnitude change attributable to Abate, especially if these could be masked by strong natural variations due to the effects of seasonal abiotic factors (ELOUARD *et al.*, 1979).

4 The large quantity of data collected both on treated and untreated rivers, during five years of monitoring enable us to answer these two questions. However, up to now, and because of the too many factors involved in the natural ecosystem variations, we cannot prove any long terme effect of the temephos on the non-target fauna with the classical methods of data analysis. For such data, we think we need multifactorial analyses which allow to point out and to grade easily the main factors interferring. Such analysis match very well the problems induced by the monitoring data. Indeed the heading word "multifactorial analysis" covers several methods. Two of them have been tried: the principal component analysis (PCA) tested by the Salford mathematical team and the Factorial correspondence analysis (FCA) tested by the Bouaké hydrological team. Up to now, only this last method has given valuable results as far as temephos is concerned.

5 It is not the purpose of this publication to detail the results obtained through the AFC method, but the aim is to prove, with example, the usefulness and reliability of such a method in the interpretation of the data collected in the African river monitoring programme. We shall expose the results of the temephos impact in a further publication.

1. METHODS

1.1. Choice off sampling station and data to be utilized

6 The principal criterion in the choice of stations was the existence of pre-and post-treatment surveillance data. Consequently the data studied were selected from:

- Danangoro site (DAN) (Maraoué) under surveillance since 1975. The data considered for this study are from 1976 to 1980, say 3 years before and one year after the treatment which began in March 1979.
- Entomokro site (ENT) (Maraoué) under surveillance since 1978 and under treatment since March 1979.
- Semien site (SEM) (Sassandra) under surveillance since late 1977 and regularly treated since 1978.

7 An empirical knowledge of the field situation has permitted the selection of the most appropriate months and sampling techniques. Later, certain more objective criteria, based on sample counts and homogeneity of data, justified our empirical choice of techniques (DEJOUX *et al.*, 1980).

8 Thus, the months of January, February, March, November and December of each year were chosen for analysis. In Ivory Coast, they correspond to the period of decreasing stream level and the beginning of the dry season. At that time, the benthic fauna is at maximum density and species richness. To illustrate the correspondence analysis method, we only consider in this paper the data obtained from Surber samples. Only the 18 taxa presented below occuring in sufficiently high numbers were used in the analysis.

Ephemeroptera		**Diptera**	
Baetidae	(BAE)	*S. damnosum s. l.*	(DAM)
Caenidae	(CAE)	Simuliidae*	(SIM)
Tricorythidae	(TRI)	Chironomini	(CHI)
		Tanytarsini	(TAR)
Trichoptera		Orthocladiinae	(ORT)
Hydropsychidae	(PSY)	Tanypodinae	(POD)
Ecnomidae	(ECN)	Other Diptera	(OTH)
Hydroptilidae	(PTI)		
Leptoceridae	(LEP)	**Neuroptera**	
Philopotamidae	(PHI)	Sisyridae	(SIS)
Polycentropodidae	(POL)		
		Acarina	
Lepidoptera		Hydracarina	(HYD)
Pyralidae	(PYR)		

* Other than *S. damnosum*

1.2. Nature and quality of the data

9 Within the surveillance protocol adopted, the fauna coliected with the different methods was identified to various taxonomic levels depending on the group considered. This level ranges from species (*S. damnosum*) to family (Baetidae) or even sub-class (Acarina).

10 The data used for the present analysis were grouped in a faunistic table representing the relative abundance of the 18 taxa. The physical variables used only served for the identification of the samples (stations, dates of collection and eventual treatment with temephos). The row of the table represents the different observations or sample collections while the columns represent the taxa.

11 The quality of these data is influenced by the level of identification chosen for each taxon and depends upon the sampling techniques. Surber samples, for example, permitted an estimation of the density of invertebrates on the rocky substrates but the precision of this estimation depends on the size of the sample (number of replicates) and on the distribution of the organisms collected (regular, random or aggregated distribution). In lotic environments aggregated distributions predominate and in this case 5 replicate samples, such as we used, only provide low precision.

12 In addition to the direct toxic action of temephos on certain taxonomic groups there may also be an indirect action of temephos caused by modification of the food web or by territorial competition. The pesticide can favor or disadvantage certain groups, thereby, profoundly changing the structure of communities. It appears to us to be more suitable to study the structure of invertebrate communities rather than the change in density of isolated taxa; these studies are based on the variations of the relative abundance. Papers on similar studies have already been presented (VERNAUX et TUFFERY, 1967; CHUTTER, 1971) establishing a classification of water quality by means of synthetic indices.

13 Factor analysis are well adapted to this category of statistical material because they permit the description of multidimensional reality.

14 Under the heading factor analysis there are several techniques presenting a common group theory. Among these we have preferred the correspondence analysis (FCA) which applies well to contingency table (or crossed tables) (BENZECRI & *coll.*, 1973; HILL, 1974). The algorithms and the logical programmes used are those described for the AFC in LEBART *et al.*, 1977.

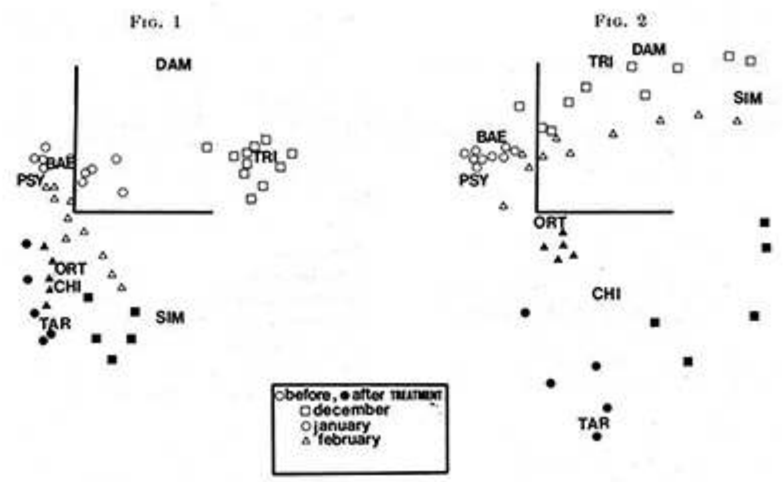

FIG. 1. — Sample collections of Entomokro. Factorial plane 1 x 2
FIG. 2. — Sample collections of Entomokro. The variable Tricorythidae (TRI), and the sample collections or Semien as supplementary element. Factorial plane 1 x 2

2. RESULTS

2.1. Analysis on raw data

15 The group consisted of 185 Surber samples, in which the numbers of the 18 selected taxa have been recorded, constitutes a matrix of 185x18. The first analysis (FCA I) was carried out on these non-transformed data. The projection of the points on the factorial plane 1x2 is represented in fig. 1. In order to assure the best legibility, the sampled points were split according to the stations. The darkened symbols correspond to samples collected during the treatment. The samples of the three stations Danangoro, Entomokro and Semien contribute to the results in the FCA I but only the Entomokro site is represented in the graph.

16 In the FCA I, the first two factors building the first two axes account for 40.3% of the total variance. Let us emphasize, however, that this percentage gives a pessimistic idea of the part of information supplied by the factors. On the first axis, there is a strong opposition between the variables TRI and SIM to PSY. The other variables occur to a lesser extent in the axis. The second axis is positive for variables Baetidae and Hydropsychidae and negative for Tanitarsini.

17 On the other hand, the samples are clearly separated as a function of the treatment. The points corresponding to the periods under treatment appear more often negative on the second axis. However, we must also consider the influence of traditional fishing using botanical poisons which takes place each year at the same time on the Maraoue. The larvicidal effect of fish poisoning is similar to the marginal effect of Abate, and this explains the similar positions of samples collected in February before and after the beginning of temephos application.

18 If the interpretation of this analysis is satisfactory, one can examine the stability of established typology. In fact the investigation of the absolute contributions of variables on the axis show that the variable Tricorythidae (TRI) with an absolute contribution of 62% to the primary axis is an element which threatens the stability of the analysis (

ESCOFFIER & LEROUX, 1976). This variable which is strongly associated with the month of December (cf. fig. 1) is an important seasonality factor. Thus, for axis 1, where the influence of Tricorythidae is a deciding factor, the effect of treatment and of the season interfere with one another.

19 In order to have a very solid base of interpretation it is usual to suppress such elements. They no longer act in the construction of the factorial axis, but can be projected on the new axes. They are then considered as supplementary elements.

20 One can thus expect clarification by introducing the variable Tricorythidae as a supplementary element. Likewise, in this second analysis (FCA II), the samples of the Semien station were introduced as supplementary elements. The variables Simuliidae, Tanytarsini, Hydropsychidae and Baetidae provide a balanced contribution on the first axis (fig. 2). The contrast is clear: one finds the species which are indifferent to the action of Abate negatively represented thereby holding the populations relatively constant, before and after treatment; in contrast the sensitive taxa, Tanytarsini, Simuliidae, are positively represented. Their presence means that they may be favoured or disadvantaged by the treatments. *Simulium damnosum,* Chironomini and Orthocladiinae, react in the same way, although they represent a smaller contribution to this axis.

21 In this second analysis, the first axis therefore expresses a constant factor of stability. The second axis discriminates between the favored taxa (essentially Tanytarsini and Chironomini) and the other sensitive taxa (*S. damnosum* and Simuliidae) which disappear after the action of temephos. We are hence in the presence of a veritable “axis of treatment” with the samples corresponding to the treated period appearing in the lower part of the figure. Again the situation is more confused for the month of February. The effects of traditional fishing and pollution due to Abate are hard to separate.

2.2. Coded data (FCA III)

22 Coding is often practised by data analysts. Though it involves a loss of information, results may be obtained which are very close to those provided with raw data (FRONTIER & IBANEZ, 1974; FRONTIER & VIALE, 1977). The established typology is the same, but the percentages of inertia of factors are weaker. This confirms the pessimistic character of this measurement as already discussed.

23 A step of this nature is well adapted to our problem. In fact we have underlined the lack of confidence which must agree with the absolute values obtained so far as estimations of population levels. It was therefore attempted to substitute computations with a code corresponding to the usually adopted semi-quantitative scale: 0 for absence, 1 for 1-9 individuals, 2 for 10-99, 3 for 100 or more. Thus the transformed table was analysed maintaining all of the individuals and variables (no supplementary elements).

24 Figure 3 where the samples are only referenced to by the absence or the presence of treatment is significant. Axis 1 constitutes an axis of treatment. The almost central position of the point *Simulium* other than *S. damnosum* appears to indicate that the treatment has no effect on this taxon. Sample analysis carried out at a species level, will allow a better understanding of this strange situation. The variables Trichorythidae and Orthocladiinae only appear in the second axis probably connected with the seasonality and the differences between stations.

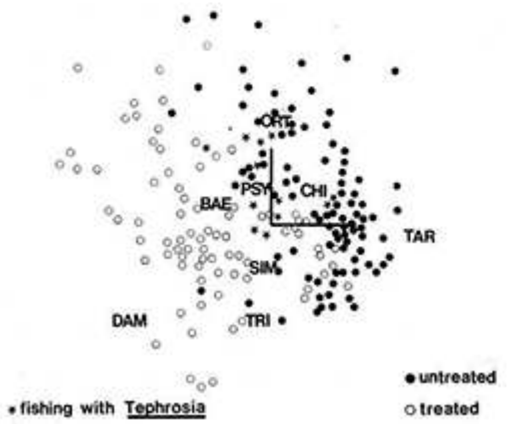

FIG. 3. — Sample collections of Danangoro, Entomokro and Semien. Analysis with coded data. Factorial plane 1 x 2

2.3. Pollution index

25 The discriminant ability of the first axis translating the susceptibility allows the construction of a variable, which is a linear combination of the analysed units, and can be considered as an indicator of Abate pollution.

26 This function has the same discriminant power as the discriminant linear function of Fisher (BENZECRI, 1976). From a description of a sample according to a semi-quantitative scale, one can then reveal, in calculating this synthetic index, the impact of Abate.

27 Such an index based on the results of a factorial analysis was used with samples of Algae by DESCY (1976). The result is very close to the "Saprobic index" but based on objective and precise criteria.

28 The qualities of such an instrument of analysis described by LEBART *et al.* (1977) are: precision, stability, easy utilization and large susceptibility.

29 This linear combination corresponds for one replicate to its projection on axis 1 and the coefficients affected to each variable are the component score on axis 1. If a_j is the coefficient corresponding to the variable j, k_{iy} is the abundance of species j in the sample i and IND (i) is the index for sample i:

30 $IND(i) = \Sigma_j a_j k_{ij} x I/k_i$

31 The term $1/k^1$ corresponds to the weighting by the total count of the sample. Based on the analysed data it is possible to calculate the coefficient of each taxa as following:

BAE =	— 0.3
CAE =	— 0.1
ECN =	— 1.2
PSY =	— 0.1
PTI =	— 0.2
LEP =	— 0.4

DAM =	— 1.1
PHI =	0.2
CHI =	0.2
TAR =	0.8
POD =	0.2
SIS =	1.2
HYD =	0.3

32 Because of the too central position of the five other taxa, their coefficient value are equal to zero and are not taken into account.

33 Of course this index needs further confirmation which will be done by analysis of samples collected at other sites. The result is more reliable when the calculated value differs from zero. This appears clearly in fig. 3 where some points related to an untreated period can have a positive value on this axis. Thus, these samples are not correctly ranked but the value of the index is near zero.

2.4. Comparison with coded data classified to a specific level

34 The third analysis of data gives a stable typology on the factorial plane 1 x 2 (fig. 3) which demonstrates the action of Abate on the structure of the benthic invertebrate populations. A control can be done by the same analysis carried out on the same data identified to a specific level. As an example, we have analysed 111 replicates from Danangoro and Entomokro sites (1978 to 1980). The 21 following taxa have been selected.

EPHEMEROPTERA

Tricorythus sp. 1	(E01)
Pseudocloëon berlrandi	(E21)
Pseudocloëon sp. 1	(E29)
Centroptilum sp. 1	(E31)
Caenomedea sp	(E54)
Caenodes sp	(E57)

DIPTERA

Simulium damnosum s.l	(DAM)

Simulium adersi	(ADE)
Simulium Iridens	(TRI)
Simulium schoutedeni	(SCH)
Antocha sp	(TIP)
Strictochironomus sp	(CC5)
Cricotopus quadrifasciatus	(CO2)
Ablabesmyia pictipes	(CP1)
Tanytarsus sp	(CT1)

TRICHOPTEHA

Cheumatopsyche falcifera	(T01)
Cheumatopsyche digitata	(T10)
Amphipsyche sp	(T02)
Orthotrichia sp	(T14)
Chimarra petri	(T16)
Protomacronema sp	(T29)

35 The counts are transformed according to the semi-quantitative scale described above. The factorial plane 1 x 2 of the analysis is presented in fig. 4 and in this case too, the primary axis is the treatment axis. The analysis of variables which contribute to it will permit the position of certain taxa to become clearer in analyses I, II and III. If the general positions of principal taxa are not modified on the treatment axis, it appears that the central position of SIM (Simuliidae other than *S. damnosum s.l.* results from differences in susceptibilities of the species which represent this taxonomie group. For example *S. schoutedeni* is largely favoured by Abate whereas *S. adersi,* a sensitive species, disappears. Only *S. Iridens* is effectively unaffected. The same phenomenon can be described for the variable Baetidae, which is slightly negative on axis I in the FCA III. This point is the center of mass of three species; two of them (E31 and E29) are very susceptible and disappear after treatment and one (E21) is unaflected with a very similar reaction to those of Hydropsychidae (PSY). One can note, on the other hand, that all the species of this taxon occupy central positions.

36 The adoption of a rough level identification conceals therefore certain variations of the faunistic composition. However it does not imply aberrations in the typology. The substitution of one species for another, phenomenon from which the central position of

simulidae results, can be considered minor in the global balance-sheet; all depends on the nature of the “ecological equilibrium” that one expects to safeguard.

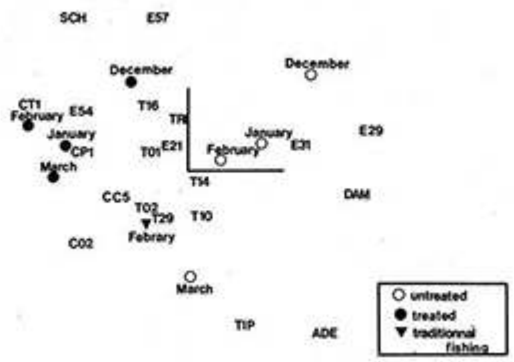

FIG. 4. — SAMPLE COLLECTIONS OF DANANgORO AND ENTOMOKRO. DATA ARC CLASSIfIED TO A SPECIfic LEVEL AND CODED. FACTORIAL PLANE 1 X 2

3. DISCUSSION

37 The correspondence analysis demonstrates stable typology allowing the description of variations in structure of the benthic communities from rocky substrates. In the case of raw data, the first axis translates the stability of the ecosystem and axis 2, the susceptibility to temephos. When coded data are used, the factorial plane of axis 1 and 2 remains identical whereas the susceptibility axis does not occupy the same range. The third axis corresponds to seasonal variations in population structure.

38 The correspondence analysis made on the data classified according to the specific taxonomie level supplied a similar type of information, although of a more precise nature. Axis I corresponds in this case to the axis of pollution by temephos. The projection of the different species on this axis leads to a classification related to the susceptibility of the species to the insecticide.

39 Thus *S. damnosum* s.l. (DAM) and *Trichorytus sp.* (EO1) are present at one of the extremities of the axis whereas *S. schoutedeni* (SCH) and *Tanytarsus sp.* (CT1) are at the other. The extreme position of *S. damnosum* s.l. on this susceptibility axis explains the success of the Onchocerciasis Control Programme. Amongst the abundant organisms we can define three groups in regard to their susceptibility to Abate treatments under the conditions of the programme. (It means the position of their projection on the axis of susceptibility).

SUSCEPTIBILITY GROUP

Simulium damnosum s.l	(DAM)
Tricorylhus sp	(EO1)
Simulium adersi	(ADE)

LESS SUSCEPTIBILITY GROUP

Cheumalopsyche falcifera	(TO1)
Cheumalopsyche digitata	(T10)
Pseudocloëon bertrandi	(E21)

UNAFFECTED GROUP OR TAXA WITH LOW SUSCEPTIBILITY

Strictochironomus sp	(CC5)
Cricotopus quadrifascialus	(CO2)
Tanytarsus reductus	(CTI)
Simulium schoutedeni	(SCII)

40 Now, it has been confirmed that this classification of taxa obtained by FCA is very similar to the scale of relative susceptibility obtained *in situ* with gutter tests. We had obtained in fact, at the time of an experiment *in situ* with temephos (Abate 200 CE):

Tricorythus sp	87.3% of detaching	
Cheumatopsyche falcifera	14.9%	—
Cheumatopsyche digitata	51.6%	—
Stictochironomus sp	35.2%	—
Simulium schoutedeni	3.4%	—
Cricolopus quadrifascialus	18.1%	—
Tanytarsus s.p	3.4%	—
Simulium adersi	Not tested	

41 The axis of susceptibility to temephos obtained by the factor analysis corresponds therefore to the real sensitivity of the organisms. Lets us recall here that the correspondence analysis comes to this result by taking account not of the numbers but of the relative frequencies, that is to say the structures of the communities.

42 In the case of coarser taxonomie identification realized in the surveillance programme, we obtained a relative scale of susceptibility similar to that obtained for the species. Only the disappearance of *S. adersi* and the appearance of *S. schouledeni* are unnoticed because

they compensate each other. We note likewise that the disappearance of species of Baetidae is evident.

43 In these conditions, the analysis performed (FCA) on the surveillance data expresses a tangible reality and the adjustment to an index of pollution (based on coded data) established from the most representative taxa appears to be necessary and suitable. This index would permit a rapid estimation of river pollution by temephos and allow one to control its evolution, efficiently and in a simple way.

4. CONCLUSION

44 The statistical treatment of the surveillance data by correspondence analysis demonstrates evidence of long term effects of temephos on the rocky substrat invertebrates.

45 The well known short term effect on populations as it was anticipated, has repercussions on the structure of communities after repeated weekly treatments.

46 This proof of the long term impact of temephos responds implicity then to the second question formulated at the beginning of this paper. The protocol for surveillance of benthic fauna on rocky substrates provides a sufficiently precise method for pointing out the toxic effect of the insecticide employed in the Onchocerciasis Control Programme during the months of decreasing stream level and at the beginning of the dry season.

47 If the identification level adopted in the monitoring protocol effectively permits the estimation of a certain action of Abate, the specific identification is necessary to point out substitution of species.

48 The calculation of the index of pollution based on coded data simplifies and lightens the computations. It allows the reduction of the number of taxa studies, saving time for fundamental research. As a matter of fact, the interpretation of monitoring results cannot be done without first knowing the ecology, susceptibility, voltinism and seasonability of species.

49 We have in the prsent work only treated a part of the surveillance data in order to demonstrate:

- the long term effects of Abate;
- the operational value of sampling methods utilized;
- the possibility of demonstrating the effects of temephos by factor analysis.

50 The treatment of all the collected data in the whole area under surveillance should provide other information on the impact of temephos on non-target fauna.

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NOTES

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ABSTRACTS

During six years of insecticide application against Simulium damnosum s.l., *the side effects on the non-target macroinvertebrate fauna have been monitored. Using the correspondence analysis (reciprocal averaging), the authors point out the evidence of a long term effect of the pesticide (temephos) and consider the validity of the methods used for invertebrate sampling.*
The correspondence analysis of the data from Surber samples shows a modification in the population structure occurring after temephos treatment as well as a seasonal pattern. The substitution of an abundance quotation (i.e. codification) for the original counts does not modify the established typology.
The target species Simulium damnosum s.l. *and the species* Tricorythus sp. *(Ephemeroptera) appear to be the most sensitive organisms, and the density of Chironomidae and especially Tanytarsini rise markedly after treatment. The proportions of Baetidae and Hydropsychidae are lightly affected by the pesticide.*
Parallel studies carried out al a specific level of identification have shown that certain phenomena are masked in the monitoring results by the grouping of several species within the same taxon. The central position on graphs and thus the presumed insensitivity of Simuliid species other than S. damnosum *is, in fact, the result of a variation in susceptibility amongst the species.* S. adersi *being sensitive,* S. schoutedeni *resistant and S. tridens* apparently *unaffected.*
The use of a calculated index of pollution resulting from the factorial axis of susceptibility obtained with coded data in the correspondence analyses, is proposed.

En Côte d'Ivoire, après six années de surveillance des milieux lotiques traités au téméphos dans le cadre du programme de lutte contre l'Onchocercose dans le bassin de la Volta, les auteurs mettent en évidence, à l'aide de l'analyse factorielle des correspondances, un impact à long terme du pesticide et s'interrogent sur la validité du protocole d'échantillonnage choisi pour la récolte des invertébrés.

Une analyse factorielle des correspondances effectuées sur des données recueillies à l'échantillonneur de Surber montre une modification de la structure des peuplements après traitement au téméphos ainsi qu'une saisonnalité marquée. L'utilisation d'une cotation d'abondance ne perturbe pas la typologie établie. L'«espèce» cible Simulium damnosum s.l. *et un Éphéméroptère,* Tricorythus sp. *apparaissent être les plus sensibles, par opposition aux Chironomidae et plus particulièrement aux Tanitarsini, dont la proportion dans les échantillons augmente notablement après traitement; Baetidae et Hydropsychidae réagissent peu à l'insecticide.*

Il est également démontré que certains phénomènes sont masqués du fait du regroupement de plusieurs espèces dans un taxon. Ainsi, la position centrale dans les représentations graphiques et donc l'insensibilité présumée des Simulies autres que S. damnosum *résulte de la sensibilité variable des espèces concernées, sachant que l'on peut qualifier* S. adersi *de sensible,* S. tridens d'indifférente et S. schoutedeni *de résistante.*

Les auteurs proposent l'utilisation d'un indice synthétique de pollution issu de l'axe factoriel de sensibilité obtenu avec des données codées.

INDEX

Keywords: Onchocerciasis Control Programm, Temephos, Non-target fauna, Lotic communities, Correspondence analyses, Pollution index, Ivory Coast, Africa

Mots-clés: Programme de Lutte contre l'Onchocercose, Temephos, Faune non cible, Communautés lotiques, Analyse des correspondances, Indice de pollution, Côte d'Ivoire, Afrique

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Impact du téméphos (*Abate*) sur les invertébrés non-cibles

B. Un indice biocénotique pour mesurer l'action du téméphos sur la faune lotique non-cible des rivières traitées dans le cadre du Programme de Lutte contre l'Onchocercose[1] / B. A biocenotic index to monitor the impact of temephos on lotic non-target fauna of treated rivers in the onchocerciasis control programme

Impact of temephos (abate) on the non-target invertebrate fauna

Jean-Marc Elouard et Jean-Marie Jestin

1. INTRODUCTION

1 L'onchocercose humaine est une filariose endémique de l'Afrique de l'Ouest, sévissant essentiellement en zone tropicale. Outre un certain nombre de lésions bénignes, elle occasionne chez le malade ayant une charge microfilarienne élevée, une cécité irréversible. En plus des problèmes cliniques et humains que cette affection soulève, il faut également considérer l'impact économique qui en résulte ; une partie non négligeable de la population active devient improductive par perte de la vue.

2 La transmission de cette affection, d'homme à homme, est assurée par un petit Diptère : la Simulie. En Afrique de l'Ouest, seul le complexe d'espèces *Simulium damnosum* est vecteur. Les larves et les nymphes de ces Insectes se développent dans les eaux courantes (milieu lotique).

3 Pour des raisons stratégiques, la lutte entreprise contre l'onchocercose, se fait par rupture du cycle de transmission en contrôlant par voie chimique les effectifs des populations larvaires de *S. damnosum s.l.,* écophase qui est la plus grégaire et la plus localisée, donc la plus accessible.

4 L'insecticide organophosphoré utilisé est le téméphos (Abate®), présenté en concentré émulsifiable et appliqué par voie aérienne. En raison de la brièveté du cycle de

développement de la larve, un épandage hebdomadaire s'avère nécessaire pour contrôler de façon efficace les populations du vecteur.

5 Aux doses de 0,1 et 0,05 ppm/10 mn employées dans les conditions de programme, le téméphos est totalement efficace contre les larves de *S. damnosum s.l.* Cet insecticide étant cependant assez peu sélectif, une destruction de la faune aquatique non-cible est à craindre.

6 Nous avons déjà montré que le téméphos était toxique à court terme pour les invertébrés aquatiques (DEJOUX, 1977 a ; DEJOUX, 1977 b ; DEJOUX & ELOUARD, 1977 ; ELOUARD & FORGE, 1977 ; DEJOUX, 1978 a, b ; ELOUARD, 1983). Il est logique de penser que ses effets à court terme ont des répercussions à long terme et modifient notablement et durablement les équilibres des populations en altérant les structures des communautés aquatiques.

7 Les épandages répétés de téméphos constituent une action polluante telle que l'a définie WASSON (1980) : « la pollution est l'altération d'un écosystème, naturel ou non, ou d'une fraction d'un écosystème, sous l'action d'un ou plusieurs agents polluants de nature variée, produit directement ou indirectement par l'homme ou à la suite de ses activités ».

8 L'action polluante du téméphos est de courte durée et localisée dans l'espace mais son caractère répétitif et la durée prévue des traitements (20 ans) font que les risques de déséquilibre sont réels.

9 Les organismes vivants dans un écosystème donné sont normalement, (sauf présence accidentelle) adaptés aux composantes physico-chimiques du milieu, et la présence d'un organisme particulier dans un milieu est la résultante des relations souvent étroites entre ses exigences écologiques et les facteurs mésologiques. Corrélativement, toute modification du milieu a des conséquences sur l'écophysiologie, voire l'existence même de l'organisme considéré. Cependant, tous les organismes peuplant une biocénose ne présentent pas le même degré d'exigence vis-à-vis de certains facteurs du milieu. De même tous ne manifestent pas la même sensibilité écologique vis-à-vis de l'insecticide. Certains vont donc être éliminés du milieu, d'autres verront leur densité simplement réduite, enfin certains seront indirectement favorisés par les épandages, bénéficiant de la réduction du nombre des prédateurs ou d'une moindre compétition spatiale.

10 L'existence d'une sensibilité différentielle des organismes à la pollution ainsi que de structures bien définies dans les peuplements, ont engendré le concept d'indicateurs écologiques. Un indicateur écologique est une population ou un ensemble de populations en interrelation, qui réagissent sélectivement de façon qualitative ou quantitative à des modifications, parfois minimes, d'un ou plusieurs paramètres de l'écosystème dont ils font partie.

11 En se basant sur cette notion de structure évolutive, deux voies principales de recherche ont été explorées par les écologues depuis le début du XX[e] siècle, pour mettre en évidence l'action d'un agent polluant. Ce sont :

- la recherche d'espèces indicatrices,
- l'étude et la caractérisation des changements de structure biocénotiques que l'on exprime par des indices empiriques ou mathématiques.

12 Nous rappelerons brièvement ces différentes méthodes, puis formulerons un nouvel indice biocénotique plus spécifique, applicable à la surveillance des rivières traitées au téméphos dans le cadre du Programme de Lutte contre l'Onchocercose.

2. PRINCIPALES MÉTHODES DE MISE EN ÉVIDENCE D'UNE POLLUTION DES EAUX DOUCES

2.1. Méthodes utilisant des espèces indicatrices

13 La méthode des saprobies de KOLWITZ & MARSON (1908), quoique très ancienne, fut l'une des plus employées. Les auteurs ont classé les animaux aquatiques en fonction de leur tolérance à la pollution organique et ont ainsi défini les associations ou saprobies suivantes :

- polysaprobes : ce sont les associations des eaux très polluées regroupant les animaux saprobiontes (ou espèces saprobies) strictement inféodées à ces milieux ;
- mésosaprobes α et β : associations spécifiques des eaux moins polluées. Les organismes colonisant ces milieux sont dits saprophiles ;
- oligosaprobes : biocénoses d'eau propre, non polluée. Les organismes sont dits saproxènes.

14 Selon le type de communauté trouvé, on peut donc déterminer grossièrement l'état de pollution d'un cours d'eau. Cette méthode présente cependant plusieurs inconvénients :

- un comptage rigoureux de tous les organismes est nécessaire ;
- la détermination des organismes doit se faire jusqu'au niveau spécifique. En effet, à l'intérieur d'un même genre ou d'une même famille, les sensibilités des espèces vis-à-vis de la pollution peuvent être très différentes ;
- l'écologie de tous les animaux doit être connue, la présence ou l'absence d'une espèce pouvant être fonction de la variation de paramètres autres que la pollution (saison, hydrologie...).

15 Cette méthode demande donc pour être appliquée avec rigueur, une connaissance approfondie des espèces ; elle est de ce fait difficilement applicable à grande échelle. Pour tourner la difficulté, certains auteurs ont étudié les modifications biocénotiques induites par les pollutions. TUFFERY & VERNEAUX (1967), TUFFERY (1980) construisent empiriquement, après de nombreuses études, un indice biotique caractérisant la qualité de la macrofaune invertébrée en place, ainsi que sa diversité spécifique. Cette méthode simple considère des niveaux d'identification variable selon les groupes, mais fixés quasi définitivement pour toutes les études. Elle est sensible aux variations saisonnières mais présente l'inconvénient de ne prendre en considération que l'épibenthon, et de n'être applicable qu'aux petits cours d'eaux. Enfin, les coefficients appliqués pour chaque groupe sont déterminés empiriquement, ce qui demande une grande connaissance du milieu et des effets de la pollution.

2.2. Méthodes utilisant des indices de diversité

16 En plus de leur composition taxinomique, les peuplements peuvent être caractérisés par leur *densité* ainsi que leur *richesse* et leur *diversité spécifique.* Ce dernier paramètre pourrait être défini « comme la mesure de la composition en espèces d'un écosystème, en terme de nombre d'espèces, et de leurs abondances relatives » (LEGENDRE & LEGENDRE, 1979). L'intérêt des indices de diversité est de permettre des comparaisons globales de peuplements différents. D'après PIELOU (1975), la diversité est l'équivalent, pour la variable non ordonnée espèce, de la variance pour les variables continues.

17 La mesure de la diversité spécifique se fait à l'aide d'indices appartenant à deux catégories distinctes selon qu'ils reposent ou non sur la théorie de l'information. Citons pour mémoire, l'indice de Shannon (1948) et l'équitabilité (MARGALEF, 1958 ; LLYOD & GHELARDI, 1964) pour les indices informatiques. La mesure non informationnelle de la diversité a été étudiée par de très nombreux indices dont ceux de FISHER, CORBET & WILLIAMS (1943), PRESTON (1948), PATRICK (1949), SIMPSON (1949), MARGALEF (1951), ODUM, CANTLON & KORNICKER (1960), MENHINICK (1964), MCINTOSH (1967), SANDERS (1968), HURLBERT (1971), PEET (1974)...

18 De cette longue énumération des principaux indices de diversité, il appert qu'il en existe de nombreux, pas toujours comparables et qui ne mesurent bien souvent pas la même chose. Cela est surtout vrai en ce qui concerne les espèces rares. Elles comptent pour partie négligeable dans certains indices et possèdent par contre des poids considérables dans d'autres. Certains auteurs tel HURLBERT (1971) préfèrent donc le terme de « non-concept of diversity » pour parler de la diversité spécifique.

19 Chacun de ces indices présente donc des avantages et des inconvénients qui le plus souvent limitent leur application à la mise en évidence d'un problème écologique particulier. Ils semblent cependant mal adaptés aux problèmes posés par les pollutions, l'inconvénient majeur étant toujours qu'ils nécessitent :

- une détermination des organismes au niveau spécifique. ÉCHAUBARD & NEVEU (1974-75) étudient cependant la pollution de la Couze Pavin (ruisseau du Massif Central) à l'aide de l'indice de Shannon. Selon eux, dans ce cas précis, l'indice de Shannon traduirait de façon plus évidente la pollution que l'indice biotique de TUFFERY & VERNEAUX. Il faut cependant garder à l'esprit que dans cette application, l'indice de diversité de Shannon a été calculé sur les familles et de ce fait, il n'est pas sûr que les conclusions tirées soient conformes à la théorie de l'information qui requiert un niveau spécifique d'identification taxinomique. Pourtant HUGHES (1978) travaillant sur les communautés lotiques d'une rivière du sud du Pays de Galles a montré que l'indice de Shannon calculé sur les niveaux taxinomiques du genre, de la famille ou de l'ordre donnait des valeurs proches de celles obtenues au niveau taxinomique de l'espèce ; résultat qui nous paraît toutefois contestable ;
- un comptage fastidieux.

20 Il faut en plus retenir que la plupart de ces indices sont fondés sur les fréquences relatives des espèces, ce qui fait que deux structures différentes de communauté peuvent donner des indices semblables. De même à effectifs égaux, les remplacements d'espèces ne peuvent être perçus. Ces indices sont donc aveugles.

21 En résumé, l'interprétation des valeurs obtenues pour les différents indices pour mesurer des changements survenus dans une biocénose, est délicate et s'avère peu adaptée aux problèmes de surveillance à grande échelle.

3. PROPOSITION D'UN INDICE BIOCÉNOTIQUE APPLICABLE A LA SURVEILLANCE DES RIVIÈRES TRAITÉES AU TÉMÉPHOS

3.1. Caractéristiques requises par un indice de pollution dans ce contexte

22 Le traitement au téméphos des rivières d'Afrique de l'Ouest, dans le cadre du programme régional de lutte contre l'onchocercose, doit se poursuivre durant 20 années, sur un réseau hydrographique considérablement étendu (plus de 1,5 millions de km^2). La surveillance des milieux aquatiques doit donc être la plus aisée possible afin de pouvoir être réalisée sur une grande échelle, par un personnel peu qualifié. Les indices formulés pour suivre l'action du téméphos devront donc tenir compte des impératifs suivants :

- l'identification taxinomique doit être facile ;
- le comptage des organismes doit être rapide ;
- l'indice proposé doit être sensible aux variations d'abondance ainsi qu'aux variations de structure ;
- la formulation de l'indice doit être suffisamment générale pour s'appliquer sans modifications à différentes rivières voire à différents bassins hydrographiques ;
- le calcul doit être simple et ne doit pas nécessiter de moyens sophistiqués ;
- enfin, l'indice obtenu doit être suffisamment sensible pour suivre les variations fines de la pollution et séparer distinctement les périodes traitées à l'insecticide des périodes non-traitées ;

3.2. Choix des données prises en compte dans l'analyse

3.2.1. CHOIX DES STATIONS

23 Le principal critère retenu pour le choix des stations a été l'existence, en nombre suffisant, de données recueillies avant et après traitement. Seules deux stations de la Maraoué répondaient pleinement à cette exigence.

24 Danangoro (DAN), sur la Maraoué, au N.W. de Bouaflé, était sous surveillance depuis 1975. Cependant, seuls les résultats des années 1976 à 1980 ont participé à l'analyse. L'ensemble des prélèvements concerne trois années avant et une année après que ne débutent les traitements qui commençèrent en mars 1979.

25 Entomokro (EK) sur la Maraoué au S.E. de Bouaflé, est échantillonnée depuis 1978 et sous traitement depuis mars 1979.

26 Les données provenant de ces deux stations ont été regroupées et ont fait l'objet d'une analyse unique.

3.2.2. CHOIX DES MOIS

27 La construction de l'indice proposé a été basée sur les analyses des prélèvements réalisés à l'échantillonneur de Surber (faune saxicole). Cette méthode n'est cependant guère applicable durant la crue, les surfaces rocheuses sur lesquelles se pratiquent ces échantillonnages étant profondément immergées. De même, durant certains mois de

début ou de fin de crue, les niveaux des fleuves varient très vite d'un jour à l'autre, ce qui perturbe énormément les peuplements en place et rend cette technique peu fiable. A l'opposé, lors de l'étiage, les écosystèmes lotiques présentent une certaine stabilité, l'hydraulicité du milieu étant elle-même peu variable. C'est durant une telle période, allant généralement de décembre à mars, que l'impact d'une pollution sera le plus facilement mise en évidence.

3.2.3. NIVEAU D'IDENTIFICATION ET CHOIX DES TAXONS

28 D'une manière générale, la systématique des invertébrés aquatiques des rivières africaines, est très mal connue et dans bien des cas il est pratiquement impossible de différencier les jeunes stades larvaires d'espèces ou de genres affins. Tris et comptages ont donc été réalisés à un niveau taxinomique supraspécifique nommé taxon. Le choix a été effectué en fonction des difficultés d'identification ainsi que de l'importance de ce taxon dans la biocénose. Selon les groupes taxinomiques considérés, les taxons choisis sont le genre, la tribu, la famille voire même l'ordre. Seule *Simulium damnosum s.l.* reste identifiée en tant qu'espèce tandis que le taxon « Simulies autres » regroupe le reste des espèces du genre *Simulium.* Dans le tableau I sont répertoriés les 16 taxons retenus qui présentent à la fois la plus grande abondance et une forte occurence.

TABLEAU I. **Analyse factorielle des correspondances (AFC I) des effectifs codés de 16 taxons récoltés à l'échantillonneur de Surber. Coordonnées sur l'axe F 1 et contributions absolues en ‰ à l'axe F 1**

	Coordonnées	Contributions abs. en ‰
Baetidae	- 0.19	39
Caenidae	0,23	22
Tricorythidae	- 0,41	81
Hydropsychidae	0.01	0
Hydroptilidae	0,02	0
Leptoceridae	- 0.58	37
Philopotamidae	0,25	19
Simuliidae	0,01	0
S. Dammosum	- 0,80	313
Chironomini	0,33	79
Tanytorsini	0,88	323
Orthocladiinae	0,16	26
Tanypodiinae	0.22	21

Diptères autres	- 0.17	10
Pyralidae	- 0.13	10
Hydracariens	- 0.34	20

3.2.4. CHOIX DU TYPE DE DONNÉES À ANALYSER

29 Si l'indice observé doit être sensible aux variations d'abondance des organismes ainsi qu'aux modifications de structure de peuplements, il devra intégrer d'une façon ou d'une autre les effectifs des taxons sensibles, insensibles ou favorisés par l'insecticide. Une étude quantitative était donc nécessaire. Le comptage simple a été réalisé dans un premier temps ; il a cependant l'inconvénient d'être long et fastidieux. En outre, il a souvent été prouvé en écologie qu'une analyse prenant en considération des comptages numériques dégageait moins facilement les tendances générales que le dénombrement utilisant une métrique de type logarithmique. Cette méthode à l'avantage de réduire les grandes différences numériques inhérentes à l'échantillonnage des distributions agrégatives. La codification, 1+mantisse du logarithme, a été proposée. L'emploi d'une telle cotation d'abondance pour le dénombrement apporte par ailleurs un gain de temps et de moyens considérable permettant ainsi de multiplier si nécessaire, le nombre de sites étudiés et le nombre des relevés.

3.3. Construction de l'indice

30 L'élaboration d'un indice de pollution a été dicté par trois exigences fondamentales :

- il est nécessaire de tenir compte de l'identité de chaque taxon dans le calcul de l'indice, afin que deux structures de communauté différentes ne donnent pas la même valeur de l'indice ;
- il était également nécessaire de tenir compte de la structure des communautés lotiques et de l'importance réelle de chaque groupe taxinomique qui les constituent. Pour ce faire, on affecte, à chacun des taxons intervenant dans le calcul de l'indice, un coefficient (ou poids) fonction de leur sensibilité au téméphos, mais qui, contrairement aux indices biotiques proposés par KOLWITZ & MARSON ou TUFFERY & VERNEAUX qui utilisent des poids empiriques, ne nécessitent pas une connaissance importante de la bioécologie et de la biosensibilité des taxons.
- enfin, les coefficients affectés à chacun des taxons, doivent être calculés une fois pour toute sur un grand nombre de données pour l'ensemble de l'aire du programme ou plus modestement pour chaque bassin hydrographique. Les calculs des valeurs de l'indice ne doivent nécessiter que l'application des coefficients aux effectifs codés des taxons récoltés dans chaque prélèvement.

3.3.1. RAPPEL DE LA MÉTHODE

31 L'analyse factorielle des correspondances (AFC) appliquée aux effectifs codés permet de dégager les structures des communautés et leurs variations en fonction de l'insecticide (ELOUARD & JESTIN, 1982). Les valeurs transformées ou codées sont consignées dans un tableau de contingence où les variables sont constituées par les taxons et les objets par les prélèvements récoltés aussi bien sur les rivières traitées que sur les rivières non-traitées

à l'insecticide. Selon les analyses considérées, les effectifs de différentes stations et de différents mois ont été pris en compte. Dans notre analyse, la typologie établie séparait clairement sur le premier axe factoriel les relevés ayant subi l'insecticide de ceux ne l'ayant pas subi (ELOUARD & JESTIN, 1982). Par ailleurs, les taxons sensibles étaient opposés sur cet axe factoriel aux taxons écologiquement résistants à l'insecticide. Selon cet axe, il est donc possible de construire une échelle de pollution et à partir de celle-ci, un indice de pollution qui soit une combinaison linéaire des effectifs codés des taxons ayant contribués à l'analyse. Les coordonnées sur l'axe F1 ou axe de pollution seront retenues comme poids (a_1) pour chacun des effectifs considérés. Le coefficient sera d'autant plus fort que la position du taxon sera plus excentrée par rapport à l'origine. L'indice proposé est donc de la forme :

$$(1)\quad IND_{(j)} = \sum_{i=1}^{n} x_i a_i / \sum_{i=1}^{n} x_i$$

où $\sum_{i=1}^{n} x_i$ représente le total des effectifs codés.

32 Afin de pouvoir appréhender de façon simple et rapide les variations de la pollution, il est intéressant que les valeurs de l'indice, obtenues pour chacun des prélèvements, varient entre les valeurs 1 et 10. L'indice prendra la valeur 1 lorsqu'aucune pollution n'interviendra et la valeur 10 quand la pollution sera maximale. Nous avons retenu comme plage de variation correspondant à 10 unités de pollutions la distance (d) entre les relevés extrêmes de part et d'autre de l'origine de l'axe 1, distance qui a été observée en 5 années sur une rivière particulièrement bien étudiée avant et après les traitements au téméphos : la Maraoué.

33 Un coefficient C = 10/d doit donc être introduit dans la formule (1) à laquelle il convient d'ajouter 5 pour que les valeurs de l'indice varient entre 1 et 10 et non entre —5 et +5. La formule finale retenue est de la forme suivante :

$$(2)\quad IND_{(j)} = \left[\left(\sum_{i=1}^{n} x_i a_i / \sum_{i=1}^{n} x_i \right) \times C \right] + 5$$

34 Seule la partie entière du résultat est considérée. Si la valeur de l'indice $IND_{(j)}$ est inférieur à 1 on l'assimilera à 1, de même si la valeur de l'indice est supérieure à 10, on l'assimilera à 10.

4. RÉSULTATS

4.1. Détermination des poids et du coefficient C

35 La typologie obtenue dans une première analyse factorielle des correspondances appliquée aux données de Danangoro et d'Entomokro (AFC I) oppose sur le premier axe factoriel, l'espèce cible sensible au téméphos : *S. damnosum s.l,* au taxon Tanytarsini favorisé par l'insecticide (fig. 1). Les contributions absolues de ces deux taxons, à la construction du premier axe factoriel, sont très fortes et respectivement de 313 et 323 ‰. Le premier axe factoriel explique 16 % de l'inertie totale des données (valeur toujours sous estimée dans ce type d'analyse). Les poids affectés aux variables sont consignés dans le tableau I et le coefficient C à appliquer est C = 60.

TABLEAU II. **Analyse factorielle des correspondances (AFC II) des effectifs de 6 taxocènes récoltés à l'échantillonneur de Surber. Coordonnées et contributions absolues sur l'axe factoriel F 1**

	Coordonnées	Contributions abs. En ‰
Baetidae	-0.2	44
Tricorythidae	-0.5	120
S. Damnosum	-0.8	325
Chironomini	0,3	79
Tanytarsini	0.9	385
Orthocladiinae	0.2	47

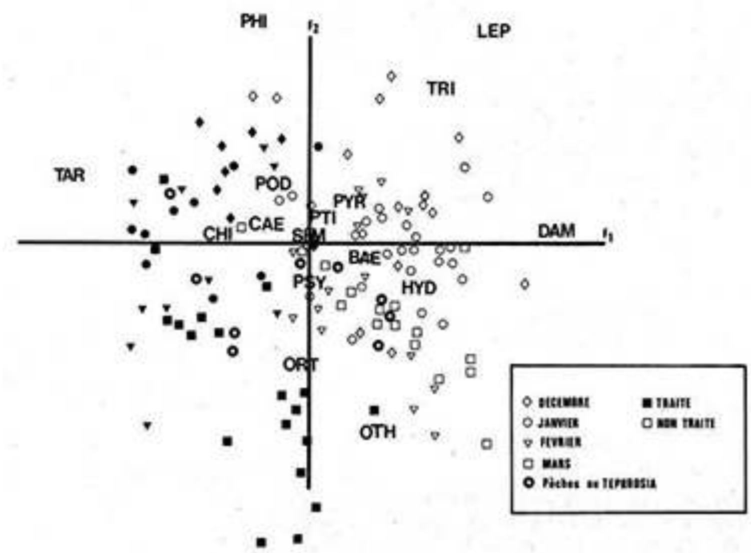

FIG. 1. — Analyse factorielle des correspondances des données codées récoltées sur les stations de Danangoro et d'Entomokro (AFC I). Plan factoriel F1 x F2 et 16 variables actives. Le premier axe traduit la pollution par le téméphos.

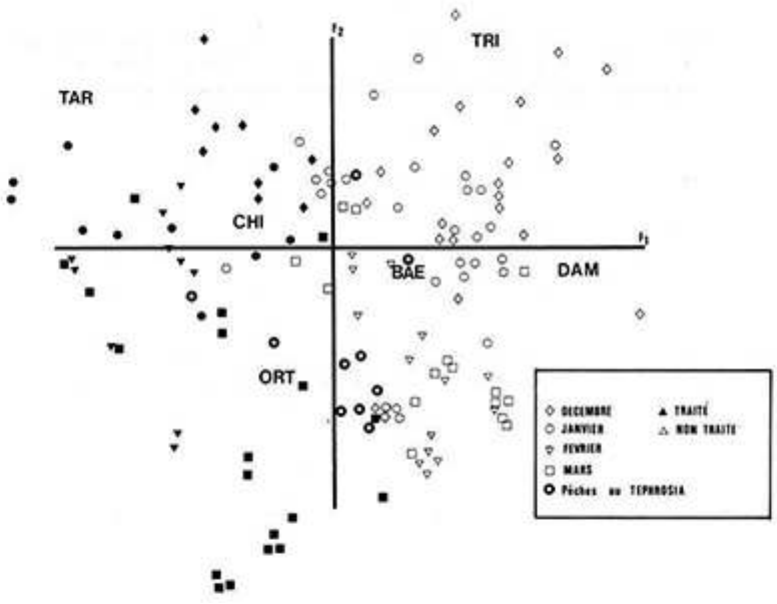

FIG. 2. — Analyse factorielle des correspondances des données codées récoltées sur les stations de Danangoro et d'Entomokro (AFC II). Plan factoriel F1 x F2 et 6 variables actives. Le premier axe traduit la pollution par le téméphos.

36 Certains taxons tels que les Hydroptilidae et les Simuliidae autres que *S. damnosum s.l.*, possèdent une contribution nulle dans la construction du premier axe factoriel. Il est inutile de les prendre en compte dans le calcul de l'indice de pollution. De même, les taxons présentant des effectifs faibles ainsi que des contributions peu élevées sont éliminés. Une seconde analyse (AFC II) portant seulement sur les six taxons suivant : Baetidae, *S. damnosum s.l.*, Tanypodiinae, Tanytarsini et Orthocladiinae a été réalisée (fig. 2). La typologie obtenue est à peu près identique à celle établie lors de la première analyse. Les contributions des différents taxons ainsi que leur coordonnées sur l'axe 1 sont consignés dans le tableau II. Les variables *S. damnosum s.l.* et Tanytarsini ont encore une contribution prépondérante dans la construction du premier axe factoriel qui représente 40 % de l'inertie totale du nuage.

4.2. Performance des indices

37 Le meilleur indice biocénotique est celui qui présente le plus haut taux de réussite dans la séparation des mois sans traitement des mois ayant subi des traitements au téméphos. Dans les tableaux III et IV sont reportés les moyennes par échantillon des indices biocénotiques (dans la plupart des cas, un échantillon représente une série de 5 prélèvements réalisés le même jour dans les mêmes conditions). L'astérisque repère les mois avec traitements tandis qu'un point noir signifie que le résultat obtenu n'est pas celui escompté. Le taux de réussite dans le classement est plus élevé lorsque les coefficients et les poids issus de l'AFC II sont utilisés. Ce taux est calculé en faisant le rapport du nombre de mois bien classés au nombre total de mois. Il est de 90 % dans la première analyse et de 95 % dans la seconde qui est donc plus performante puisqu'elle permet de mesurer avec une plus grande certitude l'effet des épandages de téméphos. Il ne nous paraît cependant pas certain que la surveillance d'un si petit nombre de taxons permette de suivre à long terme et sur une grande échelle les variations de structure des communautés de grands bassins souvent très différents les uns des autres. Notre

jugement de l'impact du pesticide pourrait être faussé s'il apparaissait des changements notables dans la biosensibilité de certains groupes taxinomiques. Il semble donc prématuré d'opter pour l'application des coefficients issus de l'AFC II tant que des vérifications plus importantes dans le temps et dans l'espace n'ont pas été réalisées.

TABLEAU **III. Tableau des indices biotiques moyens calculés à partir des coefficients issus de l'AFC I dans laquelle 16 taxons sont pris en compte (le point signale les individus mal classés ; l'astérisque signale les traitements)**

DANANGORO	Nov.	Déc.	Jan.	Fév.	Mars	Avr.	Mai
1976	-	-	• 5,8	4,4	1,0	-	-
1976-1977	4,2	1,0	2,0	1,0	1,0	3,4	• 5,6
1977-1978	-	4,2	1,0	-	-	• 6,6	-
1978-1979	-	-	1,0	1,0	* 6,8	* -	* -
1979-1980	* 7,4	* 7,3	* 8,4	* 9,4	* 7,8	* 8,2	* 10,0
ENTOMOKRO	Nov.	Déc.	Jan.	Fév.	Mars	Avr.	Mai
1977-1978	-	1,0	1,8	7,6	3,2	• 9,8	4,2
1978-1979	-	1,0	1,0	3,8	* 5,6	* -	* -
1979-1980	* 9,8	* 9,8	* 10,0	* 9,4	* 9,2	* 9,4	* 10,0

TABLEAU **IV. Tableau des indices biotiques moyens calculés il partir des coefficients issus de l'AFC II tenant seulement compte de 6 taxons (cf. tabl. III)**

DANANGORO	Nov.	Déc.	Jan.	Fév.	Mars	Avr.	Mai
1976	-	-	4,8	3,8	1,0	-	-
1976-1977	4,2	1,4	4,4	1,4	1,6	3,6	4,4
1977-1978	-	2,0	1,0	-	-	• 7,4	-
1978-1979	-	-	1,0	1,2	* 6,6	* -	* -
1979-1980	* 7,2	* 6,0	* 7,6	* 8,2	6,6	* 7,8	* 9,8
ENTOMOKRO	Nov.	Déc.	Jan.	Fév.	Mars	Avr.	Mai
1977-1978	-	1,0	2,6	7,8	3,8	• 10,0	3,4
1978-1979	-	1,0	1,2	5,0	* 5,8	* -	* -
1979-1980	* 10,0	* 7,8	* 10,0	* 9,6	* 9,0	* 8,6	* 9,4

4.3. Vérification de la validité de l'indice

38 Les indices biocénotiques ont été vérifiés sur un ensemble de 327 prélèvements qui n'ont pas participé à l'AFC. Ces prélèvements ont été récoltés sur les stations de Danangoro, Entomokro, Sémien et la Léraba., durant les mois de décembre à juin. Deux calculs différents du taux de réussite ont été effectués selon que l'on considérait l'ensemble des mois ou simplement la période comprenant les mois de décembre à mars. Les résultats permettent de conclure que la formule de l'indice biocénotique de pollution issue de l'analyse à 6 variables (AFC **II)** est la plus performante et bien que l'écart soit faible, la période intégrant les mois de décembre à mars est mieux classée que l'ensemble des mois (tabl. V).

TABLEAU V. **Pourcentage de réussite dans la séparation des mois traités et non traités au Téméphos, au moyen de l'indice biotique. % I = % de réussite par rapport à l'ensemble des mois.% II = % de réussite par rapport à la période décembre-mars**

	16 variables		6 variables	
	% I	% II	% I	% II
Entomokro	91	98	92	100
Danangoro	87	93	90	98
Sémien	71	74	80	80
Léraba	100	100	98	98

39 Les stations de Danangoro et d'Entomokro ont un taux égal de réussite voisin de 90 % pour l'ensemble des mois et de 100 % si l'on ne considère que les mois de la période de décembre à mars. Le taux de réussite pour les prélèvements de la station de Sémien étant plus bas et oscillant entre 70 et 80 %.

40 Toutefois, l'obtention de valeurs « anormales » de l'indice de pollution pour certains relevés n'est pas nécessairement due à son inaptitude. En effet, durant les périodes avec traitements, il peut arriver qu'un site soit incorrectement traité, auquel cas les peuplements peuvent rapidement en bénéficier et se rapprocher en composition et en structure de ceux existant en milieu vierge. Inversement, des pollutions de diverses origines peuvent affecter un site non traité au téméphos et en modifier les équilibres dans un sens défavorable. Certains sites enfin, peuvent présenter une typologie particulière et naturelle de leurs peuplements saxicoles qui induit alors systématiquement une moindre signification de l'indice. C'est le cas observé sur la station du Sassandra (Sémien) où nous avons pu remarquer l'absence quasi totale et naturelle de *S. damnosum s.l.* sur les faciès rocheux ; le biotope de prédilection étant de cette espèce la végétation aquatique ou semi-aquatique présente dans les rapides.

41 Pour de tels gîtes, il serait utile d'effectuer une analyse séparée afin de déterminer leurs structures biocénotiques. Une telle analyse n'est cependant possible que si l'on dispose suffisamment de données récoltées avant et après le début des traitements antisimulidiens, ce qui n'est pas le cas pour la station de Sémien. Quoi qu'il en soit, dans de tels cas de configurations biocénotiques différentes, seuls les coefficients pondérant les effectifs sont affectés, la forme générale de l'indice restant inchangée.

4.4. Période à retenir

42 Les indices biocénotiques calculés pour les périodes de décembre à mars ont un taux de réussite plus élevé que ceux calculés pour les autres mois. Les taux de réussite pour chacun des mois, calculés à partir des coefficients et des poids issus de l'AFC I sont les suivants :

Novembre 94 %	Janvier 93 %	Mars 96 %	Mai 75 %
Décembre 91 %	Février 100 %	Avril 70 %	Juin 90 %

43 Les mois d'avril et de mai paraissent les moins aptes à traduire une pollution en se fondant sur la structure des communautés. D'autre part, pour des raisons tant d'hydraulicité fluctuante des rivières que de faibles effectifs totaux des peuplements, il

semble préférable de ne pas juger l'état de pollution d'une rivière sur les indices biocénotiques obtenus dans les mois de novembre et de mai. La meilleure saison pour surveiller la faune des rochers se situe donc durant les mois de décembre, janvier, février et mars.

4.5. Incidence de l'effectif total du peuplement sur le calcul de l'indice biocénotique

44 Dans le rapport DEJOUX *el al.* (1980), il était préconisé de ne pas tenir compte des relevés présentant un effectif trop faible. Une vérification *a posteriori* de cette hypothèse montre qu'il existe une liaison assez forte entre la proportion mensuelle des relevés mal classés et le pourcentage de relevés mensuels dont l'effectif total est inférieur à 100 individus. Globalement les chances sont de 51 % d'obtenir un mauvais indice lorsque l'effectif total d'un prélèvement est inférieur à 100 individus contre moins de 10 % lorsque l'effectif total est supérieur à 100. Il faut cependant garder à l'esprit que la réduction extrême des effectifs peut être la conséquence d'une pollution extrême. Les données présentant des effectifs trop faibles devront donc être jugées objectivement avant d'être écartées.

5. CONCLUSION

45 L'utilisation régulière de l'indice biocénotique de pollution que nous venons de proposer nous semble être un outil fiable et adéquat pour une surveillance extensive et à long terme des effets toxiques du téméphos sur les peuplements d'invertébrés des rivières ouest-africaines. Bien entendu il ne dispense pas d'études ponctuelles approfondies mais permet d'émettre un jugement rapide sur la situation d'un cours d'eau. En effet, son calcul simple ne demande aucun moyen sophistiqué. L'identification des taxons n'exige pas de connaissances approfondie de systématique et le comptage à l'aide de l'échelle de type logarithmique est à la fois rapide et aisé. Nous avons vu qu'il était performant pour les rivières de Côte d'Ivoire, cependant une vérification de son adéquation pour d'autres cours d'eau ouest-africains devrait être entreprise pour qu'il devienne un moyen normalisé pour rechercher rapidement d'éventuels déséquilibres faunistiques.

46 Toutefois, si ces aspects pratiques ne sont pas contestables, il est cependant évident que le niveau d'identification des taxons adopté n'est pas sans risques. Des substitutions d'espèces peuvent apparaître à l'intérieur d'un taxon. Celles-ci passeront alors inaperçues de l'observateur si le remplacement s'opère à quantité égale. Ainsi, il a été noté que la position centrale du taxon « Simulies autres » observé dans les AFC I et II, correspond au centre de gravité de 3 principales espèces aux comportements très différents vis-à-vis de l'insecticide. *S. adersi* est sensible au téméphos, *S. tridens* insensible et *S. schouledeni* « favorisé » par ce dernier (ELOUARD, 1983). La disparition totale d'une espèce et son remplacement par une autre à l'intérieur d'un même taxon, peuvent donc être totalement masquées. Enfin, il ne faut pas perdre de vue que cet indice tient compte dans sa construction de la sensibilité d'un certain nombre de taxons choisis dans le cadre de l'utilisation d'un insecticide donné : le téméphos. Il n'est donc pas sûr qu'il soit applicable, sans ajustements des coefficients et des pondérations, à d'autres insecticides ou à d'autres pollutions de diverses origines. Seule la structure générale peut en toute sécurité être retenue. Il faut enfin garder à l'esprit que les valeurs obtenues pour l'indice ne constituent que des indications de santé des rivières.

47 Toute variation importante doit provoquer une enquête approfondie sur le terrain pour infirmer ou confirmer les « anomalies » constatées grâce au système d'alerte que constitue l'indice de pollution.

REMERCIEMENTS

48 Nous tenons ici à remercier tous ceux qui nous ont aidé dans l'élaboration de ce document ainsi qu'à la récolte des données de surveillance. Que M. MOUSSA BIHOUM trouve ici l'expression de notre reconnaissance, pour avoir trié pendant de longues journées les échantillons analysés dans ce travail.

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NOTES

1. Ce travail a été financé par une convention OMS-OCP dans le cadre du Programme régional de Lutte contre l'Onchocercose humaine dans le bassin de la Volta.

RÉSUMÉS

Les auteurs proposent un indice biocénotique pour surveiller te degré de pollution des rivières traitées au téméphos dans te cadre du Programme de Lutte contre l'Onchocercose humaine en Afrique de l'Ouest. Après une revue critique de différents indices biocénotiques et de diversité proposés dans la littérature, ils déterminent les qualités nécessaires à un indice biocénotique applicable à la surveillance écologique des rivières ouest-africaines. La formule proposée est une combinaison linéaire pondérée des effectifs de certains taxons surveillés. Une vérification de la validité de l'indice proposé et de ses performances est réalisée.

A biotic index is proposed to monitor the degree of pollution in rivers treated with temephos for blackfly control in West Africa.
After a critical review of the differents biotic or diversity index used previously in aquatic monitoring, the authors point out the qualities requiered by an index which could be useful in the context of a monitoring programme of west african rivers.
The proposed index is a linear combination of the coordonates on the first factorial axe in the reciprocal averaging analyses for the taxa numbers. Examples are given in order to check the validity and the efficiency of the proposed index.

INDEX

Keywords : Pollution, Temephos, Lotic invertebrates, Biotic index, Reciprocal averaging analyses, Onchocerciasis, Ivory Coast
Mots-clés : Pollution, Téméphos, Invertébrés lotiques, Indice biocénotique, Analyse des correspondances, Onchocercose, Côte d'Ivoire

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Homologie d'évolution de peuplements benthiques soumis aux épandages d'insecticides antisimulidiens

Homology of evolution of the benthic communities subject to antiblackfly insecticide spraying

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INTRODUCTION

2 Les études de toxicité permettent de mesurer, en laboratoire ou sur le terrain, la toxicité générale des insecticides envers les organismes aquatiques. Néanmoins, les modifications des abondances des populations ou de structure de peuplement qui apparaissent à long terme, ne sont pas directement déductibles de la sensibilité des organismes (CAIRNS, 1986). Les modifications de chaîne trophique, de compétition spatiale et de prédation se surajoutent à la mortalité due à l'emploi des pesticides. Le phénomène de mortalité compensatoire (PETERSEN et PETERSEN, 1988) joue également son rôle. Il réduit l'ampleur de l'impact *in situ* par rapport à celui mesuré en laboratoire alors que la répétition hebdomadaire des expositions, telle que celle opérée dans le Programme de Lutte contre l'Onchocercose (OCP), l'augmente. Dans ce contexte, les tests fournissent une indication de la toxicité générale des produits mais ne permettent pas de déduire les modifications risquant d'apparaître à long terme. Il en va de même pour les intoxications chroniques qui s'opèrent souvent à faibles doses, celles-ci ne se traduisant pas par des réactions directes, aiguës ou mortelles.

3 Les écosystèmes aquatiques des rivières savanicoles d'Afrique de l'Ouest sont relativement homogènes pour chaque grande zone biogéographique. Au niveau taxinomique de la famille et de la tribu, voire du genre, les peuplements sont identiques

dans leur *composition.* Toutefois, l'ensemble des paramètres physico-chimiques varie d'une rivière à l'autre voire d'une station à l'autre ce qui fait que les *structures* sont différentes. Dans un tel contexte, un programme de surveillance se doit de mesurer l'impact des insecticides épandus par l'OCP, sur chaque rivière. Ceci n'est bien évidemment pas réalisable (« too much time, too much people, too much money » selon l'expression anglo-saxonne) ; seules quelques stations peuvent être suivies.

4 Or, l'étude des modifications structurales, engendrées à long terme dans les peuplements de faune saxicole par les insecticides employés par l'OCP, a permis de mettre en évidence une typologie caractéristique de chaque produit utilisé (Abate, chlorphoxime, B.t., carbosulfan et perméthrine) (ELOUARD, 1983 ; ELOUARD et JESTIN, 1982 ; FAIRHURST et *al.,* 1986 ; ELOUARD et FAIRHURST, 1990).

5 Toutefois, afin d'envisager une surveillance allégée, nous avons voulu savoir si les modifications quantitatives de l'abondance des populations, observées sur une station donnée, étaient extrapolables à l'ensemble des rivières savanicoles. Une telle hypothèse semblait réaliste, car il a été mis en évidence que les structures de peuplements engendrées par les insecticides évoluent de façon semblable, d'une station à l'autre et d'une rivière à l'autre (ELOUARD et SIMIER, 1990) alors que les peuplements d'origine différaient.

6 Si la similarité d'évolution des effectifs des populations se vérifiait, un modèle empirique pourrait être construit. Il s'agirait alors de mesurer les modifications engendrées par les insecticides sur une station de référence, puis d'appliquer les relations obtenues pour chaque insecticide, aux effectifs des taxons comptés sur d'autres rivières. Ce modèle empirique permettrait de prédire l'évolution de la faune sur un grand nombre de rivières traitées par le Programme ; la vérification de sa validité ne demanderait alors que quelques prélèvements de sondage.

MATÉRIEL ET MÉTHODES

7 Dans cette étude, seule la faune saxicole est prise en compte, à l'exclusion de la dérive, car de précédentes analyses effectuées sur des périodes de traitement au téméphos (ELOUARD, 1983), ou à d'autres insecticides (FAIRHURST *et al.,* 1986), il découle que les typologies qu'elle fournit sont les plus typées et mettent bien en évidence les modifications des structures cénotiques engendrées par les insecticides.

8 La récole de la faune saxicole a été réalisée mensuellement au moyen de cinq prélèvements à l'échantillonneur de Surber (20 x 20 cm^2) dans les zones de rapides (0,8 <v <1,4 $m^{s-1)}$ sur les rochers couverts de Podostemaceae. La période sélectionnée s'étend de la décrue à l'étiage, c'est-à-dire des mois de décembre à mars. En effet, l'utilisation du Surber n'est pas significative durant la période de montée des eaux (avril, mai) du fait de l'impossibilité de connaître la date d'immersion des rochers et par conséquent la durée de leur colonisation par les insectes lotiques. De plus, l'échantillonnage de la faune saxicole est impossible durant la saison des hautes eaux en raison de l'immersion profonde des substrats rocheux.

9 Les critères de sélection des stations ont été l'existence de données de références prétraitement conséquentes, suivies de périodes de traitement à l'un ou à plusieurs des insecticides employés par le Programme de Lutte contre l'Onchocercose, également conséquentes. Les stations de Danangoro et d'Entomokro sur la rivière Marahoué (Bassin

du Bandama, Côte-d'Ivoire) ainsi que la station d'Amou-Oblo sur l'Amou (bassin du Mono, Togo) et la station d'Asubende sur la Pru (bassin des Volta), répondaient à ces critères. Les données disponibles pour certaines stations couvrent plus de 13 années de surveillance.

10 Le Programme de Lutte contre l'Onchocercose emploie de façon opérationnelle cinq insecticides qui sont le téméphos ou Abate® et le chlorphoxime (organophosphorés), le *Bacillus thuringiensis* (B.t., insecticide biologique), le carbosulfan (carbamate) et la perméthrine (pyréthrinoïde). Pour des raisons de forte toxicité envers les organismes aquatiques, l'emploi des deux derniers est restreint à la période des hautes eaux.

11 Afin d'obtenir des typologies stables, peu marquées par les taxons rares ou sporadiquement abondants ou encore par trop saisonniers, nous n'avons retenu que les huit taxons : Baetidae, Caenidae, Tricorythidae, Hydropsychidae, Chironomini, Tanytarsini, Orthocladiinae et Tanypodinae. Les Simulies (*Simulium damnosum s.l.* et Simulies autres) ont été négligées car trop caractéristiques des périodes prétraitement.

RÉSULTATS

12 La station d'Entomokro sur la Marahoué a été retenue comme station de référence car nous disposions 1) de nombreuses données de référence ; 2) des données concernant des séquences importantes d'utilisation des trois insecticides épandus de décembre à mars.

13 L'évolution de l'abondance des populations pour les trois insecticides étudiés (Abate, chlorphoxime, B.t.) est mesurée à l'aide du rapport :

$$R/P = \frac{\log APTi}{\log AVT}$$

14 où log APTI est le logarithme de **l'effectif moyen pour la période de traitement** à l'insecticide i et où log AVT est le logarithme de **l'effectif moyen avant traitement.**

15 Pour chaque taxon, ainsi que pour la faune totale on obtient donc un rapport Abate (R/P ABA), un rapport chlorphoxime (R/P CHL) et un rapport B.t. (R/P B.t.) *(tableau 1).*

16 Les valeurs de ces rapports obtenus pour la station d'Entomokro sont ensuite appliquées aux effectifs récoltés avant traitement sur les stations de Danangoro (Marahoué), d'Amou-Oblo (Amou) et d'Asubende (Pru). Les valeurs obtenues (valeurs calculées = C) sont comparées aux données recueillies dans le cadre de la surveillance des rivières (valeurs observées = 0) *(tableau 1).*

17 Pour la faune totale, l'écart entre les valeurs calculées et les valeurs observées est très faible *(fig. 1)* ; le coefficient de corrélation entre les deux séries de valeurs étant r = 0,98.

18 L'étude par taxon montre que la prédiction de ce modèle est excellente pour les Caenidae et les Hydropsychidae, bonne pour toutes les valeurs sauf une en ce qui concerne les Baetidae, les Chironomini, les Tricorythidae et les Orthocladiinae. Elle est par contre médiocre pour les Tanypodinae et les Tanytarsini.

Tableau 1. Moyennes des valeurs mesurées (O) et valeurs estimées (C) pour chacun des insecticides utilisés, à partir des données d'Entomokro. ***Table 1. Means of measured (O) and estimated (C) population abundances for each insecticides.***

	BAE	CAE	TRI	PSY	CHI	TAT	OCL	TAP	Total
EK AVT	219,5	15,1	340,6	423,2	47,6	16	39,5	26,4	1127,9
EK ABA	47,4	36,5	5	771	479,4	673,6	291,9	38,3	2343,1
EK CHL	24,2	14	0,2	6	264,5	250,2	270,7	5,7	835,5
EK B.t.	311,7	40,2	8,4	660,9	93,1	5,7	25	9,4	1154,4
LOG AVT	2,34	1,18	2,53	2,63	1,68	1,2	1,6	1,42	3,05
LOG ABA	1,68	1,56	0,7	2,89	2,68	2,83	2,47	1,58	3,37
LOG CHL	1,38	1,15	−0,7	0,78	2,42	2,4	2,43	0,76	2,92
LOG B.t.	2,49	1,6	0,92	2,82	1,97	0,76	1,4	0,97	3,06
DAN AVT	398	8,7	17,3	476	13,4	20	51,4	17,1	1001,9
LOG DAN AVT	2,6	0,94	1,24	2,68	1,13	1,3	1,71	1,23	3
DAN ABA O	694,1	18	5,8	995	79,1	127,1	162,3	10,5	2091,9
DAN ABA C	72,6	17,6	2,2	877,4	63,2	1137,7	438,4	23,6	2055,9
DAN CHL O	44,7	4,2	0,3	72,8	557,5	30,7	395,6	15,2	1121
DAN CHL C	34,4	8,2	0,5	6,2	42,4	390,2	404,3	4,5	745,9
DAN BT O	397,7	16,4	1,5	700	80,7	8,1	53,2	12,6	1270,2
DAN BT C	587,5	19	2,8	749,8	21	6,6	31,5	7	1025
AMOU AVT	12,1	2,3	1,5	5,1	19,2	23,8	119,2	1,5	184,7
LOG AMOU AVT	1,08	0,36	0,18	0,71	1,28	1,38	2,08	0,18	2,27
AMOU BT O	16,4	0,9	3,2	24,4	17,2	6,5	139,3	30	237,9
AMOU BT C	14,2	3,1	1,2	5,8	32,1	7,3	65,8	1,3	187,9
PRU AVT	53,1	4,8	5,4	10,1	7,7	3,6	32,8	2,4	119,9
LOG PRU AVT	1,73	0,68	0,73	1	0,89	0,56	1,52	0,38	2,08
PRU BT O	14,2	0,45	16,9	34,2	13,2	0	38,4	3,72	121,1
PRU BT C	68,8	8,5	1,9	12	11	2,2	21,2	1,8	121,8

ABRÉVIATIONS :
TAXONS :
BAE = Baetidae
CAE = Caenidae
TRI = Tricorythidae
PSY = Hydropsychidae
CHI = Chironomini
TAT = Tanytarsini
OCL = Orthocladiinae
TAP = Tanypodinae
STATIONS :
EK = Entomokro
DAN = Danangoro
AMOU = Amou-Obio
PRU = PRU
INSECTICIDES :
ABA = Abate ou téméphos
CHL = Chlorphoxime
B.t = Bacillus thuringiensis
AVT = Avant traitement
(= Before treatment)
R/P = log APTi/log AVT

DISCUSSION ET CONCLUSION

19 Les valeurs des rapports entre les effectifs dénombrés après et avant l'action d'un insecticide sont tantôt supérieures, tantôt inférieures à l'unité *(tableau 1).* Lorsque ce rapport est supérieur à l'unité, cela signifie qu'il y a une augmentation des effectifs durant la période soumise aux épandages d'insecticides antisimulidiens. Or, les mesures faites lors des tests des insecticides montrent que tous les produits sont plus ou moins toxiques vis-à-vis de la faune non-cible. Une relation directe entre la toxicité directe et l'abondance des populations devrait se traduire par une réduction de toutes les

populations, réduction plus ou moins proportionnelle à leur sensibilité, ce qui n'est pas le cas. Les réactions des populations mesurées *in situ* ne correspondent pas toujours à la toxicité évaluée lors des tests.

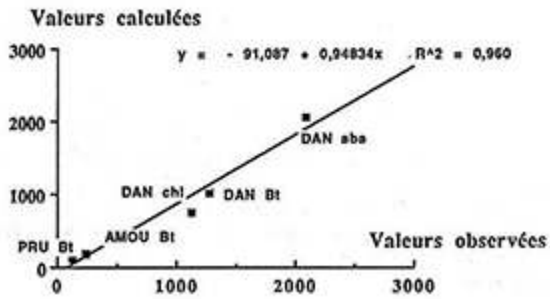

Figure 1. Corrélation entre les valeurs observées et les valeurs théoriques calculées selon le modèle empirique, des populations des taxons peuplant les stations de Danangoro (D), d'Asubende (As) et d'Amou-Oblo (Am). Prédiction pour l'Abate (a), le chlorphoxime (chl) et le B.t. (bt).
Correlation between the observed and the theoretical values following the empirical model, for the populations of the taxa living on the Danangoro (DAN), Asubende (PRU) and Amou-Oblo (AMOU) sampling sites. Prediction for Abate (a), chlorphoxim (chl) and B.t. (bt).

20 Mais on notera que les valeurs des rapports sont les plus proches de l'unité pour le B.t., insecticide considéré comme le moins toxique envers la faune non-cible (DEJOUX *et al.,* 1985), alors qu'elles sont les plus fortes pour le chlorphoxime relativement plus toxique (DEJOUX et TROUBAT, 1976 ; DEJOUX *et al.,* 1982) les valeurs du rapport pour l'abate étant intermédiaires comme l'est aussi sa toxicité (ELOUARD, 1983). La mesure de la toxicité directe permet donc de préjuger de l'ampleur des modifications qui risquent d'apparaître dans le milieu, mais pas de les quantifier.

21 La prédictibilité du modèle empirique pour les effectifs de la faune totale est excellente, ce qui constitue un résultat très prometteur dans le cadre des études d'impact global des insecticides sur la faune invertébrée. Toutefois, étant donné que la prédiction peut s'avérer médiocre pour certains taxons, il est possible que les résultats concernant l'ensemble de la faune soient artéfactuels. Ce point mériterait d'être vérifié.

22 Les raisons justifiant le mauvais ajustement du modèle pour les Tanytarsini et les Tanypodinae nous échappent. Cette inadéquation pourrait provenir de leurs modes de distribution (distribution agrégative vis-à-vis de paramètres du milieu non pris en compte dans cette étude) ou de problèmes de prolifération de leurs populations lors de la libération de certaines niches. Enfin, pour les Tanytarsini, il se peut que nous n'ayons pas affaire aux mêmes espèces d'une station à l'autre, vu leur nombre dans la zone du programme (plus de 30 espèces présentes).

23 Il faut également retenir que les calculs ont été faits en considérant que seuls les insecticides utilisés durant la période décembre-mars, régulaient les populations de la faune saxicole. Il est bien évident que certaines années, il perdure une pertubation engendrée par la perméthrine utilisée durant la saison des hautes eaux (ELOUARD et SIMIER, 1990). Des recherches approfondies sur les périodes de recrutement, la durée des cycles et les potentiels de recolonisation sont nécessaires pour déterminer l'incidence de tels épandages sur les valeurs mesurées durant la décrue et l'étiage et donc sur l'adéquation du modèle à tous les taxons.

24 En ce qui concerne ce modèle empirique, nous concluons qu'il constitue une bonne approche de la réalité puisque, dans la majorité des cas, l'estimation est satisfaisante et toujours du même ordre de grandeur que la valeur observée. Il constituera donc un outil

permettant d'estimer, au moins pour certains taxons, l'évolution des populations sur des rivières peu surveillées. Ces résultats nous informent en retour, et bien que cela semble un truisme, que les mêmes causes produisent les mêmes effets, à savoir que les populations de la majorité des taxons évoluent de façon semblable quand elles sont soumises aux mêmes insecticides.

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NOTES DE FIN

1. Les commentaires seront reçus jusqu'au 15 septembre 1992.

RÉSUMÉS

L'utilisation hebdomadaire d'insecticides pour lutter contre les Simulles modifie l'abondance des populations benthiques. Les variations ne sont ni de la même ampleur ni du même signe, selon l'insecticide (Abate, chlorphoxime, B.t.) et les taxons.
Parmi les dizaines de rivières savanicoles traitées aux insecticides antisimulidiens, seules quelques unes font l'objet d'une surveillance de la faune non-cible. La problématique de ce travail est de savoir s'il est possible de généraliser à l'ensemble des rivières, les fluctuations d'abondance des populations observées sur une station de référence.
Sur la station de référence, l'évolution de l'abondance des populations pour les trois insecticides étudiés est mesurée à l'aide du rapport : R/P = log APTi/log AVT, où log APTi est le logarithme de l'effectif moyen pour la période de traitement à l'insecticide i et où log AVT est le logarithme de l'effectif moyen avant traitement. Pour chaque taxon ainsi que pour la faune totale on obtient donc un rapport Abate, un rapport chlorphoxime et un rapport B.t.
Les valeurs de ces rapports obtenus pour la station d'Entomokro, sont ensuite appliquées aux effectifs récoltés avant traitement sur trois autres stations. Les valeurs calculées sont comparées aux valeurs observées dans le cadre de la surveillance des rivières.
Pour la faune totale, l'écart entre les valeurs calculées et les valeurs observées est très faible, r = 0,98.
L'étude par taxon montre que la prédiction de ce modèle est excellente pour les Caenidae et les Hydropsychidae, bonne pour les Baetidae, les Chironomini, les Tricorythidae et les Orthocladiinae, médiocre pour les Tanypodinae et les Tanytarsini.

The weekly utilization of insecticides sprayed by the Onchocerciasis Control Programme to control *Simulium damnosum* larvae modifies the abundance of the non target benthic populations. These insecticides (B.t., Abate and chlorphoxim) have neither the same toxicity nor the same selectivity for the principal taxa. In consequence, the long-term variations of the abundance of the populations are neither of the same importance nor of the same sign (some of them remain the same or decrease while others increase), according to the product and the taxa. In long term, these variations being mainly the consequence of direct toxicity but also take into account the duration of the life cycles as well as spatial and trophic competitions.
In this study, only the saxicolous fauna is taken into account. It has been collected on rocks with the Surber sampler during the low water period (December to March).
Among the ten savannah rivers treated with antiblackfly insecticides, only few are the object of a non-target fauna monitoring. The object of this work is to find out if it is possible to generalize to all the rivers, the variations in population abundance observed for an insecticide on a control station.
In the reference station, the variation of population abundance for each of the insecticides is calculated by means of the ratio R/P = log APTI/log AVT, where log APTi is the logarithm of the average for the period treated with the i insecticide, and where log AVT is the logarithm of the average before treatment. For each taxa as well as for the total fauna, Abate, chlorphoxim and B.t. ratio were defined.
The values of these ratio obtained for the Entomokro station on the Marahoué river in the Ivory

Coast are then applied to the data collected before treatment on three rivers or stations (Amou-Oblo on the Amou river in Togo, Asubende on the Pru river in Togo and Danangoro on the Marahoué river in the ivory coast). The calculated values are then compared to the values collected after treatment in the river monitoring programme.

In the reference station the ratio of the populations collected before and after treatment are sometimes greater, sometimes lower than unit (when the ratio is greater than unit, it means that there is an increase of the abundance of populations during the Processing period).

The ratios are very close to the one for the B.t. which is the less toxic insecticide against the non target fauna, the greatest for the chlorphoxim which is the more toxic product and intermediate for Abate which has an intermediate toxicity. It appears that the more insecticides were estimated to be toxic in gutter tests, the more population abundance differ from the reference ones.

For the total fauna, differences between the values calculated ans observed were very low (r = 0,98). For the taxa, the prediction of this model is excellent for Caenidae and Hydropsychidae, good for Baetidae, Chironomini, Tricorythidae and Orthocladiinae, but mediocre for Tanypodinae and Tanytarsini. The authors submit some hypotheses to explain the bad adequation of the model for these two taxa; hypotheses based on taxonomic problems and niche releasing.

INDEX

Mots-clés : insecticide, benthos, impact, modèle, onchocercose, Afrique de l'Ouest

Keywords : insecticide, benthos, impact, model, onchocerciasis, West Africa

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Longitudinal zonation of lotic insects in the Bandama River System (Ivory Coast)

François Marie Gibon and Bernhard Statzner

Introduction

1 Recently Lévêque *et al.* (1983) evaluated the longitudinal zonation of the macrofauna in the Bandama basin, a river System extending about 1000 km from north to south through the Ivory Coast (West Africa). They distinguished three zones: (1) headwaters and small affluents, (2) a considerable, long mid-reach, which was relatively uniform and (3) a relatively short lower reach (estuary). With regard to invertebrates the mid-reaches in the main courses of the Bandama basin were mainly characterized by the caddisfly family Hydropsychidae, particularly by *Cheumatopsyche falcifera* and *C. digitata* at periods of low discharge (see Fig. 15 and 16 in Lévêque *et al.*, 1983).

2 This is an odd zonation pattern compared to those found in other geographical areas (Illies & Botosaneanu, 1963; Hawkes, 1975; Botosaneanu, 1979). A recent study of the taxonomy and the distribution of the Hydropsychidae in the Ivory Coast (Statzner, 1984; Statzner & Gibon, 1984) allows a more detailed definition of the above zonation. Results on Hydropsychidae and two other lotic insect groups so far available, the caddisflies Philopotamidae (Gibon, 1984) and the blackflies of the *Simulium damnosum* complex (Vajime & Quillévéré, 1978; Quillévéré, 1979 and person. commun.) give evidence, that the long mid-reaches exhibit no uniform faunistic stream zone.

Methods and the area studied

3 The papers mentioned in the introduction, as far as they deal with the Ivory Coast, contain detailed information on the methods used and the area studied. Thus only the essentials will be briefly reviewed here.

4 The distribution patterns of the:

1. *Simulium damnosum* complex are exclusively based on benthic samples from 35 localities,
2. Hydropsychidae are mainly based on benthic samples, for some localities additional information from light trap samples was available. Only a few places were surveyed by light trap sampling. The number of sampling stations in the Bandama basin exceeded 50,
3. Philopotamidae are exclusively based on light trap samples, since there are still unsolved problems in the larval taxonomy. 31 localities were considered in the Bandama basin.

5 8 monitoring points in the Bandama System were sampled for Trichoptera at monthly intervals over more than one year, and therefore more information was available for them than for the other stations. The main courses of the Bandama basin were more intensely studied than the smaller affluents. The differences in the sample techniques, discussed in detail by Statzner (1984), lead us to treat the results as follows: even if a species was missing at a certain locality it was considered to be 'present' there, if we found it north and south of this locality in the appropriate affluent at some time. Thereby we exclude the north-south-north-shifts of species (Quillévéré, 1979; Elouard, 1983), related to the climatic annual cycle, from our considerations, and deal with the 'maximal distribution' reported in any of the above groups. It should be noted that the uppermost distribution limits of the Trichoptera species are less precise (Statzner, 1984), since (1) it was not always possible to sample the northern parts of the streams due to cessation of the flow and (2) no samples were available from the upper Bandama and upper N'Zi from the period before regular insecticide treatment of these stream reaches were introduced through the Onchocerciasis Control Programme. The latter may have affected the presence of Macronematinae in those regions. No data exists from the period before the construction of the Kossou reservoir. We assume that the species composition in the reach now dammed was similar to that we found upstream of the lake. Taabo reservoir was build after the entomological sampling programme in the area.

6 The most obvious longitudinal gradient in the abiotic factors of the Bandama system is to be found in the discharge pattern. On average the northernmost streams in the basin are running waters for only just over half the year. In the Southern direction the period without flow becomes shorter and shorter, until the stream is a permanent one. Due to large variations in the annual climate the periods without flow as well as the borderline between temporary and permanent stream are not fixed. In stream reaches in which flow ceases water usually remains in pools during the dry season.

7 Thus, in contrast to nearly all streams so far studied with regard to the longitudinal zonation of the macrozoobenthos, the main channel and all its large affluents do not start with a well-defined source. Therefore, for most of the year, all streams originate in a lower reach where the slope is gentle.

8 Compared to the key factor 'discharge pattern' all other abiotic factors so far considered as important in stream zonation studies exhibit relatively weak longitudinal gradients in the Bandama system (Lévêque *et al.,* 1983).

Results and discussion

9 The longitudinal distribution of the three groups under study (coded in Table 1) gives evidence that the 'zone à Hydropsychidae' sensu Lévêque *et al.* (1983) is not homogeneous in the Bandama River system (Fig. 1). In smaller streams situated west (Niouniourou) or

east (Agneby) of the Bandama these three lotic insect groups extend further south than in the Bandama itself, where no rapids occur in the lower reach.

10 In the main courses of the Bandama basin a steady increase in the species number was generally observed from north to south and almost no loss of northern species on the way south downstream to the lowest riffles of the Bandama. If a species disappears on the way south in the 'zone à Hydropsychidae' it is certainly a rare one, e.g., *Aethaloptera sp.* (2), *Polvmorphanisus sp. I* (3) and *II* (4), or *Protomacronema pubescens* (K). The latter was found further south in other stream Systems. Apparently major changes in the Trichoptera distribution take place at locations sampled at monthly intervals over a longer period (monitoring points!), and isolated occurrences were usually reported from these localities. The distribution patterns of these rare or isolated species are rather uncertain. The smaller affluents of the Bandama basin contain a fauna similar to that of the headwaters of the main rivers of the system. The example included in Figure 1 is a combination of data from various tributaries studied at various distances from the main courses and therefore represents an ecological and not a purely geographical classification. A typical species of small tributaries is *Macrostemum inscriptum.*

11 The general pattern of species distribution from 'sources' downstream to the lowest rapids of the Bandama is not a clear species replacement, as is usually found in benthic macroinvertebrates (see reviews referred to in the introduction), but a steady increase of species numbers on the way downstream due to the occurrence of additional species. We assume this to be the typical zonation pattern of lotic insects in streams running from

north to south over a considerable distance, having no well-defined source, and a gentle slope in this part of Africa.

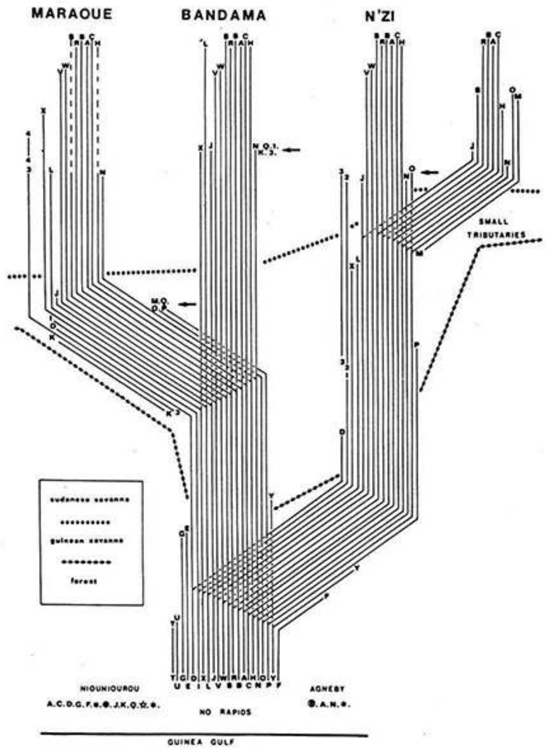

Fig. 1. Schematic presentation of the longitudinal distribution reported for three lotic insect groups in the Bandama River system before the construction of reservoirs as well as their occurrence in smaller streams situated west (Niouniourou) or east (Agneby) of the Bandama. Presence of species in small tributaries of the Bandama System consisting of samples from several streams. The streams generally flow from north to south. starting in the sudanese savanna. crossing the guinean savanna, and then entering the forest area. The length of the Bandama is approximately 1000 km. Codation of species in Table I. Arrows: monitoring stations (higher sampling frequency); dot behind the species code: isolated occurrence.

Table 1. **Code for Hydropsychidae. Philopotamidae *(Chimarra spp.).* and Simuliidae *(Simulium spp.)* used in Fig. 1. Letters: adults and associated larvae; numbers: unassigned larvae; graphic symbols: species found only in small stream west or east of the Bandama System.**

A	*Cheumatopsyche digitata*
B	*C. copiosa*
C	*C. falcifera*
D	*C. albomaculata*
E	*C. gibbsi*
F	*C. akana*
G	*C. sexfasciata*
1	*C. sp VIII*

■	*C. sp XI*
H	*Amphipsyche senegalensis*
I	*A. herneri*
J	*Protomacronema barnardi*
K	*P. pubescens*
L	*Macrostemum alienum*
M	*M. inscriptum*
●	*M. pulcherrimum*
N	*Aethaloptera dispar*
2	*A. sp*
O	*Polymorphanisus angustipennis*
P	*P. hargreavesi*
Q	*P. elisabethae*
3	*P. sp I*
4	*P. sp II*
◌	*Leptonema sp II*
R	*Chimarra sassandrae*
S	*C. petri*
T	*C. intextu*
U	*C. prodhoni*
*	*C. triangularis occidentalis*
V	*Simulium damnosum ss.*
w	*S. sirbanum*
X	*S. soubrense*
Y	*S. sanctipauli*
O	*S. vahense*

12 Specific differences in the ability of Hydropsychidae to survive periods without flow as larvae have been reported elsewhere (Statzner, 1982). In addition, whether adults are capable of surviving droughts or recolonizing a stream reach after the resumption of flow by immigration from other localities will mainly determine, how far a species extends to the north. In the southernmost rapids of the Bandama basin several species occur which are generally found in streams of the forest area. In this region the guinean savanna is extending further south than alsewhere in the country and the species distribution indicate a zone of faunistic transition.

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ABSTRACTS

The longitudinal zonation of three lotic insect groups, viz. Hydropsychidae (Trichoptera), Philopotamidae (Trichoptera), and the *Simulium damnosum* complex (Diptera) in the Bandama River System (West Africa) is anomalous when compared to the patterns known from other geographical areas: In general from the highest to the lowest riffles downstream there appears to be no clear species replacement. Instead, there is a steady increase of species numbers due to the occurrence of additional species. These distributions are related to the gradient in the length of the period without flow from the north (temporary streams) to the south (permanent running waters), the consequent lack of well-defined sources and the relatively gentle slopes of the channels.

INDEX

Keywords: tropical river, faunistic stream zonation, Hydropsychidae, Philopotamidae, Simulium, Africa

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Impact of combined large-scale ivermectin distribution and vector control on transmission of *Onchocerca volvulus* in the Niger basin, Guinea

Impact de la distribution à grande échelle d'ivermectine associée à la lutte antivectorielle d'Onchocerca volvulus dans le bassin du Niger (Guinée)

P. Guillet, A. Sékétéli, E.S. Alley, H. Agoua, B.A. Boatin, Y. Bissan, L.K.B. Akpoboua, D. Quillévéré and E.M. Samba

Introduction

1 Before ivermectin became available, onchocerciasis control was mainly directed against the vectors of the disease. The basic strategy of the WHO Onchocerciasis Control Programme in West Africa (OCP) consisted in interrupting transmission of the disease by continuing to destroy the vector larvae until the parasite reservoir in humans died out. For this to be achieved, 12-15 years of uninterrupted vector control was necessary (*1, 2*).

2 The characteristics of ivermectin (single dose, prolonged microfilaricidal effect, and absence of harmful side-effects) make it highly suitable for controlling the disease effectively. During the early treatment trials it was noted that ivermectin also reduced transmission of the *Onchocerca volvulus* parasite. This was established, first, by gorging blackflies on treated onchocerciasis patients (*3-6*); subsequently, community trials confirmed the reduction of transmission under field conditions in West (*7, 8*) and Central Africa (*9*) as well as in Central America (*10*). In African savanna areas, this reduction is reflected immediately after treatment by a 70-85% decrease in blackfly infectivity (No. of

infective *O. volvulus* larvae per 1000 parous flies) and in natural transmission, without any vector control (*7, 20*).

3 In holoendemic areas, even though a reduction in the transmission of onchocerciasis of about 80% is considerable, it is not sufficient to lead to the extinction of the parasite in humans within a reasonable time. Ivermectin alone does not therefore seem capable of replacing larvicides for control of onchocerciasis transmission, at least at present. On the other hand, it rapidly improves the well-being of patients and stops almost completely the progression of some ocular lesions. A period of 7-10 years of vector control is required to obtain the same result. However, some beneficial effects of ivermectin have been called into question following a trial in Sierra Leone (*11, 12*).

4 Ivermectin has been widely distributed in the OCP area as an adjunct to vector control, and even used on its own in some areas (northem part of the western extension). Priority has been accorded to areas where there was a high risk of blindness and where vector control had just started (western and south-eastern extensions).

5 In the western extension, the first large-scale distribution of ivermectin began in 1988 in the Milo basin in Guinea and continued subsequently at a frequency of one treatment per year, gradually extending the areas under treatment. At first, the distribution was entrusted partly and then completely to the national health personnel of the countries concerned. For 1991 alone, more than 505 000 persons were treated in the OCP western extension area. At the same time, vector control operations were being carried out normally.

6 The present article evaluates the impact of this type of distribution on the transmission of onchocerciasis under operational conditions. Since there were no data on the large-scale use of ivermectin alone, its impact was evaluated in combination with vector control. For this purpose, results obtained in the Niger basin in Guinea, where ivermectin has been combined with vector control, were compared with those obtained previously in the original OCP area through vector control alone.

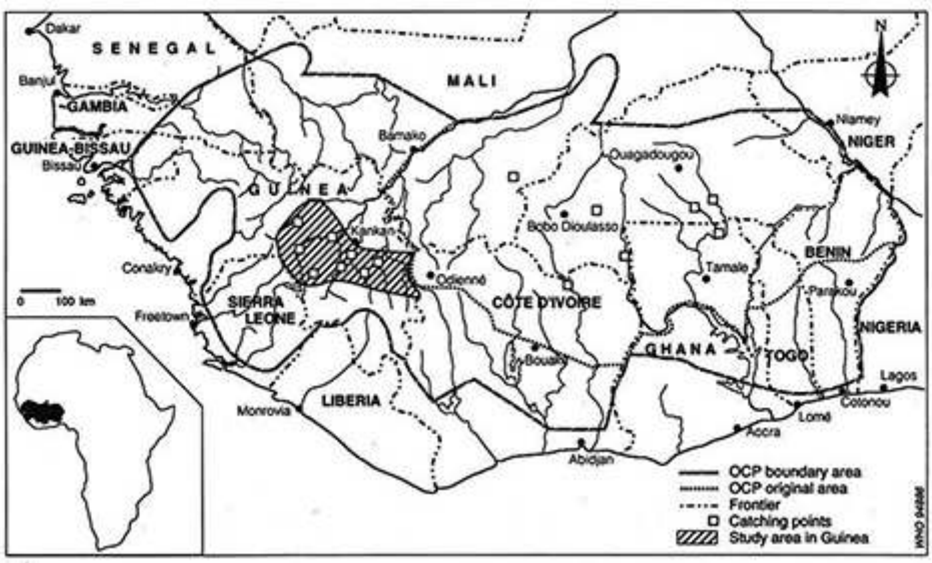
Materials and methods

7 All the 16 catching points selected for the study lie in savanna areas in holoendemic foci: eight in the Niger basin in Guinea and eight in the original OCP area (Mali, Côte d'Ivoire, Ghana, and Burkina Faso (Fig. 1)). The principal vectors were *Simulium sirbanum* and *S. damnosum* s.s. However, in Guinea, in the southern part of the area considered, other species such as *S. squamosum* or geographical forms of *S. soubrense* (Milo and Menankaya) have played a seasonal role in the transmission. In this area, some of the catching points selected were highly reinvaded up to 1986 *(13).* These reinvasion phenomena were almost brought under control in 1987 and 1988 and completely overcome in 1989 through the extension of larviciding to the west and south-west, notably in Sierra Leone (*23*). On the other hand, all the points located in the original OCP area were outside the classic reinvasion sectors, and the corresponding entomological results therefore reflect fully the impact of vector control alone on transmission, without any skewing caused by reinvasion. Larviciding began on some rivers in the original OCP area in 1975 but was fully operational by 1977.

8 The catches were made 1-2 days per week, 52 weeks per year, according to the standard procedures defined by OCP (*14, 15*). Of the various entomological criteria normally used for quantifying transmission (*16*), the following were used:

- biting rates (calculated weekly (WBR), monthly (MBR) or annually (ABR));
- transmission potentials calculated from infective larvae in the head (L_3/H) and expressed weekly (WTP), monthly (MTP) or annually (ATP);
- percentages of infective parous flies (with thirdstage larvae in the head); and
- number of L_3/H per infective bite.

Fig. 1. Map showing the locations of the catching points In the OCP original area (larviciding) and In Guinea where larviciding and large-scale Ivermectin treatment were combined.



9 The MTPs or ATPs can be used to quantify transmission, while the other criteria are used to evaluate the extern of blackfly infection by the larval stages of *O. volvulus.* Of the various developmental stages, only L_3/H were used because they could be counted precisely during routine OCP operations. The entomological results were calculated on a monthly basis from May to April because the attackphase larviciding started in Guinea in May 1989. Also, May coincided with the usual end of the annual ivermectin distribution campaigns. Seasonal larviciding was carried out in Guinea from March to July in 1987 and 1988 as part of studies of the reinvasion phenomena *(13).*

10 The epidemiological data were collected and analysed according to the standard procedure developed by OCP (*17, 18*). The criteria adopted were prevalence of microfilariae (mf) in the skin (mfs) and the mean community microfilarial load (CMFL), i.e., the geometric mean mf count per snip (mf/s) among persons aged ≥20 years. Epidemiological surveys conducted beforehand were used to evaluate the endemicity level in the whole western extension before the beginning of the operations, especially in Guinea (*19*); for the eight villages close to the catching points selected, the initial mean prevalence of mfs was 74.4% and the CMFL, 42.2 mf/s. In the original OCP area, close to the selected catching points, the initial mfs was 70.3% and the CMFL, 34.9 mf/s.

11 Ivermectin was distributed at a dose of 150 µg/kg, respecting the exclusion criteria adopted by the manufacturers. The zone treated in Guinea stretches across the greater part of six river basins, covering almost 80 000 km^2 (Sankarani to Tinkisso river basins).

The Milo river basin has been treated since 1988 (five annual treatments), the Niandan, Mafou and Niger basins since 1989, and the Sankarani and Tinkisso basins since 1990. By 1990 large-scale ivermectin distribution had started in all the basins in the study zone in Guinea.

Results

Ivermectin distribution

12 Between 1990 and 1992, the number of villages covered by ivermectin treatment increased by a factor of three and the number of people by a factor of six, the latter reflecting a significant increase in treatment compliance. In 1992 a total of 91 840 persons were treated in 550 villages.

13 The treatment was generally very well received. Most of the frontline villages in the zone, or those having a CMFL >5 mf/s, are currently being treated. Unfortunately, there are very few data on trends in the prevalence of mfs and in microfilarial loads because of the inconvenience of making repeated skin snips. However, there are data for four villages that had two successive treatments. These data were collected 12 months after ivermectin treatment, just before the following treatment. As expected, the prevalences of mfs have not decreased much; however, the CMFLs have decreased by 60–80% relative to the pre-treatment level (Table 1).

Transmission

14 For the original area, the study covered 10 years during which 34 492 blackflies were caught at the eight selected points, of which 87.8% were parous. Unfortunately, for the original area no baseline entomological data were collected before the start of vector control operations. On the other hand, for Guinea, the data for 1985–86 and 1986-87 were collected before any form of intervention. In Guinea, the intervention covered the period 1987–92, and a total of 206 765 blackflies were caught before the 1989-90 season and 33 432 since then, of which 55% and 91%, respectively were dissected; the proportion of parous females was 74.9% before the control operations and 82.1%, subsequently. To facilitate the presentation of the results, we grouped all the entomological data for the original area according to the number of years of vector control. Thus, the data for 1975-76 group together the means observed after a year of control for all eight catching points, although the operations began gradually over the period 1975-77.

Table 1: Mean prevalences of microfilariae in the skin (mfs) and CMFLs[a] in the Niger basin, Guinea, calculated 12 months after the first two ivermectin treatments

Village	Precontrol: Prevalence (%)	Precontrol: CMFL (mf/s)	After 1st treatment: Prevalence %	After 1st treatment: CMFL (mf/s)	After 2nd treatment: Prevalence (%)	After 2nd treatment: CMFL (mf/s)
Bindougou	71.3	35.1	65.2	19.1	63.6	7.5
Faroro	73.2	15.9	39.7	2.6	42.7	5.7
Wassaya	58.6	13.4	27.2	1.3	NA[b]	NA[b]
Keniedougouba	62.7	38.1	59.8	19.3	67.8	10.3

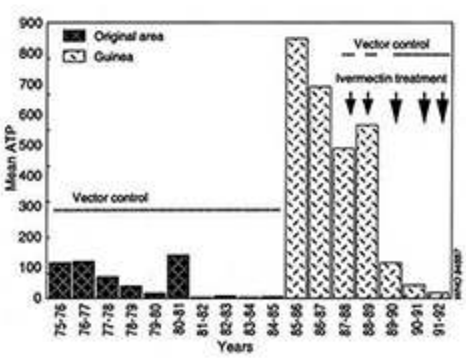
[b] Not available.

15 A preliminary significant decrease in transmission and even in the infectivity of the blackflies was observed in Guinea after the first two seasonal applications of larvicide in 1987 and 1988 (Fig. 2). These larvicide applications, which were aimed at interrupting reinvasion of the original OCP area, were carried out during the period of highest transmission. Furthermore, the Milo form of *S. soubrense,* which generally had high infectivity, had been virtually eliminated as a result of these larvicide applications.

16 A marked decrease in transmission was observed in the First year of continuous vector control in 1989-90 in Guinea (Fig. 2). From the second year, transmission was, at all the points, reduced below the tolerable threshold (100 L_3/H per person per year). It is difficult to dissociate the effect of ivermectin from that of vector control; however, in the original area, with vector control alone, it took longer to obtain the same result.

17 More significant is that the proportion of infective flies decreased rapidly from the first ivermectin treatments. After 2 years of large-scale ivermectin treatment (full coverage), the reduction was 64.6% (Fig. 3). At the same time, the number of L_3/H per 1000 parous flies had fallen by 75.7%. Compared with the 1986-87 data, before treatment began, the decrease was 78.8% for the infective flies and 82.9% for L_3/H. In the original OCP area, it took 6 years of vector control for the blackfly infection to start to fall, and 9 years before it reached the values observed in Guinea after only 3 years of combined use of larvicides and ivermectin.

Fig. 2. Mean annual transmission potential (ATP) for selected catching points in the OCP original area and in Guinea (8 catching points in each zone). The small arrows indicate that the ivermectin treatment was limited to only a few villages; and the big arrows, the large-scale ivermectin treatment.



18 The mean parasite load of infective flies for each year remained relatively stable for 9 years in the original area and for 6 years in Guinea (Fig. 4). The mean values (1.5-2) are quite representative of the species involved in the transmission (*S. sirbanum* and *S. damnosum* s.s.). However, a decrease in the load occurred in both areas after 9 years of vector control and 3 years of combined use of larvicides and ivermectin. In the latter case, the decrease was perhaps due to ivermectin, but it is too early to draw a conclusion from this.

Impact of ivermectin treatment during a breakdown in vector control in 1992

19 In February and March 1992, because of the use of a defective larvicide, the entomological situation deteriorated quickly in some river basins under vector control in the original

OCP area and in Guinea. Six catching points were chosen in the most affected basins, including three along the western limit of the original area and three in Guinea. The results were for a total of 9390 flies caught over 2 months, 53% of which were dissected. In both cases, the *O. volvulus* strain, the vectors, the larviciding period, and the insecticide used were the same. Also, the parous rates were similar.

Fig. 3. Number of infective flies and larvae per 1000 parous flies in the OCP original area and Guinea (arithmetic mean calculated for 8 catching points in each zone).

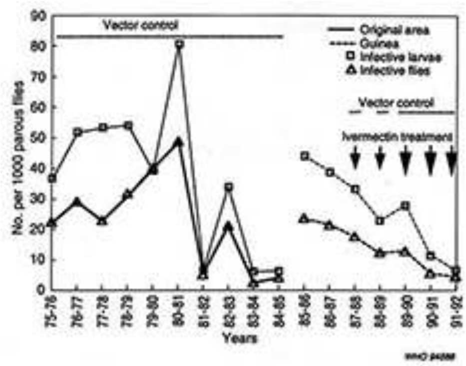
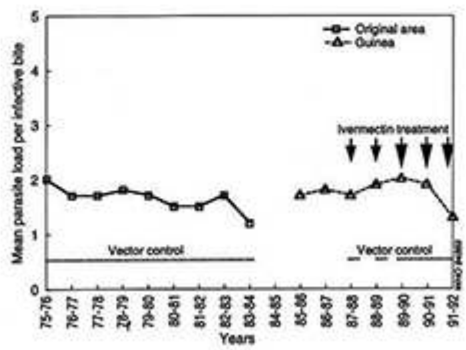


Fig. 4. Number of Infective *Onchocerca volvulus* larvae (L_3/H) per infective bite (arithmetic mean calculated for 8 catching points in each zone).



20 The average transmission potential observed during this period in Guinea was 7.3 compared with 93.7 for the original area (Table 2). For the same number of blackflies caught, transmission in the original area was 5.6 times higher; for parous flies alone, the difference was 5.7 times. Consequently, while the breakdown on control had little effect in Guinea, for two of the three selected points in the original area, the acceptable transmission potential for 1 year (100 L_3) had been exceeded in only 2 months. Such a level of transmission in the original OCP area is perhaps surprising since it should have been under control for more than 12 consecutive years. However, the three catching points selected were located in initially highly reinvaded areas. Only after 1987 were the reinvasion problems overcome and transmission interrupted, even though it had been greatly reduced previously.

Discussion

21 The combined use of vector control and ivermectin has rapidly enabled transmission of onchocerciasis to be interrupted almost completely from the Niger basin in Guinea. The

direct effect of ivermectin on transmission is perceptible in the infectivity of the flies. After only 3 years, the proportion of infective flies and their total parasite load (L_3/H) are as low as those obtained after 7-10 years of effective and continuous vector control in the absence of reinvasion. The results that we have described here for the original area are for eight catching points, all of which are located in zones where vector control has fully attained its objectives within the initially planned time limits.

22 In the original OCP area, the blackfly infestation has followed the same trend as the prevalences of mfs. For the latter, a plateau level is observed for 6-8 years followed by a rapid fall, while the CMFLs decrease more regularly (7). Between two annual ivermectin treatments, a considerable proportion of the treated individuals presented very low microfilarial loads for several months. The values calculated 12 months after treatment led to a large underestimation of the impact on the epidemiological indicators and explain why the proportion of infected flies caught throughout the year decreased much more rapidly than the observed prevalences of mfs.

Table 2: Impact of ivermectin treatment during breakdown in vector control in 1992[a]

Treatment	For female blackflies: No. caught	No. infective/1000 P[b]	L_3/1000 P[c]	Transmission potential
Larvicides alone				
Madina Diassa	1192 (87.3)[d]	3.8	6.6	55
Niamotou	1160 (84.5)	5.2	10.4	110
Vialadougou	3717 (85.0)	4.1	4.1	116
Total	6069 (85.6)	4.4	6.3	
Larvicides + ivermectin				
Tere	1704 (84.4)	0	0	0
Morigbedougou	900 (91.3)	1.6	3.2	22
Sansanbaya	717 (92.1)	0	0	0
Total	3321 (88.3)	0.5	1.1	

[a] Shown are the entomological results tor February and March 1992 for catching points located in the original OCP area (only larvicides) and in Guinea (larvicides + ivermectin).

[b] No. infective per 1000 parous flies.

[c] No. of L3 larvae per 1000 parous flies.

[d] Figures in parentheses are the % of flies that were parous.

23 Also, ivermectin may have a cumulative effect. In the Asubende area in Ghana, the microfilarial loads measured 12 months after treatment decreased by 50%, 70%, 75% and 85%, respectively, following the first four treatments (*7, 20*).[1] This cumulative effect could therefore indicate that the entomological results obtained in Guinea will improve if the present therapeutic coverage can be maintained.

24 The spontaneous participation of the populations in the control activities, which has been excellent, could decrease as the disease regresses, particularly the skin manifestations. Similarly, the distribution teams may become less active with time. With a view to taking these potential risks into account, the following measures have been taken in the OCP area to offer the best guarantees of sustainability: reinforced awareness-raising among villagers; involvement of national health personnel, and adoption of other methods of distributing ivermectin, notably by the village communities themselves.

25 Will ivermectin therefore replace vector control in OCP? The major benefit of vector control is that it leads to the exhaustion of the parasite reservoir in humans by interrupting transmission; this, however, requires 12-15 years. Subsequently, blackflies can recolonize the freed zones without transmission of onchocerciasis being resumed.

Since ivermectin does not completely interrupt transmission and does not kill the adult *Onchocerca* worms, it is not yet possible to forecast for how long it should be distributed and at what coverage level. Simulations made using the epidemiological model ONCHOSIM have shown that ivermectin would have to be distributed for more than 20 years to achieve the same results as vector control (*21*). Whatever the effect of ivermectin on the transmission of onchocerciasis, there is no evidence that the period of vector control should be shortened because of the combined use of ivermectin. For this to be the case, ivermectin would have to affect the lifespan of the adult worms; however, this has not yet been established, even though ivermectin exhibits some toxicity after repeated treatments at short intervals (*22*).

26 The larviciding problems encountered by OCP in February and March 1992 demonstrate clearly the impact of ivermectin on the success of the operations. For the same level of transmission, many more flies can be tolerated in areas that have been treated with a combination of larvicides and ivermectin. This impact provides an appreciable safety margin in the management of vector control operations, and makes it possible to envisage decreasing larviciding by a greater selectivity: fewer stretches of river treated and fewer larviciding cycles. In this context, distribution of ivermectin has been extended recently to some zones in the original OCP area, notably those that have been reinvaded, not only because of its direct impact on the level of morbidity but also because of its contribution to transmission control.

27 Larvicides and ivermectin are now two closely complementary tools in OCP activities. Use of this combination has created favourable new prospects that will undoubtedly influence the programme in the coming years.

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ENDNOTES

a. Geometric mean community microfilarial load.

ABSTRACTS

As part of the WHO Onchocerciasis Control Programme in West *Africa (OCP), the attack phase of operations in the Niger basin in Guinea began in 1989 with the simultaneous use of ivermectin and vector control. Larvicide applications coupled with annual large-scale ivermectin distribution have greatly reduced blackfly infectivity (by 78.8% for the number of infective larvae per 1000 parous flies). The combination of vector control and ivermectin has permitted excellent control of transmission. In the original OCP area, it took 6-8 years of vector control atone to obtain an equivalent decrease in blackfly infectivity. For the same number of flies caught, transmission was much higher in areas where ivermectin had not been distributed. The combined use of ivermectin and vector control has opened up new prospects for carrying out OCP operations with, notably, the possibility of reducing larviciding operations.*

Dans le cadre du Programme OMS de lutte contre l'onchocercose en Afrique de l'Ouest, la phase d'attaque des opérations dans le bassin du Niger en Guinée a démarré en 1989 avec l'utilisation simultanée de l'ivermectine et de la lutte antivectorielle. Parmi les seize points retenus pour cette étude, tous situés dans des foyers holoendémiques de savane, la moitié sont répartis dans le bassin du Haut Niger en Guinée et les autres dans l'aire initiale du Programme.
Après trois à quatre années d'opérations avec une distribution annuelle d'ivermectine à grande échelle, on note en Guinée une forte réduction de l'infestation des simulies (78,8 % pour la proportion de mouches infestantes et 82,9 % pour le nombre de larves infestantes pour 1 000 femelles pares). La prévalence des microfilaires a peu diminué; en revanche, les charges microfilariennes des communautés qui mesurent l'intensité de l'infestation ont chuté de 60 à 80 % par rapport aux données de prétraitement. La combinaison de la lutte antivectorielle et de la distribution de masse de l'ivermectine a permis un excellent contrôle de la transmission. Par contre, dans l'aire initiale du programme, il a fallu 6 à 8 ans de lutte antivectorielle seule pour

obtenir une réduction équivalente de l'infestation des simulies. A nombre égal de mouches capturées, la transmission est beaucoup plus forte dans les zones où l'ivermectine n'est pas distribuée, même après plusieurs armées de lutte antivectorielle efficace et ininterrompue.
L'utilisation combinée de l'ivermectine et de la lutte antivectorielle ouvre des perspectives nouvelles dans la conduite des opérations avec notamment une possibilité d'allégement de la lutte antivectorielle.

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Blackfly control: what choices after onchocerciasis?

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AUTHOR'S NOTE

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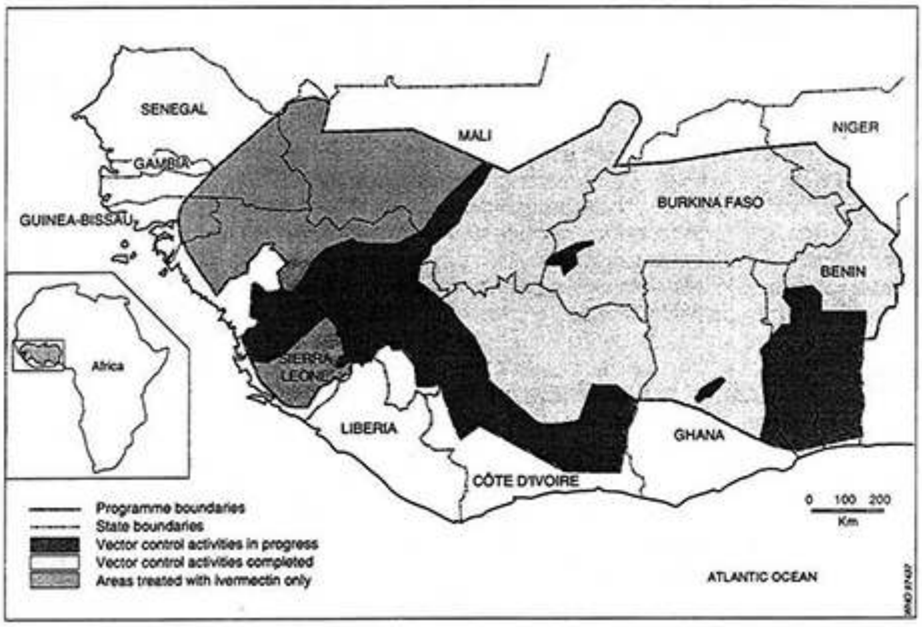
1 Above a certain level of aggressiveness, biting insects are a major inconvenience to people and may hinder socioeconomic development. This is particularly true in the intertropical zone, where aggressive behaviour by biting insects usually continues throughout the year. Most of the countries in this zone lack the resources needed for effective, long-term, environmentally friendly insecticidal campaigns.

2 Blackfly control has been practised in West Africa since 1975 at the regional level, as part of the Onchocerciasis Control Programme, whose objective is to eliminate onchocerciasis as a public health problem and an impediment to socioeconomic development. The transmission of *Onchocerca* can be interrupted by destroying the blackfly vector, *Simulium damnosum* in its larval stage. Development of the insect from egg to nymph rarely takes longer than a week, and spraying is therefore carried out at weekly intervals. Treatments are predominantly applied from the air because of the large number of breeding sites and the difficulty of reaching most of them overland. Vector control remains the preferred approach to onchocerciasis control in regions long subject to reinfection by blackflies from untreated areas and in areas where the disease is hyperendemic.

3 Aerial spraying has, however, ceased in the regions where the risk of contracting onchocerciasis has declined almost to zero, as indicated on the map. Between now and

2002, when the activities of the Programme are expected to end, 25 million hectares of fertile agricultural, agroindustrial or stock-raising valleys will become available for repopulation and use, meeting the food needs of 17 million people. On the evidence so far available, the return of blackflies does not present an obstacle to development in some areas.

Vector control in West Africa: the current situation



The control of blackflies is not necessarily the best way of ensuring the long-term development of areas freed from onchocerciasis.

4 However, in other areas the blackfly nuisance is likely to prevent progress if the threshold of bite tolerance is considerably exceeded.

Control of the blackfly nuisance

5 In the early 1990s, shortly after vector control had ceased, the blackfly bite rate again reached high levels in some areas that had been cleared of onchocerciasis infection. The Programme encouraged individual action against the blackflies, not only because of the nuisance they represented but also to reassure the public, who associated these insects with transmission of the disease. Low-cost control techniques on the ground for dealing with breeding sites were standardized *(1)* and “on the job” training was given to technicians in mobile health teams, nurses in health centres, community health workers, agricultural supervisors and personnel attached to socioeconomic development projects. Blackfly control operations on the ground have now been transferred to development units, among them the oil palm plantations on the Boubo and the Sourkoudougou ranch in Côte d’Ivoire, and the rice-growing plains upstream of the Sélingué dam on the Sankarani and the irrigated perimeter of Baguinéda on the River Niger in Mali.

6 The intensification of such operations, however, raises questions that should be considered before the Programme ends. A successful transfer of activities can only take place if the users are entirely familiar with the technique they have been taught and if they are aware of its limitations. The Programme should speedily advise the participating countries of the dangers inherent in uncontrolled larvicide application on the ground. How long the applications will persist is unknown, and problems may eventually arise in connection with the toxicity, mode of action and cost of the insecticides.

7 In the Onchocerciasis Control Programme, blackfly control is based on seven insecticides used in rotation *(2).* This strategy, intended to avoid the problem of resistance, requires precautions that protect non-target aquatic fauna *(3).* Real-time knowledge of the discharge rates of treated rivers is necessary, as is a relatively high degree of specialization among users. Larvicide application performed by non-specialists for an indeterminate period would carry an enormous risk of environmental pollution.

8 Two of the insecticides, however, present no environmental hazards: *Bacillus thuringiensis* H-14 is a product of biological origin, and temephos is an organophosphorus compound. Temephos, the chemical with which the Programme began its vector control work in 1975, unfortunately gave rise to physiological resistance in blackflies, and this would probably have spread if other insecticides had not been introduced in its place. The use of temephos therefore cannot be encouraged for long-term treatment on the ground. On the other hand, the sensitivity of blackflies to *Bacillus thuringiensis* H-14 has not diminished at all after 14 years of intensive use. For this reason the Programme has supported certain blackfly control initiatives by making this product available without charge. This cannot continue indefinitely because of the growing demand for insecticides and the impending cessation of the Programme's work. After 2002, unless public or private capital is forthcoming, village communities wishing to engage in agricultural or agropastoral activities will have to suspend blackfly control. There is a risk that these communities will use the insecticides that are available locally, in particular agricultural pesticides, with the attendant danger of causing resistance and contaminating the environment.

> ***The blackfly nuisance can be expected to intensify as the Programme draws to a close and more sites of socioeconomic interest are identified.***

Self-protection

9 The above remarks suggest that, with certain exceptions, the control of blackflies is not necessarily the best way of ensuring the long-term development of areas freed from onchocerciasis. An alternative solution is for individuals to use repellents or wear protective clothing. Numerous products intended mainly to protect against mosquitos, usually based on pyrethroids, are on the market. Their effectiveness against blackflies remains to be proved, and in any case their price is too high for most of the communities concerned. The Programme is therefore preparing an inventory of locally used repellents with a view to selecting those that perform best, ascertaining the traditional recipes for their manufacture, and promoting their wide availability.

10 The use of specially designed clothing hardly seems feasible. The hot humid conditions of the areas in question are not favourable for clothes which cover the body almost completely. Moreover, the acquisition of protective clothing, even the purchase of a long-

sleeved shirt, is likely to represent a major financial outlay in most of the communities concerned. When insects are biting in large numbers the usual response is to make the best possible use of ordinary clothing.

A rational strategy

11 The blackfly nuisance can be expected to intensify as the Programme draws to a close and more sites of socioeconomic interest are identified. It is essential to encourage a realistic attitude to the problem, and this requires three categories of site to be distinguished:

- sites where blackflies do not impair development and where no control is desirable;
- sites with the potential for providing substantial income and where advantage is likely to be gained from long-lasting treatments on the ground together with additional measures such as the training of personnel;
- traditional agricultural sites where the use of insecticides is not recommended because resources are inadequate and users are untrained.

12 In most cases the nuisance caused by blackflies does not justify control. Before the Programme ends, a public awareness campaign should be concentrated on those village communities in whose vicinity increasing numbers of blackflies can be expected. It should explain the non-infective nature of the insects in areas where onchocerciasis has been eliminated as well as the possibilities for control and self-protection.■

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ABSTRACTS

Blackflies are reappearing in areas of West Africa where they used to be controlled with insecticides because they were vectors of the parasite Onchocerca volvulus. *Even though they no longer transmit onchocerciasis in these areas they can hinder optimal land use through their biting behaviour. The authors*

discuss the problems associated with resuming the use of insecticides to control the blackfly and recommend that ground treatment be restricted to areas where it is likely to be effective on a continuing basis. In communities lacking technical and financial resources the only alternative consists of individual protection through the use of repellents or protective clothing.

Eliminating Onchocerciasis after 14 Years of Vector Control: A Proved Strategy

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2 Despite advances in therapy and vaccine research, the control of vectorbome diseases is still dependent, to a large extent, on vector control. This strategy helps to slow down or stop transmission and to reduce or eliminate the clinical manifestations of the disease. It is sometimes difficult to apply because of the complexity of some parasitic cycles or immune mechanisms. The control of human onchocerciasis, the most serious manifestations of which are blindness and incapacitating skin lesions [1], lends itself more to a vector-control strategy. Indeed, the frequency and severity of the symptoms are correlated closely with the number of microfilariae of *Onchocerca volvulus,* which depend on the number of adult filariae. This, in turn, is governed by the number of

infective larvae received by the subject and therefore by the number of bites received from infected blackflies in relation to the length of time spent in an endemic area. Since onchocerciasis is an accumulative disease, clinical onchocerciasis manifests itself only after an accumulation of infections over several years (the development of *O. volvulus* in the vector blackfly results in a reduction of parasite quantity), and, with regard to eye lesions, blindness generally does not appear before age 30 years, although it may occur at an earlier age in inhabitants of certain hyperendemic areas.

3 By reducing the blackfly population at its larval stage through aerial application of selective insecticides on infested rivers [2], the transmission of infecting *O. volvulus* larvae and the appearance of microfilariae and macrofilariae are reduced greatly. The full interruption of transmission during a period exceeding the reproductive life span of the adult worm, which is ~12-14 years [3], leads to the graduai extinction of the parasite in man and therefore to the elimination of onchocerciasis in the area of vector control. The Onchocerciasis Control Programme (OCP) in West Africa [4], which was launched in 1974, helped to validate this assumption. The first aerial treatments began in February 1975 and by the end of 1977 were extended gradually to cover a “core” area of ~700,000 km^2 spread over 7 countries, where the incidence of blindness was highest (Burkina Faso, southeastern Mali, southwestern Niger, the northern parts of Côte d’Ivoire, Benin, Ghana, and Togo). In some villages, the prevalence of infected individuals sometimes exceeded 80%, and the rates of onchocercal blindness could reach 9%. Moreover, the frequency and level of evolution of ocular lesions and of onchocercal blindness were among the highest in the world.

4 Since evidence supported that the borders of this area were affected by infective blackflies originating from beyond these borders, the hyperendemic regions suspected to be sources of invasion were put progressively under aerial treatment, to control onchocerciasis and thus to protect the border of the core area from any exogenous parasitic contamination. However, the size of the core area was such that the regions located at its center were protected continuously since the inception of the OCP. This is why, a quarter century after the vector control operations began and 10 years after their cessation, the parasitic “insularity” of these landlocked regions has facilitated the elimination of onchocerciasis. Although the vector retumed to precontrol densities, several hyperendemic areas of several thousand square kilometers thus were “freed” without further intervention, particularly through the use of drugs, usually ivermectin, which is a microfilaricide now used widely for onchocerciasis control [5].

5 Although onchocerciasis unquestionably is no longer a public health problem (the clinical manifestations of onchocerciasis, which were still noted even during the first years of vector control, are no longer reported by health centers), the complete absence of the parasite is yet to be confirmed. Indeed, it would appear that, in the particular case of these areas, there has been no more transmission of the parasite over the past 10 years and that onchocerciasis has been completely and permanently eliminated. The entomological and parasitological data collected since 1975 have been used for a dynamic and detailed review of the situation, 10 years after the end of insecticide treatments, to analyze the validity of the vector-control strategy.

Methods

6 *Study area.* The study area (figure 1) is located in the middle of the core area that was treated starting in 1976. Its size is equivalent to that of Switzerland (~40,000 km^2). A major part of the study area is located in Burkina Faso, southeast of Ouagadougou, in the upstream basins of Sissili, Nazinon, Nakambé, and Koulpéolgo. It is a Sudan savanna region, which is relatively homogeneous with respect both to blackfly species (*Simulium sirbanum* exclusively) [6] and to parasite strains ("savanna" strain of *Onchocerca volvulus*) [7].

7 *Vector-control operations.* Insecticide treatments were conducted exclusively in the study area with temephos, a cheap and efficient organophosphorous insecticide, with insignificant impact on nontarget aquatic invertebrates and vertebrates. The spraying, which was carried out essentially by aircraft, began in 1976 and was completed in late 1989 (i.e., 14 years after the beginning of control operations). All the rivers and tributaries of the study area that hosted larval stages of *S. sirbanum* were treated on a weekly basis each time the hydrological conditions were favorable for the development of blackfly larvae.

8 *Entomological evaluation.* An entomological evaluation network was established shortly before the beginning of control operations, to ensure a permanent follow-up of the efficiency of insecticide spraying. The catching points were selected from accessible sites where transmission would be noticeable and, if possible, near villages earmarked for epidemiological evaluation. About 30 catching points thus had been gradually put in place in the study area. For the purpose of longitudinal monitoring, we selected 4 of these catching points, where pretreatment data collection was the most complete: Kampalaga and Ziou Zabré on the Nazinon, Loaba on the Nakambé, and Bitou on the Nouhao, which is a tributary of the Nakambé (figure 1). Among the entomological indices monitored over these years, we essentially retained the annual biting rates (ABRs), the annual transmission potential (ATP), and the infectivity rate. ABR is the annual number of bites that a man located at an area heavily exposed to blackfly bites, 12 h a day and 12 months a year, theoretically would receive. ATP is the index used most frequently to quantify transmission. It is defined as the theoretical number of infective *O. volvulus* larvae that would be received by an individual placed at a catching point during the same period of time. An ATP >800 infective iarvae per person per year is associated with clinical signs of hyperendemicity [8], An ATP <100 infective larvae per person and per year indicates that onchocerciasis transmission has been interrupted and that the disease is no longer a major public health problem. The infectivity rate is a way of expressing the intensity of transmission and is independent of blackfly density and corresponds to the number of infectious female blackflies (carrying infective larvae in the head) per 1000 female blackflies caught. This index was used mostly to evaluate the residual transmission after the complete cessation of insecticide spraying, because the collection of field data entailed less operational constraints, compared with those for the calculation of ATP. The infectivity rates were recorded before treatment, then twice a year, and 10 years after discontinuing insecticide spraying at 2 catching points, Ziou Zabré and Loaba.

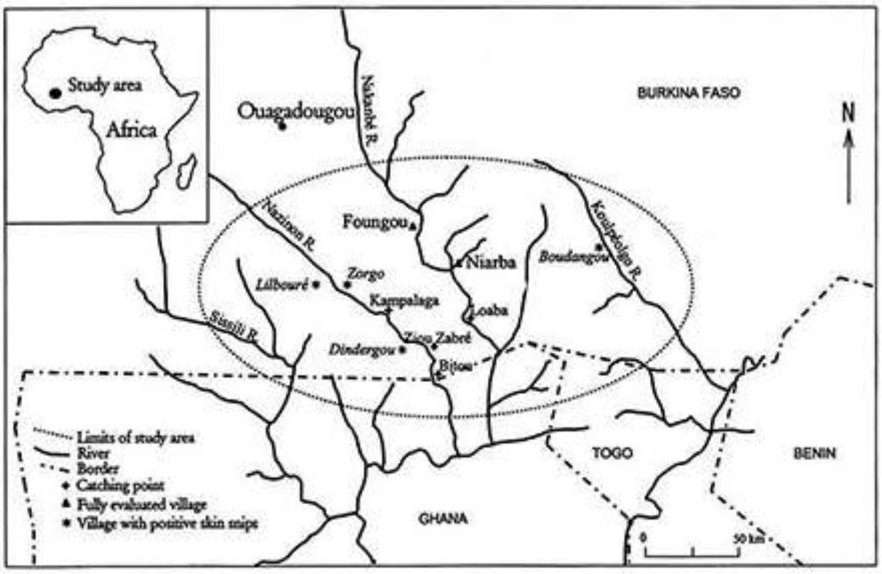

FIGURE 1. Map of the area showing the location of the catching points and evaluated villages

9 *Parasitological evaluation.* The parasitological evaluation is based on the count of the microfilariae contained in 2 skin-snip samples taken from the iliac crests. The parasitological indices considered were the prevalence [9] of subjects carrying microfilariae and the community microfilarial load (CMFL) [10]. Prevalence is a composite indicator that depends on incidence, population movements, and deaths and births. It indicates, at a given time, the magnitude of parasite infestation among the population. CMFL is clearly more sensitive than prevalence and is considered to be the best indicator of onchocercal endemicity. It corresponds to the geometric mean of the number of microfilariae per skin snip in subjects ≥ 20 years old, including those who are not or are no longer infected with microfilariae. Incidence is the number of new cases recorded every year in a selected population. A new case is an individual with microfilariae whose skin-snip samples were negative during the preceding 2 examinations or an individual born after control started who was found to have microfilariae. Incidence is theoretically nil in the absence of transmission. The data available to us were obtained from a cohort of individuals whose skin-snip samples were found to be negative shortly after the end of vector control operations (1990) and who then had been monitored 4 other times (1995, 1996, 1998, and 2000).

10 *Opthalmological evaluation.* The opthalmological evaluation helped to determine the consequences of the infection as ocular manifestations of the disease. Seventeen villages, preferably located near the entomological catching points, were selected in the study area before the beginning of vector control. Two of these 17 villages, Niarba and Foungou, which are located along the Nakambé river and have the highest blindness rates, were selected for this evaluation (figure 1). The examination was limited to individuals ≥5 years old and consisted of measuring visual acuity, looking for and counting microfilariae in the cornea and in the anterior chamber of the eye, detecting early lesions (punctate keratitis), recording the lesions of the anterior and posterior segments of the eye (sclerosing keratitis, iridocylitis, optic atrophies, and choroido-retinitis), and determining the nature of blindness of onchocercal origin. For the study area, we collected detailed data for the village of Foungou, which was monitored 5 times, before the beginning of treatments and after 5, 7, and 10 years of vector control. This village also underwent the

latest opthalmological survey in March 2000 (i.e., 11 years after treatment was discontinued).

11 *Demographie surveys.* During the periodic epidemiological surveys, the individuals found infected in the freed areas were subjected to perfunctory migration questionnaires. The individuals interviewed were asked specifically to give their profession, place of birth, and movements over the past 15 years, as well as a number of questions related to onchocerciasis. The questionnaires of 10 individuals who were distributed among the 17 villages of the study area were analyzed. They had been found to have positive skinsnip samples during the most recent parasitological surveys (from 1996 to 2000).

Results

12 *Entomological evaluation.* ABR and ATP values recorded at Ziou Zabré, Kampalaga, Bitou, and Loaba before the beginning of operations varied from 5865 to 11,879 bites and from 309 to 880 infective larvae per person per year (table 1 and figure 2). Figure 2 also shows, for the same catching points, ATP evolution from 1975 through 1991; ATP values remained <100 infecting larvae per person per year, at least during the last 8 years of vector control. These figures were correlated with the ABR values (table 1), which were very low compared with those recorded in the untreated periods (1975 and 1991). The infectivity rates recorded at Ziou Zabré and Loaba varied from 33.05 and 37.48 per 1000 infectious female blackflies caught before vector control to 0.25 and 0.17, respectively, in 1991. Ten years after the complete cessation of vector control, the infectivity rates remain nearly nil, with only 1 infectious female blackfly of 18,600 blackflies caught at Ziou Zabré and no infectious female blackflies of 8500 blackflies caught at Loaba.

13 *Epidemiological evaluation.* Table 2 shows the very high level of onchocercal endemicity in the 17 villages surveyed in the study area before the beginning of operations. An average of 7 of 10 individuals was infected with microfilariae in the study area in 1976 (2622 individuals of 3830 examined tested positive for microfilariae). Figures 3 and 4 show the epidemiological trends (prevalence, CMFL, and blindness rates) in the populations of Foungou and Niarba from 1975 and 1976 through 2000. At Niarba, 162 individuals, on average, were examined at each passage (of 226 listed; i.e., 72% participation) versus 262 at Foungou (of 350 listed; i.e., 75% participation). In the 2 villages, prevalence and CMFL started to decrease significantly after the fourth year. At Foungou, the prevalence of infection in the population examined the year when treatments were stopped was only 7.7%, and CMFL was 0.16. At Niarba, the values were close to 0 (0.6% prevalence and 0.1 for CMFL). The downward trend was confirmed the years after, with values negligible to nil through 2000.

Table 1. Values of annual biting rate before, during (average of 14 years), and after vector control.

Catching point	Precontrol period. 1975	Control period, 1976-1989	Postcontrol period, 1991
Ziou Zabré	11.879	1465	30,739
Kampalaga	7821	351[a]	—
Bitou	5865	233[a]	—

Loaba	6090	238	8617

[a] Average on 8 years only (data after 1983 not available).

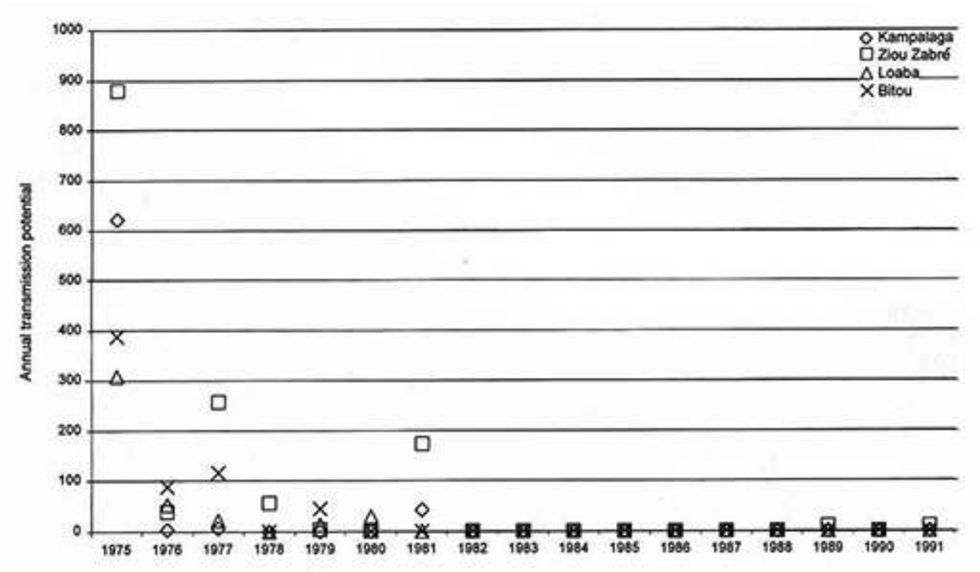

FIGURE 2. Trend in annual transmission potential of infective *Onchocerca volvulus* larvae in 4 catching points of the study area (Kampalaga, Ziou Zabré, Loaba, and Bitou), 1975–1991.

14 With regard to the incidence of infection, the cohort selected in November 1990 at Foungou included 257 individuals: 129 female blackflies and 128 male blackflies. At Niarba, it included 117 individuals: 70 female blackflies and 47 male blackflies. No new infection was detected during the later surveys conducted in these 2 cohorts (374 individuals in total) from November 1995 through March 2000; therefore, the incidence of infection during the 10 years after the complete cessation of insecticide treatments therefore was nil at Foungou and Niarba. In April 2000, an opthalmological survey in these 2 villages gave the opportunity to practice skin snipping, as well as patch tests using diethylcarbamazine (DEC) [11]. None of the 168 individuals examined at Niarba and none of the 335 individuals at Bétaré were found to be positive via either skin snip or DEC patch tests.

15 *Opthalmological examinations.* Before the beginning of vector-control operations, 66.9% of the individuals examined at Foungou had ocular problems, including 3.4% with punctate keratitis, 38.1% with microfilariae in the cornea and/or the anterior chamber of the eye, and 25.4% with severe and irreversible onchocercal ocular lesions [12]. In March 2000 (i.e., 11 years after the definitive cessation of insecticide spraying), we observed neither punctate keratitis nor microfilariae in the cornea and/or the anterior chamber, which had virtually disappeared after 10 years of vector control [13]. Among the 7.19% of severe ocular lesions, we only observed cicatricial ocular lesions but no inflammatory or progressive lesions. Opthalmological examination also was conducted in March 2000 in 2 other villages of the study area, Niarba and Bétaré. The results are identical to those from Foungou.

16 *Migration surveys.* Of the 10 individuals with positive skinsnip samples, 3 were found to have positive samples in 1996 at Zorgo on the Nazinon, whereas the others were found to have positive samples in 1998 (4 individuals at Dindergou near the Nazinon, 2 at Lilbouré

near the Nazinon, and 1 at Boudangou near the Koulpéolgo). At Zorgo, a 34-year-old man, a native from the village, lived 10 years in the humid forest zone of Côte d'Ivoire. A 27-year-old woman spent 10 years in the forest zone of Côte d'Ivoire, whereas her daughter spent the first 5 years of her life there. At Dindergou, a 40-year-old man and a 35-year-old woman, who were village natives, had recently lived for ~10 years in the humid forest zone of Ghana and Côte d'Ivoire. The other 2 individuals, a 36-year-old man and a 31-year-old woman, said that they had never left their village of origin. At Lilbouré, the 38-year-old man was a native from the village, whereas his 10-year-old daughter was born in Côte d'Ivoire in a forest zone where both of them had spent 5 years. A 34-year-old man from Boudangou, a native from that village, claimed that he had not left the village over the past 15 years. The results of the parasitological examination of the 3 individuals who seem to have not left the village or, at least, the study area are shown in table 3.

Table 2. Prevalence, community microfilarial load (CMFL), and onchocercal blindness rates in the study area before the beginning of vector-control operations.

Village	Basin	No. examined[a]	Prevalence, %	CMFL	Blindness rate, %
Niarba	Nakanbé	122	71.9	21.76	9.03
Foungou	Nakanbé	253	76.6	40.57	8.85
Wayen	Nakanbé	275	52.7	12.79	6.66
Bangasse	Koulpéolgo	108	71.1	89.05	6.36
Loaba	Nakanbé	222	77.0	53.83	6.28
Yakala	Nakanbé	177	67.5	16.89	6.28
Boudangou	Koulpéolgo	311	76.4	47.00	6.01
Kounou	Sissili	78	75.1	17.81	5.74
Zorgo	Nazinon	193	73.0	31.85	4.42
Lamiougou	Koulpéolgo	500	68.1	21.41	3.51
Dindergou	Nazinon	115	79.2	74.88	3.34
Tili	Nazinon	285	91.8	24.45	3.09
Nianlé	Nakanbé	375	65.8	27.25	2.42
Natiédougou	Sissili	149	83.6	45.48	2.27
Koumbili	Sissili	144	71.1	47.20	1.86
Betaré	Nazinon	294	63.2	28.12	1.41
Lilbouré	Nazinon	229	58.1	15.57	0.00

[a] Subjccis ≥*20* years old.

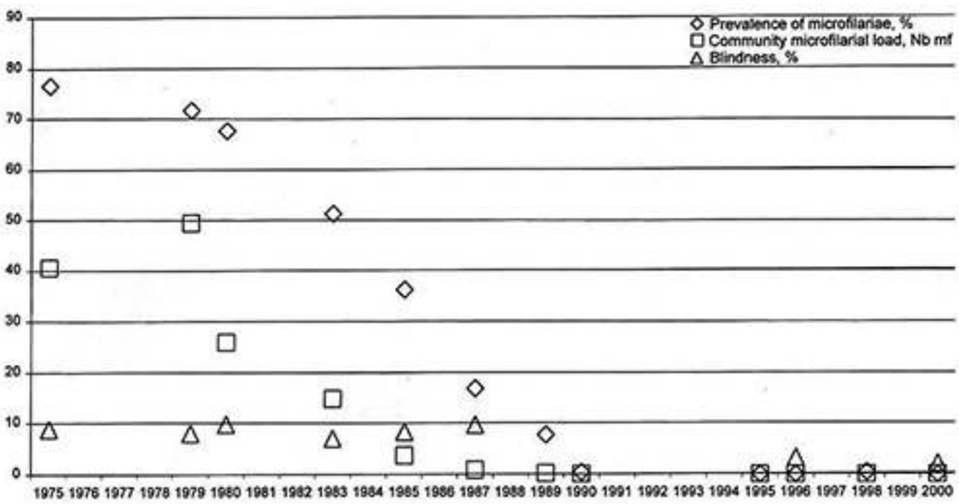

Figure 3. Epidemiological trend or prevalence of microfilariae, community microfilarial load, and blindness at Foungou village, 1975-2000. Nb mf, no. of microfilariae.

Discussion

17 As shown in figure 2, a drastic and sustainable reduction of transmission has been achieved throughout the duration of vector control and after, since ATP values remained unchanged despite the return of blackfly densities to pretreatment levels (table 1). The measurement of infectivity rates after discontinuing vector controi confirms these good results: with infectivity rates <0.5 infectious female blackflies per 1000 female blackflies caught, transmission is considered to be virtually interrupted, and no controi measure is required [14].

18 The results of table 2 show that the area under study is located, according to the parasitological criteria defined by Prost and Prod'hon [9], in an area of onchocercal hyperendemicity, thus confirming the high ABR and ATP values recorded during the precontrol period. The very low transmission after vector-control operations is confirmed by the parasitological level in the 2 villages longitudinally monitored (figures 3 and 4), with clearly declining trends in terms of prevalence of microfilariae and of CMFL (prevalence and CMFL were close to 0 in 1990 before becoming nil during the following years). These results also are confirmed by the incidence surveys conducted in those 2 villages, as well as by the opthalmological survey conducted independently in 3 villages of the area in April 2000 (old lesion that did not redevelop since vector controi was discontinued).

19 Answers to questionnaires given to the 10 individuals more recently found to be carrying microfilariae indicate that 7 of these 10 individuals probably were infected during stays outside the study area, specifically in neighboring countries—Ghana and Côte d'Ivoire—in humid forest zones, where onchocerciasis with less incidence of blindness prevails [15]. For the 3 individuals who claim that they did not leave the study area (table 3), a first interpretation could be that this infection was due to an artifact of skin snip reading, particularly for the 2 patients of Dindergou who had very low parasite loads and who were found to test positive for the first time in 1998. A second interpretation would be the unreliability of the account given by the individuals interviewed (omission of information or bad understanding of the message delivered). The last assumption could be the maintenance of a residual transmission, which sometimes is not insignificant; for example, at Boudangou, the individual positive for *O. volvulus* carried out 13 microfilariae per skin-snip sample.

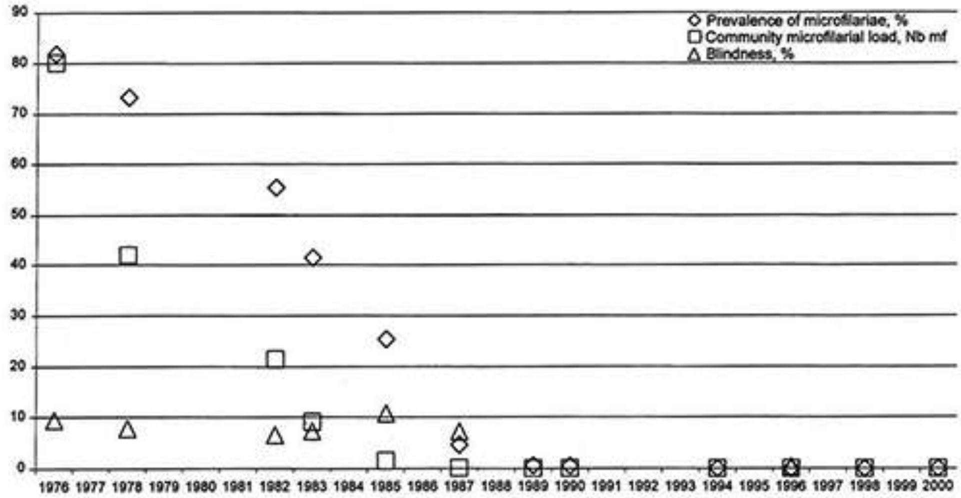

FIGURE 4. Epidemiological trend of prevalence of microfilariae, community microfilarial load, and blindness at Niarba village, 1974–2000. Nb mf, no. of microfilariae.

In conclusion, the review of entomological and parasitological results for the whole study area confirms the strong and sustainable reduction of the transmission of *O. volvulus* after the complete cessation of vector control in the absence of ivermectin distribution. The absence or, at least, the very low level of transmission of *O. volvulus* by *S. sirbanum* in so wide an onchocercal area that used to be hyperendemic is quite spectacular, given that OCP was used as a control rather than as an eradication program. The results suggest that the initial vector-control strategy, which was developed in the early 1970s on the basis of a few pilot studies [16], was a realistic option, since the treated areas had been protected permanently from parasite reinfestation of human populations or infected blackflies. Unfortunately, this opportunity was not offered everywhere, as witnessed by some residual foci reported within the core area. Therefore, particular attention was paid to these foci, which led to the identification of the factors of failure as climatic (e.g., migration of Aies), logistic (e.g., inappropriate location of catching points), or demographic (e.g., migrants in search of new farmlands) [17]. The creation of the Bagré dam in 1992 on the Nakambé, upstream of the Loaba catching point, is a good example of such foci to be carefully surveyed. This important water dam, essentially meant for fishing and agriculture, causes new migratory flows in the subregion that are potential sources of parasite reintroduction. Therefore, monitoring of the evolution of transmission is advised in this onchocerciasis-freed zone, especially since this region has developed considerably over the past few years. Residual activities of surveillance and control are now in the hands of the participating countries, since OCP ends in December 2002, after a 5-year process of phasing out.

Table 3. Average load of *Onchocerca volvulus* microfilariae (mf), by analysis of skin-snip samples from 3 subjects who did not leave the study area.

Year of survey	Average parasite load, mf		
	Dindergou		Boudangou
	Subject 1 (male; 1963)	Subject 2 (female; 1965)	Subject 3 (male; 1964)
1978	—	—	45.0
1982	—	—	43.0
1985	—	—	12.5
1992	0.0	0.0	—
1994	0.0	0.0	0.0[a]
1998	1.0	0.5	13.0

[a] Subject 3 probably was treated with ivermectin.

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ABSTRACTS

From 1976 through 1989, weekly aerial spraying operations against blackflies were carried out along the rivers of a wide savanna area of West Africa (~700,000 km^2) where onchocerciasis was hyperendemic. The level of endemicity began to decrease significantly after 4 years of vector control and became very low in 1989. This situation has been maintained without any vector control activity or chemotherapy, and no incidence of any new cases has been detected. An ophthalmological study carried out in 2000 has confirmed these good results, showing only cicatricial ocular lesions in the examined population. These results led to the conclusion that 14 years of vector control may achieve long-term elimination of onchocerciasis, even in the absence of chemotherapy, provided that the treated areas are not subjected to any contamination by exogenous parasites carried in infected humans or flies.

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Combating onchocerciasis in Africa after 2002: the place of vector control

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1 Since the launching of the African Programme for Onchocerciasis Control (APOC) at the end of 1995, all 30 African countries affected by endemic onchocerciasis have been involved in a merciless fight against the disease. Already, onchocerciasis is no longer a public-health problem or an obstacle to socio-economic development in the 11 countries monitored by the Onchocerciasis Control Programme in West Africa (OCP; Molyneux, 1995). This should soon be the case in the 19 APOC countries (Dadzie, 1997), as an efficient and self-sustainable System of community-directed treatment with Mectizan® (ivermectin, MSD) is established.

2 In West Africa and most of the APOC countries, *Simulium damnosum* s.l. is the only species responsible for transmission of *Onchocerca volvulus.* In some foci in East Africa, however, this filarial parasite can be transmitted by species of the *S. neavei* group as well as those of the *S. damnosum* complex. Whichever the *Simulium* species involved, their elimination depends on spraying insecticide on the rivers where the pre-imaginal, rheophilic stages develop (Walsh, 1985). The periodicity of such treatment varies with the life-span of the larvae targeted: generally every week for *S. damnosum* s.l. and every 2-4 weeks for *S. neavei* s.l.

3 Between 1974 and 1989, such vector control, carried out in seven West African countries, was the only means of combating onchocerciasis. It successfully freed many regions of the disease, by minimising transmission for a period longer than the longevity of the adult worm in man (about 14 years). Today, vector control still remains a favoured control method in the OCP extension areas (Hougard *et al.,* 1993), but it is now combined with treatment of communities with Mectizan, the distribution of which became widespread as early as the beginning of 1990. For the remaining OCP countries and for the APOC countries, chemotherapy with Mectizan is now the main tool used against onchocerciasis. It is used both to treat and prevent the clinical manifestations of the disease, particularly onchocercal blindness and skin lesions (Chippaux *et al.,* 1995).

4 If all goes to plan, 31 December 2002 will be a landmark date in the history of blackfly control in Africa. This is the day set for phasing out all OCP activities. In fact, the current, combined strategy of chemotherapy and vector control should help to clear almost all the basins treated by the OCP before this deadline. In addition, most of the vectorelimination projects necessary in APOC countries have already been identified. They should start before the end of this century and most of them are likely to be completed by 2002. Few campaigns against the vectors of *O. volvulus* are therefore likely to be conducted in Africa beyond 2002. Feasibility studies for new vector-elimination projects in APOC countries, which may be implemented after 2002, have yielded convincing results (APOC funding by the donor community should continue until 2007). However, sustainable elimination of the vector (the APOC strategy) may only be possible in those rare hydrological basins where blackfly immigration is not possible or likely. Interruption of the transmission of the parasite by vector control for many years (the OCP strategy) is now considered to be a too heavy financial investment, both by the donors and the countries concerned the estimated cost of treating the 14 000 km of rivers in the framework of the OCP's operations in 1996 amounted to U.S.S7 650 000, just for insecticides and flight-hours). The development of the APOC, a programme essentially based on sustainable, community-based distribution of Mectizan, is a clear demonstration that affected countries no longer wish to become involved in long-term, vertical programmes of the OCP type, no matter how successful such programmes might be.

5 The only vector-control operations likely to survive after 2002 will be those needed for 'mopping up' in areas previously covered by OCP or/and APOC operations. In some basins in the OCP area, such as the Dienkoa basin in Burkina Faso, control of transmission was delayed (Hougard *et al.,* 1997) and insecticide spraying may have to be continued for some months or even 2-3 years after 2002, until epidemiological results are totally satisfactory. This should not be a major problem in Dienkoa, as the aim there will be to continue the low-cost, land-based treatment which is already managed by local health services. It may be more of a problem in the tributaries of the Oti river in northern Togo, as all the human, material and financial resources needed would have to be found, larviciding could not be carried out from the ground, and the amounts of insecticides to be sprayed would have to be fairly large because of the hydrological pattern of the local rivers.

6 To conclude, whatever the number and significance of the larviciding operations conducted after 2002, they will play an insignificant role in onchocerciasis control compared with that of chemotherapy. Mectizan is likely to be the main control agent but other microfilaricidal drugs, or even a macrofilaricidal drug, may be in routine use by 2002. Control of blackflies to limit their biting rather than disease, although not to be encouraged, may increase in significance, especially in the OCP area. As larviciding by the OCP ceases, there will be a surge in the number of blackflies and in the number of bites in communities which have become unaccustomed to such a nuisance.

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Focus. Insecticide Resistance in the Onchocerciasis Control Programme

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1 The Onchocerciasis Control Programme (OCP) in West Africa aims to reduce the transmission of onchocerciasis to the point where the disease is no longer a serious public health problem[1,2]. The Programme relies on weekly applications of larvicides to the riverine breeding sites of the immature stages of *Simulium damnosum* s.l., the only vector in West Africa. As a result transmission has been virtually eliminated over about 80% of the Programme area. But suppression of the vector populations must continue for about 15 years in order to eliminate the disease, because the parasite is long-lived in the human host and is not amenable to mass treatment with currently-available drugs.

2 From the beginning of treatments in early 1975 until early 1980, temephos was the only larvicide used in the OCP, and it was entirely effective. During most of this period, treatments were directed mainly against the 'savanna' species of the *Simulium damnosum* vector complex (*S. damnosum* s.s. and *S. sirbanum*). In late 1978 and early 1979, larvicide treatments were extended to the south, to include the large-river breeding sites of two 'forest' species –*S. soubrense* and *S. sanctipauli.* This was done primarily to eliminate savanna species in the same breeding sites which invaded areas further north, but it also implied a commitment to control the forest species.

Treatment Failures

3 In April 1980 a series of treatment failures on the lower Bandama River in Ivory Coast were shown to be due to resistance to temephos[3]. Resistance was limited to S, *soubrense/ sanctipauli*[*] but it spread to include all the previously-known distribution of these species in the treated part of the Ivory Coast the extreme south of Burkina Faso (mid-1981), and western Ghana (early 1982). The resistance rendered temephos virtually useless, even at several times the routine dose. It produced a 50-100 x increase in the LC 100 in susceptibility tests.

4 When temephos resistance was first detected, the role of *S. soubrense/sanctipauli* as a vector of the blinding form of the disease was uncertain. It was therefore judged necessary to maintain a high level of control. The first response was to apply chlorophoxim, another organophosphate, which was the only alternative that had successfully completed screening at the time. But within one year, the same population of S. *soubrense sanctipauli* developed full resistance to chlorophoxim[4]. It was then replaced by a formulation of the biological insecticide *Bacillus thuringiensis (Bt)* serotype H14. Although this product could be effective, it was impossible to maintain control in the wet season because of poor dispersal and the relatively large amount of which had to be added per cubic metre of river discharge (3 - 20 x the quantity of temephos). Only a few small areas could be treated year-round, and that required considerable extra expense by the use of large aircraft Even at moderate river discharge, the use of Bt is several times more expensive than temephos, taking into account the additional flying time and ground transport of insecticide. Only at very low discharge are the two types of treatment similar in cost

Reversion to Susceptibility

5 In 1982, it was found that some populations resistant to chlorophoxim reverted to a normal susceptibility some months after use of the product stopped. This reversion is strongest at the western edge of the treated area and is probably due to immigration of susceptible individuals. In 1983 and 1984, reasonable year-round control of S. *soubrense/ sanctipauli* was achieved by alternating *B. thuringiensis* at low discharge and chlorophoxim in the rainy season.

6 In mid-1984, the completion of longterm species-specific transmission studies on S. *soubrense/sanctipauli* in the resistance zone combined with the earlier observation that blinding onchocerciasis was never found when only these species were present[5-6], led to the decision to abandon attempts to control this species pair in the forest zone. This policy is now being implemented and tested. If it is proved that not controlling *S. soubrense/sanctipauli* has little influence on transmission of blinding onchocerciasis, the significance of the insecticide resistance will be considerably reduced.

7 At the same time, however, evidence appeared that the much more dangerous savanna species (*S. damnosum* s.s. and *S. sirbanum*) can also develop resistance. In late 1982 and early 1983, an isolated population of savanna species on the lower Bandama River in southern Ivory Coast displayed a progressive regression of susceptibility to temephos, Before this reached the stage of full resistance, blanket *Bt* H14 treatments were introduced in the zone and continued until the end of 1984. The treatments were effective and savanna species virtually disappeared. Savanna species reappeared in April 1985, after 4 months' suspension, They were tested and found to have a normal level of susceptibility However, it was judged prudent to avoid the use of organophosphate compounds in that area.

8 Due to the long pre-patent period of onchocerciasis, it is still too early to evaluate the impact of insecticide resistance on the epidemiological results. As control had only been going on for one year when resistance was noted, it may not even be possible to see the effects. There was certainly a large increase in biting rates of the resistant forest flies, although often this was not accompanied by much transmission. Even where

transmission did occur, it may have been transmission of the more benign form of the disease, which unfortunately can only be distinguished from the blinding form by its developed clinical features in the human host.

Elimination of Savanna Species

9 The resistant species became predominant in areas in the northern part of its range where they were previously in the minority, but they never surpassed their original distribution. Their predominance was due to the elimination of the savanna species by the temephos treatments which continued. However, as *S. soubrense/sanctipauli* moved away from its normal forest habitat, restricted numbers and increased zoophily reduced its vectorial capacity which can be nil at the northem part of its range.

10 There may have been some transmission by savanna species during the total treatment suspensions which were undertaken for susceptibility testing or trials of new products as the methods for combating the resistance were developed.

11 Development of resistance in savanna species is a serious risk to the OCP, because even if replacement compounds are fully tested, they will undoubtedly be more difficult and more expensive to use than temephos. This risk will increase as the programme is extended because reservoirs' of susceptible individuals presently unexposed will come under treatment. For this reason, close surveillance of susceptibility is continuing, along with an intensive screening programme for hew compounds. Already, a pyrethroid (permethrin) and a carbamate (carbosulfan) have reached the level of large-scale operational trial. In general, however, the influence of resistance on OCP results is limited. Against savanna species, which are the major vectors of the grave form of the disease, temephos is still completely effective over virtually all of the OCP areas.

AERIAL APPLICATION OF INSECTICIDES TO RIVERINE BREEDING SITES* OF SIMULIUM DAMNOSUM *S.L. IN THE ONCHOCERCIASIS CONTROL PROGRAMME

Acknowledgements

12 The authors would like to thank the many OCP staff members who contributed to the development of this paper.

Environmental Monitoring of Rivers in the Onchocerciasis Control Programme

13 The Onchocerciasis Control Programme was established with a strategy that recognized the importance of social, geographical and environmental aspects, as well as the fundamental vector control and epidemiology work. Depopulation of the river valleys in West Africa in favour of neighbouring areas with poorer soils has been profound, and a return following successful control of onchocerciasis requires careful national and international planning and support. Clearly, a control programme designed around a weekly insecticide treatment of rivers in a 700 000 km^2 area should bear in mind the longterm effects on non-target organisms in the rivers, safeguarding against substantial changes in productivity leading to a reduction of fish stocks.

14 An Ecology Panel, comprised of internationally recognized and independent experts was established to plan the environmental monitoring programme, and progress is reported at Hydrobiologists' meetings each year. Any changes in protocol have to be agreed or suggested by them.

15 The initial monitoring protocol was inevitably a compromise between academic desirability and practical possibility. The selection of sites was carried out by personnel from the ORSTOM laboratory in Bouaké, Cote d'Ivoire, the Institute of Aquatic Biology, Achimota, Ghana, and the Department of Biology, University of Salford. Methods involved were day and night drift net samples, modified Surber substrate samples and artificial substrates for invertebrates, and drift and gill nets of five mesh sizes for fish. Standard forms for ease of computer storage and analysis were introduced. Sites were to be sampled every month where possible, and a hydrobiologist based in Ouagadougou was contracted to work on the Upper Volta sites. No pretreatment samples were available, so as collections in the Phase I treatment zone would follow insecticide applications, further stations outside the control area were sampled for comparison. Subsequently these areas have also been treated because of problems due to reinvasion by *Simulium* from untreated areas, and they provide an ideal before and after comparison. All data has been regularly sent to the WHO, Geneva for checking and computer entry, and independent analyses are also carried out for the monitoring teams and the Ecological Panel by a team at Salford University. Essential supporting research in the form of short-term toxicity tests and biological work on the taxonomy and life cycles of non-target organisms has been carried out – the subject of many reports by ORSTOM and IAB, who have assisted in the screening of other candidate insecticides.

16 Monitoring commenced at the end of 1974, and it was soon apparent that there were no major changes in non-target river fauna that could be attributed to applications of temephos. Therefore, reports in 1980 and 1981 suggested a reduction of monitoring intensity, by concentrating on those sites, methods and times of year which had proved particularly reliable and informative. With extension of the programme area to the west and south-east being discussed, new river stations have been established and training programmes initiated to enable more national teams to be involved.

17 A full analysis of the data collected over the ten year period is now being undertaken. The aquatic monitoring programme in the Volta Basin is probably the largest of its type ever

carried out and apart from environmental protection, has contributed greatly to our knowledge of tropical riverine ecosystems.

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ENDNOTES

***.** *Recent cytotaxonomic révision of the sanctipauli group suggests that all temephos resistant populations are in fact* S. sanctipauli, *in the sense of having a fixed diagnostic inversion which is consistently absent from* S. soubrense (*Post R.J.,* Genetica. *in press*).

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Cloning and characterization of an *Onchocerca volvulus* specific DNA sequence

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Introduction

2 There are marked geographical variations in the spectrum of clinical manifestations associated with onchocerciasis. In West Africa the incidence of blindness due to sclerosing keratitis is much higher in savanna areas than in forest areas [1–3]. Although in other endemic areas of Africa and South and Central America the epidemiology and etiology of the disease may vary considerably, there are similarities in the ocular changes found in different areas. Studies on African onchocerciasis indicate that both the vector and parasite may consist of different forms, strains or species [4-9] and it has now been established that the main vectors of onchocerciasis in Africa are sibling species of the *Simulium damnosum* complex. Immunological and biochemical studies on *Onchocerca volvulus* have done little to clarify the possible existence of different strains, forms or subspecies, although allozyme studies have shown evidence of genetic variation in worms from different geographic areas [10,11].

3 During the collection of baseline data, epidemiological studies or during the monitoring of control or intervention programs, it is important to be able to assess accurately the transmission of *O. volvulus* by vector species. Most onchocerciasis vectors are known to be zoophilie to some degree, and can thus be infected with animal onchocerca species which, as third stage larvae (L3), are morphologically indistinguishable from each other and from *O. volvulus.* At present, *Onchocerca* infections are detected by dissection of the vector, and the transmission of the disease is measured by the Annual Transmission Potential, but the presence of animal *Onchocerca* species can resuit in inaccurate transmission data.

A reliable method for detecting infected vectors and distinguishing between the *Onchocerca* species as well as between the strains of *O. volvulus* is required, especially with the implementation of large scale Ivermectin trials in West Africa [1].

4 *Note:* Nucleotide sequence data reported in this paper have been submitted to the Genbank Data Bank with the accession number J04659

5 *Abbreviations:* NET, NaCl/EDTA/Tris buffer; SSC, NaCl/Nacitrate; TE. Tris/EDTA buffer; PBS, phosphate-buffered saline; RF, replicative form.

6 Highly repeated DNA sequences which undergo rapid evolutionary change [13] have been successfully used to isolate species-specific probes for vectors and parasites [14-17]. Recently, *Onchocerca*-specific clones were described which hybridized strongly to *O. volvulus,* but also to some other *Onchocerca* species [18-19], and Erttmann et al. reported a DNA sequence specific for a forest form of *O. volvulus* [20]. We describe here a cloned DNA sequence which appears to be *O.* volvulus-specific, hybridizing with isolates of *O. volvulus* from forest and savanna areas of West Africa, Central Africa and Central America. Sequence analysis of this clone revealed that it consists of twelve examples of a 149bp repeated sequence. The sequence of this repeat is strikingly similar to those previously reported for both the *O. volvulus* forest form-specific clone reported by Erttmann et al. [20] and the *Onchocerca*-specific clone reported by Shah et al. [19]. These results suggest that this 149-bp sequence is highly repeated in the genome of *O. volvulus,* and that the repeats have evolved to include different members which demonstrate different levels of specificity.

Materials and Methods

7 *Parasite material.* The *O. volvulus* material was obtained either as nodules which had been cryopreserved in liquid nitrogen immediately after excision, or as worms which had been collagenase-digested from nodules freshly excised in the field and snap-frozen.

8 The *O. volvulus* were from either a defined savanna area (Manambougou, Missira, Foura in Mali) or forest regions (Danane, Trompleu, Abraninouin, Ivory Coast; Bo, Sierra Leone) and from habitats of unknown definition in Zaire and Guatemala. *O. gutturosa* originating from England and *O. cervicalis* from South Carolina (U.S.A.) were received in propanol after excision from the hosts.

9 Frozen nodules of *O. gibsoni* from Australia and *O. ochengi* from the Central African Republic were obtained from abattoirs.

10 *Brugia malayi* microfilariae were obtained from jirds infected intraperitoneally [21], and *Dirofilaria immitis* microfilariae were provided by Dr. A. Scott (Johns Hopkins University, Baltimore).

11 *Isolation of DNA.* The nodules which had been cryopreserved immediately after excision were allowed to thaw at 37°C. Nodules of human origin were thawed in a 0.1% SDS solution as a precaution against HIV infection. The parasites were then freed by collagenase digestion as described by Schulz-Key et al. [22] and carefully cleaned of any host tissue. The worms were then washed in PBS and NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.5) three times. Similarly, the cryopreserved or alcohol-preserved adults were carefully washed to remove host tissue.

12 The parasites were freeze-thawed three times and homogenized with a mortar and pestle. The homogenate was then transferred to a 15 ml tube in NET buffer (1.8 ml NET per adult female worm). *n*-Lauryl sarcosine (Sigma, St. Louis, MO) and proteinase K (Boehringer Mannhein, Indianapolis, IA), were added to a final concentration of 1% (w/v) and 100 µg $ml^{-1,}$ respectively. The mixture was then incubated at 37°C for up to 6 h. Ribonuclease A (Sigma) was then added to a final concentration of 100 µg ml^{-1} and the solution was further incubated for 30 min at 37°C. The DNA was extracted with phenol/chloroform and dialyzed extensively against TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

13 The yield of DNA varied from 3 µg from a small female to 13 |xg from a large female worm. The amount of host DNA contamination of the preparation was monitored as described previously [19] using an *Alu*I like human sequence or calf thymus DNA (Sigma) to probe Southern blots.

14 *Blackfly material.* DNA was extracted as described above from pooled *Simulium sirbanum* from Tienfala (Niger River, Mali) which had been either cryopreserved in liquid nitrogen or preserved in propanol.

15 *Construction of genomic libraries.* Genomic libraries were constructed in the plasmid pUC 9; Sau 3A fragments of DNA pooled from *O. volvulus* from Mali were ligated into the *BamHI* site of the polylinker [19] for the *O. volvulus* 'savanna' library. Similarly, *Sau*3A restriction fragments of worms from Danane, Ivory Coast, were ligated into the *Bam*HI site of pUC9 for the 'forest' library.

16 The libraries were screened by differential hybridization of four replicate nitrocellulose filters of the ampicillin-resistant colonies from the library. The filters were air-dried and the DNA was denatured, fixed and neutralized according to Maniatis et al. [23]. The filters were probed with ^{32}P-radiolabeled nick-translated genomic DNA from *O. volvulus* (savanna or forest), human DNA, *O. gibsoni* DNA and a cocktail of DNA from *B. malayi, Brugia pahangi* and *D. immitis.*

17 Colonies were selected if they hybridized to *O. volvulus* DNA and not to DNA from humans, bovine *Onchocerca* species or the lymphatic filariae. A second screening of the selected *O. volvulus* colonies was carried out by probing replicate filters with nick-translated DNA from *O. volvulus* savanna and forest, and second species of animal *Onchocerca.* Colonies hybridizing strongly to one of the *O. volvulus* isolates and not to the other *Onchocerca* species were investigated further.

18 Plasmids pOvs134, pOvs742 and pOvs745 from the savanna library, and pOvf4, pOvf7 and pOvf2 from the forest library, which appeared to hybridize specifically to *O. volvulus* genomic DNA, were colony-purified and the plasmid DNA containing the *O. volvulus* sequences was isolated [23].

19 *Southern and dot blot analysis.* Genomic DNA was digested with a 5-fold excess of restriction endonuclease. The completeness of the digestion was monitored by including a lambda DNA standard in an aliquot of the digestion mixture.

20 The digest products were then size-fractionated by electrophoresis on an agarose gel, and the gel was used to prepare a Southern blot, as previously described [24], Nick-translated DNA probes (2-3 x 10^6 cpm (100 ng DNA)$^{-1}$) were hybridized to the filters. Hybridization was performed at 42°C in a solution containing 50% formamide, 5 x Denhart's solution, 2.5 x SSC, 0.1% SDS and 200µg/ml^{-1} herring sperm DNA, and three washes of 30 min each in 0.1 x SSC (150 mM NaCl, 15 mM sodium citrate), 0.1% SDS at 50°C.

21 Dot blot hybridizations were carried out on nitrocellulose filters by spotting dilutions of genomic DNA onto the filter, air-drying it and then denaturing the DNA for 1 min in 0.5 M NaOH and neutralizing twice for 5 min in 1 M Tris, 1.5 M NaCl, pH 8. Before hybridization, the filters were baked at 70°C for 2 h.

22 Dot blots on the nylon membrane Gene Screen Plus (Du Pont Company, Boston, MA) were performed according to the manufacturers directions using a manifold vacuum filtration apparatus (Biorad, Richmond, CA) and the filter hybridized in a solution containing 10% dextran sulphate at 65°C.

23 The sensitivity of the selected plasmids was assessed by spotting genomic DNA from savanna *O. volvulus,* and forest *O. volvulus* in a doubling dilution series from 500 ng to 0.244 ng onto Gene Screen Plus membrane and probing the filter with the purified *Eco*RI-*Hind*III fragment of the insert of plasmid pOvs134.

24 *DNA sequence analysis.* The inserted DNA of pOvs134 was subcloned into the bacteriophage vectors M13Mp18 and M13Mp19, and doublestranded replicative form (RF) DNA was prepared. The RF DNA was then used to prepare a set of clones containing nested deletions of the pOvs134 insert using the exonuclease BAL 31, as previously described [25]. The DNA sequence of the nested deletions was then determined using the dideoxynucleotide termination method [26]. The entire sequence of the insert, in both orientations, was obtained from the overlapping deletions.

Results

25 *DNA isolation.* The isolation of high molecular weight DNA from *Onchocerca* parasites proved to be quite difficult. In order to avoid the problem of host contamination of the parasite DNA reported by Perler et al. [18], it was necessary to digest the nodular material with collagenases. However, the thawing and the subsequent long incubation of the material in collagenase resulted in degradation of the parasite DNA. If the incubation time was minimized by frequent changes (2-3-hourly) of collagenase and constant agitation. DNA of reasonable quality could be obtained. The best quality DNA was obtained from the worms digested in the field and snap-frozen. High-molecular-weight DNA was also obtained from the worms preserved in propanol, although the quality of DNA did deteriorate with increasing time of conservation.

26 *Identification of Onchocerca-specific sequences from the genomic libraries.* 800 clones of the savanna library, and 600 clones from the forest *O. volvulus* library were screened with nick-translated genomic DNA from *O. volvulus,* humans, *B. malayi, B. pahangi, D. immitis* and various bovine or equine *Onchocerca* species. Clones which hybridized strongly to the *O. volvulus* DNA, but not to the other DNAs were selected for further investigation. The plasmids pOvs134, pOvs742 and pOvs745 were isolated from the savanna library, and pOvf2, pOvf4 and pOvf7 were isolated from the forest library.

27 *Specificity and sensitivity.* To further examine the specificity of the six putatively *O. volvulus*-specific clones, the inserted DNA of each clone was used to probe Southern blots of genomic DNA of various filarial species. Using this stringent test, only one of the six clones, pOvs134, proved to be specific for *O. volvulus.* As is shown in Fig. 1, pOvs134 hybridizes strongly to *O. volvulus* DNA from both savanna and forest isolates, as well as to *O. volvulus* from Guatemala. The probe did not hybridize to genomic DNA from *Onchocerca*

gibsoni, Onchocerca ochengi, Onchocerca gutturosa, O. cervicalis, or human or vector blackfly DNA.

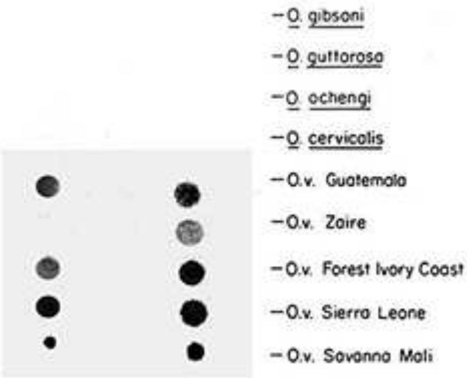

Fig. 1. Specificity. Approximately 200 ng and 400 ng of genomic DNA from *O. volvulus* isolates (O.v.). and from other *Onchocerca* species was spotted onto a nitrocellulose filter which was hybridized to the purified insert of pOvs134.

28 Southern blot analysis was then used to confirm the specificity of pOvs134 for *O. volvulus* DNA. As is shown in Fig. 2, pOvs134 hybridized strongly to genomic DNA from two isolates of *O. volvulus,* but did not hybridize to DNA from *O. gutturosa, O. ochengi, O. cervicalis, O. gibsoni, B. malayi* or *D. immitis.*

TABLE I. Specificity of the three related probes at different stringencies

	pOVS134		pFS		puOv3	
	50°C	55°C	50°C	55°C	50°C	55°C
O. volvulus forest	+	+	+	+	+	−
O. volvulus savanna	+	+	+	−	+	−
O. gibsoni	−	−	+	−	+	−

Each of the three related probes was hybridized to Southern blots containing the DNA samples shown, and the blots were washed at the temperatures indicated in the table.

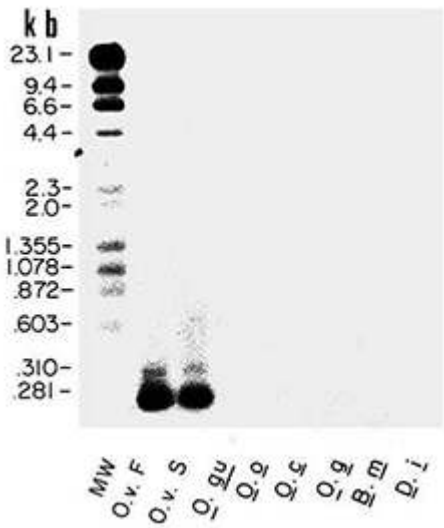

Fig. 2. Southern blot analysis of 1.5 µg of genomic DNA from *Onchocerca* and filarial species digested with *Rsa*I and probed with the purified insert of pOvs134. MW. molecular weight markers (λ *Hin*dIII, Ox *Hae*III); O.v.F, *O. volvulus* forest (Ivory Coast); O.v.S, *O. volvulus* savanna (Mali); O.gu. *O. gutturosa:* O.o. *O. ochengi;* O.c. *O. cervicalis;* O.g. *O. gibsoni;* B.m. *B. malayi;* D.i. *D. immitis.*

29 In order to test if the DNA sequences recognized by pOvs134 were restricted to a sub-population of *O. volvulus,* a Southern blot containing *O. volvulus* genomic DNA samples from several geographically distinct isolates was probed with pOvs134. As shown in Fig. 3, pOvs134 hybridized to *O. volvulus* genomic DNA from parasites isolated from the Ivory Coast, Sierra Leone, and Mali. These results, together with those shown in Fig. 1, suggest that the sequence recognized by pOvs134 is present in *O. volvulus* from both the savanna and forest regions of West Africa, as well as in isolates from the New World.

30 The sensitivity of clone pOvs134 was assessed by dot-blotting decreasing amounts of *O. volvulus* genomic DNA from the savanna and forest regions of West Africa. As shown in Fig. 4, pOvs134 was able to detect as little as 250 pg of *O. volvulus* genomic DNA.

31 *Sequence organization.* Hybridization of pOvs134 to complete *Rsa*I digests of *O. volvulus* DNA revealed two bands approximately 320 and 170 bp in size (Fig. 5). Partial digestion of the *O. volvulus* DNA gave a ladder pattern, suggesting that pOvs134 contains a sequence that is tandemly repeated in the genome (Fig. 5).

32 *DNA sequence analysis of pOvs134.* The DNA sequence of the inserted DNA of pOvs134 was determined as described in Materials and Methods. The insert of pOvs134 was found to be 1779 bp in length. It consisted entirely of 12 repeats of a 149-bp sequence which were joined head to tail. Examination of the DNA sequence of pOvs134 for similarities to other known sequences showed that the repeat was similar to both the *Onchocerca* specific clone pUOv3 described by Shah et al. [19], as well as the *O. volvulus* forest formspecific clone pFS-1 described by Erttmann et al. [20]. The sequence of the twelve repeats found in pOvs134 is given in Fig. 6, as well as the sequences of pUOv3 and pFS-1. A consensus sequence derived from the fifteen known examples of the repeat is also shown. Residues in individual repeats which differ from the consensus are outlined.

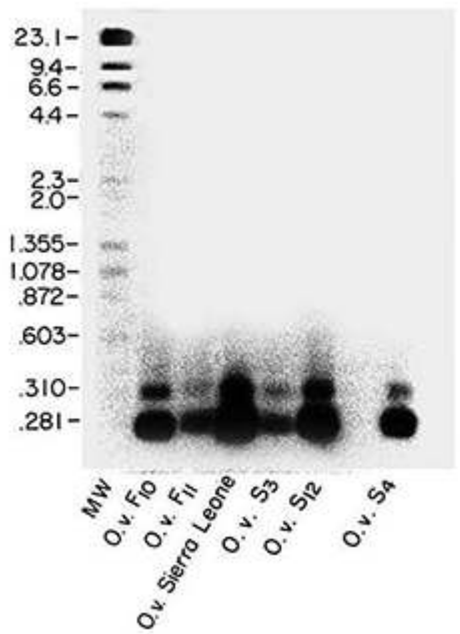

Fig. 3. Southern blot analysis of different isolates of *O. volvulus* genomic DNA digested with *Rsa*I and probed with purified insert of pOvs134. Lanes 2, 4.6 and 8 had 1.5 µg DNA and lanes 3 and 5 had less parasite DNA due to slight contamination with host DNA. MW, molecular weight markers; O.v.F10 and O.v.F11, *O. volvulus* forest isolates from Ivory Coast; O.v. Sierra Leone, *O. volvulus* forest isolate from Sierra Leone; O.V.S3, 12 and 4, *O. volvulus* savanna isolates from Mali.

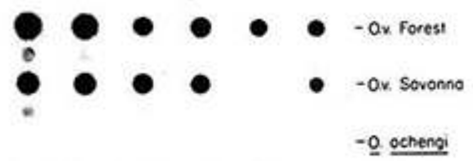

Fig. 4. Sensitivity. Genomic DNA from *O. volvulus* forest (O.v. Forest), O. *volvulus* savanna (O.v. Savanna), and *O. ochengi* were spotted in doubling dilutions from 500 ng to 0.244 ng onto Gene Screen Plus membrane and probed with nicktranslated pOvs134. Filter exposure of 90 min.

33 ***Copy number of the 149-bp repeat in the genome.*** An estimation of the copy number of pOvs134 in the genome was made by dot-blotting genomic DNA, pOvs134 and pUC9 onto Gene Screen Plus filters in dilutions from 100 to 0.1 ng, and hybridizing the filter to the nick-translated insert of pOvs134. As is shown in Fig. 7, approximately 1% of the *O. volvulus* genomic DNA appears to be related to the repeat found in pOvs134. Assuming a haploid genome size of 8 x10^7 bp [27], this resuit suggests that the repeat is present in around 4500 copies per haploid genome.

34 ***Specificity of the related DNA probes at different stringencies.*** The discovery that the DNA probes pOvs134, pFS-1 and puOv3 are variations of a 149-bp sequence which is highly repeated in the genome of *O. volvulus* suggested that the specificity of these probes might be affected by the stringency of hybridization. To test if this was the case, Southern blots containing *O. volvulus* forest and savannah form DNA, as well as *O. gibsoni* were probed with the three probes. The blots were then washed at 50°C and 55°C. pOvs134 retained its specificity and sensitivity at both temperatures. In contrast, pFS-1 lost its specificity at 50°C, and pUOV3 appeared to lose all sensitivity at 55°C (Table I).

Fig. 5. Southern blot analysis of 1.5 µg of genomic DNA digested with *Rsa*I to completion, (lane 1 and 2) or partially digested (lane 3) and probed with purified insert of pOvs134. MWM, molecular weight markers; O.c, *O. cervicalis;* O.v.S, *O. volvulus* savanna; O.v.S p, *O. volvulus* savanna partial digest.

Discussion

35 There is a real need for a method of distinguishing between the savanna and forest forms of *O. volvulus,* and also for differentiating *O. volvulus* infective larvae from other *Onchocerca* species. The Onchocerciasis Control Programme (OCP) in West Africa is committed to

reducing the level of onchocerciasis in savanna regions to a level where it is no longer a public health problem [28]. Until very recently this has been primarily a vector control program and, despite considerable success, problems remain with reinvading infective Aies and occasional new outbreaks of infection [29]. Thus it is important to be able to identify the origin or form of the parasite in order to assess the risks and plan appropriate control strategies. Erttmann et al. reported identifying a DNA sequence, pFS-1, which appears to be specific for the forest form of *O. volvulus* [20]. An *O. volvulus* savanna-specific sequence has not yet been isolated.

Fig. 6. DNA sequence of the inserted DNA of pOvs134. The DNA sequence of the inserted DNA was determined as described in Materials and Methods. The sequence of each of the twelve repeats of pOvs134 described in the text are labeled Ovsl-12. The sequence of the forest form specific clone pFS-1 is labeled pFS-1 [20], and the two repeat units found in the *Onchocerca* specifie clone pUOV3 [19] are labeled Ov3A and Ov3B. A consensus sequence derived from the fifteen examples of the repeat is labeled CONS. Residues within individual repeats which differ from the consensus are outlined.

36 The necessity to distinguish between L3 of *O. volvulus* and other *Onchocerca* species arises because domestic animais are commonly infected with *Onchocerca* species. In Africa, for example, cattle are known to carry several different *Onchocerca* species [30]. Although the vectors are not known for all species, it has been demonstrated that *O. ochengi* and *O. gutturosa* develop to infective L3 indistinguishable from *O. volvulus* in *S. damnosum* s.l. and *S. vorax* [31,32] while *Simulium* and *Culicoides* species are the incriminated vectors for the other *Onchocerca* species. In the OCP the transmission is estimated by measuring the Annual Transmission Potential [13]. The presence of infective larvae of animal origin can affect the accuracy of the transmission assessments. This is not only of importance in areas where control measures are in operation, but also in areas where trials of Ivermectin are being undertaken and an accurate assessment of the effect of the drug on transmission is crucial. Although pOvs134 still needs to be tested for cross-hybridization to the closely related species *Onchocerca dukei, Onchocerca hamoni,* and *Onchocerca*

schulzkeyi, it could be valuable as a means of distinguishing *O. volvulus* infective larvae from other morphologically similar species. Used in combination with a clone such as the forest-specific *O. volvulus* sequence described by Erttmann et al. [20], pOvs134 could also be useful in distinguishing between savanna and forest *O. volvulus* strains in vectors, or in human hosts.

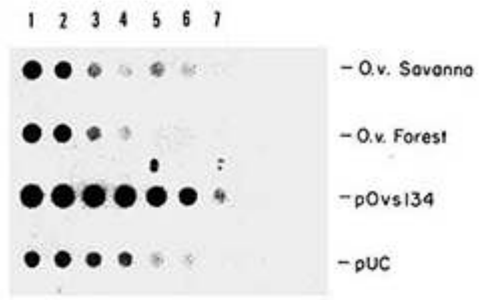

Fig. 7. Copy number. Genomic DNA from *O. volvulus* savanna and forest. plasmid pOvs134 and pUC9 were spotted onto Gene Screen Plus membrane and probed with the *Eco*RI- *Hind*III purified insert of pOvs134.1-100 ng. 2-50 ng. 3-10 ng. 4-5 ng. 6-0.5 ng. 7-0.1 ng.

37 Evolution within the genus *Onchocerca* is thought to be relatively recent, specially in the line of *Onchocerca* of African Bovidae to which *O. volvulus* belongs [33]. The relatedness of *Onchocerca* species is confirmed by rRNA sequence data [34] and is evident from the difficulties encountered in isolating species-specific repetitive DNA sequences. In this study, out of hundreds of clones only one was found to be specific for *O. volvulus,* while Erttmann et al. screened over 20000 clones in order to identify one clone that appeared to be specific for the forest form of *O. volvulus* [20]. Surprisingly, DNA sequence analysis of pOvs134 demonstrated that it was very similar to both the *Onchocerca*-specific clone pUOV3 isolated by Shah et al. [19], as well as the *O. volvulus* forest form-specific clone isolated by Erttmann et al. [20]. Comparison of the DNA sequences of these three clones may allow oligonucleotide sequences to be identified whose specificités as probes will be less dependent upon the conditions of hybridization.

38 The titration experiment described above suggests that the 149-bp repeat unit is present in approximately 4500 copies per haploid genome. This fact, when considered in light of the discovery that three independently isolated clones with differing specificites for *Onchocerca* DNA appear to contain variations of this 149-bp repeat, suggests that members of the 149-bp repeat family have diverged during the course of the evolution of the genus *Onchocerca.* This process has resulted in the production of different versions of the repeat unit. Some of the repeats, such as those found in pOvs134, have diverged to the point that they are now specific for *O. volvulus,* and at least one version appears to be found only in the forest form of *O. volvulus.* In contrast, some of the repeats, such as those found in pUOV3, are still able to recognize repeats in different species of *Onchocerca.* This situation is similar to that found with minisatellite sequences in the human genome, where closely related minisatellite DNA probes recognize different repeat sequence families within the genome [35,36]. By analogy to other highly repeated DNA sequences found in other species, it is unlikely that evolution of the 149-bp repeat family will be hindered by functional constraints. It may therefore be possible to use the sequence of examples of the 149-bp repeat to define the evolutionary relationship between closely related species of *Onchocerca,* as well as the different forms of *O. volvulus,* that would be difficult to elucidate using more slowly evolving DNA sequences.

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ABSTRACTS

A cloned sequence, pOvs134, was isolated from a genomic library prepared from *Onchocerca volvulus* of savanna origin in the plasmid pUC9. pOvs134 hybridizes to all the geographic isolates of *O. volvulus* tested from both the New and the Old World, but not to the species *Onchocerca gibsoni, Onchocerca gutturosa, Onchocerca ochengi, Onchocerca cervicalis,* the filarial parasites *Brugia malayi.* or *Dirofilaria immitis,* nor to human or simuliid DNA. As little as 250 pg of DNA can be detected on a dot blot hybridization. suggesting that pOvs134 is sensitive enough to detect a single third stage larva. DNA sequence analysis of the inserted DNA of pOvs134 revealed that it consisted of twelve examples of a 149-bp repeat. The sequence of this repeat is strikingly similar to that of two *O. volvulus* genomic clones previously described, one of which has been reported to be specific for forest form *O. volvulus.* and one of which hybridizes to genomic DNA of several species of *Onchocerca.* These results suggest that the 149-bp repeat sequence is highly repeated in the genome of *O. volvulus,* and that variants of this repeat with different specificities exist.

INDEX

Keywords: DNA probe, Onchocerca volvulus, Repeated sequence, Species determination

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Zonation ichtyologique du bassin du Bandama (Côte d’Ivoire)

Ichtyological zonation of Bandama basin (Ivory Coast)

Bernard de Merona

INTRODUCTION

1 Les milieux lotiques sont le plus souvent extrêmement complexes, ce qui a des conséquences sur la répartition l’abondance et le comportement des populations de poissons. Celles-ci présentent, dans les milieux naturels un équilibre que l’intervention humaine tend à rompre. Il apparaît actuellement essentiel de maintenir autant que possible ces équilibres, dans la mesure où il est établi que leur rupture peut avoir des conséquences graves sur l’ensemble de l’environnement. Les études de répartition des poissons apportent les éléments nécessaires pour l’aménagement piscicole et pour le contrôle des communautés naturelles.

2 Ainsi ce travail s’inscrit dans le cadre d’une surveillance des populations de poissons dans des rivières traitées à l’insecticide[1] (Pour détecter d’éventuelles modifications il était nécessaire de localiser les différentes espèces, de mettre en évidence les associations interspécifiques et de décrire les peuplements tout au long du cours.

3 Depuis longtemps les problèmes de zonation des rivières ont préoccupé les hydrobiologistes et d’excellentes mises au point ont été faites par ILLIES et BOTOSANEANU (1963) ou plus récemment par HAWKES (1975). Beaucoup de travaux, parmi les plus anciens, sont des études descriptives partielles. La rivière étudiée est partagée en un certain nombre de zones à partir de la présence d’espèces types de poissons. En raison de l’aire de distribution limitée de ces espèces types, et de la diversité des rivières, ces schémas n’ont pu être généralisés.

4 Considérant ces informations portant sur les espèces de poissons les plus abondantes, comme insuffisantes pour caractériser des portions de cours, d’autres auteurs ont tenté des descriptions plus exhaustives. Ainsi, ILLIES et BOTOSANEANU (1963) étudient la faune d’invertébrés benthiques de la Fulda et proposent un schéma théorique général sur lequel

il est possible de placer les zones piscicoles précédemment établies sur quelques rivières. L'Eucrenon et l'Hypocrenon sont les zones de source et les ruisselets qui leur font suite. Le Rhithron, séparé en Épi, Meta et Hypo-Rhithron, est la partie montagneuse qui correspond le plus souvent aux zones à Salmonides. Enfin, le Potamon, divisé lui aussi en Épi, Meta et Hypo Potamon, est la zone de plaine et englobe les zones à Barbeaux, à Brème et la partie estuarienne.

5 VERNEAUX (1976), à partir d'une analyse de données portant sur l'ensemble de la faune de plusieurs cours d'eau français et suisse, détermine un schéma théorique de 10 niveaux typologiques auxquels sont associés des groupements d'espèces.

6 Une autre approche intéressante est réalisée par ECHELLE et SCHNELL (1976) qui analysent des données sur les peuplements de poissons d'un bassin de l'Oklaoma. Des groupements d'espèces ayant une distribution voisine sont mis en évidence.

7 Par ailleurs, au lieu de décrire les peuplements par leur composition spécifique relative, il peut être suffisant d'étudier certaines caractéristiques. Quelques travaux mettent ainsi en évidence, une augmentation régulière de la richesse et de la diversité spécifique de la source à l'embouchure d'un cours d'eau (HARREL *et al.,* 1967 ; SHELDON, 1968 ; DEACON et BRADLEY, 1972 ; WHITESIDE et MCNATT, 1979).

8 Enfin, dans le souci de généraliser, les auteurs ont tenté de relier aux facteurs abiotiques du milieu, les distributions d'organismes et ont proposé des modèles. Les facteurs qui ont une influence sur la répartition des organismes sont nombreux (MACAN, 1961), mais parmi ceux-ci, la vitesse du courant semble avoir un rôle prépondérant en ce qu'elle influe sur nombre d'autres caractéristiques, et HUET (1962) définit la « règle des pentes » qui, dans une même aire géographique, associe la présence de certaines espèces de poissons à une fourchette de gradients. Dans le même ordre d'idées, VERNEAUX (1977), à la suite d'analyses d'un grand nombre de caractères abiotiques, donne une formule d'un « type écologique théorique », conjonction de quelques facteurs principaux, qui permettrait de déterminer l'appartenance typologique théorique d'un point quelconque. Là encore, l'application de ce modèle n'est valable que dans l'aire géographique où a été menée l'étude.

9 Il ressort de cet ensemble de travaux, qu'en dépit des efforts consentis, un schéma de zonation, généralisable à toutes les rivières n'ait pu être dégagé, et cela, à l'intérieur de la même zone climatique.

10 En Afrique intertropicale, les travaux sont beaucoup moins nombreux. Un certain nombre d'études ont été menées dans des rivières prenant leur source en altitude et au dénivelé important, au moins sur une partie de leur cours. Les résultats obtenus, aussi bien dans le bassin du Zaïre (MARLIER, 1954 ; MALAISSE, 1976) que dans les bassins d'Afrique du Sud (HARRISON et ELSWORTH, 1958 ; OLLIF, 1960 ; HARRISON, 1965 ; GAIGHER, 1973) ou du Zambèze (BALON, 1974), montrent un schéma de zonation bien net et HARRISON (1965) a pu appliquer avec succès la nomenclature d'ILLIES et BOTOSANEANU (1963). En revanche, dans une rivière forestière du Nigeria, SYDENHAM (1978) ne sépare qu'une zone de cours inférieur dans la forêt inondée et une petite zone de source, le reste du cours présentant un peuplement de poissons homogène.

LE MILIEU

11 Le bassin du Bandama (fig. 1) est l'un des trois grands bassins de Côte d'Ivoire. Le cours principal coule du nord au sud sur une distance d'environ 1 000 km et reçoit deux grands affluents : la Maraoué sur sa rive droite (550 km) et le Nzi sur sa rive gauche (725 km). Les rivières de ce bassin traversent plusieurs zones de végétation. Prenant naissance dans la savanne arborée, elles pénètrent progressivement dans la forêt dense en passant par des zones de forêt défrichée. Elles présentent un « régime équatorial de transition atténué » (GIRARD *et al.*, 1971). La saison des moyennes et hautes eaux s'étale de mai à novembre. Bien que, sur une grande partie du bassin, les précipitations présentent deux pics (mai-juin et septembre-octobre), la crue annuelle n'est, en général pas dédoublée. Les crues présentent une irrégularité inter-annuelle importante.

12 Le profil, tel qu'il apparaît sur la figure 2 montre que la pente est faible, de l'ordre de 0,40 m par km, et régulière sur la presque totalité du cours. Seule une portion proche de la source présente un dénivelé de 1 à 2 m par km. Cette régularité de la pente entraîne une homogénéité dans les types de milieu rencontrés tout au long du cours. De grandes étendues d'eaux calmes et relativement profondes (vasques) sont séparées par des seuils rocheux (radiers). Les vasques ont une profondeur variable suivant la saison, mais en général supérieure à 1 m. Leur fond est vaseux, parsemé de rochers, rarement sableux. Les radiers sont plus hétérogènes, comprenant des chenaux à courant d'eau plus ou moins violents, des zones de tourbillons et des plages d'eau calme peu profonde.

ÉCHANTILLONNAGE

13 L'échantillonnage des vasques est réalisé à l'aide d'une batterie de filets maillants comprenant sept filets de 12,5 ; 15 ; 17,5 ; 20 ; 22,5 ; 25, et 30 mm de maille.

14 Les filets sont posés avant la tombée de la nuit et sont relevés le matin. Chaque prélèvement porte sur deux nuits afin de compenser les aléas de la pêche. Un échantillon est constitué de la somme des prises par unité d'effort espèce par espèce (exprimée en nombre ou en poids de poisson pour 100 m^2 de surface pêchante et par nuit), pour les différents types de filets.

FIG. 1. — Hydrographie du bassin du Bandama et localisation des stations d'échantillonnage.

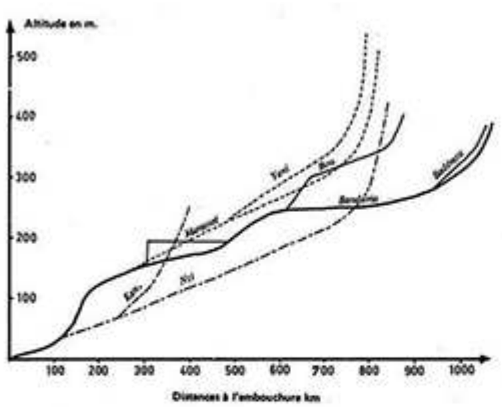

FIG. 2. — Profil en long du bassin du Bandama.

15 L'échantillonnage des milieux peu profonds est effectué à l'aide d'un matériel de pêche électrique à l'épuisette (GOSSET, 1976). Aucune unité d'effort satisfaisante n'a pu être définie tant l'efficacité de ce genre d'engin est dépendante du milieu et de l'utilisateur. Les résultats sont donc dans ce cas utilisés bruts et les comparaisons quantitatives de station à station sont impossibles.

16 L'étude porte sur 24 échantillons aux filets maillants et 43 échantillons en pêche électrique réalisés pendant la période d'étiage (novembre à mars) et couvrant la totalité du bassin (fig. 1).

TRAITEMENT DES DONNÉES

17 En raison de la faible efficacité des filets maillants et du hasard de la pêche, les captures des espèces rares sont très aléatoires, et leur absence de l'échantillon n'a pas de réelle signification. Nous avons donc éliminé, pour les comparaisons entre échantillons, les espèces dont l'effectif n'est, dans aucun prélèvement, supérieur à 5 % de l'effectif total du prélèvement.

18 En vue d'homogénéiser les variances, et de normaliser, même grossièrement les distributions, une transformation log(x+1) a été appliquée aux données brutes. Les matrices des effectifs transformés ont fait l'objet d'une analyse factorielle des correspondances (BENZECRI, 1973).

19 Les diversités dans les échantillons ont été calculées à partir des biomasses par la formule de Shannon : $I = — \Sigma\, p_1 \log_2 p_1$ où p_1 représente les biomasses relatives de chaque espèce i.

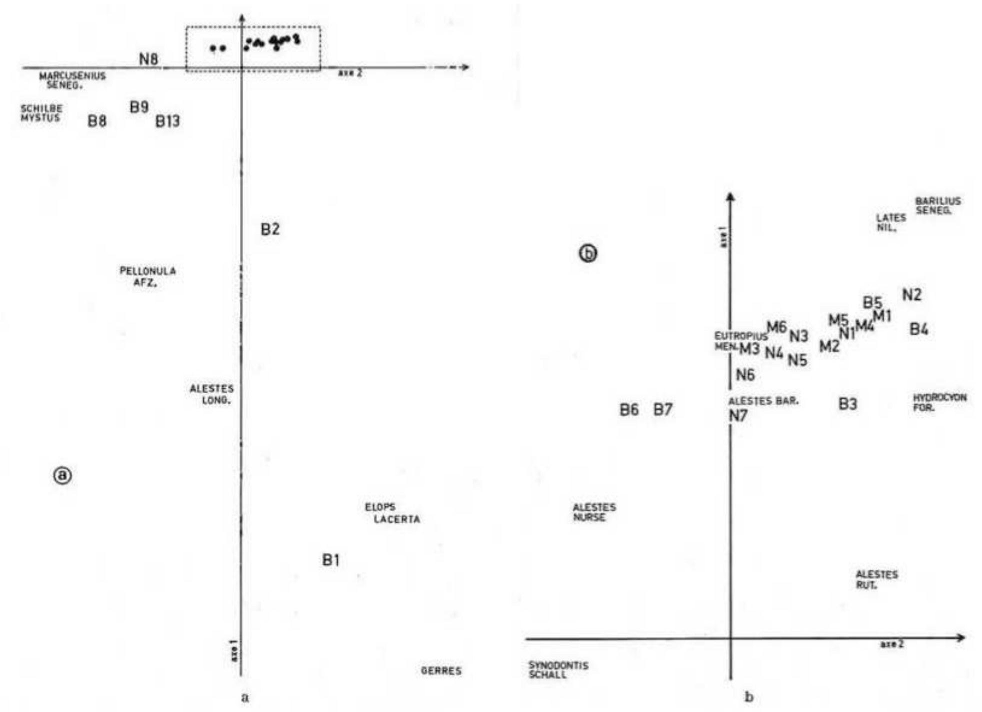

FIG. 3. — Projection des points, dans le plan des axes 1 et 2 de l'analyse factorielle des correspondances sur les prélèvements dans les milieux d'eau profonde du bassin du Bandama. a : ensemble de la projection ; b : détail de la partie encadrée. B : stations du Bandama ; N : stations du Nzi ; M : stations de la Maraoué.

RÉSULTATS

A. Milieux profonds

20 Les deux premiers facteurs de l'analyse des correspondances extraient 56 % de l'inertie totale. L'image fournie par la projection des points sur les axes 1 et 2 est donnée sur la figure 3 (a et b). La dispersion des prélèvements peut être mise en rapport avec la distance à l'embouchure comme le montre la figure 4 (a et b) qui porte les coordonnées des prélèvements sur les axes de l'analyse, en fonction de la position géographique des

stations. Le tableau I, qui donne les corrélations des espèces avec les deux premiers axes permet de dégager les espèces responsables de la dispersion des prélèvements. La répartition des espèces ayant les corrélations les plus élevées est portée sur la figure 5.

21 L'axe 1 sépare principalement les stations de la partie la plus inférieure du cours (B1 et B2) et, dans une moindre mesure, les stations les plus éloignées de l'embouchure (N8, B8, B9, B13), de l'ensemble des autres prélèvements.

22 A cette dispersion correspondent les espèces *Gerres* sp., *Elops lacerta, Alestes longipinnis, Pellonula afzeluisi, Eutropius mentalis* et *Alestes baremoze.*

23 L'axe 2 isole nettement les stations les plus septentrionales par les espèces *Marcusenius senegalensis* et *Schilbe mystus.* Les stations des cours moyens s'ordonnent également suivant cet axe (figure 3b). Les prélèvements des parties inférieures (Ml, M2, B4, B5, N1), avec des espèces *Barilius senegalensis, Hydrocyon forskahlii* et *Lates niloticus,* ainsi que ceux des portions supérieures (B6 et B7) avec les espèces *Alestes nurse* et *Synodontis schall* encadrent les prélèvements moyens correspondant aux espèces *Eutropius mentalis* et *Alestes baremoze.* En dépit de cette image générale, la succession topographique des stations dans une rivière n'est pas toujours reproduite par les coordonnées sur l'axe 2, comme le montre bien la représentation de la figure 4b. Dans le Bandama, il existe une cassure entre les stations B5 et B6. La présence du barrage de Kossou qui interrompt totalement le cours du fleuve en est probablement responsable. En effet, l'abondance, dans les stations B6 et B7 d'espèces telles que *Labeo senegalensis* est vraisemblablement liée à la proximité du lac. Dans la Maraoué, un accident dans la courbe s'observe entre les stations M3 et M4. Ce phénomène serait lié à la distribution particulière d'espèces du cours moyen qui effectuent des déplacements, provoquant ainsi des pics de leur abondance relative dans certaines stations. En l'occurrence, *Hydiocynus forskahlii,*

Petrocephalus bovei, et *Eutropius mentalis* présentent des évolutions concomitantes de leur abondance relative.

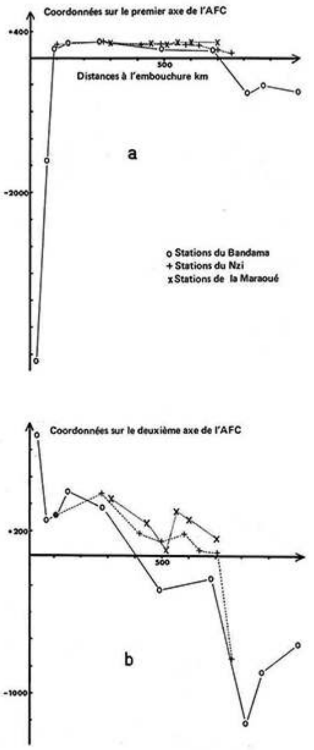

FIG. 4. — Relation entre les distances à l'embouchure et les coordonnées sur les axes de l'analyse factorielle des correspondances, des prélèvements d'eau profonde, a : premier axe ; b : deuxième axe.

TABLEAU I. Corrélations des espèces pêchées en eau profonde avec les axes I et II de l'analyse factorielle des correspondances

Espèces	Corrélations I	Corrélations II
Gerres sp.	**0,844**	0,080
Alestes longipinnis	**0,789**	0,009
Elops lacerta	**0,770**	0,071
Pellonula afzeluisi	**0,391**	0,079
Eutropius mentalis	**0,217**	0,001
Alestes baremoze	**0,117**	0,015
Chrysichthys velifer	**0,092**	0,036
Alestes imberi	**0,092**	0,042
Petrocephalus bovei	**0,086**	0,024
Marcusenius bruyeri	**0,039**	0,000
Marcusenius senegalensis	0,048	**0,793**
Schilbe mystus	0,007	**0,679**
Hydrocyon forskahlii	0,078	**0,611**
Alestes futilus	0,009	**0,563**
Alestes nurse	0,034	**0,454**
Synodontis schall	0,002	**0,408**
Labeo senegalensis	0,000	**0,288**
Barilius senegalensis	0,057	**0,160**
Lates niloticus	0,077	**0,118**
Distichodus rostratus	0,006	**0,065**
Marcusenius furcidens	0,054	**0,056**
Synodontis bastiani	0,044	**0,047**
Tilapia zilii	0,036	**0,039**

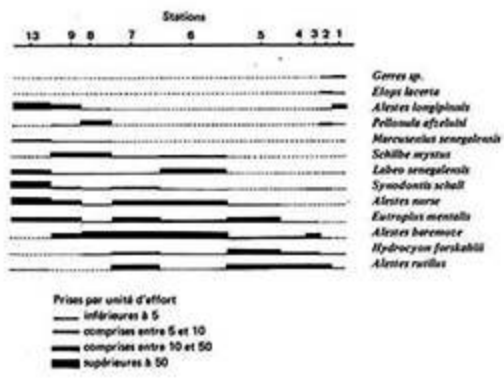

FIG. 5. — Répartition des espèces d'eau profonde les plus importantes dans le Bandama.

24 Le facteur 3 extrait encore 12 % de la variabilité, mais apparaît lié uniquement à la distribution particulière de *Pellonula afzeluisi* (40 % de contribution relative) qui sépare les stations B1 et B2 d'une part et B8 et B13 d'autre part. Le quatrième facteur n'extrait plus que 6 % de l'inertie.

25 A partir de ces résultats, il est possible de classer les espèces en quatre groupes principaux :

1. ESPÈCES ESTUARIENNES : *Gerres* sp. et *Elops lacerta.* A ce groupe peuvent être rattachées les autres espèces estuariennes qui n'ont pas participé à l'analyse de par leur rareté. Ce sont : *Belone senegalensis, Polydactylus quadrifilis, Tilapia mariae* et *Tylochromis jentinki.*
2. ESPÈCES PRÉSENTES OU PARTICULIÈREMENT ABONDANTES AUX DEUX EXTRÉMITÉS DU COURS : *Alestes longipinnis* et *Pellonula afzeluisi. Hepsetus odoe* rare dans nos prélèvements présente le même type de distribution.
3. ESPÈCES PRÉSENTES OU PARTICULIÈREMENT ABONDANTES DANS LES PARTIES DU COUPS LES PLUS ÉLOIGNÉES DE L'EMBOUCHURE : *Marcusenius senegalensis, Schilbe mystus* et *Synodontis schall,* mais aussi *Polyplerus endlicheri* retiré de l'analyse.
4. ESPÈCES À DISTRIBUTION LARGE, CONSTANTES DANS LE COURS MOYEN ET DONT L'ABONDANCE EST VARIABLE EN FONCTION DE CRITÈRES INDÉPENDANTS DE LA ZONATION. *Alestes baremoze, Alestes rutilus, Eutropius mentalis* et *Hydrocynus forskahlii* sont parmi les plus abondantes.

26 Cette classification aboutit, pour les milieux profonds, à un schéma de zonation clair : une zone de cours inférieur, sous influence estuarienne, et une zone de cours supérieur, toutes deux peu étendues, encadrant une large zone de cours moyen.

27 Aucune différence notable dans la richesse, la diversité et l'équitabilité des peuplements n'apparaît liée à cette partition du bassin (tabl. II).

B. Milieux peu profonds

28 Les trois premiers facteurs de l'analyse des correspondances sur les 43 prélèvements extraient 42 % de l'inertie totale du nuage. Les projections des prélèvements et des espèces dans les plans des axes 1 et 2 d'une part et 1 et 3 d'autre part, sont données sur les figures 6 et 7. La variabilité restante est expliquée par les 34 facteurs suivants, le quatrième n'en extrayant déjà plus que 6 %.

29 Les espèces se répartissent en trois groupes en fonction de leurs corrélations aux trois premiers axes (tabl. III). Le premier facteur qui extrait 22 % de l'inertie totale semble lié à la zonation longitudinale. Il existe une bonne corrélation entre les coordonnées des

prélèvements sur cet axe et la distance des stations par rapport à la source (fig. 8). Quelques points se situent cependant en dehors de ce schéma. Le prélèvement N11 a été effectué dans la Mafa juste au niveau du confluent avec le Nzi. L'échantillon peut donc avoir été contaminé par les espèces se trouvant normalement dans le cours principal. Par ailleurs, la position des prélèvements N3' et N4' effectués dans la même station mais dans des biotopes différents, montre que, dans une même zone, des variations importantes des peuplements peuvent exister. C'est ce qui explique vraisemblablement aussi l'isolement de la station N2.

TABLEAU II. Caractéristiques des échantillons récoltés aux filets maillants dans le bassin du Bandama en étiage

Station	B1	B2	B3	B4	B5	B6	B7	B8	B9	B13	M1	M2
Richesse	16	22	13	11	18	23	18	24	24	18	17	23
Diversité	3,265	3,765	2,789	2,327	3,210	2,868	2,397	2,977	3,093	3,268	2,453	3,564
Équitabilité	0,816	0,844	0,754	0,673	0,770	0,634	0,575	0,649	0,675	0,784	0,600	0,788

Station	M3	M4	M5	M6	N1	N2	N3	N4	N5	N6	N7	N8
Richesse	20	27	15	24	13	10	18	19	13	22	16	17
Diversité	3,547	3,516	1,974	3,408	2,428	3,005	3,182	3,553	3,029	3,898	2,541	3,225
Équitabilité	0,821	0,739	0,505	0,743	0,656	0,905	0,763	0,836	0,819	0,874	0,635	0,789

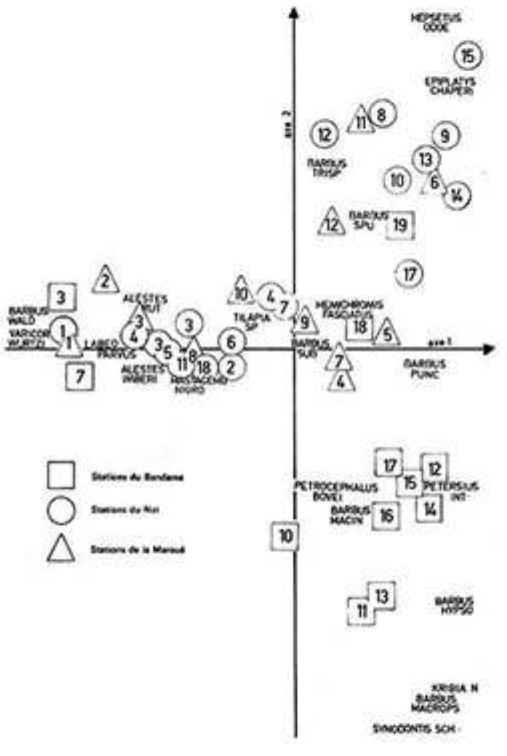

FIG. 6. — Projection des points, dans le plan des axes 1 et 2 de l'analyse factorielle des correspondances sur les prélèvements dans les milieux d'eau peu profonde du bassin du Bandama.

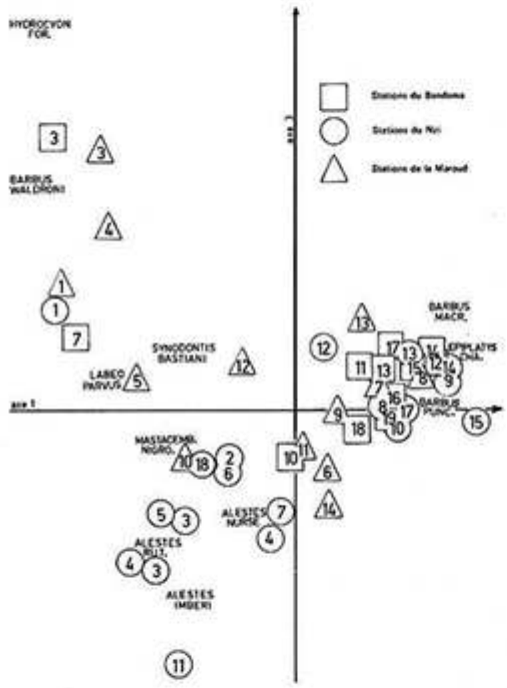

FIG. 7. — Projection des points, dans le plan des axes 1 et 3 de l'analyse factorielle des correspondances sur les prélèvements dans les milieux d'eau peu profonde du bassin du Bandama.

TABLEAU III. Corrélations des espèces pêchées en eau peu profonde avec les axes I, II, III de l'analyse factorielle des correspondances

Espèces	Corrélations I	II	III
Labeo parvus	**0,680**	0,000	0,003
Barbus waldroni	**0,588**	0,001	0,120
Mastacembelus nigromarg.	**0,576**	0,019	0,058
Varicorhinus wurtzi	**0,536**	0,002	0,227
Barbus punctitaeniatus	**0,434**	0,007	0,002
Nannocharax occidentalis	**0,390**	0,000	0,008
Petersius intermedius	**0,340**	0,190	0,019
Neoloebias unifasciatus	**0,314**	0,178	0,002
Alestes rutilus	**0,287**	0,001	0,183
Barilius senegalensis	**0,268**	0,058	0,044
Nematogobius maindroni	**0,245**	0,001	0,065
Alestes longipinnis	**0,243**	0,055	0,004
Synodontis bastiani	**0,228**	0,030	0,020
Aplocheilichthys spp.	**0,205**	0,015	0,003
Tilapia spp	**0,188**	0,040	0,061
Heterobranchus isopterus	**0,149**	0,137	0,007
Hemichromis bimaculatus	**0,117**	0,112	0,009
Clarias senegalensis	**0,097**	0,019	0,000
Pelmatochromis guntheri	**0,090**	0,002	0,075
Gobius guineensis	**0,090**	0,000	0,014
Eutropius mentalis	**0,029**	0,010	0,020
Barbus macrops	0,203	**0,545**	0,049
Kribia nana	0,167	**0,351**	0,031
Synodontis schall	0,056	**0,347**	0,007
Barbus spurelli	0,247	**0,319**	0,001
Barbus hypsolepis	0,186	**0,311**	0,013
Barbus macinensis	0,119	**0,292**	0,000
Hepsetus odoe	0,077	**0,263**	0,002
Barbus trispilus	0,012	**0,241**	0,206
Petrocephalus bovei	0,023	**0,238**	0,039
Epiplatys chaperi	0,148	**0,234**	0,011
Chrysichthys velifer	0,064	**0,204**	0,020
Micralestes occidentalis	0,071	**0,142**	0,017
Alestes nurse	0,005	**0,050**	0,044
Alestes imberi	0,320	0,004	**0,362**
Hydrocyon forskahlii	0,275	0,021	**0,360**
Hemichromis fasciatus	0,059	0,026	**0,061**
Barbus sublineatus	0,014	0,001	**0,019**

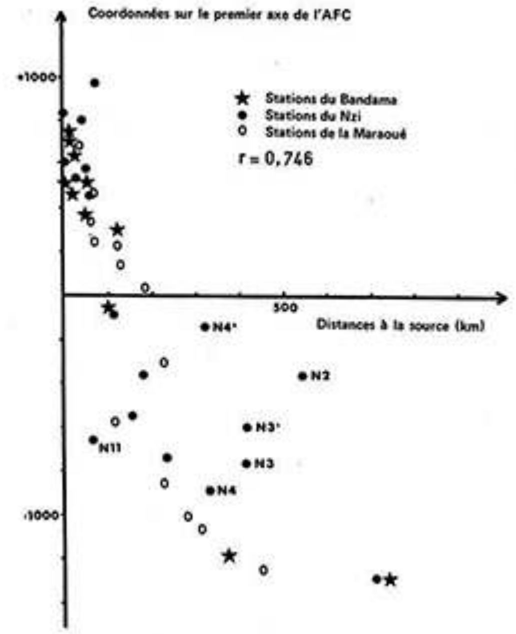

FIG. 8. — Relation entre les distances à la source et les coordonnées sur le premier axe de l'analyse factorielle des correspondances des prélèvements d'eau peu profonde.

30 Ces quelques exceptions mises à part, la représentation utilisée permet de classer grossièrement les prélèvements en deux groupes :

1. les stations du cours moyen jusqu'à une distance d'environ 150 km de la source ;
2. les stations du cours supérieur dans les 150 premiers km de cours.

31 Les espèces correspondant à cette dispersion sont celles qui ont leur meilleure corrélation avec l'axe 1. D'après leur répartition, établie dans le Nzi (fig. 9), il est possible de les séparer en trois groupes principaux :

1. ESPÈCES DE LA ZONE SOUS INFLUENCE ESTUARIENNE : *Gobius guineensis,* mais aussi *Scyscidium* sp. présent uniquement dans la station B2 et qui a été exclu de l'analyse.
2. ESPÈCES DE COURS MOYEN : les principales étant : *Labeo parvus, Mastacembelus nigromarginatus, Alestes rutilus, Tilapia* spp., *Alestes imberi.*
3. ESPÈCES DU HAUT COURS : distribution limitée à 100 à 150 km de la source : ce sont principalement, dans le Nzi : *Alestes longipinnis, Hepsetus odoe, Aplocheilichthys* spp. Mais, dans les autres rivières, on trouve également *Barbus punctitaeniatus, Petersius intermedius, Neoloebias unifasciatus...*

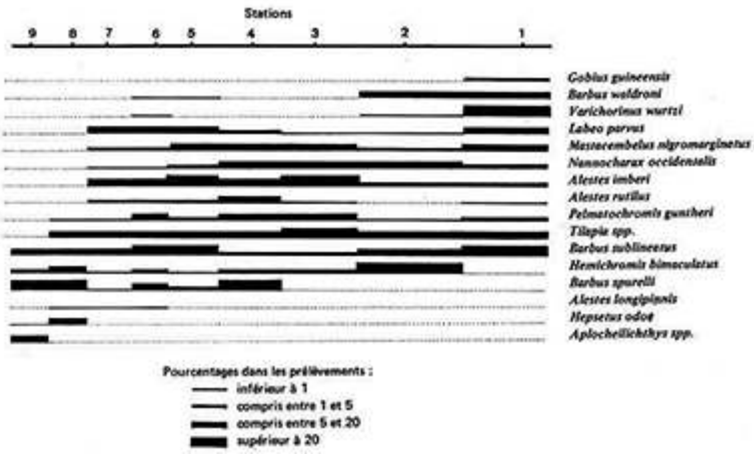

FIG. 9. — Répartition des espèces d'eau peu profonde les plus importantes dans le Nzi.

32 Ce schéma n'est bien sûr pas rigide et des répartitions intermédiaires sont observées. Ainsi, *Barbus spurelli* et *Hemichromis bimaculalus* ont une distribution plutôt supérieure, et, à l'inverse, *Barbus waldroni* et *Varicorhinus wurlzi* sont surtout abondants dans le cours inférieur.

33 Le deuxième facteur expliquant 13 % de la variabilité sépare principalement les stations du haut cours du Bandama. Les espèces correspondantes sont *Barbus macrops, Kribia nana, Barbus hypsolepis...* pour celles que l'on trouve exclusivement ou préférentiellement dans le haut cours du Bandama et *Epiplatys chaperi, Barbus trispilus, Hepsetus odoe...* pour celles présentant une distribution inverse. Le troisième facteur qui explique 8 % de la variabilité isole les stations du cours moyen du Nzi. Les espèces les mieux corrélées avec ce facteur sont *Alesles imberi* et *Hydrocyon forskahlii.*

34 Comme dans les milieux profonds, la richesse et la diversité des échantillons ne sont pas liées à la zonation longitudinale (tabl. IV).

DISCUSSION-CONCLUSION

35 Les analyses menées portent sur un ensemble de prélèvements approximativement synchrones effectués au cours d'une saison sèche. Or parmi les facteurs de variabilité des échantillons, les variations saisonnières et interannuelles peuvent avoir des conséquences importantes. Pour cette raison, dans la plupart des stations, des prélèvements ont été effectués à d'autres époques. Des variations dans les peuplements sont observées concernant en particulier l'abondance des espèces migratrices. Pourtant, des analyses de données prenant en compte l'ensemble des échantillons donnent les mêmes dispersions que celles obtenues à partir des seuls prélèvements de saison sèche. Par ailleurs, les corrélations entre prélèvements réalisés dans une même station à différentes époques de l'année sont le plus souvent élevées. Seuls quelques prélèvements effectués au moment de la crue apparaissent parfois différents.

36 Ainsi, il semble qu'il existe une certaine stabilité des peuplements dans une zone déterminée pendant la période des eaux basses ou moyennes.

37 De plus, les résultats de ces prélèvements supplémentaires nous permettent de nuancer les données acquises, et d'apporter des éléments nouveaux au schéma de zonation.

38 *Pellonula afzeluisi* paraît, dans les prélèvements étudiés, habiter les parties supérieures et inférieures des cours, à l'instar d'un certain nombre d'autres espèces. Or, à plusieurs reprises, ce poisson a été capturé en grande quantité à Marabadiassa (station B6), dans le sud de la Maraoué (station M1), et dans le cours moyen du Nzi (station N6). Bien que la biologie de l'espèce soit peu connue, il semble que ce petit *Clupeidae* puisse développer des populations importantes dans les grandes étendues d'eaux calmes (lac de Kossou : ROEST, 1974). Par ailleurs, l'existence de migrations le long des rivières n'est pas à exclure

TABLEAU IV. Caractéristiques des échantillons récoltés par pêche électrique dans le bassin de Bandama en étiage

Station	B3	B7	B10	B11	B12	B13	B14	B15	B16	B17	B18
Richesse	8	14	21	23	25	29	28	16	22	22	17
Diversité	1,325	1,897	3,206	2,998	3,646	3,526	3,207	3,126	3,409	3,597	3,077
Équitabilité	0,442	0,498	0,730	0,663	0,785	0,726	0,667	0,782	0,765	0,793	0,753

Station	B19	M1	M3	M4	M5	M6	M7	M8	M9	M10	M11
Richesse	15	18	14	20	23	23	27	12	26	19	21
Diversité	2,878	3,301	1,776	3,467	3,378	3,591	3,821	2,659	3,347	3,131	3,841
Équitabilité	0,737	0,792	0,466	0,802	0,747	0,794	0,804	0,742	0,712	0,737	0,874

Station	M12	M13	M14	N1	N2	N3	N4	N5	N6	N7	N8
Richesse	18	8	4	17	23	16	19	22	27	24	14
Diversité	3,367	1,812	1,387	2,530	3,362	3,052	2,960	2,631	3,130	2,494	2,964
Équitabilité	0,807	0,604	0,693	0,619	0,743	0,763	0,697	0,590	0,658	0,544	0,779

Station	N9	N10	N11	N12	N13	N14	N15	N17	N18
Richesse	11	16	15	8	15	12	13	23	25
Diversité	1,880	1,874	2,445	2,354	3,251	2,504	2,881	3,403	2,502
Équitabilité	0,543	0,468	0,626	0,785	0,832	0,699	0,779	0,752	0,539

39 *Schilbe mystus* a été trouvé caractéristique des hauts cours. Pourtant, à plusieurs reprises il a été capturé dans les stations B3, N1, N2 et N4. De plus, dans des bassins côtiers voisins, tels que le Boubo et l'Agnebi, l'espèce est abondante jusqu'à l'estuaire, à des salinités importantes correspondants à des conductivités de l'ordre de 1 800 micromhos (PAUGY et LÉVÊQUE, 1977 ; ALBARET et MERONA, 1978). De même, cette espèce est dominante dans le bas Sénégal jusqu'à 150 km de l'embouchure (REIZER, 1971). Cette espèce se rapprocherait donc par sa distribution de *Alestes longipinnis,* caractéristique en même temps des cours inférieur et supérieur.

40 Les mêmes remarques peuvent être faites pour *Synodonlis schall* qui, bien que présent tout au long du cours, est particulièrement abondant dans le nord du bassin, dans les bassins côtiers et qui a été trouvé en quantité dans le bas Sénégal. Une autre espèce présente le même type de distribution : il s'agit de *Hepsetus odoe* qui ne participait pas à l'analyse en raison de sa rareté dans les échantillons pris en compte.

41 A partir de ces données, nous proposons un schéma théorique de zonation du bassin (fig. 10).

42 Une zone de source, aux caractéristiques physiques très particulières précède une zone de cours supérieur liée à la présence de quelques espèces. La plus grande partie du cours

présente un peuplement homogène dominé par les *Alestes* dans les vasques et par *Labeo parvus* dans les radiers. Enfin, une zone de cours inférieur se dégage, caractérisée par la présence d'une part d'un certain nombre d'espèces du cours supérieur et d'autre part d'espèces marines et estuariennes.

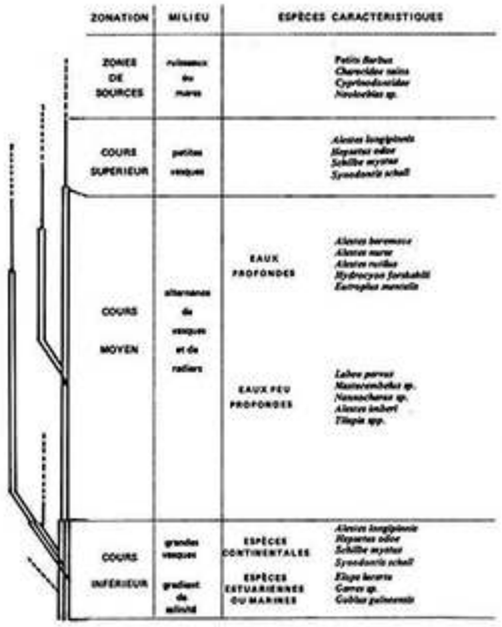

FIG. 10. — Schéma théorique de la zonation longitudinale des poissons dans le bassin du Bandama.

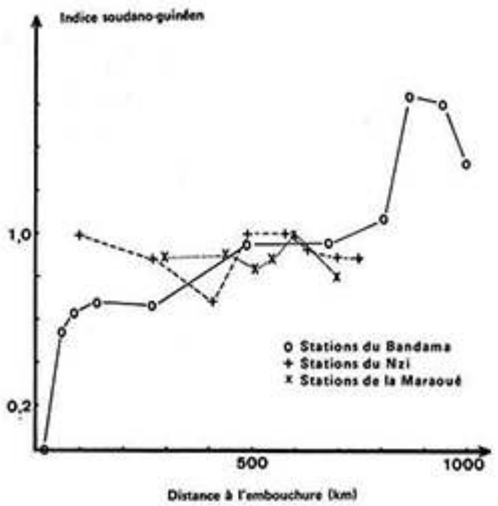

FIG. 11. — Évolution de l'indice Soudano-guinéen en fonction de la distance à l'embouchure, dans le bassin du Bandama. S : nombre d'espèces Soudaniennes ; G : nombre d'espèces Guinéennes.

43 Pour la clarté du schéma, les différentes zones sont arbitrairement séparées. En fait, comme il a été dit dans les chapitres précédents, il s'agit plutôt d'une évolution des peuplements qui s'effectue de manière plus ou moins rapide. Ainsi, la zone de cours supérieur a été déterminée par des espèces d'eau profonde, mais présente dans les milieux peu profonds des peuplements intermédiaires entre ceux de source et ceux du cours moyen.

44 Par ailleurs, des différences existent de rivière à rivière. C'est ainsi que *Mormyrus hasselquisti,* ne se rencontre que dans les milieux profonds du nord du Bandama, *Barbus*

stigmatopygus, B. leonensis, B. macrops, et *Kribia nana,* caractérisent les milieux peu profonds de cette même zone. L'absence totale de *B. macrops* dans le Nzi, de *Marcusenius isidori* dans la Maraoué contribuent également à différencier les trois rivières.

45 Les causes de cette zonation ont été recherchées. L'originalité de la zone de cours inférieur est liée à deux facteurs. Elle est due d'une part à la présence d'espèces d'eaux salées ou saumâtres, et donc à la proximité d'un gradient de salinité. Les particularités écologiques des estuaires ont été bien étudiées (ARTHUR, 1975), et des évolutions du même type dans les peuplements ont été relevées en Afrique, aussi bien dans des rivières prenant leur source en montagne (OLLIF, 1960) que dans des rivières « plates » (REIZER, 1971 ; SYDENHAM, 1978 ; PAUGY et LÉVÊQUE, 1977 ; ALBARET et MERONA, 1978). D'autre part, nous avons relevé la présence de quelques espèces continentales abondantes également dans les parties les plus hautes du cours. Aucune hypothèse satisfaisante n'a pu être avancée pour expliquer cette distribution très particulière.

46 Beaucoup de travaux ont développé l'importance des facteurs de milieu dans la zonation des poissons (MACAN, 1961 ; HUET, 1962 ; GAIGHER, 1973).

47 La rivière au niveau de la source est un ruisseau étroit, le plus souvent couvert par la végétation bordante et au fond généralement vaseux. Ces milieux sont soumis à des assèchements périodiques pendant lesquels le cours est réduit à une succession de petites mares. Les espèces que l'on y trouve présentent des adaptations à ce type de conditions. Ainsi, les mécanismes de respiration accessoire des Siluroïdae (FISH, 1955 ; ABDEL HAGID et BABIKER, 1975). Ainsi, également, les adaptations morphologiques des *Cyprinodontidae* à une alimentation de surface (bouche subère, dos plat).

48 A partir des sources, les milieux d'eau peu profonde évoluent vers des biotopes typiques de radiers. Ce sont des milieux hétérogènes à l'intérieur desquels il existe une microdistribution des poissons (MERONA et ALBARET, 1978). L'importance relative des habitats ainsi que leur variabilité en fonction des conditions hydrologiques induisent des différences non négligeables dans les peuplements qui sont mises en évidence sur la figure 8 (prélèvements N2, N3' et N4').

49 L'isolement des stations les plus septentrionales du Bandama est dû à la présence de quelques espèces à fort caractère soudanien (DAGET et ILTIS, 1965). L'indice soudano-guinéen défini par ces mêmes auteurs est nettement plus élevé dans cette partie du bassin (fig. 11). Une relation entre une zone géographique, déterminée par le climat ou la nature de la végétation, et la distribution de certaines espèces a été mise en évidence dans l'Ogun par SYDENHAM (1978). Il s'agirait ici d'un phénomène de même nature.

50 Enfin, il est à noter qu'aucune évolution régulière de la diversité des peuplements n'est relevée de la source à l'embouchure. Dans des rivières américaines plusieurs auteurs ont mis, au contraire, en évidence une augmentation de cet indice des cours supérieurs vers les cours inférieurs (SHELDON, 1968 ; DEACON et BRADLEY, 1972 ; WHITESIDE et MCNATT, 1979). En fait, la diversité des peuplements semble liée plutôt à la diversité de l'habitat (GORMANN et KARR, 1978).

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NOTES

1. Travail réalisé dans le cadre d'une Convention O.R.S.T.O.M.-O.M.S. Lutte contre l'Onchocercose. Surveillance de l'environnement aquatique.

RÉSUMÉS

A de multiples reprises, les hydrobiologistes ont tenté de dégager une zonation des rivières basée sur la distribution des organismes. Différents types de schémas ont été proposés pour décrire la répartition des organismes dans une rivière, mais aucun n'a pu être généralisé. Pourtant, le schéma proposé par ILLIES *et* BOTOSANEANU *(1963) a été appliqué à un certain nombre de rivières d'Afrique prenant leur source en altitude. Les rivières du bassin du Bandama ont un faciès très différent ; comme beaucoup de rivières d'Afrique de l'Ouest, elles présentent une pente faible sur la plus grande partie de leur cours.*
Une série de prélèvements d'étiage dans les milieux profonds comme dans les milieux peu profonds, ont fait l'objet d'analyses factorielles pour la mise en évidence éventuelle de regroupements entre les stations et entre les espèces. Les résultats de ces analyses, nuancés par la prise en compte d'échantillons supplémentaires, montrent que la zonation ichtyologique est peu marquée dans ce bassin.
A part une zone de source aux caractéristiques physiques très particulières (ruisseaux temporaires), nous distinguons une zone de cours supérieur et une zone de cours inférieur, toutes deux peu étendues, encadrant une large zone de cours moyen au peuplement homogène.
Une distribution très particulière de certaines espèces, présentes ou spécialement abondantes dans les parties inférieures el supérieures des cours est mise en évidence.

In many occasions, hydrobiologists tried to bring out a river zonation from organisms distribution. Different kinds of diagrams have been propouned to describe the répartition of organisms in a river but none of them achieved a generalization. Nevertheless, the diagram propouned by ILLIES *and* BOTOSANEANU *(1963) has been applied to some African rivers which rise in altitude. The Bandama basin rivers have a very different aspect; like many rivers in West Africa, they have a low slope gradient all over their course.*
Many samples have been done during the dry season, in the deep as in the shallow waters. The data have been computed using a factor analysis, to point out possible similarities between samples and/or between species repartition. The results of these analysis discussed with some considerations from additional samples, show that the ichthyological zonation is poorly marked in this kind of rivers.
Apart from a spring area which exhibits very particular physical characteristics (temporary brooks), we distinguish an upper course and a lower course zones, both very small, and, between them, a large zone of middle course with an homogenous fish population.
A very particular distribution of some species, which are present or more abundant in the upper and the lower course, is pointed out.

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Mots-clés : Afrique, Rivières, Ichtyologie, Écologie, Zonation
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Onchocerciasis Control in West Africa

Current Status and Future of the Onchocerciasis Control Programme

D.H. Molyneux

1 The Onchocerciasis Control Programme (OCP) in West Africa is the largest and most successful human disease control programme currently being executed[1]. The OCP celebrated its 20th anniversary in 1994 and has been subject to a series of reviews since 1974. Its initial objective was to eliminate onchocerciasis as a public health problem in seven countries [Benin, Burkina Faso (formerly Upper Volta), Côte d'Ivoire, Ghana, Mali, Niger and Togo].

2 In 1986, the programme was extended to include Guinea, Guinea-Bissau, Senegal and Sierra Leone when it was recognized that the original programme area could not be protected on a permanent basis without extension westward to eliminate the human reservoir of *Onchocerca volvulus* in areas from which savanna blackflies were invading the core area. Extension of the programme further south into Benin, Ghana, Cote d'Ivoire and Togo was initiated in 1979 through 1986. Until the late 1980s, when ivermectin was registered for use, control was based solely on the weekly larviciding of breeding sites of *Simulium damnosum* cytospecies.

3 Control operations started in late 1974 with the environmentally acceptable larvicide temephos (Abate), an organophosphate compound. However, in 1980, resistance to temephos was detected[2], a finding which followed the earlier discovery in 1976 that *S. damnosum* savanna cytospecies were capable of long-distance migrations from areas outside the limits of the programme area, carrying with them infective larvae[3]. These two events provoked reconsideration of the programme's long-term future by an Independent Commission which reported in 1981 (Ref. 4).

4 Resistance was controlled by the introduction of new larvicides, particularly the biocide *Bacillus thuringensis* sérotype H. 14 (Bt H14), which became used at low river discharges[13]. Its timely introduction when temephos resistance was becoming a problem prevented resistance spreading. Bt H14 is a larvicide with a specific toxicity for Dipteran larvae with no side effects on other nontarget invertebrates and fish. The ecological and

environmental issues involved in the programme are reviewed by an Ecological Group which oversees the activities of the National Hydrobiological Teams, whose monitoring activities ensure that the larvicides in use have no long-term environmental impact.

5 The programme's success has been documented on many occasions in terms of both humanitarian and socioeconomic results (see Box l)[5,6]. This success is recognized as being due to several factors[1,5], including clear objectives, a realistic timeframe, choice of best available technology, contracting out specialized tasks, priority given to operational research, a high degree of autonomy, delegation of authority, long-term commitment of donors and participating countries, specified mid-term goals in six-year financial planning cycles, transparency and free flow of information among constituent bodies, strong vertical management and high-quality staff. To these qualities can be added high levels of motivation, a willingness to embrace new ideas to refine microstrategy and consistent adherence to overall control imperatives.

6 The World Bank considered that the achevements of the OCP (Box I), when expressed in economic terms based on increased agricultural productivity, represent a rate of return on investment of around 20%, which far exceeds that normally associated with health projects. Hence, OCP is a success not only in health terms, but also in économic and development terms: indeed, the programme has been described as a unique 'development partnership' by the United Nations Development Programme (UNDP).

7 Given the annual expenditure of around 27 million US dollars during the current phase and the investment made to date by a consortium of some 22 donors over a 20-year period, it is vital that all aspects of programme operations are reviewed on a regular basis. This is undertaken by an Expert Advisory Committee which meets annually to review progress and report to the governing board of the programme, the Joint Programme Committee, which is made up of donors and participating countries. The programme has also been reviewed independently in 1981 (Ref. 4), 1986 (Ref. 7) and 1990 (Refs 1, 8), emphasizing that a constant evaluation process has been undertaken. All reviews have concluded that the overall strategy, ie. larviciding on a weekly basis, to reduce transmission over the maximum duration of the life of the adult worm, is the only appropriate means of control in the absence of an effective macrofilaricide to kill adult worms.

8 Larviciding alone has been proven to be successful in the original core area. However, to maintain the core area free of blinding *O. volvulus* it was necessary to extend the programme both west and south to eliminate the adult worm in the areas from which reinvasion occurs by migrating savanna blackflies, *Simulium sirbanum* and *Simulium damnosum ss*.

9 The programme was recently reviewed again during 1994 at a point midway through the (penultimate) IVth phase[9]. The review was conducted by the Expert Advisory Committee of the programme to provide the Joint Programme Committee (the goveming body of the OCP) with a report on progress since the 1990 review[1,8], and to provide a prospective of the future of the programme, predicting the end point of the programme and an estimate of the costs.

10 The programme, while initially based solely on vector control, has benefitted remarkably from the provision of Mectizan® (ivermectin), free of charge, by Merck Sharpe and Dohme. This drug, which rapidly reduces skin microfilarial loads and causes régression of

some of the ocular lesions, prevents blindness developing in some individuals who are vulnerable, in spite of vector control[10-11].

11 Mectizan®, given annually, at an oral dose of 150μgkg^{-1}, now plays a major role in reducing ocular morbidity in the extension areas and in a few problematic sites in the original programme area. Distribution is in the hands of national teams with OCP support non-governmental organizations and the communities themselves where the highest levels of compliance and coverage are achieved (65–75%).

12 In areas where Mectizan® distribution occurs concurrently with vector control, there has been a more rapid improvement in ocular morbidity compared with that achieved in the core area some years ago when vector control alone was available.

Box I. Achievements of the Onchocerciasis Control Programme

• 125000-200000 people have been prevented from going blind.
• Over 30 million people in 11 countries are protected from damaging ocular lesions due to *Onchocerca volvulus* infections[5].
• 10 million children born since the programme began have been spared the risk of blindness from *O. volvulus.*
• 1.5 million people originally infected are now no longer so.
• 25 million hectares of riverine valley and adjacent land has been made available for resettlement; enough to support some 17 million people.
• Over 400 professionals have been trained in various areas of relevant health sciences, such as epidemiology, vector biology and control, hydrobiology, ophthalmology and public health management.
• Over two million people are under ivermectin treatment within the programme area.
• As a result of 20 years of operations in the core area, the disease (as a public health problem) has been eliminated from Burkina Faso, Niger, large areas of southeast Mali, and northern areas of Côte d'Ivoire, Ghana, Togo and Benin.

13 At present, Mectizan® is given to over two million people annually in the programme, mainly in extension areas. The evidence available on the effect of Mectizan® distribution on transmission of infection is equivocal, but in some areas (Gambia river basin in Senegal) where (for epidemiological reasons) twice-yearly distribution is undertaken, there is evidence that transmission has been reduced considerably as no infections have been found in the under-five age group born since distribution began, or in adults known to have had no infection before treatment started in the area.

14 Mectizan® is also important in the context of potential recrudescence control as well as in a very restricted number of areas where larviciding has not been totally satisfactory (eg. Dienkoa, Burkina Faso; Bui, Ghana) or in areas bordering Nigeria (eg. R. Sota in N.E. Benin) where infected blackfly infiltration cannot be prevented as there is no vector control in Nigeria. Similarly in extension areas where vector control is unnecessary to protect the core area (Guinea-Bissau. Senegal), or where it is considered that transmission is due to *O. volvulus* which causes the less-severe form of the disease and the vector is non-migratory (eg. *S. leonense* in Sierra Leone), then Mectizan® distribution alone is appropriate.

15 Since 1990, the programme has made remarkable progress in enhancing its efficiency of larviciding operations[12]. There are now seven larvicides available for vector control.

Resistance to temephos has regressed. The seven larvicides approved for use are the organophosphate compounds temephos, pyraclofos and phoxim; the pyrethroid permethrin; the biocide Bt H14; the carbamate carbosulfan and, most recently, a pseudopyrethroid etofenprox. Hougard et *al.* [12] have summarized the recent development and the rationale behind the rotational use of insecticides, their relative cost-effectiveness, the conditions under which each is used, and the environmental problems inherent in each.

16 The availability of the seven compounds means that any blackfly resistance can be readily controlled and their rotational use reduces the likelihood of it occurring at all. Each insecticide is used at particular river discharge levels, and restrictions are placed by the Ecological Group on the number of consecutive cycles when permethrin and carbosulfan can be used (six). The choice of insecticide is dépendent on many parameters: river discharge levels, insecticide and transportation cost potential of development of resistance and the distance downstream from the point of application over which insecticide is effective ('carry' distance). Improvement of computer-based insecticide-delivery Systems in helicopters allied to almost instantaneously available information on river discharges is obtained via solar powered 'balises' (hydrological monitoring stations) transmitting river levels via a geostationary satellite to a ground station in Toulouse for processing before transmission back to the programme area for use by vector controllers and pilots. The OCP is the world's largest user of real-time hydrological data. Over a prolonged period, the programme has undertaken much work on blackfly cytotaxonomy and encouraged research to develop methods in identification of adult blackflies[13]. An identification method for adult flies has been developed based on morphometrics providing a reliable method for identification[14]. It is expected that DNA methods will also soon be available for this purpose.

17 Adult *Simulium* identification, together with the ability to recognize blinding and non-blinding *Onchocerca volvulus* strains using DNA methods based on PCR of different *O. volvulus* stages, has provided vital epidemiological information, enabling OCP to make major savings on vector control[15]. Differentiation between *O. volvulus* and *O. ochengi* using PCR has shown that the annual transmission potential (previously regarded as being unacceptably high in public health terms) in some savanna areas was due to *O. ochengi* L3 stages in savanna flies confusing the epidemiological picture. This differentiation has also enabled some river Systems (eg. in Mali) to be eliminated from vector control activities. *Onchocerca ochengi,* which is closely related to *O. volvulus,* has proved to be an excellent model for chemotherapy and immunological studies[16].

18 Insecticide treatment of extension areas which commenced in the late 1980s has confirmed that treatment of savanna blackfly breeding sites in Guinea and northern Sierra Leone prevents invasion of the core area by the savanna cytoforms.

Current Epidemiological Situations

19 There has been a continuing reduction in ail epidemiological parameters in areas under control by larviciding alone, ivermectin alone or by a combination of both methods. This is borne out by reduced annual transmission potentials, throughout the area of savanna blackfly transmission of blinding *O. volvulus.*

20 Classical skin snipping for parasitological diagnosis for measurement of community microfilarial loads (CMFL) is becoming less valuable. Invasive diagnostic procedures should be avoided if possible (as they pose a risk of HIV infection, and are becoming unacceptable to local populations). In addition, there is reduced sensitivity of skin snipping where microfilarial loads have been lowered where Mectizan® is distributed. The search for an immunological, or a DNAbased technique is in the process of development. A tricocktail antigen test based on an enzyme-linked immunosorbent assay (ELISA) detection System is now being evaluated before a decision is made to make it operational in the programme, while studies of PCR-based methods for detecting parasites are being pursued.

21 In reviewing the overall epidemiological situation, we must consider the role and influence of human migrants carrying patent onchocercal infection from outside the programme area in the south where non-blinding strains are present. Such populations may influence the CMFLs, annual transmission potentials and prevalence if the migratory status and strains of *O. volvulus* present are not considered. In such situations, PCR will be invaluable in determining strain identity.

22 Mectizan® distribution has continued to be expanded; over two million people have been treated annually over the past two years. OCP has been closely involved with non-govemmental organizations in Mectizan® distribution, particularly in developing community selftreatment and evaluating the epidemiological impact.

Development of a Macrofilaricide

23 The OCP has supported extensive research (through the Onchocerciasis Chemotherapy project and Macrofil, a combined OCP/TDR funding committee) to seek an effective chemotherapeutic agent which will kill adult *O. volvulus,* a macrofilaricide. Despite extensive studies, the prospect of finding a compound with the characteristics of Mectizan®, a safe drug with limited side effects which can be used via mass treatment without medical supervision, is remote within the timeframe of the programme. However, the long-term value of a macrofilaricide effective against *Onchocerca, Wuchereria* and *Brugia* is widely recognized. In the context of OCP, the mass distribution of such a compound could have reduced the duration of vector control by rapidly removing the adult worms, and hence the reservoir of infection. As things are, this is unlikely to be accomplished because only three options for a macrofilaricide are now under consideration. One of the candidate compounds (amocarzine) has been undergoing trials in Ecuador; before further clinical trials commence in Africa, a review will be made of the results of a three-day regimen (6 mg kg^{-1} oral dose daily) (total drug given, 18 mg kg^{-1}) taken with food.

24 A further product under test is a product of the University of Michigan (UMF 078). The compound has good macrofilaricidal effects and is in preclinical and efficacy trials. The development costs of UMF 078 would have to be borne entirely by the OCP/TDR Macrofil Project. Given the time necessary to get a drug into use, it was not considered by the recent review[9] that OCP should support the Macrofil programme beyond 1997. There is no likelihood that the achievement of the programme goals could be influenced by further research on a macrofilaricide, even though the availability of such a drug would be of great value outside the programme area as well as for lymphatic filariasis control.

25 The potential macrofilaricidal properties and tolerance of higher dose levels of Mectizan® are currently under investigation. However, it is important that higher dosages do not produce effects which might jeopardize the use of Mectizan® as a microfilaricide at current dose levels both within and outside OCP.

26 There is also recognition that, while the likelihood of the development of resistance to Mectizan® is limited in *O. volvulus* (due to the long duration of the life cycle), the danger of resistance developing in other nematodes (eg. *Haemonchus contortus* and the model *Caenorhabditis elegans*) is. greater. Therefore, the development of a method for detecting resistance in *O. volvulus* should be pursued. While the risk of resistance to Mectizan® developing cannot be excluded, the combination of vector control and Mectizan® distribution dramatically reduces (and may totally eliminate) its likelihood. This is an additional justification for maintaining vector control until adult worms are eliminated from the populations in areas which could act as a source of reinvasion of the core area.

Modelling

27 The OCP has benefited considerably in recent years from the development of the ONCHOSIM model[17]. This model has been used extensively to inform decisions relating to the cessation of control under different scenarios of larviciding duration and Mectizan® distribution either alone or combined with vector control. Initially the model was tested using the standard epidemiological parameters: annual transmission potential, CMFL, prevalence of microfilaria and prevalence of blindness. It was realized at an early stage that there was close correlation between predicted and observed figures, and that ONCHOSIM would provide an increasingly valuable tool to inform the decision-making process towards the programme conclusion.

28 Experience has shown ONCHOSIM is a robust predictive tool of considerable value and, on the basis of simulations on risks of recrudescence in various control scenarios, it has been concluded that the combined use of Mectizan® and larviciding was required for 12 years to reduce the risk of recrudescence to less than 1%. Hence, the period of larviciding could be reduced by two years when ivermectin was given annually and concurrently at a coverage of 65-70%. This compares with the 14-year period of larviciding alone which has been shown to be required in the core area to eliminate all adult worms[9].

29 If combined larviciding and Mectizan® distribution were maintained for only 10 years, Mectizan® distribution would have to be continued at a coverage of 65% annually for a further 10-year period to reduce the risk of recrudescence to less than 1%. If Mectizan® itself were used alone (65% coverage), continuous treatment for at least 20 years would be required to reach an epidemiological situation where the risks of recrudescence are reduced to a similar level. These predictions mean that combined larviciding and Mectizan® should be maintained for 12 years in extension areas where blinding *O. volvulus* transmitted by migrating blackflies capable of reinvading the core area occurs. To achieve this, control will need to continue until 2002.

30 ONCHOSIM simulations will be required in future to determine the possible impact of any macrofilaricidal effects of high dose Mectizan® on parasite fecundity and the requirements for the control of possible recrudescence. ONCHOSIM has also assisted in defining the parameters for resumption of treatment or unsatisfactory post-control epidemiology based on the number of L3 stages per 1000 parous females.

31 In addition to OCP research (see Box 2) hydrobiological teams have made the major contribution to our knowledge of the aquatic ecosystems of West African rivers[22]. The development of ONCHOSIM (see above) has demonstrated the value of modelling in informing control strategy. The extensive operational research in Mectizan® delivery both within and outside the programme has made the cost-effective delivery of this drug a reality. The TDR/OCP collaboration is supporting the ongoing development of an immunological test for *O. volvulus* in order to avoid skin snipping. The prospect of a non-invasive, DNAbased test is also being investigated.

Devolution

32 For many years, OCP has been seeking to devolve the responsibility for maintaining the programme achievements and skills to the participating countries to enable them to maintain the achievements of the programme when external support has finished. Devolution is the process by which OCP provides support for country activities to enhance their capacity and ensure that

Box 2. Research Requirements for the Onchocerciasis Control Programme
Applied research has been an integral part of OCP and has made a major contribution to its success. This has been sustained through contracting out necessary research as well as via maintenance of strong links with the WHO/TDR programmes. Major findings on the biology of *Simulium* and *Onchocerca* have resulted over a 20-year period:

- The phenomenon of *Simulium* migration and hence reinvasion[3];
- Resistance development and its subsequent control as a result of insecticide research and refinement of rotational larviciding strategies[12,18];
- The development of methods of blackfly identification, improved cytotaxonomy, isoenzyme, DNA methods. cuticular hydrocarbons, and morphometrics [19,20];
- Development of DNA/PCR methods for *Onchocerca* differentiation and subsequent deployment to inform control strategy[15];
- Identification of *O. ochengi* in cattle and its development as a chemotherapeutic and immunological model[16];
- Development of new insecticides, particularly the improved formulation of Bt H14 (Ref. 21);
- Better understanding of epidemiology of *O. volvulus* in the context of geographical distribution, strains and severity of ophthalmological lesions[10,11];
- The definition of new blackfly species and cytoforms[14].

33 capacity is sustainable to detect and manage recrudescence of onchocerciasis. The mandate for OCP is strictly that relating to onchocerciasis and, in that context, over 400 Fellowships have been provided, countries have developed their own devolution plans, technical manuals have been drawn up, sensitization and education activites have developed, epidemiological surveillance of sentinel villages established and Mectizan® distribution Systems have been put in place in partnership with the countries, NGOs and local organizations. These significant achievements reflect OCP's commitment to providing the health services with the most appropriate support to achieve the long-term objectives which are to eliminate the public health problem of onchocerciasis and ensure

the countries can sustain that achievement. Over recent years, however, the challenge of devolution has been how onchocerciasis surveillance and control can be incorporated into a multi-disease surveillance and control System appropriate to country devolution plans which identify particular diseases for incorporation into disease surveillance and control Systems. It is now recognized that such an approach is not within the mandate of OCP itself. OCP is specifically a vertical activity; however, the maintenance of its achievements post OCP must be within a disease-surveillance System that is affordable within the varied health Systems in the participating countries. The challenge over the forthcoming years will be how onchocerciasis surveillance is integrated, as the disease will necessarily be of limited public health importance, into health Systems which are in the process of signifiant structural change, which have excessive demands placed upon them already and which will be less able to depend on OCP technical and financial support. It is expected that WHO, via the Regional Office, will play an increasing role in supporting country disease-surveillance Systems while, in parallel, changes in the health sector driven by the World Bank will ensure that such Systems fit a policy environment which reflects the new radical approach to health care provision through decentralized structures.

34 The conclusion of the recent midterm review was that the programme has been highly successful; it confirmed the reasons for that success and considered that, to achieve its long-term goals, the programme should continue its present strategy of combined larviciding and Mectizan® until 2002. Research on macrofilaricides, while necessary in the wider context, should not be supported by OCP beyond 1997.

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Meetings on Onchocerciasis Control
The World Bank is organizing a Donors Conference to discuss funding for the African Programme for Onchocerciasis Control (APOC) in Paris in October 1995. This will be followed by two meetings to be held at the World Bank in Washington DC: (1) the first meeting of the Joint Action Forum of APOC, 4-5 December, will be attended by the Ministers of Health of onchocerciasis-endemic countries and prospective donors; and (2) the Joint Programme Committee of the Onchocerciasis Control Programme (OCP), the governing body of the OCP consisting of representatives of the 11 participating countries and the 22 donors, 6-8 December, will be hosted by the Government of the United States.

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Onchocerciasis Control

Moving towards the Millennium

D.H. Molyneux and J.B. Davies

1 Onchocerciasis in This Wormy World' of Stoll, 1947 (Ref. 1) attracts a single paragraph of seven lines! In 1947, foci of infection in mainland South America were unknown, and Stoll only refers to the 'Zone in Africa eastward from Sierra Leone... contains 57 million inhabitants'. His estimate of 19.8 million for the world total of *Onchocerca* infections in 1947 was probably an overestimate at that time. The most recent estimate of the number of infected people, provided by the World Health Organization (WHO) in 1995 (Ref. 2), is 17.516 million in Africa and Yemen and 140455 in the Americas. Since 1947, onchocerciasis has been recognized as a disease of significant public health importance and an impediment to socio-economic development. It is endemic in 37 countries; in Africa (30), in the Americas (6) and in Yemen. The disease and its vector are the target of the largest successful and sustained vector-control programme the world has known, the Onchocerciasis Control Programme (OCP), which commenced operations in 1974 in seven countries, has since been extended to encompass a total of 11 countries, and is still continuing.

2 Recent years have seen considerable progress in onchocerciasis control and a change of emphasis. The WHO Expert Committee Report[2] emphasized the opportunity presented by the registration of ivermectin (Mectizan®) as a safe and effective microfilaricide for use in humans. The commitment by the manufacturera, Merck & Co., via the Mectizan Donation Programme, to provide the drug free of charge to the port of entry for as long as necessary to control the public health problem of onchocerciasis was the incentive for the development of a partnership of Non-governmental Development Organizations (NGDOs) involved in blindness prevention, international organizations (WHO and the World Bank), governments of endemic countries and a donor consortium to establish the African Programme for Onchocerciasis Control (APOC), based on the efficacy, safety and availability of ivermectin[3]. In parallel with APOC, the programme for the elimination of onchocerciasis in the Americas (OEPA) is supporting the distribution of ivermectin in the endemic countries of Mexico, Guatemala, Venezuela, Colombia, Ecuador and Brazil[2]. In the OCP area of 11 West African countries, the original strategy of vector control alone has been modified and refined to include the distribution of ivermectin to reduce

morbidity more rapidly, while maintaining vector control for 12 years to stop transmission in areas endemic for blinding onchocerciasis[4].

3 The number of ivermectin treatments administered globally has increased rapidly over the period 1988-1996: in 1996, over 19 million treatments were provided through the Mectizan Donation Programme in 25 countries in Africa (including Yemen) and in six countries in the Americas[5]. The OCP will continue control operations until the end of 2002 (Ref. 6). Boatin *et al.* [4] reviewed the most recent approaches to control, the definition and rationale of control in areas that require special interventions (owing to, for example, infiltration from non-controlled countries in border areas, inadequate vector control or premature cessation of larviciding, the indicators used and the current epidemiological stratification of onchocerciasis in West Africa). In 1995, Molyneux summarized the status of the OCP after a review of the programme in 1994 (Ref. 6).

4 Several studies relevant to the future of the OCP have been completed since 1994. (1) Amocarzine[7] has been assessed as a potential macrofilaricide in Africa, following studies in Ecuador that indicated its efficacy against adult worms[8]. (2) The *Onchocerca ochengi* model has been developed for the evaluation of macrofilaricides[9], and has been used to demonstrate a prophylactic activity of monthly doses of ivermectin in cattle exposed to natural challenge with *O. ochengi* (V. Wood *et al.,* unpublished). (3) The diethylcarbamazine patch test has been developed and evaluated as a non-invasive tool for the diagnosis of onchocerciasis (WHO, OCP in West Africa, Expert Advisory Committee Report, OCP/EAC/18.2, June 1997) to detect recrudescence at the community level within the context of a post-OCP environment. (4) Isolated foci of onchocerciasis have been detected within the original OCP area owing to a variety of circumstances including inadequate larvicide treatment and too early cessation of larviciding[4]. (5) A new class of insecticide, a pseudopyrethroid, etofenprox (Vectron), has been introduced into the OCP vector-control strategy. Etofenprox fulfils a key operational need in the rotational insecticide application strategy, as it can be used at river discharges of 15-70 $m^3 s^{-1}$, where until now only organophosphate compounds have been effective, thus averting the danger of an operational gap should resistance develop to the existing organophosphates, temephos, phoxim and pyraclofos (see Hougard *et al.,* this issue). (6) Rapid epidemiological mapping of onchocerciasis (REMO) and rapid epidemiological assessment (REA), together with géographic information Systems, have been used extensively for targeting control in APOC countries[10].

APOC _

5 Effective control within the APOC countries (the 19 endemic countries in Africa outside the OCP) will depend on the sustainable delivery of ivermectin throügh community-directed treatment, the philosophy being to ensure adequate community involvement to sustain delivery after the support to projects has ceased after five years. The WHO Special Programme for Research and Training in Tropical Diseases has undertaken preparatory operational research for APOC through a Task Force, which has focused on developing community-directed approaches[11], evaluating the impact of ivermectin on onchocercal skin disease[12], determining the social and economic consequences of severe skïn disease and developing the use of REMO[3-10]. Recent studies[13] compared the number of spontaneous abortions in hyperendemic foci in Ecuador before and after ivermectin treatment, providing evidence that ivermectin reduces the frequency of abortions in

patients with onchocerciasis. In future, research on operational issues will be directed towards improving the efficiency of communitydirected treatments, ensuring that Systems are appropriate to developing countries and encouraging sustainability of the cost-effective delivery of ivermectin. The challenge facing health Systems in the Americas, in APOC countries and in some OCP countries is the delivery of a free drug to communities in need; if the health community cannot achieve adequate annual coverage with free ivermectin,-it is unlikely that any health intervention in resource-poor rural settings can be cost-effective. The target of cost per treatment to be reached at the end of the five-year APOC funding cycle should be around US$0.20; this figure should represent costs of any national and local (regional or district) structures required, and would largely represent the distribution cost from the port of entry, through a rapid and effective chain, to the periphery. The cost of the distribution System and of reporting on treatment will be borne by national and community structures after the five-year APOC funded period.

6 APOC[3] is based on the concept of partnerships between NGDOs and governments in the organization of the National Onchocerciasis Task Force and, at the local level, the development through NGDOs and regional or district (or equivalent) govemment of the appropriate community-directed delivery Systems. Different NGDOs are involved in different countries, but all major NGDOs with an interest in blindness prevention participate: Sightsavers, Christofel Blinden Mission, Helen Keller International, Africare, United Nations Children’s Fund, River Blindness Foundation (now incorporated into the Carter Centre Global 2000 programme), International Eye Foundation and the Organisation pour la Prevention de la Cécité.

7 APOC has indicated that initial support costs to finance programme establishment can be relatively high, but should not exceed around US$2 per person treated; treatment should be confined to hyperendemic and meso-endemic villages identified by REA and mapped through REMO[11]. Country maps are required before funding is provided through APOC. The NGDO and govemment contribution should be at least 25% of the funding requested from APOC, whether in cash or in kind. Projects would be funded against a background of a comprehensive national plan; each project should demonstrate a trend to sustainability based on the graduai reduction in cost per person treated over the five-year period to a level of around US$0.20. The concept of sustainability in this development process used by APOC refers to the ability of communities following initial external involvement to maintain the viability and continuity of the ivermectin treatment process in-the absence of extemal support. The indicators of sustainability currently used by APOC are listed in Box 1.

Ivermectin-delivery systems

8 Ivermectin has been an important intervention within the OCP area since 1989 after early trials in 1987 in Asubende, Ghana[14,15]. Initially, distribution within the OCP was based on mobile national teams, particularly in the extension areas (those that were not in the OCP initially), with local health sector personnel as well as community members also being involved. Clearly, over the coming years, OCP will move to incorporate community-directed approaches to increase sustainability, particularly in areas designated for special interventions[3].

9 As the success of ivermectin distribution requires that at least 65% of the exposed population is treated annually[3], evaluation of distribution Systems is necessary. Recent studies[16] have been undertaken in four OCP countries, Benin, Côte d'Ivoire, Ghana and Togo, to investigate coverage and operational aspects of the distribution System. This study revealed that in 130 selected villages, 97 had received treatment and 67% of the population had received ivermectin. Nearly 30% had taken the drug in all treatment rounds and the main reasons for non-treatment were: absence (54.5%); non-eligibility (young children or pregnant women) (12.2%); refusai (2.6%); and shortage of drugs (only 1.9%). In general, there was community approval for treatment, despite 26% of villages preferring the mobile delivery method. The authors of the study suggested that communities themselves could accept responsibility for distribution, after appropriate instruction and communication.

10 An extensive study in five countries (Mali, Ghana, Nigeria, Cameroon and Uganda) was carried out to identify and develop simple, acceptable and sustainable methods for community-directed treatment with ivermectin. A study was designed to ascertain whether programme-directed approaches (ie. those developed in collaboration with the programme and proposed to the community) or community-designed methods (ie. those identified by the communities themselves) could be more effective, taking account of the multiplicity of different communities involved[11]. The guidelines employed to assess the success of these two approaches were related to the process, structure and changes of methods of drug delivery; ivermectin procurement; adherence to treatment protocols (exclusion criteria and referral of adverse reactions); supervisory strategies; and issues of coverage and reporting. The study was also intended to identify factors relevant to the sustainability of drug-delivery Systems and to provide information applicable on a continent-wide scale in Africa. Similar studies in Nigeria[17] suggested that, where appropriate human resources are available at the village level, such community-based workers need to receive continued external and internai support. These studies also demonstrated that data collection is limited by literacy standards. Local leaders considered that there was a need to set payments to compensate for time and effort involved, although local resources (both human and financial) are a prerequisite for true ownership and hence sustainability in these studies. The WHO multicountry study concluded that community-directed treatment is feasible and effective, and is successful in a range of diverse settings, suggesting it is likely to be replicable. Distribution Systems designed by communities achieved better coverage than programme-designed approaches, and the distribution performance was adequate in terms of coverage achieved, adherence to exclusion criteria and dosing level[11]. The key factor in limiting sustainability was the non-availability of ivermectin at pick-up points when expected. However, better performance was associated with supervision by health service staff; cost-recovery Systems, an obligatory part of the health Systems in Cameroon, had a detrimental effect on coverage and communities had difficulty in reporting.

Box 1. Indicators of Sustainability of Community-directed Treatment

Commitment of the partners

(1) Percentage share of all costs is borne by the Ministry of Health, local NGDO(s), NGDO partner and APOC Trust Fund.

(2) The Ministry of Health budgets an amount for onchocerciasis control in the current year.

(3) Ivermectin is made available to the community.

Integration into primary health care
(1) There is a a number of targeted endemic communities in which the distribution is part of the Primary Health Care System and supervised by the Primary Health Care System.
(2) There is also a number of targeted endemic communities in which supervision is done by the Primary Health Care personnel.
Community involvement
(1) Many targeted endemic communities involve the community in decision-making on mode of distribution; selection of community-based distributors (CBDs); procurement and collection of ivermectin from central collection point; reporting and referral of cases of severe adverse reactions; decision-making on incentives and/or remuneration to community-directed distributors; and change in mode of distribution System.
(2) There are plans to increase community participation.
Estimated cost per person treated
This includes the cost of: drug delivery from port of entry to community; collecting drug from central point by the community; training CBDs; supervising CBDs; monitoring community-directed treatment with ivermectin (CDTI); and remuneration or incentives paid to CBDs by the community.
Indicators of coverage of CDTI
These include targeted communities treated with ivermectin; targeted persons treated with ivermectin; targeted communities treated the previous year; targeted persons treated the previous year; and reasons for refusals.

Vector control in Africa

11 *OCP countries.* The extensive vector-control activities in the OCP area have been regularly reported and reviewed[18,19]. The rationale for continuing vector control in key areas of the OCP area until the end of 2002 is the need: (1) to maintain vector control to prevent transmission for a period equivalent to the maximum duration of adult worm life, and (2) to reduce the risk of re-invasion of the core OCP area from infected blackflies carrying third-stage larvae (L3s) of savanna *Onchocerca volvulus.* Vector control ensures the eventual absence of a human reservoir, the only feasible approach in the absence of a safe macrofilaricide that could be delivered to the communities. Continuous effective larviciding is effectively the best available'macrofilaricide'and protects more people than other interventions by totally blocking transmission. Hougard *et al.* (this issue) review recent developments and approaches in vector control used by the OCP.

12 The change in prevalence and annual transmission potential within the OCP area between 1974 and 1996 has been reviewed by Boatin *et al.* [4]. Figures 1a and 1b demonstrate the change in prevalence during the programme's activées. Table 1 shows the change in estimated numbers of cases of infection and of onchocerciasis-related blindness between 1970 and 1995. Considerable epidemiological variation in prevalence exists over the 11 countries of the OCP. The different areas of the programme and the pre-and post-control prevalences are provided in Table 2.

13 In the whole OCP area, the numbers of people prevented from going blind between 1974 and 1995 are estimated at 125 000–200 000; 30 million people in 11 countries are protected

from damaging ocular and skin lesions, 10 million children born since 1974 are at no risk of blédness and 1.5 million people originally infected are no longer infected.

14 *Elsewhere in Africa.* Table 3 provides estimates of the number of infected and blind individuals (where known) in countries in Africa outside the OCP area.

15 Vector control has been successful in the elimination of populations of *Simulium damnosum* at the Owen Falls Dam, Jinja, in Uganda, while *Simulium neavei* was eradicated from a focus in North Nyanza, Kenya using only seven treatments of DDT at 0.5-2.0 ppm at intervals of 10 days[19]. Combined ivermectin treatment and vector control have been used with great effect in the Itwara focus, in the Kabarole District of Uganda[20]. Annual ivermectin treatments since 1991 immediately reduced infection rates in the vector *S. neavei,* but after two months, rates began to increase, indicating that transmission was still continuing. Consequently, in 1994-1995, treatments with temephos (Abate®) were applied at 29 dosing points every one or two months. Annual biting rates immediately fell from 3600 to 65 bites per person per year and the last *S. neavei* was caught on 3 August 1995, signalling the end of transmission. The ability to eradicate *S. neavei* and *S. damnosum* s.l. populations in East Africa has led APOC to include vector elimination as a part of its strategy. Clearly, if (1) *Simulium* populations are isolated and susceptible to temephos (or other insecticides), (2) treatment would cause limited environmental and ecological consequences, and (3) the vector is a specific local non-migratory cytoform that could be eliminated, then control is logistically feasible.

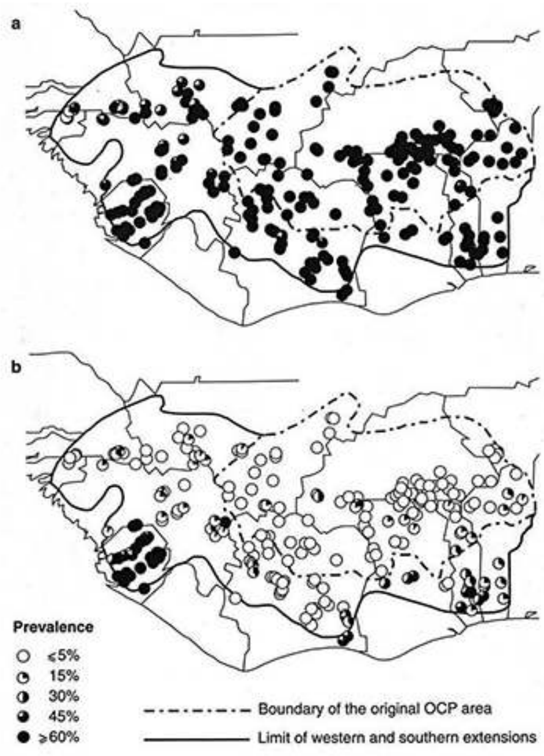

Fig. I. Prevalence of positive skin-snips in villages of the OCP before control (a) and in 1993-1996 (b) (redrawn after Ref. 4).

16 J.F. Walsh (pers. commun.) has undertaken several detailed analyses of *S. neavei* foci in Uganda, Tanzania and Malawi to evaluate the potential for elimination of *Simulium* as an alternative to long-term ivermectin delivery. These studies suggest that only a limited number of such foci can be expected to match the criteria for a vector-elimination

programme, and those that do will require appropriate advice on treatment, monitoring and operational issues, provided adequate assistance from local people is available. Clearly, vector elimination at a focus of onchocerciasis would reduce the duration of ivermectin distribution necessary; calculations suggest that, in the absence of vector control, ivermectin distribution would need to be carried out for around 15 years. The impact of ivermectin in such situations is to reduce the morbidity of ocular and dermal symptoms and, in East Africa, if confirmed, those of epilepsy, goitre and dwarfism (Nakalanga syndrome).

17 *The Americas.* Onchocerciasis in the Americas is endemic in Brazil, Colombia, Ecuador, Guatemala, Mexico and Venezuela. In Mexico, Guatemala and probably Colombia, the onchocerciasis-endemic areas are well delineated, but elsewhere, because they lie in extremely inaccessible regions, the full extent of the endemic area is still unknown. In Brazil, a new focus has been discovered at Minaçu on the Tocantins river 500 km north of Brasilia and 2500 km south of the previously known foci near the Venezuelan border. It is believed that the focus is a resuit of an influx of miners who had previously been working in onchocerciasis-endemic areas. The vector is probably *Simulium guianense* s.l.[21] A new westward extension to the Southern Venezuelan focus has been found in the Unturan mountains[22], and the Ecuadorian focus is intensifying and expanding owing to the extreme efficiency of the vector in that area, *Simulium exiguum* [23].

Table I. Estimated numbers of cases of onchocerciasis and onchocercal blindness in the seven original OCP countriesa

Year	**Total population (thousands)**	**No. infected (thousands)**	**No. blind (thousands)**
1970–1971	33093	1706	35.0
1984–1985	48250	1463	39.7
1995	61000	1219	29.3
Change since 1970	**+84.0%**	**-28.5%**	**-16.3%**

[a] Data from Ref. 2.

Table 2. Prevalences of onchocerciasis in OCP areas before and after control strategies implemented[a]

OCP areas	**Control method and commencement date**	**Pre-control prevalence (%)**	**Post-control prevalence (%)**
Area 1: western extension: Senegal, western Mali, northern Guinea and Guinea Bissau	Ivermectin only, 1988	18.8–81.9	0.0–17.0
Area 2: western extension: northern Sierra Leone	Temporarily suspended, 1987–1990	65.6–87.6	30.4-49.0

Area 3: western extension: Southern Sierra Leone	Ivermectin only, 1987–1990	60.2-88.5	39.0-74.3
Area 4: western extension: Mali, Southern Guinea	Vector control and ivermectin, 1987–1990	61.2–82.6	5.0-23.8
Area 5: Southern extension: Côte d'Ivoire	Larviciding, 1979; ivermectin, 1992	49.4-87.4	0.0-50.6
Area 6: Southern extension: Benin, Togo, Ghana	Larviciding, 1979; ivermectin distribution, 1992	60.4–85.1	14.0–64.5
Area 7: original area	1974	60.0-84.2	0.0-6.0

18 The availability of the safe microfilaricide ivermectin resulted in the establishment of the OEPA following a Pan-American Health Organization resolution in 1991, with the objective of eliminating the severe manifestations of onchocerciasis in the Americas through mass distribution. In contrast to the position in Africa, vector control has not been regarded as a credible option because the vectors inhabit either myriads of small streams or, as in Brazil and Venezuela, rivers with huge discharges. In South America, the logistics and costs of vector control preclude its consideration as an approach to reducing onchocerciasis transmission. The one exception is in the San Vicente Pacaya focus in Guatemala, where temephos in various formulations, including briquettes, was applied to control *Simulium ochraceum,* leading to Virtual eradication of the vector and cessation of transmission[24,25] . *Simulium ochraceum* has just begun to retum eight years after the end of control (J O. Ochoa, pers. commun.). In Central America, vector control is still an option in a few hyperendemic coffee estates where blackfly biting is also a serious nuisance. Vector control was also undertaken in the adjoining focus in Chiapas, in Mexico in the 1950s[19].

19 Ivermectin has thus provided a major new opportunity to reduce the morbidity associated with onchocerciasis, but the length of time that treatments will need to be continued has yet to be determined. Ivermectin distribution in the Americas is currently based on the use of communities, local structures and existing health Systems. Mexico has been using twiceyearly treatments since 1988-1989 (pre-dating OEPA) in an attempt to reduce not only morbidity but also, eventually, transmission[26]. In the Oaxaca focus, treatments have been so successful that there have been no new cases for several years. National plans in Brazil, Colombia, Ecuador and Venezuela seek to integrate ivermectin distribution into other interventions, such as hepatitis B vaccination, through the primary health care System. Although, in Guatemala, the stated intention was for distribution to be made twice a year in hyperendemic communities, in practice, this has hardly been attained, most receiving ivermectin only once a year. In these localities, it is unlikely that transmission will have been halted (R. Lujân and B. Morales, pers. commun.).

Surveillance and epidemiological assessment

20 A workshop convened by OEPA and held in Guatemala in June 1995 to examine the transmission cycle of *O. volvulus* by *S. ochraceum* s.l. in Guatemala and Mexico concluded that (1) the low infection rates in this vector (<0.2%) rendered indexes such as the annual transmission potential and infective biting rate of questionable statistical value; however, the infection rates of all *Onchocerca* stages in host-seeking flies were still a feasible parameter; (2) entomological surveillance should be an integral component of the ivermectin-distribution programme, which should be carried out in meso-endemic and hyperendemic sentinel communities; and (3) research should continue into the use of DNA probes, other alternatives to fly dissections, and the development of computer models.

21 For areas where skin-snipping (the removal of a portion of skin for parasitological diagnosis and collection of prevalence and incidence data) is no longer acceptable, a proxy measure of skin microfilarial density, the vector microfilarial uptake (numbers of microfilaria ingested by vectors) has been developed[27]. In separate studies, DNA probes on pooled samples of *S. ochraceum* [28] have been used in Guatemala to demonstrate a decrease in vector infection rates after ivermectin treatments (J.B. Davies *et al.,* unpublished). Similar techniques for use with *S. damnosum* have been described by Katholi *et al.* [29] The classical parameters of epidemiological assessment used by the OCP are increasingly being re-evaluated because of (1) the variety of complex epidemiological situations within the programme; (2) the problems posed by the impact of vector control and of ivermectin treatment, which complicates the assessment of prevalence because it has reduced the number of microfilaria in the skin (microfilidermia); (3) the need for robust parameters to inform decisions on when to stop larviciding; (4) the need to provide countries with cost-effective, non-invasive methods of community diagnosis for detecting recrudescence; and (5) the need to determine how cost-effective evaluation of transmission can be achieved using crushed *Simulium* to replace dissection.

New approaches to chemotherapy

22 *Macrofilaricides.* Vector-control strategies in the OCP area have been based on the need to prevent transmission for sufficiently long to allow the human reservoir of adult *O. volvulus* to die out because new adults are not recruited. The only intervention that could short-circuit this approach would require a safe, effective and deliverable macrofilaricide, and intensive research to identify candidate compounds with appropriate qualities has been undertaken. In recent years, the most promising target compounds have been developed through a variety of partnerships with drug companies, studied in several screens and evaluated at the Onchocerciasis Chemotherapy Centre at Hohoe, in Ghana.

23 In the early 1990s[30,31], the efficacy of amocarzine (CGP6140) was investigated in studies in Ecuador and Guatemala. The optimal dosages were established in different racial groups in the context of the relationships of food intake to the efficacy and side effects of the drug. These studies showed amocarzine to be an orally active drug. Four months after treatment with a regime of 3 mg^{kg-1} twice daily for three days (ie. a total of 18 mg^{kg-1}), 81-88% of adult female worms and 69-82% of male worms were dead or moribund, and all

intrauterine stages were degenerate. Although side effects were recorded they were regarded as acceptable[31].

24 Recently, Awadzi *et al.* [7] reported the outcome of a study of ivermectin and amocarzine treatment in 100 patients in Ghana. A detailed investigation of all clinical parameters together with a nodule biopsy after 120 days were carried out in three groups of patients receiving ivermectin alone, amocarzine alone or a combined dose of ivermectin and amocarzine. Amocarzine alone provoked Mazzoti-type allergic reactions which were more frequent than those observed with ivermectin; ivermectin pre-treatment suppressed the amocarzine-induced Mazzoti reactions. This study demonstrated that: (1) ivermectin at standard dosage produced minor macrofilaricidal effects; (2) amocarzine did not affect male worms and was a less potent macrofilaricide than was ivermectin; (3) the efficacy of a combined dose was similar to that of ivermectin alone. Awadzi and colleagues[7] concluded that 'amocarzine has no role in the treatment of onchocerciasis in Africa'.

25 *Prophylaxis.* A prospective study in Cameroon using the *O. ochengi*-cattle model (V. Wood *et al.*, unpublished) provided evidence that ivermectin given at monthly intervals has a prophylactic effect. Calves from two to eight weeks old were treated monthly with ivermectin at either 200 $\mu g kg^{-1}$ or 500 $\mu g kg^{-1}$ for 21 months. None of 15 calves treated with ivermectin at either dose developed an *O. ochengi* infection, whereas five of six control untreated animais maintained in the same herd and exposed to natural challenge of *O. ochengi* from *Simulium* became infected. A total of 54 *O. ochengi* nodules developed and all five animals developed a microfilidermia. This resuit has important implications for the use of ivermectin in the control of human onchocerciasis; if prophylactic ivermectin can be given immediately before the control period there is likely to be a more significant effect on transmission. Ivermectin is currently distributed in the USA by Merck & Co. Inc. as a prophylactic against dog heartworm, *Dirofilaria impiritis,* under the name Heartgard. Such a strategy might be equally applicable in human onchocerciasis.

Table 3. Estimates of the numbers of people infected with *Onchocerca volvulus* and the numbers of people blind due to onchocerciasis by country in Africa outside the OCP area, and in the Americas[1]

Country	Total population (millions)	No. infected (thousands)[2]	No. blind (thousands)[3]
Angola	10.0	100	2
Burundi	5.5	143	?
Cameroon	11.8	1300	26
Central African Republic	3.0	390	19
Chad	5.7	870	20
Congo	2.3	50	0.6
Equatorial Guinea	0.4	60	?

Ethiopia	49.2	929	?
Gabon	1.2	60	?
Kenya	26.0	?	?
Liberia	2.6	600	2.6
Malawi	8.8	150	?
Mozambique	16.6	?	?
Nigeria	99.0	3302	100
Rwanda	7.8	?	7
Sudan	25.8	620	10
Tanzania	27.3	650	?
Uganda	18.8	1200	?
Zaire	35.6	4565	37.5
The Americas	–	140.5	0.75
Total	**357**	**14 989**	**217.7**

26 *More effective microfilaricides.* The *Onchocerca lienalis-cattle-mouse* surrogate model[32] has been used to compare the ivermectin analogues, doramectin and moxidectin, with ivermectin for their ability to clear *O. lienalis* microfilaria from the skin; in these studies, moxidectin was up to ten times more effective as a microfilaricide than ivermectin.

Costs

27 Although OCP has been subject to probably the most rigorous financial control of any vector programme, very little mention of costs appears in the literature. A cost-benefit analysis was carried out by Benton and Skinner in 1990 (Ref. 33), who estimated that the total cost of OCP from inception in 1974 to completion in 2004 would be US$437 million (1985 dollar values) at an average annual cost of US$14 million or US$0.54 per head of the total population in the 11 OCP countries. A calculation based on the average at-risk population and a period of protection of 50 years (until 2023) yielded an annual cost of US $0.45 per person protected. More recently, Benton (pers. commun.) has calculated the annual costs of OCP to be around US$0.57 per person protected in endemic areas. This figure includes all related activites, covering administration, vector control, ivermectin delivery, training, research and development. The last item includes the search for a macrofilaricide (the'Macrofil'project). In comparison, it has already been stated that the target costs of the ivermectin-based APOC should be about US$0.20 per head per treatment.

Current status

28 Those planning onchocerciasis-control strategies face the problem that there is still no effective alternative to vector control to reduce transmission by eliminating the adult worms. Studies on repeated and high-dose ivermectin therapy have proved that this strategy has no signifícant macrofilaricidal effect[34-35]; thus, there is no effective macrofilaricide available for use in onchocerciasis-control programmes. Planners are therefore left with the necessity of employing treatment with ivermectin through government or local-community delivery Systems, together with the option of additional vector control, where feasible, to reduce transmission and to shorten the overall duration of the programme. At present estimates, ivermectin-delivery programmes that have a limited impact on transmission will need to be sustained for a minimum of 15 years.

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Note added in proof

30 Finally, the most recent information on onchocerciasis describes monitoring and ivermectin treatment in the war conditions of South Sudan[36] and in the same publication a brief note describes the endemicity and prevalence of ocular lesions amongst the Yanomami Indians of the Brazil-Venezuela border[37].

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NOTES

1. Data for APOC countries from unpublished APOC Project Document; data for Americas from Ref. 2. The total population at risk in the Americas cannot be accurately estimated.

2. Denotes that onchocercal blindness is not considered a significant public health problem.

3. Denotes that onchocercal blindness is not considered a significant public health problem.

ENDNOTES

a. Data from Ref. 4.

ABSTRACTS

The recognition of onchocerciasis as a major public health problem in the savanna belts of West Africa resulted in the establishment of the Onchocerciasis Control Programme (OCP) in 1974. Control was initially based on vector control by weekly larviciding. The OCP is now in transition towards its final phase in which repeated treatment with ivermectin, a safe and effective microfilaricide, is incorporated with vector control, or in certain circumstances is used alone. Ivermectin distribution hingeing on sustainable community Systems is the basis of a new programme in endemic African countries outside the OCP and in the Americas. David Molyneux and John Davies describe the latest trends and developments related to onchocerciasis control.

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Reproductive strategies of fishes in a tropical temporary stream of the Upper Senegal basin: Baoulé River in Mali

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1. Introduction

3 There have been many recent studies demonstrating an influence of environmental conditions on the reproductive strategies of tropical fish in Africa (Munro et al., 1990; Lévêque, 1997; Lévêque and Paugy, 1999), but the timing of reproduction of tropical riverine fishes has been investigated mainly in floodplain rivers and permanent streams. For seasonal floodplain rivers, most of the fishes have breeding periods that coincide with the annual flood (Welcomme, 1979; Lowe McConnell, 1987; Munro et al., 1990; Lévêque and Paugy, 1999), while in stable habitats such as permanent forest streams, fishes may have much more extended breeding seasons (Albaret, 1982). However, in a stream or a geographie zone, reproductive patterns may differ between species, suggesting the influence of both environmental and biotic factors. For the neotropical stream fishes studied by Winemiller (1989), it was possible to discern three different reproductive strategies among fishes: equilibrium, opportunistic and seasonal.

4 In the tropics, there is very little information on fish reproduction in intermittent streams and most of the studies are from neotropic regions (Alkins-Koo, 2000 for a review). In temporary rivers, environmental conditions are sharply contrasted and the variation amplitude of hydrological and physicochemical characteristics is high. During the dry season, there may be only a few isolated lentic pools.

5 In the Baoulé River in tropical West Africa (Mali), the remaining pools comprise only 10% of the total length of the river during the peak of the dry season (Fig. 1). During the wet season, there is a short but intense period of flooding. Finally, lotic conditions resume during some months and the cycle restarts. Given such drastic seasonal changes, it may be expected that breeding should be highly seasonal. However, it may be difficult to predict the most appropriate breeding season, even if flood conditions appear the best. Indeed, in some case, if channel shape and space limit the access of the floodplain habitats floods may even have a negative impact on juveniles or availability of food resources.

6 In this study, the reproductive strategies of 18 species of freshwater fishes were investigated in the upper reach of a temporary stream within the Baoulé drainage, with complete seasonal records on nine species. They belong to eight families, show variation in feeding guilds, and vary in size and habitat preferences (Table 1).

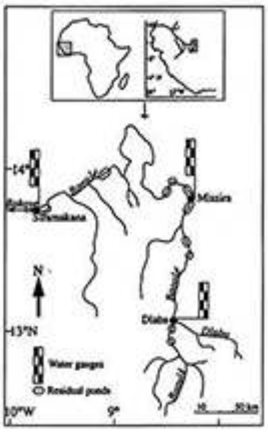

Fig. 1. Baoulé: geographical situation, position of the sample sites and residual ponds during the maximal low water.

2. The study site

7 The Baoulé River is a tributary of the upper Senegal basin in Southwest Mali (Fig. 1). The total length of the river is about 550 km with a 59,500 km^2 drainage area (for a complete description see Paugy, 1994). The river is situated in the Nilo-Sudanian zone influenced by the tropical climate, which is characterised by two main seasons, one short wet season and one long dry season.

2.1. Hydrological features

8 Climatic alternation characterises the hydrological regime that is typical of a tropical water body (Rodier, 1964). The dry season is very severe. During this period up to 90% of the stream channel dries out, leaving a few isolated pools or chains of pools which represent scarcely 10% of the total stream length (Fig. 1). At the end of the dry season, the water reaches temperatures of 35 °C, the conductivity reaches 120 µS and the oxygen levels drop to very low levels (2.1-3.0 mg/l between March and May). These conditions are reversed during the rainy season, which begins early in July and lasts about two months up to midSeptember. During this period the water temperature and the conductivity fall to 25 °C and 40 µS. At the end of the rainy season, the water level

decreases very quickly. In fact, depending on the year, the period of actual flow lasts only four to five months (July to November) (Fig. 2).

Table 1. Biological characteristics of the 18 fish species studied. MSL: maximum standard length (source: Paugy, 1994; Lévêque and Paugy, 1999), N: number of fish examined in the present study.

Species	Family	MSL(mm)	N	Feeding habits	Habitat
Hyperopisus bebe	Mormyridae	510	27	insectivorous	benthic dweller
Marcusenius senegalensis		321	280	insectivorous	benthic dweller
Mormyrops anguilloides		1 500	20	insectivorous and ichthyophagous (large adults)	benthic dweller
Mormyrus rume		870	29	insectivorous	benthic dweller
Petrocephalus bovei		100	69	insectivorous	benthic dweller; schools
Alestes baremoze	Characidae	425	256	omnivorous	pelagic dweller; schools
Brycinus leuciscus		119	151	omnivorous	pelagic dweller; schools
Brycinus macrolepidotus		530	79	omnivorous (mainly allochthonous feeder)	surface dweller
Brycinus nurse		218	229	omnivorous	pelagic dweller; schools
Hydrocynus forskalii		780	90	ichthyophagous	pelagic dweller
Labeo senegalensis	Cyprinidae	550	55	algae and 'aufwuchs' grazer	bottom dweller
Chrysichthys auratus	Claroteidae	270	269	invertivorous	bottom dweller
Schilbe intermedius	Schilbeidae	500	1 399	omnivorous	pelagic dweller; schools
Schilbe mystus		350	30	omnivorous	pelagic dweller; schools
Clarias anguillaris	Clariidae	1 500	36	omnivorous	benthic dweller
Synodontis ocellifer	Mochokidae	370	68	invertivorous	bottom dweller
Synodontis schall		400	451	invertivorous	bottom dweller
Sarotherodon galilaeus	Cichlidae	340	72	microphagous	along the river banks

2.2. Sampling sites and fish fauna

9 The lower reaches of these streams tend to be completely dry during the dry season because of a slope inversion (for details see Michel, 1973), so two perennial pools were chosen in the upper and middle course of the river, namely Dlaba and Missira (Fig. 1). No aquatic macrophytes were recorded, but a narrow gallery forest fringes both sites. Beyond a savannah zone there are only scattered small trees in short stretches constituting a characteristic Sahelian landscape.

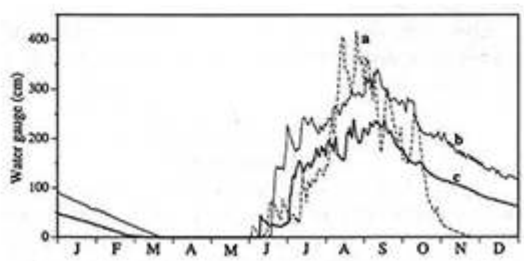

Fig. 2. Baoulé River: hydrological regime (weekly average from 1984 to 1988) for the upper, medium and lower course (see the sites position on (Fig. 1). **(A).** Dlaba (# 5 000 km^2); **(B).** Missira (11 400 km^2); **(C).** Siramakana (58 000 km^2).

10 The fish fauna of this river is typically Nilo-Sudanian (Hugueny and Lévêque, 1994; Paugy et al., 1994). In the Senegal River basin, 120 species are currently known, but fewer than 100 species have been caught in the Baoulé River (Daget, 1961 and own observations). Of these only 50 have been regularly collected during the period studied. Eighteen species were common enough to be investigated for reproductive strategies (Table 1).

3. Materials and methods

3.1. Samples

11 Fishes were collected monthly (October to June) or twice a month (July to September) from February 1985 to November 1988 using three sets of gillnets (mesh sizes of 10, 12.5, 15, 17.5, 20, 22.5, 25, 30 and 40 mm knot-toknot). All nets were 2.0 m deep and 25 m long. Sometimes, in some particular biotopes and when gillnets catches were insufficient, they were supplemented by cast nets (mesh size: 12.5 mm knot-to-knot). Catch-effort was approximately 12 hours, from 6: 00 P.M. to 6: 00 A.M. After the first night of fishing, gillnets were removed during the day and set elsewhere the second night. During the flood (July to September), when the fishes are collected twice a month, gillnets were set only one night to avoid overfishing.

12 With regard to fishes, the drift is a poorly known phenomenon. Therefore, in this study the goal is not to understand or explain the reason of the drift, but to use this method to sample young fishes. In this way a massive catch of young fishes may demonstrate that adults of species have spawned some days before. As observed for insects, there is a circadian periodicity in the drift of fishes inhabiting the rivers of western Africa (Elouard and Lévêque, 1977). According to previous observations, drift nets were used during 12 hours, from 18h30-19h00 until 6h30-7h00. The drift net is 3 m long with a 40 x 70 cm opening, and the mesh size is 1.5 mm (knot-to-knot). This gear is used only during the flood period for capturing young fishes. It is not a quantitative method and it is only used to get information about the presence of species.

3.2. Reproductive parameters

13 Size at (50%) sexual maturity was determined during the maximum breeding period (ripe or spent) when 50% of the adults stock of a species are mature. Nevertheless, when the number of specimens is insufficient for a species, the smallest specimen estimates the size at first maturity. Because males and females sometimes have a different growth pattern, we considered each sex separately.

14 All fishes caught were immediately identified, standard length measured to the nearest mm and weighed (whole fish and gonads to the nearest 0.1 g). All the fishes were then dissected and sexed. For females, gonads were weighed individually, but for males, all the gonads of a same length group are weighed because of their very low individual weight (the gonado-somatic index [GSI] always less than 2% and for numerous species less than 1%). The GSI is calculated with the following formula:

15 $GSI = GW / W - GW$ where GW is the gonad weight and W the total fish weight.

16 Because of the prediction of the climatic seasonality, data were pooled monthly across years to calculate mean GSI value (i.e., we have calculated the means of the different years for each month).

17 In order to establish the ordination of species fonction of their life-history traits, the factorial correspondence analysis (CoA) for which salient points have been described elsewhere (Benzecri, 1973; Legendre and Legendre, 1984) was used. Then a UPGMA (agglomerative algorithm: average link) distance analysis was carried out on the factorial

coordinates (species) of the CoA to check species relationships. We used the ADE-4 software (Chessel et al., 1995) for the whole analysis.

18 Oocytes are measured with a reticle fitted into an adjustable eyepiece (10 x). All oocytes larger than the minimum mature oocyte size were taken into account (i.e., only oocytes belonging to the last clutch of the frequency distribution of oocyte diameter). Ovarian fecundity was estimated using two methods: for small ovaries, complete counts were made; for large ones, a two-gram subsample was taken, counted, and extrapolated to the total ovaries'weight.

4. Results

4.1. Size at first sexual maturity

19 For most of the species discussed here, females tend to have a faster growth rate than males, and tend to have a larger body size at sexual maturity. Nevertheless, as the general pattern is approximately the same for the two sexes, we have only considered females in this study. There is a significant difference between the two sites. The median length at first sexual maturity is smaller at Dlaba (upstream) than at Missira (downstream) (Table 2 and 3). In fact, it is certainly a difference linked to the growth rate of the species between the two sites and related to most harsh conditions in Dlaba. Indeed, in this site the dry season is more severe because the refuge zones (residual ponds) are less numerous and of very small sizes. In fact, the poorer the environment is, the lower the growth rate is (greater for Missira, weaker for Dlaba).

4.2. Reproduction seasonality

4.2.1. Species with a short spawning period

20 Two main groups can be distinguished: the floodspawning species, and those which are not influenced by the flood cycle.

4.2.1.2. Fiood spawners species

21 Whatever the species, a peak in mean monthly GSI of females is identified in June-July (Fig. 3). In fact, for the six species, *Marcusenius senegalensis* (Mormyridae), *Alestes baremoze, Brycinus nurse* (Characidae), *Schilbe intermedius* (Schilbeidae), *Synodontis ocellifer* and *S. schall* (Mochokidae), the ovaries start to mature in May and continue their development up to June or July depending on the species and/or the individual. For all the species reproduction seems to be over in August, except for *B. nurse,* for which a large proportion of specimens is still mature at this date. Finally, reproduction is over by September for all species in our sample. When we compare the changes in GSI and the hydrological cycle, we note that these species lay during a short period (approximately one month) just before the maximum impact of the fiood (Fig. 3). In this fiood spawners group, most fish are egg-scattering pelagic spawners. If not ancestral, this reproductive style seems to be general and unspecialised (Balon, 1990).

Table 2. Median size (standard length, in mm) or age (year) at first maturity for 18 species of the Baoulé River, and at two other West African sites for comparison (* indicates smallest [and not median] size observed). RCI: Côte d'Ivoire. Sources: (1) Bénech and Dansoko, 1994; (2) Albaret, 1982.

Species	Baoulé at Dlaba	Baoulé at Missira	Niger at Mopti (1)	RCI (2)
Hyperopisus bebe	255*	324*	320	
Marcusenius senegalensis	140	150		
Mormyrops anguilloides		346*		210
Mormyrus rume	252*	325*	330	
Petrocephalus bovei	one year	one year		one year
Alestes baremoze	140	185		175
Brycinus leuciscus	one year	one year		
Brycinus macrolepidotus		190		185
Brycinus nurse	100	95		80
Hydrocynus forskalii		165		100*
Labeo senegalensis	206*	201*	210	175
Chrysichthys auratus	86	108		
Schilbe intermedius	100	110		100
Schilbe mystus		185*		
Clarias anguillaris		288*	150	235*
Synodontis ocellifer	135*	151*		
Synodontis schall	105	175		150
Sarotherodon galilaeus	111*	137*	140	145*

22 For some species, data are insufficient (lack of data for some months) to give a general survey of the GSI variations. But, we expect that *Mormyrus rume, Hyperopisus bebe* (Mormyridae), *Brycinus leuciscus* (Characidae) and *Labeo senegalensis* (Cyprinidae) belongs to the same flood spawners category (Fig. 3). Finally, from the scarce data collected on *Petrocephalus bovei* (Mormyridae), it seems that this species has the same type of reproduction.

Table 3. Maximum median gonado-somatic index for females and males of 18 species in the Baoulé river.

Species	Females	Males
Hyperopisus bebe	8.0	0.2
Marcusenius senegalensis	18.7	0.5
Mormyrops anguilloides	7.5	
Mormyrus rume	11.8	0.2
Petrocephalus bovei	21.4	0.4
Alestes baremoze	13.4	1.3
Brycinus leuciscus	17.0	1.2
Brycinus macrolepidotus	19.8	6.7
Brycinus nurse	26.1	2.0
Hydrocynus forskalii	9.4	2.1
Labeo senegalensis	17.2	2.0

Chrysichthys auratus	27.5	0.7
Schilbe intermedius	23.4	1.1
Schilbe mystus	16.2	
Clarias anguillaris	14.4	
Synodontis ocellifer	26.3	1.5
Synodontis schall	16.7	2.1
Sarotherodon galilaeus	4.8	

4.2.1.2. Other species

23 Two Characidae, *Hydrocynus forskalii* and *Brycinus macrolepidotus* don't belong to the flood spawners species. In contrast to the species mentioned above, the predatory tiger fish, *H. forskalii,* seems to reproduce both before (March to April) and at the end (August to September) of the high water period (Fig. 3). We may expect an adaptive strategy that allows the predator to benefit from an important diet supply. Indeed, juveniles of the predator feed on insects or large invertebrates (Lauzanne, 1975). Two or three months later, the young tiger fish have reached a sufficient size to feed on the newborn juveniles of the other species.

24 The spawning period of *Brycinus macrolepidotus* is not clearly delimited. Mature individuals are found in each sample period, from February to September, yet each individual spawns only once a year. Nevertheless, we observe a peak in May just at the end of the dry season. Among large common species, *B. macrolepidotus* is the only known species that does not spawn during a restricted period just before the flood.

4.2.2. Small-brood spawners

25 In the Baoulé River, small-brood spawners seem to be limited to a few Cichlid species. Among the five Cichlidae inhabiting the area two are rather common but reproduction data are available only for *Sarotherodon galilaeus* (Fig. 4). The mean monthly GSI of this mouth brooder is always reduced, and the standard deviation generally high. Further more, throughout the year ripe and spent individuals are found and the frequency distribution of oocytes in the ovaries is multimodal. All these factors strongly suggest a multiple spawning strategy.

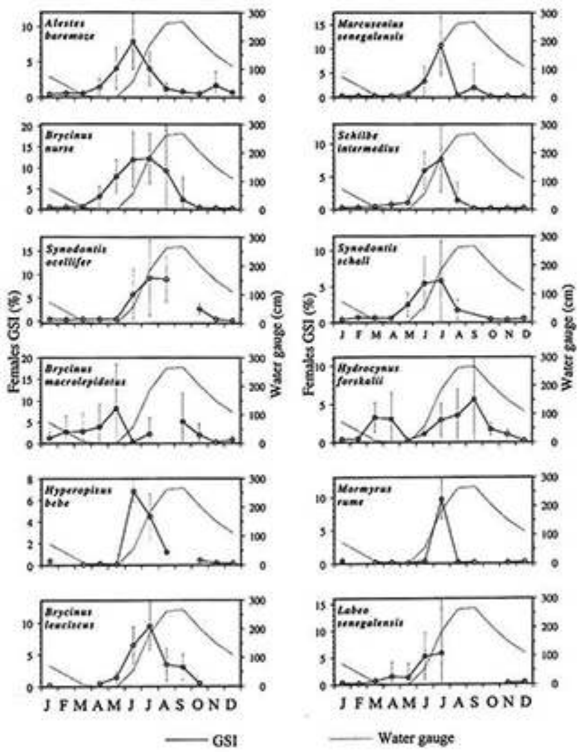

Fig. 3. Baoulé River: hydrological cycle and changes in females gonado-somatic index (GSI) of different 'non-guarding' fishes.

4.3. Fecundity

4.3.1. Egg diameter

26 Most of the species encountered in the Baoulé River have small eggs (Fig. 5). Within a given family, egg size is more or less similar for all the species. There is an inverse relation between egg size and fecundity. The optimal egg size is that which maximises the number of offspring to become reproductively active. So, egg size could be considered as a tradeoff between fecundity and young survival. Bigger eggs, which produce larger larvae, seem to be an adaptational advantage when food supply is variable or limited. Conversely, in the Baoulé River the minimisation of egg size would tend to maximise fecundity.

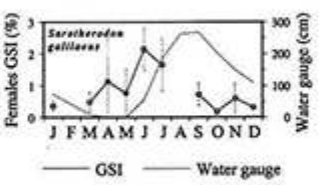

Fig. 4. Baoulé River: hydrological cycle and changes in females gonado-somatic index (GSI) of the 'small-brood' spawner *Sarotherodon galilaeus.*

4.3.2. Size distribution of oocytes

27 There are three main patterns of the size distribution of oocytes within the ovaries of the different species. The first group shows a unimodal distribution (Fig. 6). Most species

belong to this group: Characidae, Cyprinidae and catfishes (*Chrysichthys, Synodontis* and *Schilbe*). The second group, which includes the Mormyridae (Fig. 6) has a multimodal distribution of eggs, with a peak of high diameter values and a second batch of very small oocytes. We have no information about the structure of the ovary during the non-breeding season so that we do not know if the smaller eggs develop during a second breeding season, or whether they are held in reserve for the following year. Finally the third group is that of the multiple spawners species (Fig. 6). In the particular case of the Baoulé River this group is represented by Cichlidae like *Chromidotilapia guntheri* (multimodal structure).

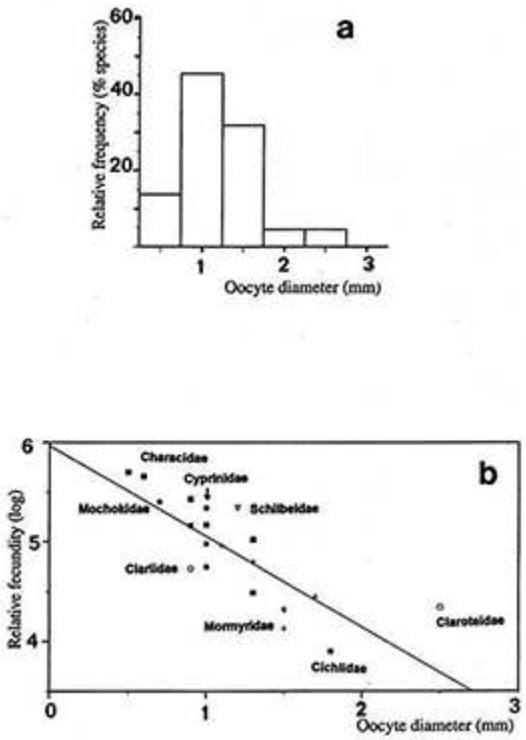

Fig. 5. **(A)** Frequency distribution of oocyte mean diameter; **(B)** relationship between relative fecundity and oocyte mean diameter for species from the Baoulé River (r^2 = 0.648).

4.4. Drift

28 Following the hydrograph, drift of organisms begins in July and lasts up to November-December. Nevertheless, if the catches are mainly composed of very young fishes (10-35 mm SL) at the beginning, sub-adults are more and more numerous from October-November. In fact, the maximum of juveniles drift from July to September with a mean of 100-150 individuals per night (Table 4).

29 The composition of drift catches differs from that in gillnets. Cyprinidae are dominant in driftnets while Characidae, Mormyridae and Mochokidae are more numerous in gillnets. Juveniles first appear during the flood, confirming the conclusion that spawning is timed by the flood period.

5. Discussion and conclusion

30 For most of the species, sizes at first maturity observed in the Baoulé River are comparable to other West African areas with similar climatic regimes (Albaret, 1982; Bénech and Dansoko, 1994). We may therefore consider that in the Baoulé River, the species reach their (50%) sexual maturity at the similar lengths – and ages – than in other zones of West Africa.

31 For African species, the GSI is generally higher for females than for males (Munro et al., 1990; Paugy and Lévêque, 1999). This observation is confirmed for the fishes of the Baoulé River. For most species the females had a GSI higher than 10% except for Cichlidae, where the value reaches only about 5%.

32 Based on the relative frequency distribution of oocytes in the ovaries, two main styles of spawning strategies (sensu Lowe McConnell, 1987) may be distinguished among the considered species. The first, the 'total spawners', generally have a short annual spawning period. The second, the 'small-brood spawners', give parental care and produce small batches of eggs at frequent intervals for most of the year. In the Baoulé River, the two species of Cichlidae studied, *Sarotherodon galilaeus* and *Tilapia zillii,* belong to this second group while the remainder belong to the 'total spawners' group; most of the species spawn just before the flood.

33 The ichthyophagous *Hydrocynus forskalii* seems to reproduce both before (March to April) and at the end (August to September) of the high water period. For an open water feeder (Jackson, 1961; Winemiller and Keslo-Winemiller, 1994), there is a huge advantage of being bom some months before other species to reach a sufficient size to eat juveniles when they leave their birth sites. This reproductive pattern of predatore is also observed in temperate regions where the pike (*Esox lucius*) spawns some weeks before the others species (Billard, 1996). In the Chad basin (lake and rivers), *H. forskalii* has also two spawning periods (Srinn, 1976; Bénech and Quensière, 1985). The first takes place in March and concerns most of the individuals (more than 90% of the females); the second takes place in August and concems the remaining population. In the rivere of Côte d'Ivoire the frequency distribution of oocytes in the ovaries is multimodal (Albaret, 1982), which suggests that each female is able to spawn twice a year if the environmental conditions are favourable.

34 Most of the species encountered in the Baoulé River have small eggs. This minimisation of egg size would tend to maximise fecundity. That is to say, that for most of the species, food supply would not be a limitative variable during the flood. This resuit is generally similar to that observed for European freshwater species, which also show a frequency distribution skewed towards the smaller diameters (Wootton, 1991). Nevertheless, some European species have eggs, which diameter (up to 6-7 mm) has never been observed in West Africa, where the maximum egg size is 2.5 mm for *Chrysichthys auratus.* Generally speaking, two major groups in which the ripe females have more or less the same gonado-somatic index may be distinguished: the first, the 'egg-number group', which invested heavily in higher fecundity (West African Characidae, for example), and the second, the 'egg-size group', which invests in egg size (West African *Chrysichthys,* for example).

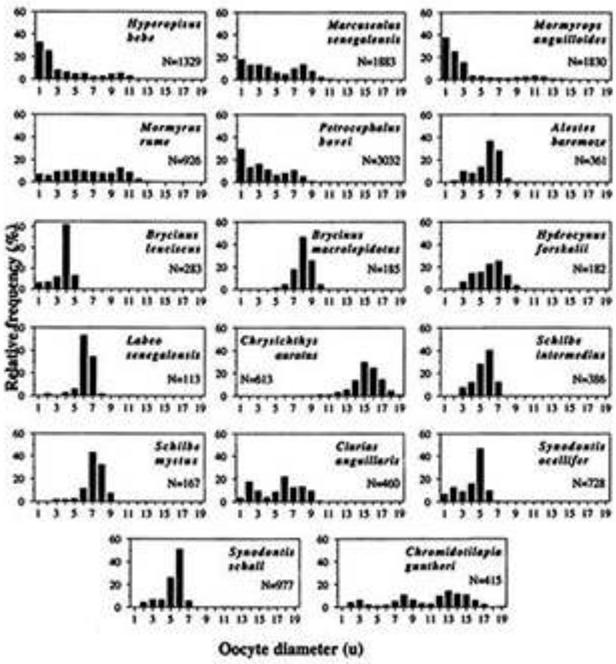

Fig. 6. Relative frequency distribution of clutches sizes. Unit of x-axis (u = 0.165 mm) is that of the reticle fitted into an adjustable eyepiece (10 x).

35 Most of the species inhabiting the Nilo-Sudanian region exhibit a breeding strategy in relation to flood seasonality. This type of strategy (high density-independent mortality), called “r-selection” (McArthur and Wilson, 1967), is common in harsh and unpredictable environments and selects early reproduction, high fecundity and short life expectancy. According to Balon (Balon, 1977), ‘r’strategists are more or less similar to altricial species. Finally, as a resuit of previous studies Winemiller (1989 and 1992) and Winemiller and Rose (1992) identified three types of strategies fitted into a triangular continuum: 1) small size, rapidly maturing, short-lived fish (opportunistic strategists); 2) larger, highly fecund fish with longer lifespans, often associated with spawning migrations (periodic strategists); and 3) fish that often exhibit parental care and produce fewer but larger offspring (equilibrium strategists). These different strategies are evaluated from different life-history traits.

36 Concerning the Baoulé River, 12 variables (see appendix) related to life-history theory were measured, coded or estimated (bibliographic data from the same zone when original data were unknown) for 18 species. In order to establish the ordination of species function of their lifehistory traits, we carried out a correspondence analysis (CoA) on log-transformed data [Log (*x*+1)] so as to normalise the variances. Then a UPGMA (agglomerative algorithm: average link) distance analysis was carried out on the factorial coordinates (species) of the CoA to check species relationships.

37 Clustering of 18 species based on Euclidean distances resulted in two groups (Fig. 7). The first corresponds to developed parental care, large oocytes and low fecundity. Two species, *Chrysichthys auratus* and *Sarotherodon galilaeus,* belong to this ‘equilibrium’ strategy. Others species have a ‘seasonal’ strategy which is adapted to the harsh local conditions. In fact most fishes in the Nilo-Sudanian area appeared to be associated with a seasonal life-history strategy that exploits an annual expansion of aquatic production. The seasonal strategy is characterised by high fecundity, absence of parental care, Iimited breeding season and, for some species, large adult size (i.e., *Alestes baremoze, Synodontis*

schall, Hyperopisus bebe) and upstream spawning migrations to floodplains (Daget, 1954, 1957; Bénech and Quensière, 1985).

38 The lack of 'opportunistic' species has to be underlined. The 'opportunistic' life-history strategy in fishes appears to place a premium on early maturation, frequent and extended spawning, rapid larval growth, and rapid turnover rates. This type corresponds to small fishes with early maturation with generally small eggs and small clutches. This suite of life-history traits permits efficient recolonisation of marginal habitats over relatively small spatial scales. Small, short-lived fishes, like *Fundulus* (killifishes) or *Gambusia* (mosquitofishes) in North America, typically have an 'opportunistic' strategy (Winemiller and Rose, 1992). In Africa, killifishes like *Aphyosemion, Epiplatys* and *Aplocheilichthys* likely belong to this group. The smallest meshes employed in our set of gillnets do not allow us to catch species as small as killifishes. The lack of 'opportunistic' life-history strategy in fishes of Baoulé River is therefore related to a sample bias. Nevertheless, species that have this type of strategy are not very common in large rivers and are encountered in very small tributaries.

Table 4. Monthly drift (number of fishes per night) in the Baoulé River.

Species	Family	July	August	September	October
Polypterus bichir	Polypteridae	0.4	0.3		
Mormyrops anguilloides	Mormyridae		0.3		6.0
Mormyrus rume		0.6	0.3		
Alestes baremoze	Characidae			0.5	1.0
Brycinus leuciscus		51.8	75.3	4.5	
Brycinus nurse			7.7		
Distichodus rostratus	Distichodontidae		5.0	0.5	
Nannocharax sp.		0.6		0.5	
Barbus cf *macinensis*	Cyprinidae			125.5	
Barbus macrops				16.5	
Barbus punctitaeniatus				6.0	
Barbus sp.			0.7		
Labeo senegalensis			1.7		
Labeo sp.		7.4	24.0	1.5	
Raiamas sp.		8.6	3.0	0.5	3.0
Bagrus docmac	Bagridae	0.2	0.3		
Auchenoglanis occidentalis	Claroteidae		6.3		
Chrysichthys auratus		1.4			
Schilbe intermedius	Schilbeidae	1.6	4.0	0.5	
Clarias anguillaris	Clariidae	6.0	3.7	0.5	
Heterobranchus longifilis		17.8	1.3		
Malapterurus electricus	Malapteruridae		0.3	0.5	1.0
Synodontis sp.	Mochokidae	3.8			
Epiplatys sp.	Cyprinodontidae	3.4			
Lates niloticus	Centropomidae	3.0	1.0		
Hemichromis sp.	Cichlidae	0.8			
Sarotherodon galilaeus		1.4	2.0		
Tilapia zillii		1.0			

39 If conditions favourable for species durability are periodic and occur at frequencies smaller that the lifespan, selection will favour the synchronisation between reproduction and optimal climatic conditions for a good recruitment of offspring without parental care. Hydrological conditions of the Baoulé River are very restricting, so most of the species are of course periodic. "In fact, the periodic strategy maximises age-specific fecundity (clutch size) at the expense of optimising turnover time and juvenile survivorship" (Winemiller and Rose, 1992). Large size of adults enhances their survivorship during poor conditions and permits storage of energy for the next bout of reproduction. Finally, species such as *Alestes baremoze* execute longdistance upstream migrations to spawn during the wet season. Among the studied species, 89% have a periodic strategy and only

11% an equilibrium strategy. The Baoulé River is a nice example where fishes have proved their ability to adapt life histories to very drastic environmental conditions.

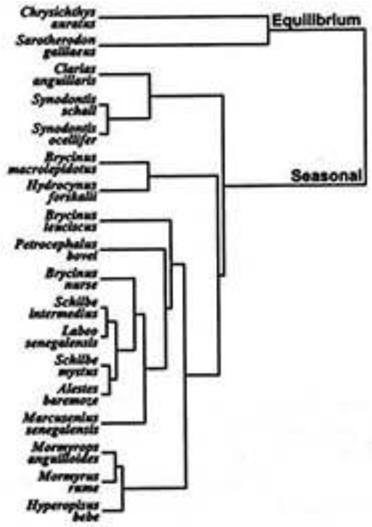

Fig. 7. Hierarchical classification (average link on Euclidean distances) of the center of mass of the factorial coordinates (CoA) of the life-history traits for each species.

40 The pattern of traits related to the life history of species of the Baoulé River is very close to that observed for example in seasonal environments for neotropical fish species from the Venezuelan llanos (Winemiller, 1989). Most of the fishes have a ‘seasonal’ life-history strategy associated with synchronised reproduction at the early wet season, high fecundity and absence of parental care. The second important group is that of species that have an ‘equilibrium’ life-history strategy associated with parental care, low fecundity and large eggs. Finally, the less important group is that of fishes that have an ‘opportunistic’ life-history strategy associated with early maturation and small clutches.

41 This study doesn’t concern all the species of the System but it constitutes a contribution to a more global matrix where life-history traits will be the foundations of a comparison between different geographical and climatic Systems (intercontinental comparison, for example). In this way an interesting goal would be a macro-ecological approach to study the relations between the diversity of the biological strategies (heritability and adaptation) and the environmental variability.

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APPENDIXES

Appendix

List of life-history traits used in statistical analysis.

Females standard length at maturation (mm)

Maximum length observed (mm)

Longevity in years (from literature)

Maximum clutch size: i.e.; the largest batch fecundity reported (number of eggs per kg)

Oocyte size: mean diameter of mature ovarian oocytes (to nearest 0.01 mm)

Range of oocyte sizes (to nearest 0.01 mm)

Number of spawning bouts per year

Parental care: quantified as ($x1 + x2$) where

$x1$ = 0 (no special placement of zygotes); 1 (zygotes are placed in a special habitat); 2 (zygotes and larvae are placed in a nest)

$x2$ = 0 (no parental protection of zygotes or larvae); 1 (parental period of protection)

Hatch duration:

0 = 1 to 3 months

1 = 4 to 6 months

2 = 7 to 9 months

3 = 10 to 12 months

One-year length (mm)

Maximum GSI

Structure of the ovary: size-frequency distribution of oocytes:

0 = one mode; 1 = two modes; 2 = more than two modes

ABSTRACTS

In the tropics, there is very little information on fish reproduction in intermittent streams and most of the studies are from the neotropic regions. In temporary rivers, environmental conditions are sharply contrasted and the variation amplitude of hydrological and physicochemical characteristics is high. In the Baoulé River (Mali), during the peak of the dry season, the remaining pools comprise only 10% of the total length of the river. During the wet season, there is a short (three months) but intense period of flooding. In this study, the reproductive strategies of 18 species of fishes were investigated in the upper reach of the Baoulé drainage. They belong to eight families, show variation in feeding guilds, and vary in size and habitat preferences. Fishes were collected from February 1985 to November 1988 using gillnets. Two main types of spawning strategies may be distinguished among these species. The first, the 'total spawners', have generally a short annual spawning period just before the flood. The second, the 'small-brood spawners', produce small batches of eggs at frequent intervals for most of the year. Most of the species inhabiting the Baoulé River exhibit a breeding strategy in relation to the flood seasonality. This type of strategy, common in harsh but predictable environments, favours a synchronous reproduction in phase with the optimal environmental conditions and the production of large number of juveniles that require no parental care. Concerning the Baoulé River, 12 variables related to life-history theory were measured for 18 species. Clustering of these species based on Euclidean distances resulted in two groups. The first corresponds to developed parental care, large oocytes and low fecundity (about a hundred eggs). Two species, *Chrysichthys auratus* and *Sarotherodon galilaeus,* belong to this 'equilibrium strategy'. Others species have a 'seasonal strategy', which is adapted to the harsh local conditions. In the Nilo-Sudanian area, most fishes appeared to be associated with a seasonal life history strategy that exploits an annual expansion of aquatic production. In the Baoulé River, the 'seasonal strategy' is characterised by high fecundity, absence of parental care, limited breeding season and for some species large adult size and upstream spawning migrations to floodplains.

Stratégies de reproduction des poissons d'un cours d'eau temporaire du bassin du haut Sénégal (la Baoulé au Mali). En zone tropicale, il existe peu d'informations qui concernent la reproduction de poissons peuplant les cours d'eau temporaires et la majorité des études existantes concerne la région néotropicale. Dans ce type de rivière, les conditions environnementales sont très contrastées car l'amplitude des variations hydrologiques et des caractéristiques physico-chimiques est généralement très importante. Sur la Baoulé (Mali), au maximum de la saison sèche, les mares résiduelles ne constituent plus 10% de la longueur totale de la rivière. Au moment de la saison des pluies, la crue est courte (trois mois) mais intense. Dans cette étude, les stratégies de reproduction de dix-huit espèces de poissons d'eau douce ont été examinées dans le cours supérieur du Baoulé. Elles appartiennent à huit familles, ne font pas partie des mêmes guildes alimentaires, ne fréquentent pas les mêmes habitats et sont de taille différente. Les poissons ont été collectés à l'aide de filets maillants de février 1985 à novembre 1988. Ces espèces présentent deux principales stratégies de reproduction. Les espèces "à ponte annuelle unique" possèdent généralement une période de reproduction assez courte qui se situe juste avant la crue; les espèces "à pontes multiples" pondent des petits lots d'œufs à intervalles fréquents pendant presque toute l'année. La majorité des espèces qui peuplent la Baoulé ont une stratégie qui leur permet de se reproduire au moment de la période d'inondation. Ce type de stratégie, la plus fréquente dans des environnements contraignants mais prévisibles, favorise le synchronisme entre la reproduction et la période durant laquelle les conditions environnementales sont optimales. Une telle stratégie est généralement accompagnée d'une fécondité élevée et d'absence de soins à la descendance. Douze variables ont été identifiées pour chacune des dix-huit espèces. L'analyse des distances ordonnée selon une classification hiérarchique ascendante basée sur des distances Euclidiennes permet de distinguer deux groupes. Le premier correspond aux espèces qui prodiguent des soins parentaux, possèdent de grands ovocytes et ont une faible fécondité (quelques centaines d'œufs). Deux espèces, *Chrysichthys auratus* et *Sarotherodon galilaeus* présentent ce type de "stratégie équilibrée". Les autres espèces ont une stratégie de type "saisonnière" qui est parfaitement adaptée aux contraignantes conditions locales. Dans le secteur Nilo-Soudanien, la plupart des poissons semblent adopter une stratégie vitale saisonnière pour mieux exploiter la meilleure production annuelle. La stratégie périodique se caractérise dans la Baoulé par une fécondité élevée, l'absence de soins parentaux, une période de ponte réduite et pour quelques espèces par des adultes de grande taille qui effectuent des migrations anadromes de reproduction.

INDEX

Keywords: Freshwater fishes, Environment, Reproductive strategies, Senegal basin, Western Africa

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Écologie des poissons tropicaux d'un cours d'eau temporaire (Baoulé, haut bassin du Sénégal au Mali) : adaptation au milieu et plasticité du régime alimentaire

The ecology of fishes in a temporary tropical stream (Baoulé, a tributary of the upper Senegal R. in Mali): environmental and diet adaptation

Didier Paugy

1 Les pressions sélectives qui s'exercent sur un peuplement tendent à imposer aux espèces qui le composent une stratégie adaptative optimale. En terme de valeur adaptative, cela implique, notamment, l'exploitation la plus efficace possible des ressources alimentaires disponibles.

2 Les habitudes alimentaires des poissons montrent une vaste gamme d'adaptations puisqu'ils occupent pratiquement tous les niveaux trophiques depuis les détritivores jusqu'aux carnivores. La plupart des espèces font preuve d'une importante plasticité alimentaire qui leur permet de s'adapter au mieux aux conditions environnementales, même si leur morphologie (forme du corps et/ou de la bouche, position de celle-ci, dentition...) est parfois adaptée à un régime spécifique.

3 Il est généralement admis que les animaux partagent leurs ressources selon trois axes principaux qui sont l'habitat, les ressources alimentaires et le temps (PIANKA, 1969 ; Ross, 1986). En ce qui concerne les poissons, Ross (1986), montre que la structure de leurs communautés paraît plus influencée par un facteur trophique (57 %), que par un facteur temporel (11 %) ou lié à l'habitat (32 %). Quelques exemples illustrent l'aspect primordial du facteur trophique. En Amérique centrale, par exemple, certaines espèces « réduisent » le spectre de leur alimentation ce qui restreint le chevauchement de leur niche durant l'étiage lorsque les ressources alimentaires deviennent moins abondantes (ZARET, RAND, 1971). Il a aussi été montré que le chevauchement des niches est moindre dans les régions

où la productivité est faible (GASCON, LEGGETT, 1977). La pression sélective de l'alimentation et le partage des ressources permettent également de fournir des informations sur les relations qui lient les espèces et leur habitat (WERNER *et al.*, 1981), et cela durant tout leur cycle biologique ou leur ontogénèse.

4 La capacité qu'ont les espèces d'élargir leur niche trophique est certainement la meilleure stratégie qu'elles peuvent mettre en œuvre pour s'adapter aux conditions extrêmes. Ainsi, en zone tropicale les conditions, tant d'habitat que d'alimentation, peuvent devenir très difficiles pour les poissons, notamment à l'étiage lorsque ne subsistent plus que quelques mares résiduelles où demeurent concentrés les individus susceptibles de repeupler la rivière lors de la remontée des eaux.

5 Le but de cette note est de préciser et de mettre en exergue la plasticité de comportement alimentaire que peuvent développer les espèces face aux conditions difficiles qu'elles sont susceptibles de rencontrer. Pour cela, nous avons pris l'exemple des peuplements de quelques mares résiduelles de faible superficie qui subsistent lors de l'étiage du Baoulé, affluent du haut Sénégal au Mali.

MATÉRIEL ET MÉTHODES

Présentation du milieu

HYDROGRAPHIE

6 Le Baoulé (bassin versant de 59 500 km^2) se forme à l'extrémité sud-est du plateau Mandingue (Mali), à une trentaine de kilomètres au nord de la vallée du Niger. Il coule d'abord plein nord, puis, au-delà du 14^{e} parallèle nord, il dessine une grande boucle et prend une direction sud-ouest pour se jeter dans le Bakoye (fig. 1).

7 Le Baoulé supérieur est un ancien bassin endoréique dont le cours se perdait dans la vaste cuvette de sédimentation du Hohd, près d'une longue vallée morte « La vallée du Serpent ». Cette dernière n'est toutefois pas un ancien lit du Baoulé car son fond s'élève en s'éloignant du cours actuel de la rivière actuelle (MICHEL, 1973). L'actuelle « grande boucle du Baoulé correspond certainement à un coude de capture » (DARS, 1955), qui se serait produite par déversement, probablement après la phase aride de l'Ogolien (— 18 000 B.P. : Paléolithique moyen). Sa vallée étant barrée par les ergs ogoliens, le Baoulé se serait alors jeté, lors d'une très forte crue dans la vallée d'un petit affluent droit du Bakoye, le Goumaniko (MICHEL, 1973b).

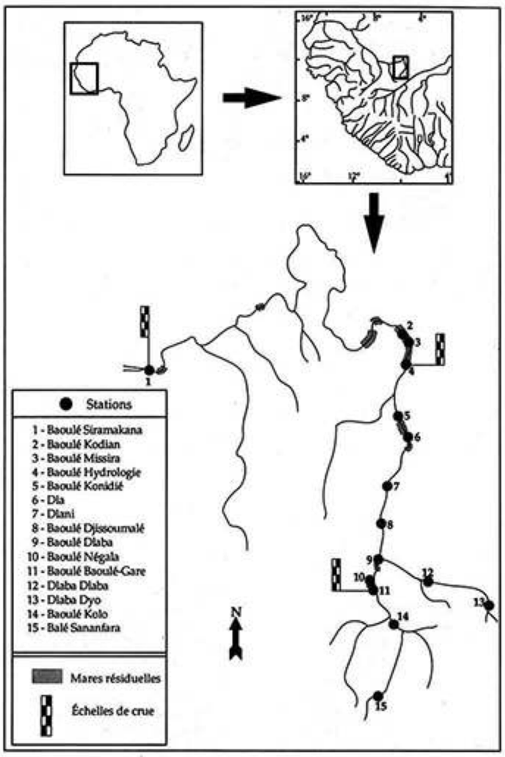

FIG. 1. — Baoulé : situation géographique, emplacement des stations échantillonnées, des échelles de crue et des mares résiduelles durant l'étiage maximal.
Baoulé : geographical situation, position of the sample sites, water gauges and residual ponds during the maximal low water.

CLIMAT ET HYDROLOGIE

8 En zone sahélo-soudanienne, certaines rivières cessent de couler ou s'assèchent plus ou moins partiellement durant l'étiage. Tel est le cas du Baoulé, affluent du haut Sénégal (fig. 2), qui, à la fin de la saison sèche, n'est plus constitué que d'un chapelet de mares de superficies et de profondeurs variables dont la somme ne représente plus qu'une collection d'eau d'environ 50 km, soit moins de 10 % de la longueur totale du cours d'eau (fig. 1).

9 L'ensemble du bassin du Sénégal se situe dans le domaine tropical *sensu stricto,* de l'hémisphère boréal, caractérisé par l'alternance de deux grandes saisons annuelles : la saison des pluies d'été et la saison sèche d'hiver. Cette alternance climatique marquée caractérise le régime hydrologique de ce fleuve qui peut être considéré comme « tropical pur » (RODIER, 1964). Dans son ensemble, le cours du Baoulé est situé entre les isohyètes 750 mm au nord et 1 000 mm au sud (MICHEL, 1973), ce qui le situe entièrement dans la zone sahélo-soudanienne qui se caractérise, entre autres, par une absence de pluie durant plusieurs mois consécutifs. Cette absence, jointe à de fortes températures et à une évaporation élevée engendre une importante sécheresse qui se répercute aussi bien sur l'écoulement des eaux que sur la nature et la densité du couvert végétal. Ainsi, le long du cours, l'étroite forêt-galerie, composée d'espèces hygrophiles verdoyantes, se limite le plus souvent à un rideau d'arbres localisé le long des berges, contrastant avec les branchages défeuillés de la végétation de la savane desséchée qui le jouxte.

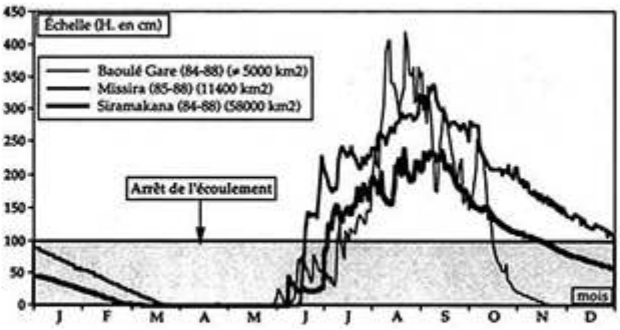

FIG. 2. — Baoulé : régimes hydrologiques dans le haut, le moyen et le bas cours (voir emplacement des stations en fig. 1).
Baoulé : hydrological regime for the upper, medium and lower course (see the sites position on fig. 1).

10 Dans cette région, la décrue s'amorce dès que les pluies diminuent, soit à partir de septembre. En théorie, les vitesses de tarissement sont pondérées par les apports de la nappe alluviale qui soutiennent, en partie, les débits des fleuves au cours de la période d'assèchement. Toutefois, depuis quelques années les déficits pluviométriques enregistrés n'ont pas permis un renforcement suffisant de la nappe phréatique, qui va semble-t-il en s'amenuisant. De ce fait son rôle de soutien à l'étiage est moindre, ce qui accroît la vitesse de tarissement et, fait grave, provoque un étiage précoce indépendant, ou presque, de la hauteur et de la durée de la crue (OLIVRY *et al.*, 1993). Outre une décrue plus rapide et donc certainement préjudiciable à la vie aquatique, il se pose également un problème au niveau de la végétation qui souffre de cette carence, les racines ne trouvant plus les ressources en eau nécessaires et suffisantes dans une nappe déficitaire.

11 Dans un cas extrême comme celui-ci, les poissons sont évidemment soumis à des conditions très drastiques puisque, selon les années, l'écoulement des eaux ne dure que quatre à cinq mois (juillet-novembre) dans le cours moyen, cette période pouvant être réduite à trois, voire même deux mois, dans le haut cours (fig. 2). Dès les premières pluies, la montée des eaux est assez rapide, ce qui permet aux poissons de repeupler les biefs asséchés, tant par l'amont que par l'aval, phénomène primordial pour les hauts cours totalement asséchés.

12 Enfin, en ce qui concerne le degré d'anthropisation du milieu, la quasi-totalité du cours du Baoulé se situe dans un parc national, ce qui *a priori* exclut tout prélèvement de faune. Néanmoins, quelques pêcheurs braconniers effectuent des pêches aux filets maillants en fin d'étiage. Nous avons pu constater qu'il s'agissait de pêcheurs itinérants possédant des véhicules équipés d'une glaciaire de plus de 1 mètre cube et passant d'une mare à la suivante. L'impact qu'exerce ces pêcheurs existe, mais n'est pas drastique, seules les grandes espèces commerciales les intéressant. On peut donc considérer que l'impact sur la communauté ichtyologique est relativement faible et que les conditions demeurent presque naturelles.

ICHTYOFAUNE

13 De par sa composition spécifique, l'ichtyofaune du bassin du Sénégal, dont fait partie le Baoulé, appartient à la région biogéographique sahélo-soudanienne (ROBERTS, 1975 ; HUGUENY, 1990 ; HUGUENY, LÉVÊQUE, 1994 ; PAUGY *et al.*, 1994). Cet ensemble comprend les grands bassins de la zone sahélienne que sont le Niger-Bénoué, le Tchad, la Volta et la Gambie, auxquels se rapprochent également les bassins atlantiques que sont l'Ouémé,

l'Ogun et la Cross. Jusqu'à présent, 120 espèces ont été recensées pour l'ensemble du bassin du Sénégal. Un certain nombre ne se rencontre que dans le bas cours dulcaquicole, d'autres sont inféodées aux milieux saumâtres. Ainsi, en théorie, un peu moins de cent espèces sont susceptibles d'être capturées dans le Baoulé, mais en réalité seules une cinquantaine d'espèces sont rencontrées régulièrement.

Techniques d'échantillonnage

14 En période de hautes eaux, les prélèvements sont traditionnellement effectués à l'aide de trois batteries de filets de différentes tailles de mailles (deux batteries de multi- et une batterie de monofilaments). Mais lorsqu'il ne reste plus que des petites mares résiduelles, nous effectuons un échantillonnage plus exhaustif, en utilisant en premier lieu des petites sennes, type senne de plage, puis un ichtyotoxique à base de roténone. Les poissons sont capturés avant qu'ils ne meurent dès leur remontée à la surface, ce qui permet de connaître assez précisément leur bol alimentaire avant que la digestion ne soit trop avancée. Pour les plus gros spécimens, la mort n'intervient cependant qu'avec un certain retard. Dans ce cas, les spécimens sont évidemment pris en compte pour l'analyse de la composition du peuplement en place, mais les contenus stomacaux ne sont pas analysés.

Analyses des données

15 La plupart des résultats ont été traités par Analyses Factorielles des Correspondances (CHESSEL, DOLÉDEC, 1992) **à** partir des matrices « importance pondérale des espèces de poissons / date et lieu des prélèvements » en ce qui concerne les peuplements et des matrices « pourcentage d'occurrence des proies / espèces de poissons par prélèvement », en ce qui concerne l'alimentation de ces derniers.

16 Nous avons éliminé les espèces de poissons représentées par un seul exemplaire ainsi que les taxons proies peu consommés (< 5 % de l'occurrence totale). Nous avons ensuite effectué une Analyse Factorielle des Correspondances (CHESSEL, DOLÉDEC, *op. cit.*) sur un tableau comprenant 44 échantillons (espèces/prélèvements) et 18 types de proies, résultats exprimés en pourcentage d'occurrence pondérée (centrage par ligne pour que chaque espèce pèse d'un poids égal dans l'analyse).

La diversité alimentaire a été calculée en utilisant l'indice de Brillouin ($Hb = \frac{(\ln N! - \sum \ln ni!)}{N}$) qu'il est recommandé d'employer lorsque les calculs sont effectués à partir de collectes estimées (MAGURAN, 1988). Mais comme le type d'indice de diversité en usage est fonction à la fois de la richesse spécifique et du nombre d'effectifs de chaque espèce, il convient de calculer aussi la régularité[1] (SCHERRER, 1984 ; FRONTIER, PICHOD-VIALE, 1991), qui représente l'équirépartition des effectifs pour chacune des espèces présentes. Ce paramètre ($R = \frac{Hb'}{Hb'_{max}}$, avec Hb' : diversité observée et Hb' max : diversité maximale pour un nombre de taxon identique soit $\log_2 S$) est obligatoire lorsque les échantillons ont des richesses et des effectifs différents. En effet des peuplements à physionomie apparemment très divergente peuvent néanmoins présenter une diversité identique.

LES PEUPLEMENTS DE POISSONS ET LA SUPERFICIE DES MARES

17 En fin d'étiage, les mares ont des caractéristiques morphologiques différentes. Quelques-unes conservent une dimension (longueur ≥ 1 km) et une profondeur (≥ 2 m) respectables, d'autres sont plus modestes (de l'ordre de l'hectare) et moins profondes (de l'ordre de 1 m en moyenne), ou petites (quelques 100 m^2) et très peu profondes (quelques dizaines de cm). Enfin, certaines d'entre elles s'assèchent totalement.

18 La composition et la structure des peuplements de poissons demeurant dans les mares les plus profondes sont sensiblement identiques à ce que l'on observe en périodes de hautes et de moyennes eaux dans le lit majeur (observations personnelles). Bien que très peu nombreuses (deux ou trois), elles servent certainement de réservoir principal permettant aux espèces de recoloniser le milieu lors de la remontée des eaux.

19 Les résultats que nous présentons proviennent de prélèvements qui, pour la plupart, ont été réalisés dans deux mares de Missira (fig. 1), de taille, petite (750-900 m^2 ; 0,3-0,5 m) et moyenne (6 000-9 000 m^2 ; 1 m), situées à quelques centaines de mètres l'une de l'autre, pour éviter un biais lié à la distribution des espèces le long du bassin versant. Trois prélèvements ont été effectués en plein étiage dans chacune des deux mares. Dans la plus importante, qui s'isole complètement en avril, l'eau subsiste en permanence. La richesse spécifique des trois prélèvements que nous avons effectués en avril (1986, 87 et 88), varie entre 35 et 37 espèces (fig. 3). Pour l'autre mare (la plus petite), située quelques centaines de mètres en aval, qui s'isole beaucoup plus tôt puis qui se tarit dès avril-mai, nous avons échantillonné trois fois en mars, mais à des niveaux d'assèchement différents selon les années. En effet, si la surface inondée n'était plus que de quelques centaines de mètres cubes en 1985 et 1986, elle était encore importante en 1987. En terme de richesse spécifique, les deux prélèvements effectués peu avant l'assèchement étaient assez pauvres (17 espèces en 1985 ; 15 espèces en 1986). Celui effectué plus tôt dans l'année (1987), avec une surface en eau encore assez importante, était presque deux fois plus riche (30 espèces). L'analyse factorielle des correspondances (cf. p. 161) montre bien qu'il y a d'abord un effet station, puisque deux récoltes effectuées dans des mares de peu d'importance situées beaucoup plus en amont (Djissoumalé et Négala) s'isolent par rapport aux deux stations de Missira (fig. 4). De même, les captures des deux mares en voie d'assèchement (Missira 2, mars 1985 et mars 1986) s'isolent, tandis que le prélèvement effectué sur le même site, alors que la mare était moins asséchée (Missira 2, mars 1987), fait la transition avec les collections d'eau plus importantes (Missira 1, avril 1986, 1987 et 1988). La richesse et la composition spécifiques varient selon la surface d'eau disponible (fig. 5) et vraisemblablement suivant les conditions environnementales qui lui sont associées (fig. 3). En ce qui concerne les espèces, on remarquera la totale disparition des *Labeo* spp., abondants en conditions normales, encore relativement nombreux lorsque la quantité d'eau est suffisante et que des rochers parsèment le biotope, puis totalement absents lorsque l'eau devient rare et que le substrat n'est plus qu'un mélange de sable et de vase. En ce qui concerne ce genre, il est difficile de dire si sa raréfaction puis sa disparition sont liées au manque de substrat adéquat, source unique de nourriture par le périphyton qui s'y développe, ou s'il s'agit d'une carence en oxygène, puisqu'il a déjà été constaté que *L. senegalensis* était une espèce sensible à un déficit en oxygène (BÉNECH, LEK, 1981). L'autre phénomène important est la diminution puis la disparition de la quasi-

totalité des espèces de Mormyridae, et nous avons pu observer cela dans une autre station, située 100 kilomètres en amont, échantillonnée à l'aide de filets maillants. En ce qui concerne ce groupe, on sait que la plupart des espèces sont sensibles à la diminution de la concentration en oxygène dissous, puisque cela a déjà été mis en évidence en Afrique tropicale pour certaines espèces de cette famille (BÉNECH, LEK, *op. cit.*).

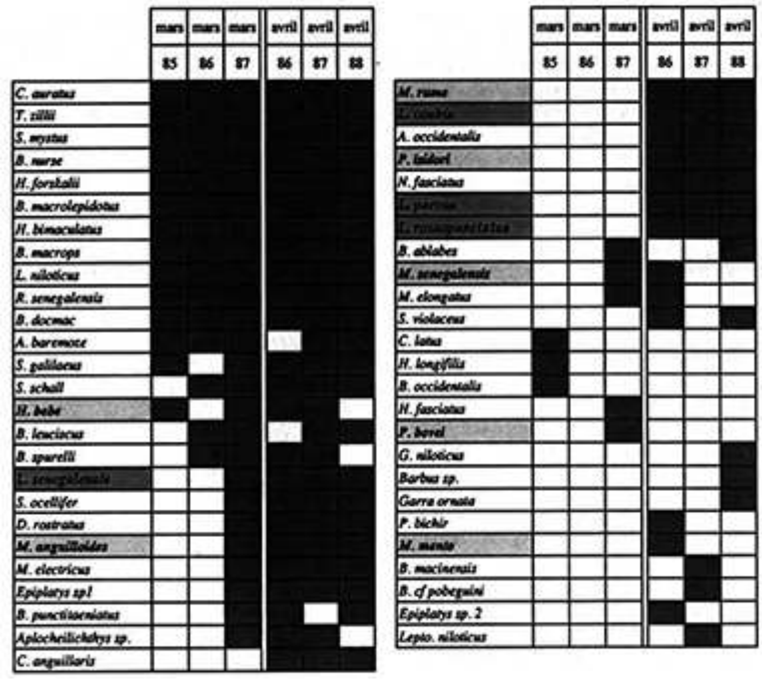

FIG. 3. — Baoulé : présence (grisé) ou absence (blanc) des espèces en fonction des biotopes (mares distinctes) et des dates d'échantillonnage (volumes en eau disponible différents). Cadre gris foncé (Cyprinidae), cadre gris clair (Mormyridae).
Baoulé : presence (greyish) or absence (white) of species in the isolaled ponds in relation with sampling date (different available volumes of water). Dark grey (Cyprinidae), pale grey (Mormyridae).

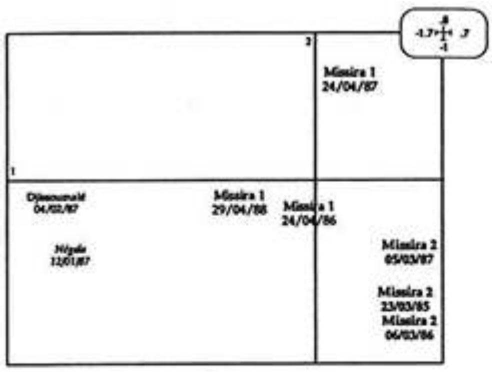

FIG. 4. — Baoulé : peuplements ichtyologiques de quatre mares résiduelles (voir localisation des stations en fig. I). Analyse factorielle des correspondances effectuée à partir de huit échantillons distincts (dates et/ou lieux).
Baoulé : ichthyological assemblages of four residual ponds (see the sites position on fig. 1). Correspondence Factorial Analysis computed for eight distinct samples (dates and/or sites).

COMPOSITION TROPHIQUE DES COMMUNAUTÉS DE POISSONS

Relation longueur de l'intestin/longueur standard des espèces continentales d'Afrique intertropicale

20 On a souvent écrit et montré pour de nombreux groupes de vertébrés qu'il y avait généralement une relation positive entre la longueur de l'intestin et le type de régime alimentaire des espèces (GRASSÉ, DEVILLERS, 1965). En ce qui concerne les poissons, les travaux insistant sur la relation entre la longueur du tube digestif et la nature de la nourriture sont assez nombreux, quels que soient les milieux considérés (pour une revue assez complète, on pourra se reporter à KAPOOR *et al.,* 1975 et GEISTDOERFER, 1981).

21 Toutefois, si les travaux ne sont pas rares, peu concernent encore les espèces tropicales, africaines en particulier. De ce point de vue, seuls les Cichlidae du lac Tanganyika ont été particulièrement bien étudiés. FRYER et ILES (1972), comme les autres auteurs, montrent qu'il existe également une relation entre la longueur relative de l'intestin et le régime alimentaire des espèces (fig. 6). Il est cependant difficile de généraliser ces observations aux lacs de la Rift Valley, où l'ichtyofaune est très diversifiée et d'un point de vue alimentaire très spécialisée, et aux cours d'eau africains en général où la spéciation est moindre et les régimes souvent plus généralistes. Une des raisons de cette différence réside certainement dans le fait que, dans les grands lacs, la nourriture est disponible tout au long de l'année, alors qu'elle est plus saisonnière dans les cours d'eau intertropicaux (LOWE MCCONNEL, 1991). Cela est d'autant plus vrai que l'on se situe en zone sahélo-soudanienne, comme c'est le cas pour le Baoulé, et que l'étiage très prononcé correspond généralement à une période où les ressources en nourriture se raréfient ou manquent, au moins en ce qui concerne certains groupes « proies ».

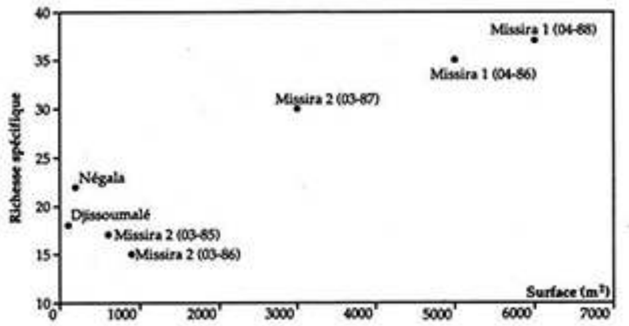

FIG. 5. — Baoulé : relation entre la surface des mares échantillonnées et leur richesse spécifique respective.
Baoulé : relation between the pond area and the fish specific richness.

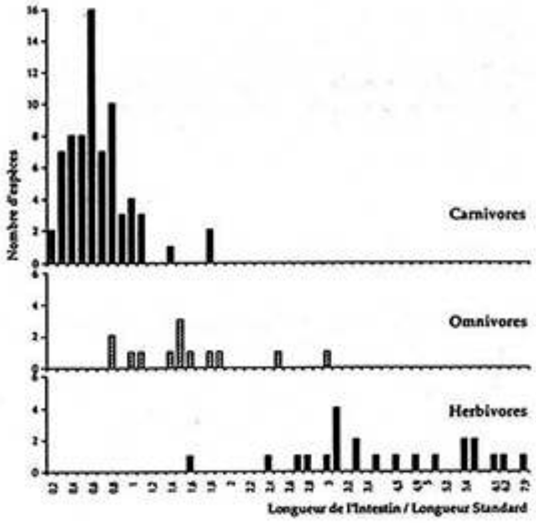

FIG. 6. — Lac Tanganyika : relation entre la longueur relative de l'intestin et le régime alimentaire chez les Cichlidae (d'après FRYER et ILES, 1972).
Lake Tanganyika : relation between the relative gut length and the diet of Cichlid fishes (after FRYER and ILES, 1972).

22 Nos propres observations, ajoutées à ce que nous avons pu trouver dans la littérature, nous ont permis de rassembler des connaissances concernant le régime alimentaire et la longueur intestinale de 80 espèces africaines inter-tropicales, pour la plupart fluviales (annexe I).

23 D'après nos observations tirées de l'analyse des contenus stomacaux des espèces rencontrées en Afrique occidentale, nous avons réparti les espèces en six grandes catégories trophiques : limnivores (7 espèces), micro- et macrophytophages (21 espèces), zooplanctophages (3 espèces), omnivores (15 espèces), invertivores (28 espèces) et ichtyophages[2] (6 espèces). Il ressort assez clairement que les limnivores et les phytophages possèdent, comme il fallait s'y attendre, un intestin plus long que les autres catégories (fig. 7). Pour les autres guildes, il n'existe, en revanche, pas de différence significative entre les longueurs relatives moyennes des intestins, même si les omnivores possèdent généralement un tractus un peu plus long. Si l'on considère que les omnivores comptent certainement parmi les espèces les mieux adaptées pour survivre en cas de disette (en terme de diversité et non d'abondance), on doit alors admettre que ce sont certainement les limnivores et certains phytophages qui ont le moins de chances de survivre dans les milieux prospectés lors de cette étude, leur régime et leurs adaptations morphologiques étant par trop spécifique. Ainsi, la structure de la bouche et des lèvres des *Labeo* les contraignent de toute évidence à un broutage périphytonique. Cela explique certainement la différence importante qui existe entre la morphologie intestinale de ces espèces et celle des autres. En effet, certaines espèces microphages comme les *Citharinus* ou certains Cichlidae *(Tilapia s.l.)* possèdent des dents qui leur permettent à l'occasion de se nourrir d'autres éléments. Cette possibilité qui leur est donnée peut, en partie, expliquer que la dimension moyenne de leur intestin est plus faible.

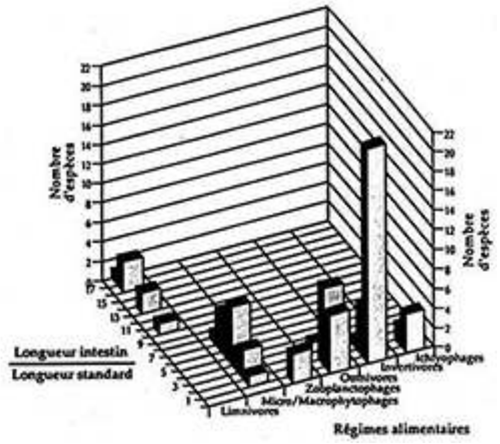

FIG. 7. — Relation entre la longueur relative de l'intestin et le régime alimentaire chez différentes espèces rencontrées en Afrique (hors du lac Tanganyika) (voir Annexe).
Relation between the relative gut length and the diet of different african fish species (except Lake Tanganyika) (see Annex).

24 Si certaines espèces, comme les Cichlidae des grands lacs d'Afrique de l'Est par exemple, paraissent *a priori* présenter un haut degré de spécialisation alimentaire, l'examen de leur anatomie fonctionnelle met en évidence leur capacité d'adopter un régime plus large que ne le suggère leur apparente spécialisation (LIEM, 1980), qui peut de ce fait paraître parfois simplement facultative. C'est ainsi que plusieurs auteurs se posent la question de savoir pourquoi certaines espèces se spécialisent alors qu'elles sont en mesure de s'alimenter à partir de ressources multiples. Une réponse possible est qu'en cas de raréfaction de certains aliments, les individus capables de se nourrir à partir d'une ressource non accessible aux autres en tirent forcément un avantage adaptatif. Ainsi, l'évolution favoriserait les organismes s'offrant la possibilité de survivre dans des conditions de sévère compétition intraspécifique (RIBBINK, 1990). Dans ce cas, certains Cichlidae réputés spécialistes peuvent aussi être généralistes ce qui leur procure un double avantage. De telles adaptations n'ont semble-t-il pas encore été mises en évidence ailleurs, ce qui conduit d'autres auteurs à estimer qu'en fonction des circonstances, le fait d'être spécialiste ou généraliste permet de tirer ou non des avantages (LOWE-MCCONNEL, *op. cit.*).

25 En ce qui concerne les mares que nous étudions, outre les problèmes peut-être liés à l'hypoxie, on peut donc certainement imaginer que les *Labeo* ont un certain mal, voire une impossibilité, à survivre, dû aux difficultés de se nourrir dans certains biotopes durant de trop longues périodes (trois à quatre mois). Cela d'autant que ces espèces en difficulté (nourriture et habitat) deviennent certainement une cible plus facile pour les prédateurs. À l'inverse, on doit pouvoir s'attendre à voir subsister plus facilement toutes les espèces capables d'adapter leur régime aux ressources disponibles.

Plasticité du régime alimentaire des poissons de l'Afrique sahélio-soudanienne

26 Les poissons montrent souvent une importante faculté d'adaptation aux conditions extérieures. Ainsi, par exemple, contrairement à d'autres groupes (insectes, oiseaux, mammifères) dont la croissance est déterminée (ce qui signifie que les adultes ont une taille fixée), celle des poissons est beaucoup plus variable et le plus souvent dépendante

des caractéristiques du milieu. Un des paramètres de cette qualité du milieu est, entre autres, la quantité et la qualité des ressources alimentaires disponibles. Si l'on considère les espèces généralistes, celles-ci ont la possibilité de se nourrir à partir de presque toute ressource vacante. Au contraire, les espèces spécialisées peuvent, si leur alimentation spécifique fait défaut, soit disparaître, soit migrer vers un habitat où les conditions alimentaires sont plus favorables (ce qui nécessite évidemment la possibilité de se mouvoir librement), soit enfin dans certaines limites, adapter leur régime en fonction des proies disponibles.

27 Les changements de régime sont assez bien connus chez les poissons généralistes (KEAST, 1978), mais ils existent aussi chez des espèces réputées plus spécialisées. Ainsi, en zone tropicale, dans le lac Victoria certains Cichlidae typiquement zooplanctophages, insectivores ou détritivores, ne se nourrissent pratiquement plus que de Diatomées, lorsque celles-ci deviennent surabondantes (WITTE, 1984). D'autres exemples existent, ainsi des études menées en laboratoire montrent que certaines espèces *(Cichlasoma minckleyi)* possèdent des morphes trophiques différentes uniquement en période de disette (LIEM, KAUFMAN, 1984). Essayons donc de savoir ce qui se passe dans les mares résiduelles des bassins sahélo-soudaniens.

28 Nous avons analysé les contenus stomacaux des espèces capturées dans trois mares du Baoulé (station de Missira) et dans une mare du Niger (station de Souldougou, 20-30 kilomètres en aval de Bamako). Tous ces prélèvements ont été effectués entre mars en juin (période d'étiage), dans des mares de superficie et de profondeur différentes.

29 Il ressort assez clairement que la diversité alimentaire des différentes espèces est essentiellement sous la dépendance du milieu et donc des proies disponibles. Cela est aussi vrai si l'on considère toutes les espèces que si l'on ne prend en compte que les espèces communes aux différentes mares (fig. 8 et 9). Cela montre que les espèces, pour survivre dans des conditions parfois extrêmes, adaptent, dans certaines limites, leur régime aux ressources dominantes disponibles. Ainsi, nous pouvons voir que la diversité de l'alimentation en rapport avec la richesse en proies ingérées est vraisemblablement dépendante des proies disponibles (fig. 10). En ce qui concerne l'alimentation des espèces confinées dans les petites mares du Baoulé, on note toujours une régularité élevée ($\geq$ 0,83), souvent égale à un, notamment lorsque la richesse est peu élevée. Si l'on tient compte de l'ensemble des échantillons ($n = 44$), on note une corrélation négative significative entre la richesse spécifique et la régularité. En revanche, si l'on ne considère que ceux dont la richesse est supérieure à huit (espèces d'une mare ayant ingéré plus de huit catégories de taxons-proies, soit 26), on ne note plus de corrélation entre la régularité et la richesse spécifique. Cela semble donc signifier que face à une faible variété de nourriture, liée également à une moindre quantité, les prédateurs utilisent la ressource la plus abondante, et qu'à l'inverse si les proies sont qualitativement, et certainement quantitativement, plus abondantes, les poissons s'alimentent à partir de beaucoup plus de types de proies. On peut donc dans le premier cas craindre une compétition d'autant plus accrue que la quantité et la qualité de la ressource est faible.

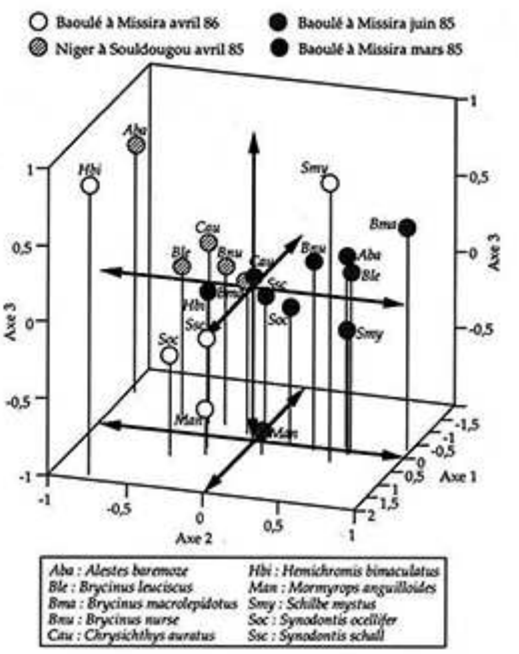

FIG. 8. — Baoulé : analyse factorielle des correspondances à partir d'une matrice de 20 échantillons (espèces/prélèvement) et 18 types de proies. Représentation spatiale sur les trois premiers axes des espèces communes à différentes mares.
Baoulé : Correspondence Factorial Analysis computed from the matrix of 20 samples (species/catches) and 18 preys taxa. Spatial representation on the three first axes of common species from different pools.

DISCUSSION - CONCLUSIONS

30 Dans un système comme celui du Baoulé, les poissons doivent, durant l'étiage, faire face à trois contraintes majeures : les diminutions de la teneur en oxygène et de la quantité de nourriture disponible, et l'augmentation de la prédation[3]. Lorsque la surface en eau diminue, la concentration en poissons s'élève, au moins au début, ce qui favorise évidemment la prédation par les poissons carnassiers. Par ailleurs, la diminution de la couche d'eau facilite certainement aussi une prédation externe, de la part des oiseaux par exemple.

31 Bien qu'en règle générale les poissons tropicaux paraissent, par rapport aux espèces des zones tempérées, plus tolérants à l'hypoxie (WOOTTON, 1991), nous avons vu que certaines formes, plus que d'autres, pouvaient souffrir d'une diminution de concentration en oxygène. Pour contourner cette difficulté, certaines espèces, comme les *Clarias* ou les *Ctenopoma,* ont développé des adaptations particulières qui leur permettent d'avoir une respiration plus ou moins aérienne. Toutefois, ce mode d'accommodation n'existe pas chez la majorité des espèces. Certaines ont cependant la faculté d'utiliser le film d'eau de la couche superficielle qui est enrichi en oxygène en raison des échanges gazeux qui existent avec l'atmosphère. Les poissons qui agissent ainsi ne présentent pas forcément d'adaptations morphologiques particulières. Néanmoins, il est vraisemblable que l'utilisation optimale de ce mince apport oxygéné est favorisé si les poissons possèdent certaines caractéristiques telles qu'une tête relativement aplatie, une bouche tournée vers le haut et une petite taille (KRAMER, 1983).

32 Parmi les espèces les plus courantes, nous avons vu que deux groupes, *Labeo* spp. et Mormyridae, semblaient plus particulièrement sensibles et disparaissaient lorsque les conditions devenaient par trop extrêmes. Toutes les espèces appartenant à ce genre ou à cette famille possèdent une bouche infère. Cette particularité morphologique rend difficile une utilisation pratique du film superficiel enrichi en oxygène, d'où peut-être leur raréfaction puis leur disparition. Ce qui, éventuellement, ne serait pas le cas des *Synodontis* qui possèdent également une bouche infère mais qui ont la particularité de nager souvent sur le dos et de venir souvent en contact avec la surface. La carence en oxygène est certainement importante mais il existe un autre facteur limitant majeur, la diminution de nourriture disponible tant quantitativement que qualitativement.

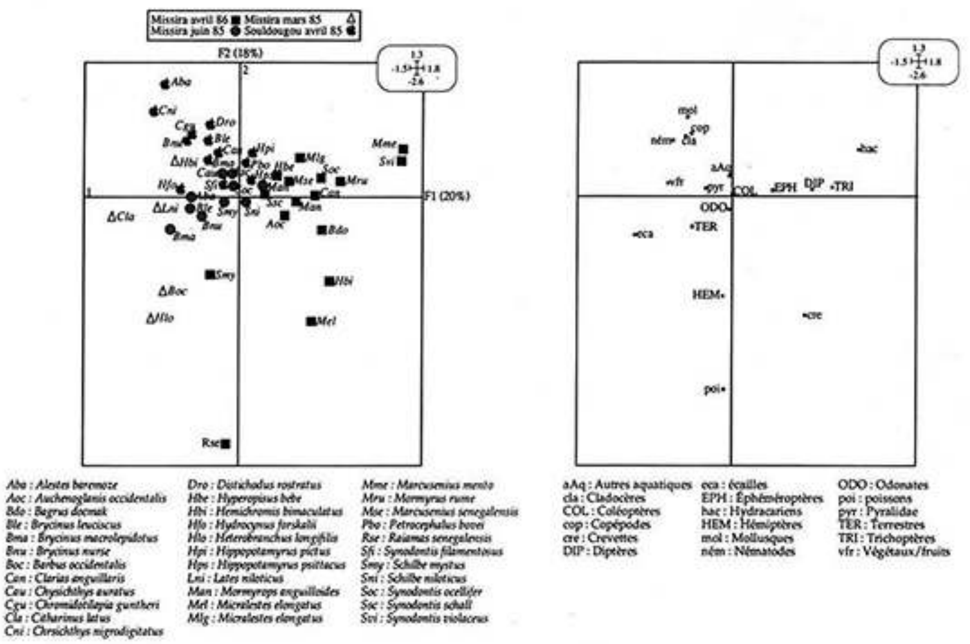

FIG. 9. — Baoulé : analyse factorielle des correspondances effectuée à partir d'une matrice de 44 échantillons (espèces/prélèvement) et 18 types de proies. A gauche : représentation des espèces/prélèvement ; à droite ; représentation des proies.
Baoulé : Correspondence Factorial Analysis computed from the matrix 44 samples (species/catches) and 18 preys taxa. Left : species/catches representation ; Right : preys representation.

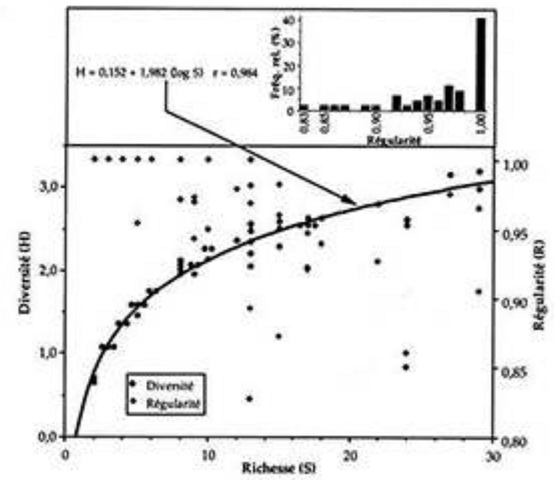

FIG. 10. — Baoulé ; relation entre la diversité (H) ou la régularité (R) de l'alimentation et la richesse (S) en proies ingérées.
Baoulé : relation between the diversity (H) or the regularity (B) of feeding and the richness (S) of ingested preys.

33 Nous avons pu voir que chez certaines espèces, la morphologie de l'intestin (ici la longueur relative) reflétait une base alimentaire spécifique. Bien que quelques travaux aient démontré que chez différentes populations (ODUM, 1970), voire au sein d'une même population (SIBLY, 1981), la longueur relative de l'intestin pouvait varier avec un changement de régime alimentaire, il n'en demeure pas moins vrai qu'à chaque fois il s'agit d'espèces plus ou moins omnivores et que les variations constatées sont assez limitées même si elles sont significatives. La longueur relative de l'intestin est une conséquence du régime qui, lui, est lié à d'autres caractéristiques morphologiques, de la bouche ou des dents par exemple. En ce qui concerne la longueur relative de l'intestin, on peut distinguer trois grandes catégories : la première dont l'intestin est au moins dix fois supérieur à la longueur standard comprend les *Labeo* spp. qui sont des limnivores (périphytonophages) stricts ; la seconde, dont l'intestin est deux à huit fois supérieur à la longueur standard comprend les herbivores *(Tilapia s.l., Citharinus, Distichodus)* ; enfin la troisième dont la longueur de l'intestin est moins de trois fois (quatre fois pour certains omnivores) supérieure à la longueur standard comprend toutes les autres espèces. Ce sont évidemment celles de ce dernier groupe et à un moindre degré celles appartenant au précédent, qui, d'un strict point de vue alimentaire, s'adapteront le mieux puisqu'elles semblent les moins spécialisées et donc capables de tirer au mieux parti de ressources quantitativement limitées. Les ichtyophages, qui peuvent être considérés comme des spécialistes, ne rencontrent pas de problèmes de nourriture, car la concentration en espèces augmente avec la diminution de volume d'eau qui limite les refuges potentiels des proies. On doit donc s'attendre à voir subsister dans ce type de biotopes les espèces capables d'adapter leur régime en fonction des ressources alimentaires disponibles. Cela est primordial car l'enjeu n'est pas simplement la survie de quelques individus mais surtout la capacité d'engendrer une génération future « (...) organism can be seen as playing a game against Nature, in which success is not judged by how large the winnings are, but rather by how long the player can stay in the game » (SLOBODKIN, RAPOPORT, 1974).

34 En ce qui concerne le Baoulé lorsque l'étiage s'installe, les mares résiduelles vont évoluer selon quatre grandes tendances. Compte tenu des deux facteurs essentiels, nourriture et

oxygène, les peuplements en poissons présenteront une structure et une diversité que l'on peut résumer comme suit :

- scénario n° 1 : à l'étiage maximal, la superficie (plusieurs hectares) et la profondeur (1-3 mètres) de la mare demeurent importantes. Dans ce cas, toutes les espèces sont capables de survivre ;
- scénario n° 2 : à l'étiage maximal, la superficie (de l'ordre d'un hectare) et la profondeur (en moyenne de l'ordre de un mètre) de la mare sont moyennes. *A priori,* pas de problèmes majeurs d'oxygène, en revanche il est possible que la disponibilité en nourriture soit insuffisante. Les espèces à régime spécialisé (*Labeo* spp. par exemple) peuvent disparaître ;
- scénario n° 3 : à l'étiage maximal, la superficie (quelques centaines de mètres carrés) et la profondeur (quelques dizaines de centimètres) de la mare sont faibles. Ici, ne subsistent que les espèces généralistes tolérantes à une assez faible teneur en oxygène ;
- scénario n° 4 : à l'étiage maximal, la mare finit par se tarir et toutes les espèces (sauf peut-être *Protopterus*[4]) disparaissent.

35 Le scénario n° 1, qui est le plus favorable, est malheureusement le moins fréquent puisque nous n'avons recensé que cinq à six mares de ce type sur l'ensemble du cours.

36 La densité de populations humaines est quasiment nulle le long de cette rivière. De plus, comme nous l'avons déjà dit, la pêche y est théoriquement interdite. Ainsi, l'ichtyofaune est protégée et conservée durant les deux à trois mois très critiques durant lesquels elle doit survivre. On peut facilement imaginer ce que provoquerait assez rapidement une pêche intensive dans ces milieux peu nombreux, de petite taille et très fragiles.

37 Indépendamment d'autres facteurs, un cycle climatique marqué doit avoir un effet limitant vis-à-vis de la disponibilité en nourriture. Cette restriction trophique explique alors peut-être en partie que la richesse spécifique est, proportionnellement, plus faible en Afrique sahélo-soudanienne qu'en Afrique forestière ou en Amazonie. Ainsi, pour survivre les poissons sont amenés à être plus généralistes. Cette augmentation du nombre des généralistes entraîne de fait un élargissement de la niche trophique de chacun d'eux, ce qui accroît les chevauchements et en conséquence augmente la compétition. À l'inverse, une nourriture toujours disponible limite la largeur de la niche trophique et favorise la diversification du régime et donc des espèces spécialistes. À cet égard, il a déjà été montré, notamment en zone tropicale, qu'il y avait une corrélation positive entre la diversité des guildes trophiques et la pérennité des ressources alimentaires (ANGERMEIER, KARR, 1983). Le facteur trophique est certainement une des raisons ayant favorisé la spéciation et donc la richesse spécifique dans les grands lacs africains (LOWEMCCONNELL, *op. cit.*)[5]. Donc, la diversité spécifique des prédateurs peut être accrue si la taille des niches trophiques respectives est réduite (fig. 11). Dans le cas des lacs tropicaux, il existe évidemment un facteur espace, mais on peut supposer aussi une influence alimentaire puisque la quantité de nourriture est toujours plus ou moins constante. On doit donc considérer que la disponibilité en nourriture, c'est-à-dire la pérennité de la ressource, est un des facteurs cruciaux responsable de la diversité spécifique et ainsi lui associer la persistance du milieu. D'ailleurs, la pérennité de l'environnement est un des facteurs importants favorisant la spéciation et donc la diversité (WOOTTON, *op. cit.*).

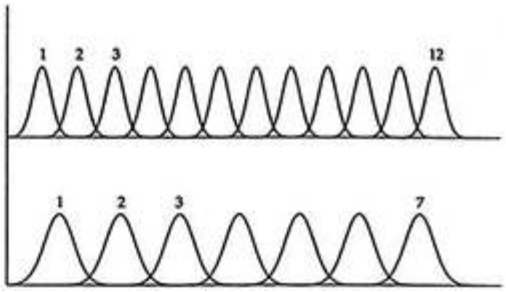

FIG. 11. — Relation entre la diversité spécifique et la dimension des niches trophiques.
Relation between specific diversity and trophic niche size.

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ANNEXES

ANNEXE

Longueur relative de l'intestin de différentes espèces africaines (hors lac Tanganyika). Données originales et bibliographiques. *Relative gut length of different fish species (except Lake Tanganyika). Original and bibliographical data.*

	Espèces	Familles	Guilde	LI/LS	Sources
1	*Acapoeta tanganicae*	Cyprinidae	Micro/macrophyt.	5,00	Matthes, 1963
2	*Alestes baremoze*	Characidae	Omnivore	0,89	Rowntree, 1903 et présente étude
3	*Amphilius atesuensis*	Amphilidae	Invertivore	0,51	*Présente étude*
4	*Auchenoglanis occidentalis*	Bagridae	Invertivore	0,88	*Présente étude*
5	*Barbus anoplus*	Cyprinidae	Omnivore	0,80	Cambray, 1983
6	*Barbus macrops*	Cyprinidae	Omnivore	0,82	*Présente étude*
7	*Barbus sacratus*	Cyprinidae	Omnivore	1,63	*Présente étude*
8	*Brienomyrus brachyistius*	Mormyridae	Invertivore	0,45	King, 1989
9	*Brycinus macrolepidotus*	Characidae	Omnivore	1,16	Rowntree, 1903 et présente étude
10	*Brycinus nurse*	Characidae	Omnivore	1,31	*Présente étude*
11	*Chelaethiops elongatus*	Cyprinidae	Zooplanctophage	0,75	Matthes, 1963
12	*Chelaethiops minutus*	Cyprinidae	Zooplanctophage	0,70	Matthes, 1963
13	*Chromidotilapia guntheri*	Cichlidae	Omnivore	3,01	*Présente étude*
14	*Chrysichthys auratus*	Bagridae	Invertivore	1,98	Nwadiaro & Okorie, 1987 et prés. ét.
15	*Citharidium ansorgii*	Citharinidae	Micro/macrophyt.	5,67	Daget, 1962a
16	*Citharinops distichodoides*	Citharinidae	Micro/macrophyt.	4,48	Daget, 1962b
17	*Citharinus citharus*	Citharinidae	Micro/macrophyt.	6,13	Daget, 1962b
18	*Citharinus congicus*	Citharinidae	Micro/macrophyt.	4,40	Daget, 1962b
19	*Citharinus eburneensis*	Citharinidae	Micro/macrophyt.	7,00	Daget, 1962b
20	*Citharinus gibbosus*	Citharinidae	Micro/macrophyt.	5,29	Daget, 1962b
21	*Citharinus latus*	Citharinidae	Micro/macrophyt.	6,78	Daget, 1962b
22	*Citharinus macrolepis*	Citharinidae	Micro/macrophyt.	4,46	Daget, 1962b
23	*Clarias anguillaris*	Clariidae	Omnivore	0,86	*Présente étude*
24	*Coptostomobarbus wittei*	Cyprinidae	Zooplanctophage	0,80	Matthes, 1963
25	*Distichodus engycephalus*	Distichodontidae	Micro/macrophyt.	4,71	*Présente étude*
26	*Distichodus niloticus*	Distichodontidae	Micro/macrophyt.	2,77	Verigh.&Med., 1968
27	*Distichodus rostratus*	Distichodontidae	Micro/macrophyt.	2,79	Verigh.&Med., 1968 et prés. étude
28	*Garra congoensis*	Cyprinidae	Micro/macrophyt.	4,50	Matthes, 1963
29	*Garra dembeensis*	Cyprinidae	Micro/macrophyt.	4,50	Matthes, 1963
30	*Haplochromis adolphifrederici*	Cichlidae	Invertivore	1,68	Ulyel, 1991
31	*Haplochromis astatodon*	Cichlidae	Micro/macrophyt.	2,84	Ulyel, 1991
32	*Haplochromis gracilor*	Cichlidae	Omnivore	1,56	Ulyel, 1991
33	*Haplochromis graueri*	Cichlidae	Invertivore	1,92	Ulyel, 1991
34	*Haplochromis kamiranzovu*	Cichlidae	Micro/macrophyt.	3,05	Ulyel, 1991
35	*Haplochromis nigroides*	Cichlidae	Omnivore	1,88	Ulyel, 1991
36	*Haplochromis olivaceus*	Cichlidae	Micro/macrophyt.	3,32	Ulyel, 1991
37	*Haplochromis paucidens*	Cichlidae	Invertivore	1,42	Ulyel, 1991
38	*Haplochromis scheffersi*	Cichlidae	Omnivore	1,72	Ulyel, 1991
39	*Hemichromis fasciatus*	Cichlidae	Ichtyophage	0,78	*Présente étude*
40	*Hemistichodus mesmaekersi*	Distichodontidae	Invertivore	0,42	Daget, 1968
41	*Hemistichodus vaillanti*	Distichodontidae	Invertivore	0,37	Daget, 1968
42	*Heterobranchus isopterus*	Clariidae	Omnivore	1,32	*Présente étude*
43	*Hydrocynus forskalii*	Characidae	Ichtyophage	0,85	Rowntree, 1903 et présente étude
44	*Hydrocynus vittatus*	Characidae	Ichtyophage	1,06	Ulyel, 1991
45	*Hyperopisus bebe*	Mormyridae	Invertivore	0,67	*Présente étude*
46	*Ichthyborus besse*	Distichodontidae	Ichtyophage	1,10	Daget, 1967
47	*Labeo horie*	Cyprinidae	Limnivore	16,75	Matthes, 1963 et Girgis, 1952
48	*Labeo lineatus*	Cyprinidae	Limnivore	16,10	Matthes, 1963
49	*Labeo niloticus*	Cyprinidae	Limnivore	16,90	Matthes, 1963
50	*Labeo parvus*	Cyprinidae	Limnivore	10,00	*Présente étude*
51	*Labeo roseopunctatus*	Cyprinidae	Limnivore	13,39	*Présente étude*
52	*Labeo senegalensis*	Cyprinidae	Limnivore	13,36	*Présente étude*
53	*Labeo variegatus*	Cyprinidae	Limnivore	17,00	Matthes, 1963
54	*Lates niloticus*	Centropomidae	Ichtyophage	0,52	*Présente étude*
55	*Leptocypris modestus*	Cyprinidae	Invertivore	0,93	Matthes, 1963
56	*Leptocypris niloticus*	Cyprinidae	Invertivore	0,52	*Présente étude*
57	*Marcusenius mento*	Mormyridae	Invertivore	0,55	*Présente étude*
58	*Marcusenius senegalensis*	Mormyridae	Invertivore	0,74	*Présente étude*
59	*Mormyrops anguilloides*	Mormyridae	Invertivore	0,46	*Présente étude*
60	*Mormyrus rume*	Mormyridae	Invertivore	0,78	*Présente étude*
61	*Nannocharax fasciatus*	Distichodontidae	Invertivore	0,43	Daget, 1961 et présente étude
62	*Nannocharax lineomaculatus*	Distichodontidae	Invertivore	0,37	Daget, 1961
63	*Nannocharax occidentalis*	Distichodontidae	Invertivore	0,34	Daget, 1961
64	*Nannocharax ansorgii*	Distichodontidae	Invertivore	0,52	Daget, 1961
65	*Nematogobius maindroni*	Gobiidae	Invertivore	0,37	*Présente étude*
66	*Opsaridium chrystyi*	Cyprinidae	Invertivore	0,73	Matthes, 1963
67	*Oreochromis mossambicus*	Cichlidae	Micro/macrophyt.	6,80	Hofer, 1988
68	*Papyrocranus afer*	Notopteridae	Omnivore	0,18	Teugels et al., 1992
69	*Parachanna obscura*	Channidae	Ichtyophage	0,55	*Présente étude*
70	*Paradistichodus dimidiatus*	Distichodontidae	Micro/macrophyt.	1,83	Daget, 1958
71	*Petrocephalus bovei*	Mormyridae	Invertivore	0,68	*Présente étude*
72	*Raiamas moorei*	Cyprinidae	Invertivore	0,73	Matthes, 1963
73	*Raiamas senegalensis*	Cyprinidae	Invertivore	0,38	*Présente étude*
74	*Raiamas steindachneri*	Cyprinidae	Invertivore	0,32	*Présente étude*
75	*Sarotherodon galilaeus*	Cichlidae	Micro/macrophyt.	6,54	Lauzanne &Iltis, 1975
76	*Schilbe mystus*	Schilbeidae	Omnivore	0,86	*Présente étude*
77	*Synodontis ocellifer*	Mochokidae	Invertivore	2,18	*Présente étude*
78	*Synodontis schall*	Mochokidae	Invertivore	1,96	*Présente étude*
79	*Tilapia zillii*	Cichlidae	Micro/macrophyt.	5,03	*Présente étude*
80	*Xenocharax spilurus*	Distichodontidae	Omnivore	1,83	Jacobshagen, 1913 et Daget, 1960

NOTES

1. Plusieurs auteurs préfèrent le terme de régularité (traduction littérale de *evenness* dû à Pielou *in* FRONTIER, PICHOD-VIALE, 1991) à celui d'équitabilité qui peut prêter à confusion à cause d'un autre paramètre de diversité nommé *equitability.*
2. Il s'agit d'espèces dont les adultes possèdent un régime ichtyophage strict.
3. Puisque le Baoulé est entièrement situé dans un parc national, l'ensemble de la faune est théoriquement protégé de la pêche et de la chasse.
4. Signalée du bassin du Sénégal, nous n'avons personnellement jamais capturé cette espèce.
5. On peut aussi citer l'exemple des *Orestias* du lac Titicaca.

RÉSUMÉS

La présente étude porte sur la structure trophique des communautés de poissons subsistant dans quelques mares résiduelles du Baoulé (haut bassin du Sénégal) au Mali.
Il est d'abord montré que les espèces limnivores et phytophages possèdent un intestin significativement plus long que les espèces des autres catégories trophiques, notamment les carnivores et les omnivores. Ce sont ces dernières espèces qui, d'un strict point de vue alimentaire, devraient s'adapter le mieux puisque peu spécialisées. On doit donc s'attendre à voir subsister dans ce type de biotopes des espèces généralistes ou opportunistes capables d'adapter leur régime aux ressources alimentaires disponibles.
De fait, en ce qui concerne le régime alimentaire des espèces subsistant dans les mares résiduelles, il ressort assez clairement que les affinités de régime alimentaire pour différentes espèces sont plus fortes à l'intérieur d'un même prélèvement qu'entre mêmes espèces d'échantillons différents. Cela signifie que pour survivre dans des conditions extrêmes, les espèces adaptent, dans certaines limites, leur régime aux ressources dominantes disponibles. On peut ainsi montrer que face à une faible variabilité de nourriture, liée également à une moindre quantité, les espèces utilisent la ressource la plus abondante, et à l'inverse lorsque les proies deviennent nombreuses, les poissons s'alimentent à partir d'un nombre de taxons plus important.

We have studied the trophic structure of the fish assemblage which remains in the residual ponds of the Baoulé river in Mali (upper Senegal basin).
There is a correlation between the diet and the gut length relative to body length: the relative gut length of limnivorous and phytophagous species is significantly longer than that of other trophic groups such as carnivores or omnivores. In terms of diet, generalists (i.e. omnivores) or species which may exhibit some trophic flexibility could have a better adaptive advantage than others. Consequently, the more opportunistic species, with respect to the available resources, are the best adapted to survive in residual ponds.
The study of the feeding habits of the fish species which remain in the residual ponds indicates that there is a higher degree of diet affinities between different species of the same sample than between the same species in different samples. Therefore to survive in severe environmental conditions, the species choose to adapt, within limits, their diet lo the dominant available resources. We demonstrated that, when the food is scarce, opportunistic fishes can feed upon the more abundant resource, and, conversely, when the food is abundant, individuals can feed upon a greater prey variety.

INDEX

Keywords : Tropical, Inland waters, Fishes, Ecology, Adaptability
Mots-clés : Tropical, Eaux continentales, Poissons, Écologie, Adaptation

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Vingt Ans de Lutte contre l'Onchocercose : Bilan Écologique

D. Paugy, K. E. Abban et Y. Fermon

Introduction :"…La maladie des villages du bout de la piste qui touche les plus pauvres des pauvres[3]…"

1 L'onchocercose[4], filariose très invalidante pour les populations et… pour l'économie des pays affectés a fait l'objet d'une très vaste campagne de lutte en Afrique de l'Ouest dès le milieu des années 1970. Sous l'égide de l'Organisation Mondiale de la Santé (OMS), le Programme de Lutte contre l'Onchocercose (OCP) fut mis en œuvre, en décembre 1974, dans le bassin des Volta, un des plus vastes foyers de la maladie. Initialement prévu pour une durée de 20 ans[5], ce programme concerne aujourd'hui 11 États, soit une superficie de plus de 1 300 000 km^2, à l'intérieur desquels 50 000 km de rivières sont susceptibles d'êtres traités et deux à trois millions de riverains d'être protégés. Au commencement de ce Programme de Lutte, le Burkina Faso, la moitié nord du Bénin, du Togo, du Ghana et de la Côte d'ivoire ainsi que le Sud Mali étaient inclus (Fig. 1). Les premiers traitements débutèrent en février 1975 dans la partie centrale de l'aire couverte par l'OCP, puis s'étendirent progressivement vers le sud, l'est et l'ouest (Hougard *et al.,* 1993 ; Samba, 1995).

Le cycle du parasite

2 Seule la femelle de *Simulium damnosum* pique l'homme. Après la fécondation, elle a, en effet, besoin d'un repas de sang pour la maturation de ses œufs. Lorsqu'elle pique une personne déjà fortement infectée, la femelle de simulie peut ingérer jusqu'à plusieurs centaines de microfilaires. La plupart sont digérées et disparaissent, mais quelques-unes peuvent traverser la paroi stomacale et gagner la cavité générale, puis les muscles thoraciques de la mouche, où elles vont se développer. Après plusieurs métamorphoses, les microfilaires se transforment en larves infectantes (six jours en moyenne) qui gagnent les pièces buccales de *S. damnosum* pour être éventuellement transmises à l'homme lors

d’un prochain repas sanguin. Le cycle parasitaire (filaire) est toujours plus long que le cycle gonotrophique (simulie) (en moyenne, respectivement 7 et 4 jours). De ce fait, le deuxième repas de sang de la mouche n’est jamais infectant.

3 La filaire d’*Onchocera volvulus,* responsable de la maladie, ne se développe que chez l’homme. Lorsqu’elle pique un individu pour prendre son repas de sang, la simulie dépose autour de la piqûre des larves infestantes (650µ de long) qui cheminent sous la peau et se développent dans le derme. Une à trois années plus tard se forme un kyste fibreux qui contient un à plusieurs couples de vers, qui, devenus adultes se reproduisent. La femelle donne naissance à des embryons, les microfilaires, de 330µ en moyenne. Chaque femelle produit annuellement 500 000 à 1 million de microfilaires. Après six mois, les larves devenues adultes, mâles et femelles, peuvent s’accoupler soit à l’intérieur de kystes fibreux (onchocercomes ou nodules), soit en restant libres dans le derme. Les vers adultes fertiles vivent en moyenne 8 à 12 ans. Ainsi, une personne fortement parasitée peut héberger 50 à 200 millions de microfilaires localisées dans le derme et dans les yeux.

La maladie

4 Les manifestations cliniques de l’onchocercose sont essentiellement dues aux microfilaires et ne s’observe qu’à la suite d’une accumulation d’infections résultant de plusieurs années d’exposition. Il existe deux types principaux de lésions. Les lésions cutanées se traduisent par une éruption érythémateuse accompagnée de violentes et persistantes démangeaisons. Il se produit une perte de fonctionnalité de la peau lorsque les lésions sont étendues. Cette répercussion fonctionelle est également le plus souvent accompagnée d’un retentissement psychologique. Les lésions oculaires, manifestation la plus grave de la maladie, résultent de l’invasion des différentes parties de l’œil par les microfilaires. En cas d’infection intense et prolongée, il se constitue des lésions permanentes. En zone d’hyper endémicité (60 % de la population est porteuse de microfilaires dermiques), la cécité peut apparaître dès l’âge de 30 ans.

Le vecteur

5 Les femelles de *Simulium damnosum* pondent dans les courants rapides de 200 à 800 œufs qui se fixent sur des supports immergés sous environ 5 cm d’eau.

6 Les œufs éclosent au bout de 36 à 48 heures. Les jeunes larves toujours fixées se nourissent en captant les particules en suspension dans l’eau grâce à leurs soies mandibulaires. La durée de vie larvaire est de 8 à 10 jours et se raccourcit lorsque la température s’élève. Au bout de cette période, la larve se mue en nymphe, toujours fixée, qui ne se nourrit plus et qui se transforme en adulte au bout de 2 à 4 jours.

7 Les simulies adultes mâles se nourissent exclusivement de sus végétaux et ne jouent donc aucun rôle dans la transmission de l’onchocercose. Les simulies femelles sont, elles, hématophages. Les prises de sang conditionnent le développement des ovaires. Au bout de 3 à 5 jours en moyenne, les œufs sont pondus et la femelle peut reprendre un nouveau repas de sang moins de 24 heures après la ponte. Chaque femelle peut ainsi accomplir jusqu’à cinq cycles de ponte (ou gonotropique) au cours de sa vie.

8 Les lieux de repos des simulies adultes sont restés longtemps inconnus. Les travaux menés au sein du programme de lutte semblent désormais indiquer qu’il n’existe pas de

lieux de rassemblement privilégiés et que les simulies occupent la totalité de la galerie forestière riveraine.

9 En dépit de leur petite taille, les simulies sont capables de voler sur une distance de 80 km en 24 heures. Avec l'aide des vents de mousson, elles peuvent même effectuer des migrations de plusieurs centaines de kilomètres, ce qui confère un pouvoir de ré-invasion très important.

10 On estime qu'il existe dans le monde une vingtaine de millions d'individus atteints d'Onchocercose, valeur très certainement sous-estimée. En Afrique, l'aire d'endémie se situe globalement entre les 15e parallèles nord et sud. À l'ouest du continent, plus précisément du Bénin au Sénégal, se trouvent des foyers à la fois graves et étendus. Cette région est le centre de la zone du Programme de lutte contre l'Onchocercose (OCP) (Fig. 1). En revanche, en Amérique du Sud et Centrale, comme en Péninsule arabique, les foyers, peu nombreux et le plus souvent de faibles dimensions, concernent seulement quelques milliers d'individus.

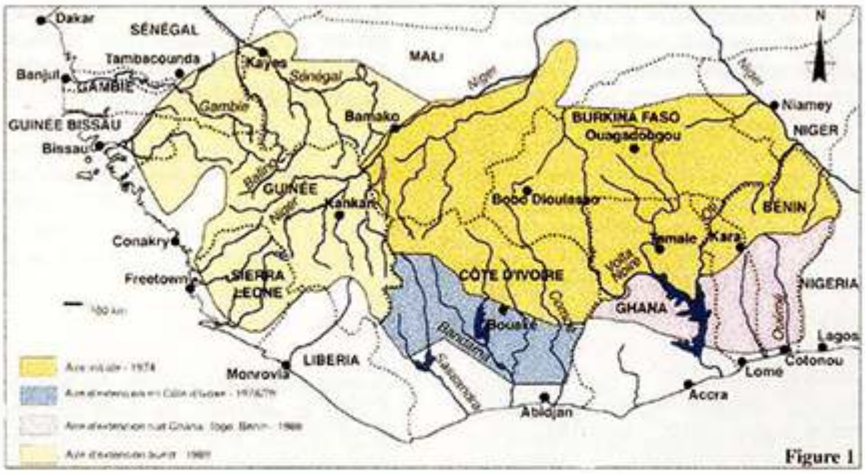

FIGURE 1. Extension maximale du Programme de Lutte contre l'Onchocercose en Afrique de l'Ouest.

LA LUTTE INSECTICIDE ET SA STRATÉGIE

11 Lors de la mise en place d'OCP, aucune thérapie de masse satisfaisante ne pouvait être aisément mise en place en raison des risques secondaires associés à l'utilisation des médicaments existants. Il était donc indispensable de recourir au contrôle des populations du vecteur, seule chance raisonnable de limiter la transmission de la maladie. En dépit de nombreuses recherches réalisées, les aires de repos des femelles adultes n'avaient, à l'époque, pas encore été clairement identifiées (voir encadré "*Vecteur*"), ce qui excluait une lutte contre les adultes d'autant que celle-ci, comme tout contrôle de ce type se serait avérée certainement très polluante pour l'environnement et assez aléatoire, les populations imaginales étant généralement très dispersées. Face aux nombreux échecs engendrés par ce type de lutte, il fut donc décidé d'utiliser des insecticides destinés à contrôler les populations larvaires qui sont aquatiques et restreintes aux rapides des rivières. Ce genre d'opération impliquait cependant de déverser, dans les rivières de la zone à traiter, des quantités très importantes d'insecticides.

La sélection des insecticides utilisés et leur utilisation respective

12 Pour lutter contre le vecteur, OCP a mis en place une programme destiné à sélectionner les insecticides. Deux critères primordiaux ont dicté le choix de la sélection. D'une part le larvicide devait être totalement efficace contre les Simulies (morbidité des larves au moins égale à 90, voire 95 %). D'autre part il ne devait pas être trop toxique à l'encontre de la faune non cible, notamment des poissons. Parmi les insecticides potentiellement candidats, jamais aucun organochloré, tel le DDT (dichloro - diphényl - trichloroéthane) pourtant efficace ou la Dieldrine, n'a été employé en campagne durant toutes ces années de lutte. Au commencement, le programme n'utilisait qu'un unique organophosphoré qui, au fur et à mesure de l'apparition de souches résistantes de simulies, dut être suppléé par divers insecticides appartenant à plusieurs familles. Le premier larvicide utilisé, le téméphos (organophosphoré), après de nombreux tests, s'avéra, et se révèle encore être le meilleur, d'une part grâce à son coût peu élevé et à son efficacité contre les simulies et d'autre part à cause de sa relative innocuité à l'égard de l'environnement. Après les premiers signes de résistance (décembre 1979 pour les cytotypes[6] de forêt et 1986 pour ceux de savane), un autre organophosphoré fut utilisé, le chlorphoxime. Si son action sur les mouches s'est avérée satisfaisante, il s'est montré, en revanche, plus toxique que le téméphos pour la faune non-cible. Étant de la même nature chimique que le téméphos, un phénomène de résistance croisée à ce pesticide se manifesta assez rapidement. Le *Bacillus thuringiensis* (*B.t.* H14), insecticide dit "biologique", fut alors également utilisé. Il est spécifique de la lutte contre les moustiques et les simulies et peu toxique pour la faune non-cible, mais il doit être employé en importante quantité, ce qui d'un point de vue logistique limite, voire interdit, son utilisation durant la crue et rend son coût très élevé. Depuis, deux autres organophosphorés (pyraclofos et phoxime), un pyréthrinoïde (perméthrine), un pseudo pyréthrinoïde (etofenprox) et un carbamate (carbosulfan) sont venus s'ajouter aux formulations initiales (Tab. 1).

Tableau 1. Quantités d'insecticides commerciaux (en milliers de litres) utilisés par le Programme de Lutte contre l'Onchocercose.

Années	Téméphos Organo-Phosphoré	Chlorphoxime Organo-Phosphoré	*B.t.* H14 insecticide "biologique"	Permethrine Pyréthrinoïde	Carbosulfan Carbamate	Pyraclofos Organo-Phosphoré	Phoxim Organo-Phosphoré	Etofenprox Pyréthrinoïde
1975	76							
1976	130							
1977	156							
1978	216							
1979	263							
1980	185	6	0.5					
1981	130	70	1.5					
1982	163	7	233					
1983	75	36	310					
1984	77	57	257					
1985	130	6	211	3	9			
1986	120	15	385	10	20			
1987	71	30	229	10	8			
1988	84	60	380	26	11			
1989	92	65	275	51	31			
1990	109	34	407	44	36	3		
1991	76		271	46	33	32	21	
1992	47		376	23	27	55	34	
1993	76		209	20	13	45	20	
1994	48		225	29	13	45	20	5
1995	41		237	15	21	38	18	17
1996	28		205	21	6	33	24	14

13 En raison de leur toxicité relativement importante pour la faune non cible, les deux derniers ne sont employés, pendant la crue, que durant quelques cycles de traitements.

14 Ainsi, l'utilisation d'insecticides de toxicité relativement forte reste proportionnellement assez limitée (Fig. 2).

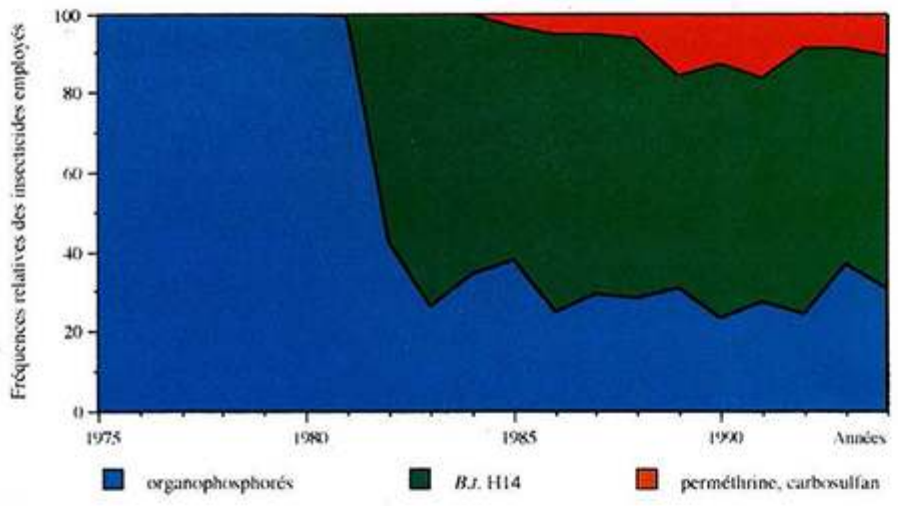

FIGURE 2. Fréquences relatives d'utilisation annuelle des différents insecticides employés lors des vingt années de traitements.

15 Actuellement, la stratégie employée par l'OCP est assez complexe (Hougard *et al.,* 1997). En premier lieu ne sont traitées que les parties de rivières où persistent des larves de simulies, en second lieu, les tactiques employées, en fonction des débits, sont multiples, variées et sont "servies à la carte" pour être à la fois les plus efficaces, les moins onéreuses et les moins menaçantes pour l'environnement.

La résistance du vecteur

16 La résistance physiologique est la faculté, pour les populations d'insectes, de tolérer des doses d'insecticides, qui, en temps ordinaire, seraient létales pour une communauté normale. Assez commun, ce type d'adaptation est survenu chez les simulies du complexe *damnosum.* Les premiers signes de résistance au téméphos sont apparus chez les populations de forêt après 60 semaines seulement de traitements (octobre 1978 à décembre 1979). Après à peine une année (43 semaines) d'utilisation du chlophoxime (un autre organophosphoré = OP), ces mêmes populations se montraient également résistantes. En général, une résistance à un pesticide entraîne le plus souvent une résistance croisée à la plupart des formulations de cette famille. Pour y remédier, le moyen le plus efficace, et certainement le plus simple, est d'utiliser un autre groupe d'insecticide ne présentant pas de parenté chimique avec le précédent. C'est une des raisons pour laquelle, l'OCP a cherché puis sélectionné plusieurs formulations pour les utiliser en alternance afin de garantir un optimum d'efficacité tout en échappant autant que possible à une résistance importante, voire irrémédiable.

LA SURVEILLANCE DE L'ENVIRONNEMENT AQUATIQUE

Historique, protocoles et méthodes

17 Du fait de la durée des traitements prévue par le Programme, les États participants, tout comme les pays donateurs, pouvaient craindre que des épandages répétés d'insecticides sur la zone contribuent à gravement endommager les écosystèmes aquatiques. Le programme se dota donc d'un organe consultatif, le Groupe Écologique[7], dont la mission principale est de s'assurer que la lutte anti-vectorielle ne constitue pas une menace importante pour l'environnement. Pour évaluer l'impact éventuel des traitements, l'OCP a, parallèlement aux traitements, mis en place un réseau de surveillance généralement constitué de chercheurs nationaux chargés de contrôler la qualité biotique des écosystèmes aquatiques. À l'époque de son lancement, ce programme de lutte anti-vectorielle a certainement été un des premiers de cette envergure à se soucier de l'impact, à long terme, des pesticides déversés dans le milieu. Pour évaluer l'ampleur de cet éventuel péril environnemental, des hydrobiologistes-écologistes ont effectué des prélèvements spécifiques suivant des méthodes et un protocole précis (Lévêque *et al.*, 1979). Ceux-ci furent définis afin de pouvoir être utilisés par l'ensemble des équipes concernées durant de nombreuses années sur une zone très étendue.

- pour les poissons : l'échantillonnage se pratique à l'aide de filets maillants de différentes tailles de mailles. On connaît la sélectivité particulière de ces engins de pêche, mais la pratique a montré qu'ils étaient les seuls à pouvoir être utilisés sans difficulté, par l'ensemble des équipes, sur l'aire entière d'OCP. Deux paramètres sont pris en compte :
  - les prises par unité d'effort destinées à refléter, grâce aux captures, la structure et le degré d'équilibre des peuplements ;
  - parallèlement, chaque poisson est pesé, mesuré et disséqué afin que soient estimée sa condition et analysé son contenu stomacal, reflet de la nourriture disponible. Son sexe, son état de maturité sexuelle sont également notés afin de contrôler l'action du pesticide sur la physiologie des individus, les plus jeunes étant, par ailleurs, généralement les plus sensibles.
- pour les invertébrés :
  - l'échantillonneur de Surber permet à un instant donné de connaître la densité de la faune saxicole (saxicole : qui vit sur les rochers) en place ;
  - à côté de cette méthode quantitative, l'utilisation de filets à dérive permet de faire un inventaire faunistique des invertébrés filant au gré du courant selon un rythme naturel nocturne (généralement trophique) ou accidentel, voire traumatique, diurne.

18 Les résultats énoncés ont été recueillis par les chercheurs de IRD (ex Orstom), les hydrobiologistes d'OCP et les équipes des laboratoires nationaux de certains pays participants qui bénéficient, pour cette surveillance, d'un soutien financier de l'OCP.

Toxicité aiguë (effets à court terme) : invertébrés et poissons

19 Comme nous l'avons déjà signalé, il existe deux critères qui justifient l'acceptation et-ou le rejet d'un insecticide. Si l'efficacité contre les larves de *S. damnosum s. lat.*, est bonne et qu'il ne provoque aucune mortalité de poissons ou de macrocrustacés, il subit un second

criblage qui consiste à tester sa nocivité à l'égard de la faune invertébrée. Les tests sont réalisés expérimentalement en gouttière (essentiellement sur les larves aquatiques d'insectes) (Fig. 3), ce qui permet d'avoir pour chaque groupe le pourcentage de dérive (donc de décrochement) et de survie.

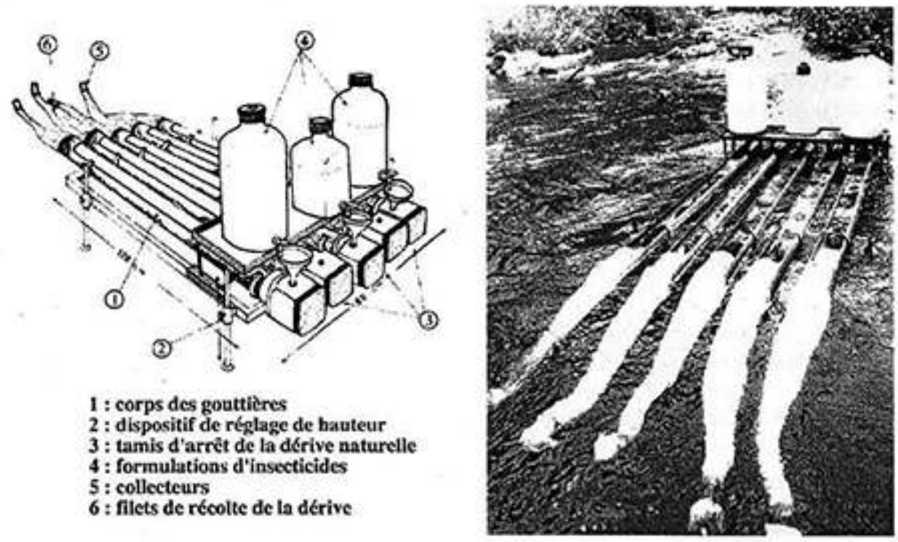

FIGURE 3. Dispositif de gouttières pour tester la toxicité, à court terme, des formulations d'insecticides sur les larves d'insectes aquatiques non-cibles (d'après Troubat, 1981).

20 En fonction des résultats obtenus, il est possible de classer les différents pesticides employés selon leur toxicité, qui dépend évidemment de nombreux facteurs biotiques et abiotiques. Quoi qu'il en soit, parmi les insecticides utilisés en campagne, on peut considérer que le plus sélectif, donc le moins toxique, est le *B.t.* H14, puis viennent ensuite le téméphos, le pyraclofos, le phoxime, le chlorphoxime, la perméthrine et enfin le carbosulfan (Yaméogo, 1994). En règle générale, on note un impact plus fort des insecticides sur les organismes lors du premier traitement. Par ailleurs, l'action des différents insecticides employés est variable selon les familles d'insectes.

21 Sur les poissons, aucun des insecticides employés pendant les vingt années de traitement ne s'est montré mortel aux concentrations employées. D'ailleurs, les individus sont le plus généralement exposés durant très peu de temps, car dès qu'ils ressentent l'arrivée du polluant, ils remontent la vague pour la traverser au plus vite. En condition expérimentale, c'est-à-dire en maintenant les poissons en eau contaminée (aquariums par exemple), on note une nette baisse de leur activité acétylcholinestérasique cérébrale (réaction chimique basale indispensable à la transmission de l'influx nerveux) après exposition aux organophosphorés (Gras *et al.,* 1982). Toutefois, aux doses employées lors des traitements, les poissons récupèrent rapidement. Si après un épandage, on pêche dans le milieu des poissons qui ont subi une vague insecticide, on remarque alors, qu'ils ne présentent aucune diminution d'activité cérébrale, ce qui prouve que l'insecticide agit peu, soit parce que les poissons le fuient, soit parce que le polluant se trouve assez rapidement dilué dans le milieu. En ce qui concerne l'accumulation dans les tissus, on a pu montrer qu'elle pouvait exister pour le téméphos mais dans des proportions très faibles et de toute façon moins importantes qu'en conditions expérimentales (Quélennec *et al.,* 1977).

22 Une question majeure demeure cependant ; est-il possible de prédire l'impact à long terme qu'auront les insecticides en se basant sur les résultats recueillis à partir d'expériences effectuées à court, voire moyen terme ? En effet, s'il était possible de dégager une telle relation, nombre de programmes pourraient, dans une certaine mesure, faire l'économie d'une lourde, parfois fastidieuse, et toujours dispendieuse surveillance à long terme. Dans ce sens, un indice a été élaboré (Elouard & Simier, 1990) pour donner une idée du niveau de modifications structurales des communautés d'invertébrés benthiques qu'engendrerait un insecticide particulier sur la faune des rochers. La relation entre cet indice et la toxicité moyenne des insecticides testés en gouttière est très hautement significative. On peut donc, à partir de résultats de tests obtenus en gouttière, déterminer le degré de perturbation que peut théoriquement engendrer un insecticide à moyen terme. Malheureusement, on ne peut généraliser ce modèle au sort qui sera réservé à long terme à chacune des composantes de la communauté, car la prédiction peut s'avérer parfois médiocre pour certains taxons. De même, ce type de test ne permet pas de prévoir l'impact produit par l'utilisation alternée de larvicides de natures différentes. Les connaissances demeurent donc encore trop imparfaites et tant que le problème ne sera pas résolu, la surveillance à long terme demeurera une activité incontournable.

Toxicité chronique (effets à moyen et long terme) : invertébrés et poissons. Le bilan de plus de vingt années d'épandages sur les communautés non-cibles

23 En phase de traitement à grande échelle, il devient évidemment difficile, notamment pour les invertébrés, de se faire une idée de l'impact réel, car on ne sait quelle fraction des populations est effectivement touchée, voire éliminée. C'est pour cette raison, et dans le cas d'une toxicité aiguë acceptable, que l'on est amené à effectuer une surveillance à long terme destinée à évaluer la toxicité chronique. Cela est aussi vrai pour les insectes non-cibles que pour les poissons. En fait, il s'agit de vérifier que, d'une part, la structure et la composition des communautés sont peu ou pas affectées et, d'autre part, pour les insectes non-cible, que la "densité" des organismes n'est pas véritablement menacée. En ce qui concerne les poissons, il faut également vérifier que leur physiologie, embonpoint (condition), croissance et reproduction, n'ont pas été modifiés. Enfin, certaines études complémentaires ont été nécessaires pour évaluer les rôles respectifs du pesticide et de l'environnement naturel.

24 Cette démarche ne consiste plus à vérifier l'impact immédiat des pesticides, mais à contrôler à long terme la réaction des communautés régulièrement exposées à des insecticides de diverses natures. Ce programme de surveillance de l'environnement se déroulant sur une vaste échelle, il est nécessaire, pour comparer les résultats dans des régions parfois assez différentes, d'adopter un protocole de référence reproductible.

Surveillance de l'entomofaune

25 Après de nombreuses années de traitements, puis l'arrêt de ceux-ci, l'état des populations d'insectes aquatiques non-cibles est la résultante, à la fois de leur résilience, mais aussi de la résistance qu'ont pu montrer certains taxons aux expositions répétées d'insecticides.

26 À l'état naturel, les communautés d'invertébrés aquatiques de la zone sont essentiellement composées de "collecteurs moissonneurs" et de "collecteurs filtreurs" (Crosa, 1996). Les autres groupes, "prédateurs", "gratteurs" et "racleurs", sont peu abondants. Après les premiers traitements, les situations peuvent changer selon les stations. Quoi qu'il en soit, après 20 années d'épandages insecticides, la tendance générale montre que les populations de "collecteurs" sont toujours affectées par les traitements larvicides, mais que le stress est différent en fonction des insecticides employés. Deux d'entre eux se montrent particulièrement peu toxiques, le téméphos et le *B.t.* H14. Tous les autres révèlent un impact plus important. En se basant sur les effets constatés sur les communautés (critères taxinomiques et fonctionnels), les différents insecticides employés ont pu être classés, de façon croissante, selon leur degré de toxicité : téméphos, *B.t.* H14, chlorphoxime, perméthrine, pyraclofos et phoxime (Crosa, 1996). Ce résultat est légèrement différent de ce que nous avions annoncé au préalable en ne tenant compte que de la toxicité aiguë. Cette échelle de toxicité doit cependant être considérée avec une extrême précaution, car la nocivité des différents produits utilisés varie selon les taxons exposés. Par exemple, le phoxime semble avoir eu un fort impact sur les communautés benthiques du Niandan (haut Niger en Guinée) et curieusement assez peu d'effets sur celles de la Pru (haut bassin de la Volta au Ghana). Mais, évidemment, les compositions faunistiques n'étaient pas les mêmes au départ.

27 Après arrêt des traitements insecticides, il se produit généralement un retour vers une structure communautaire sensiblement identique à celle que l'on observe avant les premiers épandages. Cette tendance n'apparaît cependant pas immédiatement après la suspension et le retour à une situation jugée "normale" dépend, dans une large mesure, de la durée des traitements subis par les communautés en place et de la nature des larvicides utilisés. À cet égard, il semble que des groupes sont plus sensibles que d'autres, notamment certains Éphéméroptères (Tricorythidae, Leptoceridae et Baetidae). Ce sont ces trois familles, plus, parfois, celle des Chironomini qui contribuent largement, par leur régression, à modifier la structure initiale des peuplements en place.

28 La conclusion générale rassurante du suivi de 20 années de surveillance écologique de la faune invertébrée non-cible est qu'il n'y a jamais eu d'effets drastiques du fait des larvicides employés même lorsque les peuplements ont subi des cycles de traitements longs et-ou d'assez forte toxicité. Il faut, à cet égard, tenir compte de l'attention particulière qui a été accordée, par les instances opérationnelles d'OCP, aux recommandations du Groupe Écologique, même si ce suivi scrupuleux a pu, parfois, limiter l'efficacité de la lutte proprement dite contre les simulies. Dans un souci, certainement d'économie, mais aussi d'un point de vue éthique, vis-à-vis de l'environnement, OCP limite ses épandages d'insecticides aux seuls gîtes productifs en larves de *S. damnosum.* De ce fait, par rapport aux milliers de kilomètres de rivières qui auraient pu théoriquement être traitées, beaucoup ne le furent que rarement, voire parfois jamais. Ainsi subsistent de vastes zones refuges qui permettent en cas d'arrêt des traitements une recolonisation des biefs par la faune entomique non-cible. C'est certainement une des clés de la recolonisation de certains lieux devenus faunistiquement très pauvres, et qui ont retrouvé, à la fin des traitements, leur diversité et leur richesse initiale grâce au réservoir potentiel constitué par les zones refuges.

Surveillance de l'ichtyofaune

29 La plupart des espèces considérées dans cette étude atteignent leur maturité sexuelle au bout de leur première année et ne vivent généralement guère au-delà de quatre ou cinq années (Albaret, 1982 ; Mérona, 1983).

30 En ce qui concerne les variations à long terme, pour l'ensemble des sites étudiés durant 20 années (Paugy *et al.,* 1999), l'analyse des captures totales ou regroupées par catégorie trophique montre les mêmes tendances. Cela indique, entre autres, qu'aucun groupe trophique, particulièrement les insectivores pour lesquels le principal aliment pourrait être réduit par les traitements, ne semble avoir été affecté durant la période concernée. Ce constat est meilleur que celui qui a pu être observé dans d'autres régions. Ainsi, l'utilisation du DDT pour contrôler les populations de simulies dans le Nil Victoria (Ouganda), a induit un changement important des disponibilités alimentaires pour certaines espèces comme les Mormyridae ou un manque drastique de nourriture pour une autre espèce comme *Aethiomastacembelus frenatus* (Corbet, 1958a et b).

31 La stabilité relative du coefficient de condition pour chaque espèce (Fig. 4) indique également que leur alimentation n'a pas été particulièrement affectée. Deux hypothèses peuvent être proposées. Soit les ressources alimentaires sont toujours disponibles, soit les poissons sont capables de changer leur régime comme cela a été démontrée pour d'autres rivières non traitées de la région (Paugy, 1994). Quelle que soit l'explication, il apparaît donc que les peuplements de poissons ne sont pas affectés, tant biologiquement que physiologiquement, par les larvicides employés (Antwy, 1985). Cela montre donc aussi que les insecticides qui sont utilisés n'ont pas de toxicité détectable sur le métabolisme des espèces exposées (Quelennec *et al.,* 1977).

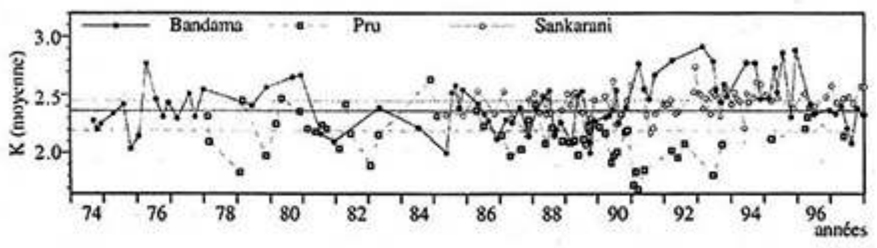

FIGURE 4. *Brycinus nurse* (Characidae) : évolution du coefficient de condition (K) dans différentes stations de la zone étudiée.

32 En considérant les différentes variables analysées dans cette étude, nous ne trouvons aucun effet détectable des pesticides sur le prises par unité d'effort (PUE), l'abondance des espèces, la structure des communautés, l'organisation trophique et la santé des poissons (facteur de condition et stratégies vitales). Cependant, on remarque qu'il existe une étroite relation entre la qualité ou la quantité des captures et le régime hydrologique des rivières (Fig. 5).

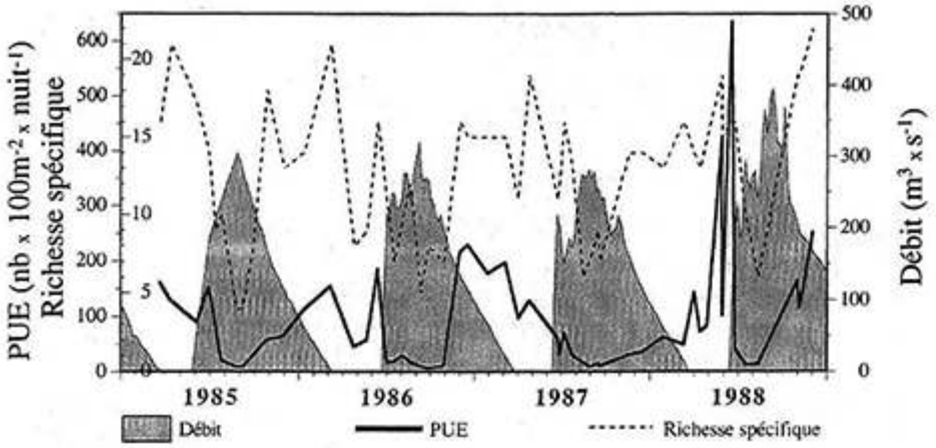

FIGURE 5. Baoulé à Missira (Mali) : influence de la crue sur les PUE (nb x 100 m^{-2} x nuit^{-1}) et la richesse spécifique.

33 En ce qui concerne les variations à long terme, on note une diminution régulière des PUE du début des traitements jusqu'à 1995. Selon les stations, les rivières ont été traitées jusqu'à 1990 ou 1993 (Fig. 6). Pourtant, la diminution des PUE a généralement persisté après l'arrêt des traitements. Ainsi, nous devons considérer que des facteurs, autres que les insecticides, ont été la cause de ce processus. Dans la zone qui nous intéresse, le débit annuel moyen des rivières a régulièrement diminué au début des années 70 (Fig. 7). Une étude récente (Hugueny, com. pers.) a par ailleurs montré qu'il existait une étroite corrélation entre la valeur des débits et les PUE durant les dix premières années de traitement (avec une année de décalage). On sait que la production de poisson fluctue dans tous les fleuves tropicaux selon le niveau de l'inondation. Lorsqu'elle est importante, elle noie des superficies plus grandes, fournissant ainsi de plus grandes quantités de nourriture disponible, tout en améliorant également les conditions de reproduction (Welcomme, 1979). Ainsi, dans le delta central du Niger (Mali), les chutes de prises annuelles et la faiblesse des crues sont hautement corrélées. Le facteur déterminant de l'abondance des stocks de poissons est donc dépendant de l'étendue et de la durée de l'inondation. En fait, 69 % des poissons de moins d'une année sont attrapés dans des filets de mailles égales ou inférieures à 20 mm (Laë, 1992). Ces observations sont en total accord avec les nôtres. Dans nos prises, l'effet observé n'est pas immédiatement visible, mais apparaît quelques années plus tard comme un effet cumulatif de mauvaises conditions hydrologiques. Inversement, l'accroissement des PUE depuis 1996 est en rapport avec l'amélioration des crues (Service Hydrologique de l'IRD, données Internet). Qui plus est, durant ces dernières années, il s'est produit une intensification de l'écoulement conduisant à un renouvellement régulier d'eau et à un rechargement de la nappe phréatique, même si ce phénomène demeure encore assez faible lorsque l'on considère le bassin dans son entier (Sircoulon, com. pers.).

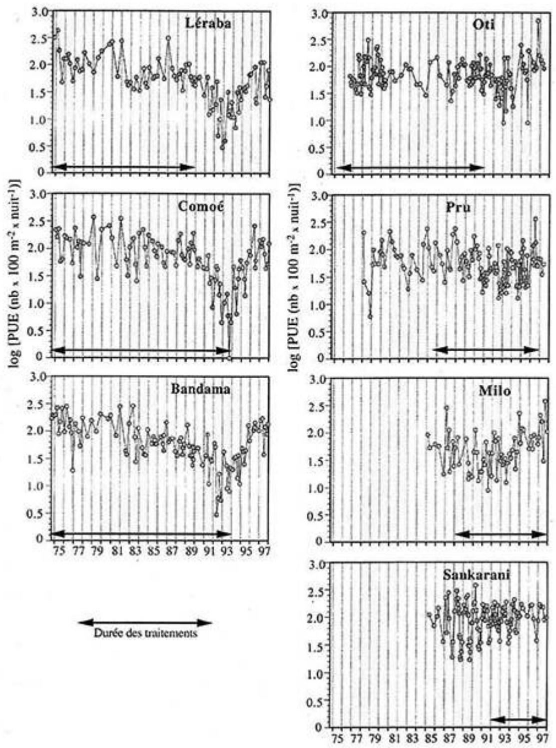

FIGURE 6. Variations des PUE (nb x 100 m^{-2} x $nuit^{-1}$) dans sept stations échantillonnées aux filets maillants.

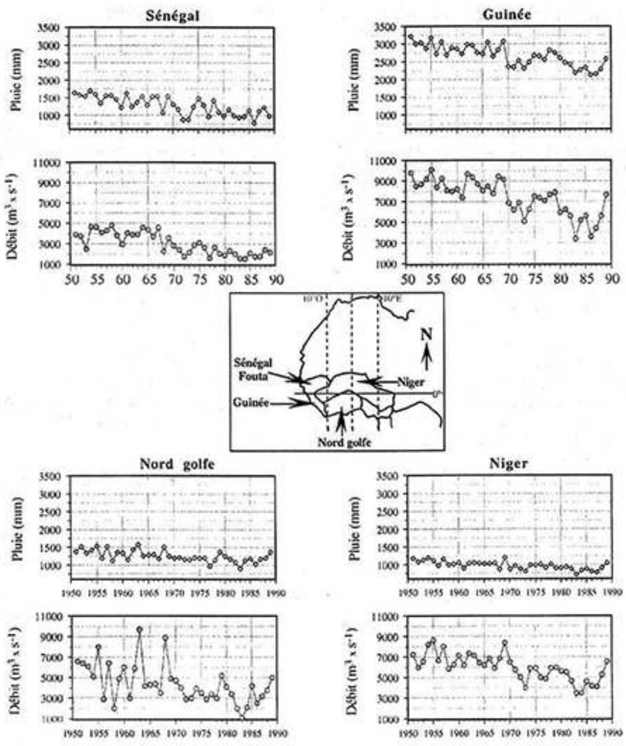

FIGURE 7. Moyenne annuelle des débits (m^3 x s^{-1}) et des pluviométrie (mm) dans quatre régions d'Afrique occidentale (d'après Mahé, 1993).

34 Parallèlement aux facteurs naturels, c'est-à-dire climatiques, certaines interventions humaines, autres que les insecticides, peuvent perturber les peuplements de poissons.

Ainsi, pour trois des stations étudiées, il semble que la construction de barrages induise des effets différents et/ou antagonistes. Dans certains cas, en dépit de mauvaises conditions hydrologiques, quelques espèces semblent favorisées par la présence du barrage. Par exemple, dans le Sankarani juste en amont du lac de Selingué, on observe une recrudescence de l'espèce pélagique *Parailia pellucida.* Parallèlement, le facteur de condition de prédateurs comme *Hydrocynus spp.* s'accroît notablement. De la même façon, il semble que le lac Volta agisse comme un "tampon" pour certains cours d'eau ghanéens sous l'influence d'une pluviométrie déficitaire. Mais, inversement, le barrage d'une rivière a généralement un effet négatif sur les espèces migratrices et particulièrement sur leur coefficient de condition comme on a pu le constater chez *Alestes baremoze* et *Schilbe mandibularis* (Paugy *et al.,* 1999).

35 Quand on compare les résultats actuels avec ceux obtenus après les dix premières années de surveillance (Lévêque *et al,* 1988), nous pouvons conclure que les pesticides épandus par l'OCP n'influencent pas la structure des communautés de poissons, la richesse spécifique et la biologie des principales espèces. Enfin, nous notons qu'aucune espèce de poisson n'a disparu à ce jour.

36 Une surveillance à long terme nécessite de prendre un certain nombre de précautions avant d'analyser et d'interpréter les résultats. Ainsi, il doit être tenu compte, en premier lieu, des changements climatiques qui jouent un rôle déterminant sur la dynamique des populations de poissons. De même, dans ce type de démarche à long terme, il est indispensable d'identifier les fluctuations naturelles des espèces pour en faire abstraction. En ne prenant en compte que les variations à court terme, nous pourrions ainsi être conduits à faire de fausses conclusions, en constatant les disparitions ou diminutions naturelles d'effectifs, souvent de faible durée, que subissent certaines espèces (*Schilbe intermedius* par exemple).

L'impact écologique de la recolonisation villageoise

37 L'objectif du programme de lutte se révèle aujourd'hui un total succès puisque les vallées fertiles initialement désertées se sont rapidement repeuplées, ce qui est assurément une réussite en termes d'essor sanitaire et économique. L'observation, dans le cours supérieur de la Léraba, de cartes d'occupation des sols entre les années 1972, soit avant le début de la campagne de lutte, et 1993, soit près de 20 années après les premiers traitements est très révélatrice (Fig. 8). En 1972, la majorité des communautés agricoles était confinée dans des villages situés à 10 km au moins de la rivière. Il faut en conclure que soit par peur de contracter l'Onchocercose, mais aussi la trypanosomiase, soit du fait de la nuisance induite par les simulies ou les glossines, les paysans préféraient exploiter leurs terres depuis des villages situés loin des rivières. En 1993, la situation montre qu'il y a eu en vingt ans une très nette expansion vers les cours d'eau de la zone de fermes et de petites exploitations. On constate également que le nombre de villages a passablement augmenté puisque, dans la zone d'étude, il est passé de trois à douze.

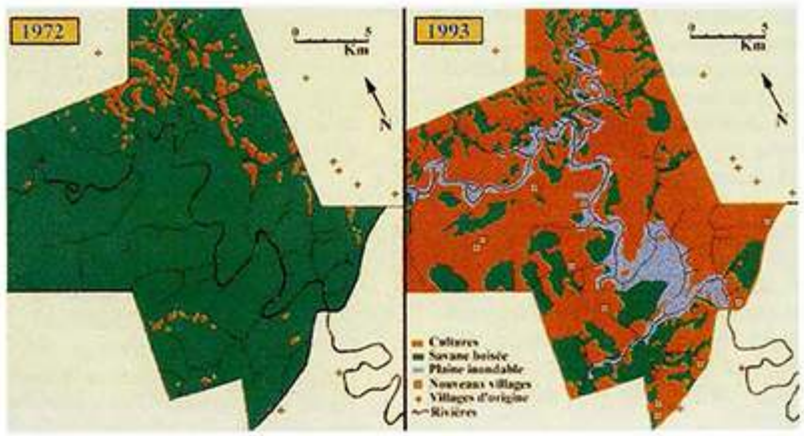

FIGURE 8. Cartes de l'occupation des sols indiquant les zones cultivées en 1972 puis 1993. Bief du cours supérieur de la Léraba au niveau de la frontière Côte d'Ivoire - Burkina Faso (redessinées d'après Baldry *et al.,* 1995).

38 Comme souvent, il convient de se demander si chaque succès n'a pas non plus son revers et si le rapide repeuplement humain des zones libérées de l'Onchocercose ne risque pas d'avoir des conséquences néfastes. Ainsi, l'augmentation progressive de la demande de nouvelles terres (agriculture, bois de construction et de chauffe, pâturages...) a naturellement entraîné d'importants changements écologiques au cours de ces vingt dernières années. On estime qu'environ 75 % de la savane boisée d'origine a été plus ou moins détruite et tout porte à penser que d'ici peu, les ressources sylvicoles seront totalement épuisées.

39 À l'époque où a été réalisée cette étude pilote, les charges organiques et les éléments nutritifs présents dans le cours supérieur de la Léraba n'étaient importants que sur des biefs limités des bras principaux (Fig.8). Là, les installations villageoises, situées près des berges, sont des sources de contamination de l'eau, mais leurs effets demeurent assez restreints, car localisés. H n'en demeure pas moins vrai que des effets locaux existent comme le montre une comparaison entre la faune observée en trois stations du même bassin, dont l'une est située près d'un village, l'autre une dizaine de kilomètres en aval et la dernière loin de toute habitation. Pour l'ensemble des paramètres, il ressort que la station à l'écart de toutes activités humaines est plus riche en poissons et en invertébrés. De plus, le fait que la station située en aval du village soit moins perturbée que celle placée à proximité immédiate est également révélateur et prouve, pour le moins, qu'une activité humaine domestique, même peu polluante dans le cas présent, est un élément perturbateur important susceptible de troubler l'écosystème environnant. De même, quelques indices récents donnent à penser que certains facteurs, autres que les épandages d'insecticides, sont susceptibles de perturber l'hydrosystème. Les sources de ces perturbations sont de deux ordres principaux :

- d'une part une dégradation chimique liée à des contaminations diverses (populations humaines, cheptel animal, engrais et pesticides d'usage agricole) ;

- et d'autre part une détérioration physique consécutive à l'utilisation du territoire (déforestation, cultures, appauvrissement et lessivage des sols...).

40 La mise en péril des forêts riveraines apparaît ainsi un danger majeur puisque celles-ci jouent un rôle important dans le maintien de la productivité des grands fleuves. On peut en résumer les principales fonctions régulatrices :

- La hauteur et la densité des arbres limite l'impact direct de la radiation solaire, ce qui présente quatre effets bénéfiques :
  - maintien de l'eau de la rivière à une température optimale pour la faune ;
  - maintien d'un niveau approprié d'oxygène dissous dans l'eau ;
  - réduction du taux d'évaporation ;
  - maintien de la température et de l'humidité optimales dans le sous-étage boisé.
- La chute des débris organiques et autres matériels biologiques fournissent une manne organique dont dépendent plusieurs espèces de poissons et d'invertébrés aquatiques.
- Les réseaux radiculaires souterrains ont deux fonctions principales :
  - stabilisation des berges, empêchant leur érosion et protégeant les bassins versants contigus ;
  - filtration des eaux de pluie et retenue de nombreux composés organiques et inorganiques.

Le bilan et les conséquences

41 Avant 1975, dans la région d'Afrique de l'Ouest actuellement couverte par OCP, il était courant de trouver des villages et vallées fertiles abandonnées. Après vingt années d'opérations de lutte que pouvons-nous conclure ? D'un point de vue sanitaire, OCP est une réussite complète, puisque trente millions de personnes sont dorénavant protégées de l'Onchocercose. Neuf millions d'enfants[8] nés, depuis 1974, dans l'aire initiale du Programme n'ont pas été infectés et ne sont plus exposés au risque de cécité onchocerquienne. Sur le plan socioéconomique, ce programme de lutte est également un succès puisqu'une partie importante des vallées autrefois abandonnées est, ou est en voie d'être recolonisée. D'un point de vue écologique, il faut distinguer deux choses. L'une est l'impact des insecticides sur la faune aquatique, l'autre est le rôle que peut jouer la recolonisation humaine sur l'hydrosystème.

42 Nous avons vu qu'au moins deux des insecticides employés se montrent relativement toxiques, principalement à l'égard de la faune invertébrée. L'analyse des données montre que certains taxons sont atteints plus que d'autres, mais elle souligne également l'avantage que tirent certains taxons peu sensibles à un insecticide donné, car en l'absence de concurrence ils sont en fait favorisés et se multiplient. Si certains gîtes furent très atteints par les traitements, la majorité d'entre eux ne le fut jamais de façon irréversible, ce qui est acceptable d'un point de vue écologique par rapport au succès sanitaire acquis. Une disparition définitive de la faune eût été catastrophique d'autant qu'elle eût touché les larves d'espèces prédatrices de simulies. En effet, à l'issue des traitements, la disparition de ces prédateurs peut entraîner une multiplication des simulies, le cycle de ces dernières étant généralement beaucoup plus rapide, d'où un déséquilibre écologique pouvant devenir inacceptable.

43 Aucune mortalité massive de poissons, due aux insecticides employés par OCP, n'a été mise en évidence, pas plus qu'une altération tant qualitative que quantitative des communautés ichtyologiques. Des modifications physiologiques, perte de condition et absence de maturation des gonades, ont parfois été constatées chez certaines espèces

durant certaines années de sécheresse. Mais ayant affecté aussi bien les populations des rivières soumises aux épandages que celles des cours d'eau non traités, il faut attribuer cet impact aux très mauvaises conditions naturelles (sècheresse notamment), plutôt qu'à l'insecticide (Lévêque *et al.,* 1988). Si la raréfaction ou la disparition momentanée d'espèces d'insectes proies a parfois entraîné une modification dans la composition du bol alimentaire des poissons insectivores, cela n'a pas eu d'effet sur leur état physiologique. H paraît clair que dans certaines limites de densité et de taille des proies, chaque espèce de poisson se nourrit à partir de ce qu'elle trouve en plus grande abondance dans le milieu.

44 En ce qui concerne la recolonisation des vallées fertiles, il semble, pour l'heure, qu'il y ait eu très peu de développement agricole dans les plaines d'inondation. De ce strict point de vue, l'incidence sur l'environnement est encore quasiment nulle, mais une surveillance particulière doit néanmoins être apportée à ce potentiel exploitable.

45 En ce qui concerne le rôle formateur tenu par OCP pour les différentes équipes en place, on peut considérer qu'il a été très positif, puisque pour l'ensemble des pays concernés de nombreux chercheurs et techniciens locaux ont été formés, notamment par les chercheurs de l'IRD (ex Orstom). Un bilan des travaux effectués, en hydrobiologie dans ce cadre, a permis de recenser 212 publications ou rapports de 1974 à 1987 (Paugy & Elouard, 1989). Notons également, pour ce qui touche au développement des connaissances, l'élaboration d'atlas, de faunes entomologiques et ichtyologiques. Ce programme de surveillance de l'environnement aquatique est semble-t-il l'exemple d'une finalité où la recherche fondamentale est mise, avec succès, au service de la coopération et du développement.

46 L'expérience de vingt années de surveillance de l'environnement aquatique dans la zone d'OCP a permis de parfaire la formation de jeunes chercheurs locaux qui avec l'expérience acquise pourront désormais conduire de leur propre initiative d'autres opérations de ce type. Par ailleurs, cette expérience unique permet, dorénavant, aux chercheurs qui ont été impliqués dans cette surveillance d'afficher une bonne expertise dans le domaine des études d'impact dont font l'objet les milieux aquatiques.

47 Enfin, rappelons ici l'énorme savoir acquis sur la base des différentes opérations conduites dans le cadre de ce programme et OCP peut se considérer comme l'élément moteur de base qui a conduit aux récents progrès obtenus en entomologie aquatique et en ichtyologie ouest africaine (faunistique, biologie et écologie).

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NOTES

3. D'après René Le Berre, un des pères fondateurs du programme OCP.
4. voir "Cycle du parasite", "Maladie" et "Vecteur"
5. On pensait alors que la longévité de la femelle adulte d'*O. volvulus* était d'environ 15 à 20 ans. Des travaux récents laissent supposer qu'elle serait en réalité de 8 à 12 ans.
6. Les parasites sont transmis d'un sujet à l'autre par de petites mouches, les simulies, qui appartiennent en Afrique de l'Ouest au complexe d'espèces *Simulium damnosum.* À l'heure actuelle, neuf espèces du complexe *S. damnosum,* toutes vectrices de l'Onchocercose humaine, ont été décrites dans l'aire d'OCP. Six d'entre elles sont cataloguées comme des espèces de forêt (*S. squamosum, S. yahense, S. sanctipauli, S. soubrense, S. leonense* et *S. konkourense*) tandis que les trois autres sont des espèces de savane (*S. damnosum* s. s., *S. sirbanum* et *S. dieguerense*).
7. Instance indépendante composée de cinq membres (initialement nommée Ecological Panel).
8. Les estimations pour l'an 2000 avancent le chiffre de 15 millions d'enfants protégés.

RÉSUMÉS

Maladie largement répandue en Afrique intertropicale l'onchocercose est un fléau, tant social qu'économique, qui provoque, à son stade ultime, une cécité irréversible. Avant l'instauration des premières mesures de lutte anti-vectorielle, on estimait à près de trois millions le nombre d'onchocerquiens en Afrique occidentale. La maladie est transmise par un petit moucheron Diptère, *Simulium damnosum,* qui présente une phase larvaire et nymphale aquatique. Ce sont les gîtes larvaires de ce vecteur, biefs à courant rapide des rivières, que le Programme de Lutte contre l'Onchocercose en Afrique de l'Ouest (OCP : Onchocerciasis Control Programme) traite lors ses campagnes de lutte. Comme toute lutte insecticide OCP représente une menace importante pour l'environnement. C'est pourquoi le programme s'est doté d'un réseau de surveillance des écosystèmes aquatiques, couvrant l'ensemble de la zone exposée aux épandages d'insecticides. Au total, près de dix équipes de chercheurs ichtyologues et entomologistes, de nationalités diverses, ont durant vingt ans effectué une surveillance régulière de la faune aquatique susceptible d'être atteinte par les traitements larvicides. C'est la première fois au monde qu'une telle entreprise a été mise en place sur une si grande échelle d'espace et de temps. Sont exposées ici les conclusions du bilan écologique.

À l'état naturel, les communautés d'invertébrés aquatiques de la zone sont essentiellement composées de “collecteurs moissonneurs” et de “collecteurs filtreurs”. Après 20 années d'épandages insecticides, la tendance générale montre que les populations de “collecteurs” sont toujours affectées par les traitements larvicides, mais que le stress est différent en fonction des insecticides employés. Après arrêt des traitements insecticides, il y a de façon générale un retour à une structure communautaire sensiblement identique à celle observée avant les premiers épandages.

L'ensemble des paramètres étudiés, qu'ils concernent la richesse et la structure des communautés de poissons ou la biologie des espèces ne montrent pas d'effets décelables de vingt années d'épandages dans les rivières traitées. En revanche, les variations observées montrent que des facteurs d'origine naturelle (hydrologie, saisons...) ou anthropique (barrages), peuvent avoir une influence particulière sur les peuplements de poissons. Ces facteurs semblent avoir une pression d'autant plus importante qu'ils se produisent en synergie. Dans un souci d'économie, mais aussi d'un point de vue éthique, vis-à-vis de l'environnement, OCP limite ses épandages d'insecticides aux seuls gîtes productifs en larves de *S. damnosum.* De ce fait, par rapport aux milliers de kilomètres de rivières qui auraient pu théoriquement être traitées, beaucoup ne le furent que rarement, voire parfois jamais. Ainsi subsistent de vastes zones refuges qui peuvent servir de réservoir potentiel et permettent en cas d'arrêt des traitements une recolonisation des biefs par la faune.

La conclusion générale rassurante du suivi de 20 années de surveillance écologique de la faune non-cible est qu'il n'y a jamais eu d'effets drastiques du fait des larvicides employés même lorsque les peuplements, notamment les invertébrés, ont subi des cycles de traitements longs et-ou d'assez forte toxicité. Il faut, à cet égard, tenir compte de l'attention particulière qui a été accordée, par les instances opérationnelles d'OCP, aux recommandations du Groupe Écologique, même si ce suivi scrupuleux a pu, parfois, limiter l'efficacité de la lutte proprement dite contre les simulies.

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Télétransmission par satellite et prévision hydrologique dans le cadre de la lutte contre l'onchocercose en Afrique de l'Ouest

Satellite transmission and flow forecasting in control of onchocerciasis in west africa

Eric Servat et Jean-Marc Lapetite

1. INTRODUCTION

1 L'onchocercose, encore appelée cécité des rivières, est transmise à l'homme par la simulie *(Simulium damnosum)*. Cette petite mouche inocule, par sa piqûre, des filaires dont l'action sur l'organisme humain entraîne, à terme, la cécité.

2 En Afrique occidentale, et plus particulièrement en zone de savane, la maladie existe à l'état endémique. Les populations en sont généralement réduites à abandonner les zones infestées, alors qu'elles sont souvent les plus fertiles car situées en bordure de rivières. Les larves de simulies se développent, en effet, dans les cours d'eau, lorsque la vitesse du courant y est suffisante.

3 Depuis 1964, l'Organisation Mondiale de la Santé a lancé un important programme de lutte contre l'onchocercose en Afrique de l'ouest, OCP (Onchocerciasis Control Program), qui couvre tout ou partie de onze états (Niger, Bénin, Togo, Ghana, Côte-d'Ivoire, Burkina Faso, Mali, Guinée, Sierra Leone, Guinée Bissau, Sénégal) (cf. fig. 1).

4 L'objectif visé par OCP est l'interruption de la transmission de la maladie par la destruction des larves de simulies, au moyen d'épandages d'insecticides dans les cours d'eau. Les équipes de prospecteurs d'OCP relevaient les hauteurs d'eau aux échelles de crues des stations hydrométriques durant leurs tournées hebdomadaires. Ces relevés, transmis aux bases de traitement, étaient utilisés pour calculer les doses d'insecticides à

injecter dans les rivières au cours d'épandages effectués par avion ou par hélicoptère la semaine suivante.

5 Bien que l'insecticide utilisé, à savoir l'Abate[R], tolère une marge d'erreur importante, certains inconvénients nuisaient à l'efficacité optimale du traitement, plus particulièrement en saison des pluies : faible accessibilité aux données et précision des dosages insuffisante en cas de variation rapide des débits.

6 A cela sont venus s'ajouter des cas de résistance à l'Abate[R] qui ont conduit OCP à utiliser, depuis 1985, de nouveaux produits dont l'emploi est plus contraignant (faible portée, impact accru sur l'environnement, spectres d'efficacité en dilution très étroits, coût élevé). Il est alors devenu indispensable d'avoir une connaissance parfaite du débit et donc de la dose d'insecticide à injecter au moment du traitement. C'est la raison pour laquelle a été mis en place un réseau de télétransmission par satellite des hauteurs d'eau enregistrées en différents points de la zone d'intervention d'OCP.

2. LE SYSTÈME DE TÉLÉTRANSMISSION UTILISÉ DANS LE CADRE D'OCP

2.1. INTÉRÊT DE LA TÉLÉTRANSMISSION

7 L'emploi de nouveaux insecticides aux conditions d'utilisation très strictes fait que l'efficacité de la méthode de traitement passe désormais par une bonne adéquation entre le débit propagé et le volume d'insecticide injecté dans le cours d'eau. Atteindre cette efficacité maximale signifie donc que les responsables des opérations aériennes de traitement des biefs doivent désormais travailler pratiquement en temps réel.

8 Au vu de l'étendue de la zone couverte par OMS/OCP, la transmission par satellite des données recueillies à partir d'enregistreurs automatiques apparaît comme un des meilleurs moyens d'atteindre cet objectif (POUYAUD et LE BARBÉ, 1987).

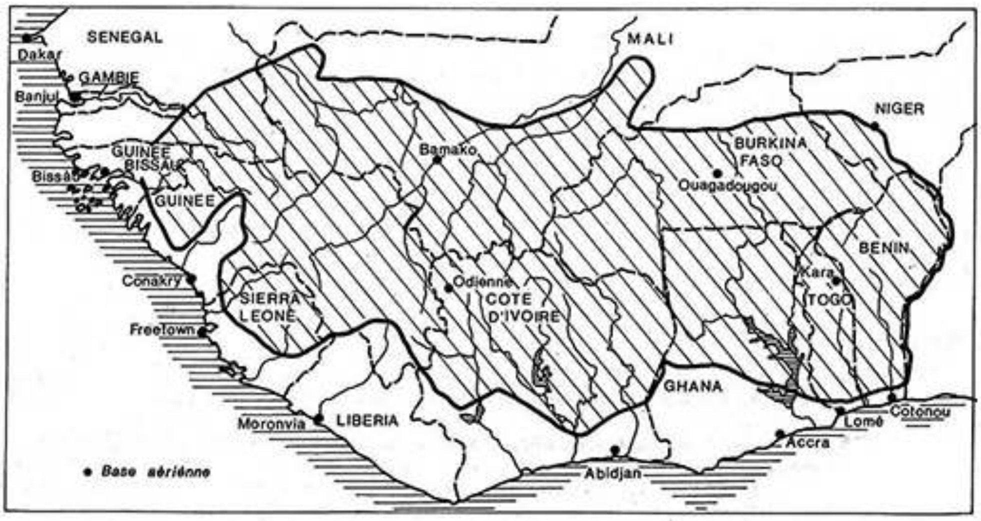

FIG. 1. — Carte de la zone couverte par le Programme OMS/OCP.

9 Cette technique offre, en effet, plusieurs avantages appréciables :

- un accès aux données garanti en toutes saisons ;

- une transmission des hauteurs d'eau quasiment en temps réel du fait des nombreux passages quotidiens des satellites relais ;
- des données plus fiables que les lectures d'échelles qui, elles, nécessitent plusieurs transcriptions qui sont autant de sources d'erreurs ;
- une centralisation rapide et simple des données par l'intermédiaire des stations de réception directe qui équipent les centres d'opérations aériennes (au nombre de deux actuellement, Odienné en Côte-d'Ivoire et Kara au Togo), et dont la fonction est de recueillir les messages relayés par les satellites.

2.2. MATÉRIEL ET MÉTHODOLOGIE

2.2.1. Description du matériel

10 En étroite collaboration avec le laboratoire d'Hydrologie de l'ORSTOM, la société ELSYDE France a mis au point une plate-forme hydrologique appelée CHLOE. Cette plate-forme est constituée d'un système de calcul de la hauteur d'un plan d'eau par l'intermédiaire d'un capteur de pression, et d'un boîtier électronique comportant une horloge et un système de codification et d'enregistrement des données sur mémoire de masse amovible. L'ensemble du système est alimenté de façon autonome par un panneau solaire et une batterie. En collaboration avec l'ORSTOM et ELSYDE France, la société CEIS-Espace a, quant à elle, intégré à cette plate-forme hydrologique une carte ARGOS permettant le transfert de données sous forme d'un message capté par un satellite relais et centralisé au niveau des stations de réception des bases de traitements aériens.

2.2.2. Méthodologie

11 Installée à proximité d'un cours d'eau, la balise de télétransmission n'est, bien entendu, opérationnelle que si la courbe d'étalonnage de la section qu'elle contrôle existe. Lorsque l'emplacement retenu ne correspond pas à une station du réseau hydrométrique national, il convient donc de procéder à un étalonnage. Le capteur de pression (SPI) est alors installé dans le même plan que les échelles limnimétriques, de façon à ce qu'il soit recouvert par l'eau même au plus fort de l'étiage. Le boîtier électronique est situé au-dessus des plus hautes eaux et à une distance du SPI n'excédant pas 50 m. Les deux éléments (boîtier électronique et capteur de pression) sont reliés par un câble souple (photo 1).

12 A intervalles de temps réguliers et réglables, il est procédé à une mesure de pression, corrigée par la température, qui permet de calculer la hauteur du plan d'eau. En outre, le SPI peut être interrogé à tout moment en dehors des instants de mesure programmés.

13 L'intervalle de temps fixé entre deux mesures de cote est d'une demi-heure. Elles sont sauvegardées sur mémoire de masse, ainsi que l'heure et la date, si la variation est supérieure à ± 1 cm par rapport à la dernière mesure. Ces cartouches de mémoire ont une autonomie d'environ une année. Hormis l'aspect télétransmission, les informations qu'elles contiennent peuvent être dépouillées automatiquement sur micro-ordinateur à l'aide d'un logiciel de gestion de banque de données hydrométriques (HYDROM) développé à l'ORSTOM (RAOUS, 1987). Les mémoires de masse peuvent alors être effacées et réutilisées.

14 Toutes les 220 secondes, la carte ARGOS envoie, par faisceau hertzien, les valeurs des quinze dernières demi-heures. Si l'un des satellites à défilement du système ARGOS passe

à ce moment là au-dessus de la balise et de la station de réception, il sert de relais au message envoyé par la balise (fig. 2).

15 La station de réception (SRDA), quant à elle, stocke et traite les messages au fur et à mesure de leur arrivée. Développée par CEIS-Espace, elle est dotée d'un environnement informatique de type IBM-AT (ou XT-286 ou compatible MS-DOS). Une telle station permet de gérer un parc d'une centaine de balises de télétransmission. Le logiciel de la SRDA crée et exploite différents fichiers, alimentés en temps réel à chaque passage de satellite. Grâce à un code de correction d'erreur, la station valide les messages reçus, en corrigeant, si nécessaire, des erreurs de transmission. Ces fichiers rangés et ordonnés peuvent faire l'objet d'édition, automatique pour certains paramètres ou commandée par l'opérateur pour d'autres. La station gère et affiche également un certain nombre « d'alertes » portant sur les paramètres de fonctionnement interne des plates-formes ou sur des seuils de hauteur d'eau, minimum ou maximum, fixés par l'opérateur (POUYAUD, 1987).

PHOTO 1. La plate-forme hydrologique et son alimentation par panneau solaire, reliée au capteur de pression, sur le Sankarani à Sanankoro, Guinée (Photo Michel GAUTIER, ORSTOM).

2.3. BILAN ACTUEL

2.3.1. Le parc de balises

16 Les conditions de vitesse du courant nécessaires au développement des larves de simulies expliquent le fait qu'on les trouve principalement dans des cours d'eau de moyenne envergure ou en têtes de bassins, dans des zones où les vitesses atteintes par l'eau sont plus rapides. En conséquence de quoi, le réseau de plates-formes hydrologiques équipées de balises de télétransmission est donc plus dense sur les hauts bassins du Niger, du Sassandra, du Bandama, de la Comoé et de la Volta Noire. Actuellement, outre les

quelques 80 balises du programme OMS/OCP, le réseau utilisé dans le cadre des traitements comprend une douzaine de balises du réseau Hydro-Niger.

2.3.2. Le bilan en terme de fonctionnement

17 Ces plates-formes hydrologiques comprenant l'ensemble « capteur-système de télétransmission » sont de conception très récente et susceptibles d'être perfectionnées d'un point de vue technologique. Néanmoins, après trois saisons des pluies de fonctionnement, et bien qu'il ait fallu faire face à différents types de problèmes (alimentation électrique, cartouches, cartes électroniques, capteurs de pression) le bilan est positif. Les difficultés rencontrées sont et doivent être à l'origine de modifications ayant pour objectif d'accroître le coefficient de fiabilité de ce matériel.

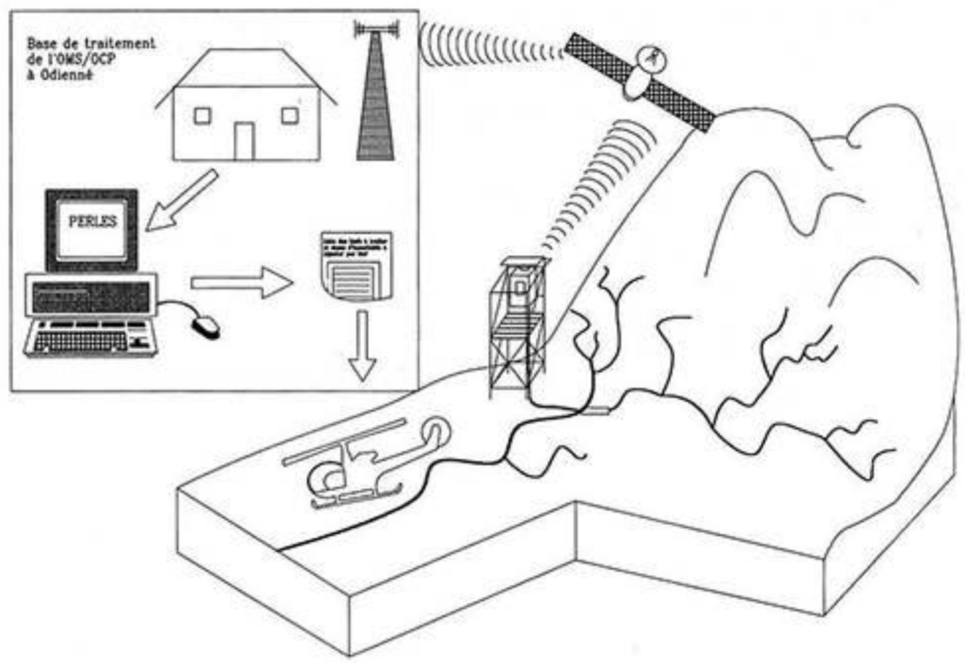

FIG. 2. — Schéma synoptique du système « mesure - télétransmission - prévision - traitement ».

18 En outre, l'expérience acquise durant les trois années écoulées sera très profitable, tant en ce qui concerne la conception future, que l'implantation et l'exploitation dans des conditions de fonctionnement difficiles (chaleur, humidité) de ce matériel technologiquement sophistiqué.

2.3.3. Amélioration du rendement des traitements et réduction des coûts

19 Avant la mise en place du protocole de traitement à partir des données télétransmises, les pilotes injectaient dans les cours d'eau des doses d'insecticides calculées à partir des lectures d'échelles de la semaine précédente. En saison des pluies, les variations de débit des rivières étant très rapides et parfois très importantes, l'adéquation « débit propagé-insecticide injecté » était souvent impossible à réaliser. L'utilisation de données hydrologiques en temps réel a permis l'obtention de dosages d'une précision rarement atteinte jusqu'alors. Hormis ce gain en efficacité des traitements, primordial pour le

succès du Programme, une importante réduction de son coût a pu être obtenue. Plusieurs explications peuvent être fournies :

- un meilleur ajustement des doses d'insecticides et, donc, la diminution des coûteux surdosages ;
- des traitements plus efficaces permettant de suspendre ceux-ci pendant une semaine ou plus ;
- la suspension des traitements dans le cas de crues trop importantes.

20 De façon à améliorer encore l'efficacité du dispositif, l'OMS/OCP a demandé à l'ORSTOM d'élaborer un logiciel de prévision des débits en chacun des biefs de traitement, qui s'appuierait sur les données télétransmises.

3. PERLES, LOGICIEL DE PRÉVISION DE DÉBITS ASSOCIÉ AUX TECHNIQUES DE TÉLÉTRANSMISSION

3.1. OBJECTIFS

21 Le logiciel PERLES (Prévisions, Etalonnages, Réception, Lectures d'EchelleS), implanté sur les micro-ordinateurs de la base des opérations aériennes d'OCP à Odienné, a été conçu pour accroître encore l'efficacité du dispositif de télétransmission.

22 Plusieurs impératifs nous ont guidé lors de sa réalisation :

- PERLES devait être en mesure d'émettre des prévisions de débits pour l'ensemble des biefs traités, à différentes échéances de temps. A cette fin, plusieurs modèles de prévision sont utilisés qui permettent d'estimer les débits aux horizons 3, 6 et 12 heures en saison des pluies, période durant laquelle les variations de débit peuvent être très rapides. En saison sèche les cours d'eau suivent un régime de tarissement et les délais de prévision sont étendus à 1, 3, 5 et 8 jours ;
- un tel logiciel, exploitant non seulement les données transmises par les balises de télétransmission, mais aussi les relevés d'échelles limnimétriques réalisés par les équipes d'OCP, devait également inclure un gestionnaire de banque de données. PERLES permet donc de gérer et d'archiver les relevés des équipes OCP ainsi que les courbes d'étalonnages de l'ensemble des stations (échelles limnimétriques et balises) suivies dans le cadre du Programme ;
- le logiciel se devait d'être parfaitement convivial, condition *sine qua non* de son utilisation par les opérateurs OCP. PERLES se présente donc sous la forme d'une succession de menus déroulants d'un emploi très simple permettant, selon les cas, d'accéder aux différents sous-ensembles du logiciel ou de sélectionner une de ses nombreuses fonctions.

3.2. STRUCTURE

23 PERLES se présente en deux parties (SERVAT et LAPETITE, 1990) : la première est installée sur la station de réception proprement dite, alors que la seconde est implantée sur un micro-ordinateur relié à la station, et sur lequel se déroulent l'ensemble des calculs de prévision et des opérations de gestion (fig. 3).

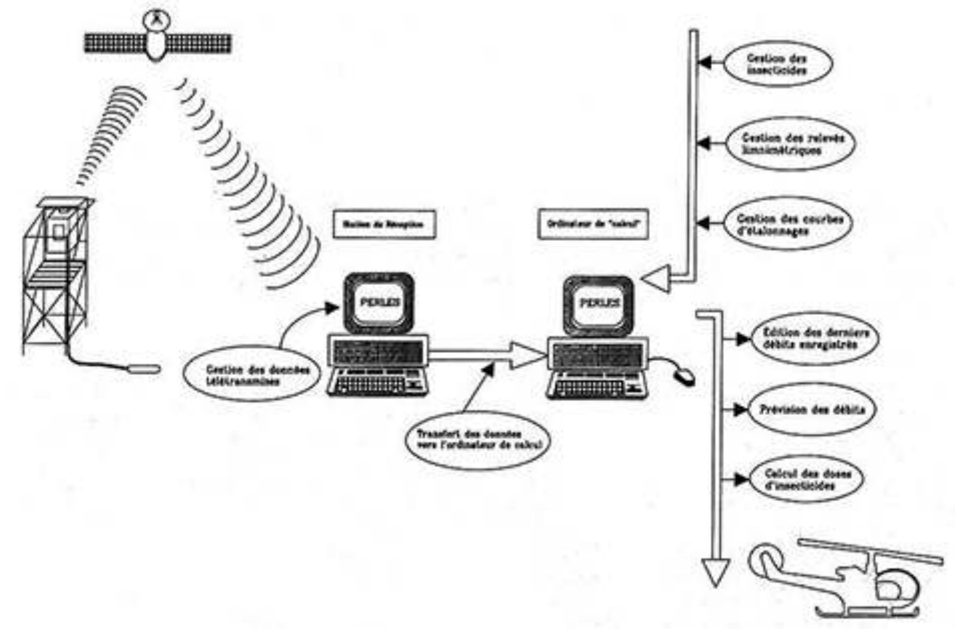

FIG. 3. — Fonctions et organisation du logiciel PERLES.

3.2.1. La station de réception

24 La partie du logiciel installée sur la station de réception a pour fonction d'organiser et de gérer le transfert des hauteurs d'eau, reçues via le satellite, vers le micro-ordinateur de calcul (fig. 4).

25 La réalisation de ces transferts est facilitée par la possibilité qu'offre PERLES de gérer des « tables de transfert » qui contiennent les numéros des balises ARGOS à prendre en compte. Lors de chaque opération de transfert des données, préalable à la phase de réalisation des calculs, et pour toutes les balises concernées, la date est mémorisée. Cela permet de ne transférer, à chaque fois, que les hauteurs d'eau postérieures à la date du dernier transfert effectué et de satisfaire ainsi une des principales contraintes, à savoir : économiser le temps, précieux dans le cadre d'une structure opérationnelle du type des bases d'opérations aériennes.

3.2.2. Le micro-ordinateur de calcul

26 La seconde partie du logiciel PERLES est installée sur le micro-ordinateur où sont effectués les calculs (fig. 5). Plusieurs fonctions y sont identifiées, que l'on peut séparer en deux groupes.

27 L'accès au premier groupe de fonctions est libre pour tout utilisateur de PERLES. Il comprend : la réception des données, la gestion des étalonnages des stations de référence et celle de la banque de données hydrométriques, le calcul et l'édition des prévisions de débits et des doses d'insecticide correspondantes pour les différents biefs à traiter. L'ensemble des opérations s'effectue par sélections successives à partir de menus déroulants.

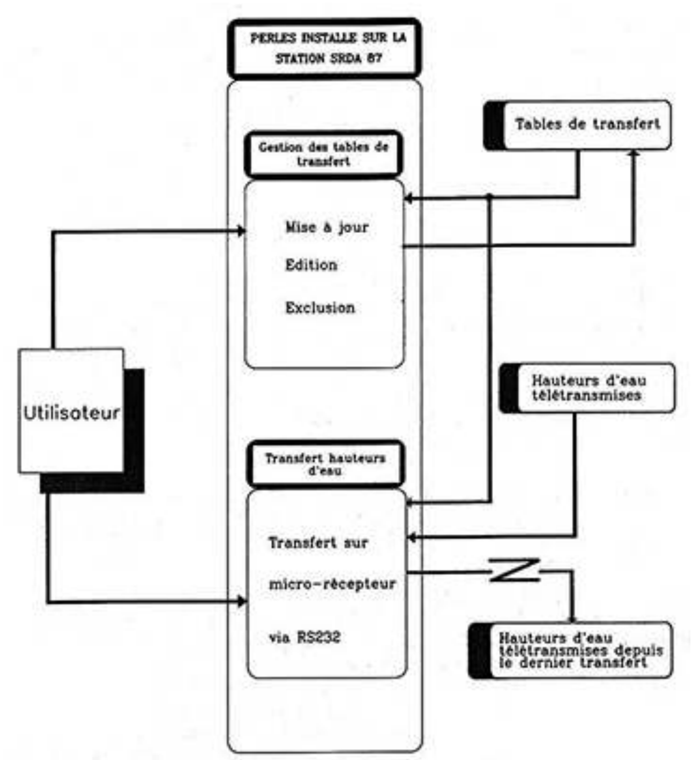

FIG. 4. — Schéma fonctionnel de PERLES installé sur la station de réception (SRDA).

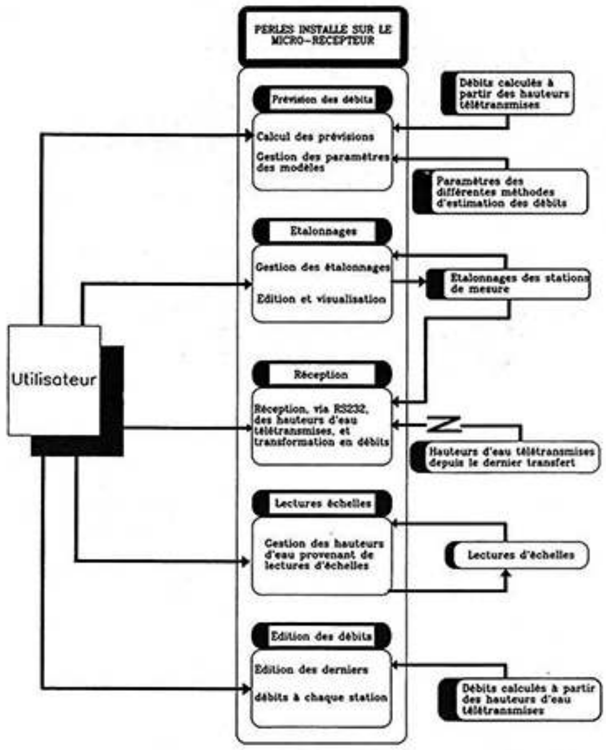

FIG. 5. — Schéma fonctionnel de PERLES installé sur le micro-ordinateur de calcul.

28 Le deuxième groupe de fonctions n'est accessible qu'aux hydrologues chargés du calage des modèles utilisés pour émettre les prévisions. Pour chacun des biefs concernés, on définit donc une gestion des paramètres qui lui sont propres. Ces paramètres

comprennent, non seulement les coefficients des différents modèles utilisés, mais aussi les coefficients de priorité qui leur sont attribués.

29 En effet, le logiciel gère, pour chaque bief, des priorités d'utilisation des méthodes de prévision. Celles-ci sont actuellement au nombre de cinq : modèle de propagation basé sur la méthode d'Hayami (VEN TE CHOW, 1959 ; QUIVEY et KEEFER, 1974), modèle auto-régressif, corrélation « balise de télétransmission-échelle limnimétrique », méthode empirique propre à OCP et modèle de tarissement. Chacune de ces méthodes nécessite des informations qui lui sont propres et, selon les cas, elle peut ou ne peut pas être appliquée. Nous avons donc défini pour chaque bief un ordre préférentiel d'utilisation de ces techniques de prévision. Si la méthode qui s'est révélée la mieux adaptée et la plus précise ne peut pas être utilisée (pour cause d'absence des données nécessaires, par exemple), on passe à la suivante et ainsi de suite. Ce processus nous permet d'être en mesure de faire une estimation du débit qui transite dans un bief dans environ 90 % des cas, quoi qu'il advienne.

3.2.3. Méthodes employées

30 Les méthodes de prévision employées dans ce logiciel sont simples et robustes, et bien connues des hydrologues. L'originalité du logiciel ayant trait, comme nous l'avons signalé, non pas aux méthodes utilisées proprement dites, mais à leur emploi en cascade et selon des priorités définies au préalable si nécessaire.

31 — Le modèle d'onde de crue diffusante est utilisé prioritairement chaque fois que cela est possible. Il consiste à négliger les termes d'inertie dans les équations de Barré de Saint-Venant. La propagation des crues obéit alors à l'équation différentielle suivante :

$$\delta Q/\delta t + C\,\delta Q/\delta x - D\,\delta^2 Q/\delta x^2 = 0 \quad (1)$$

32 avec : Q : débit,

33 C : célérité de l'onde de crue,

34 D : coefficient de diffusion de l'onde de crue,

35 x : abscisse,

36 t : temps.

37 Si C et D peuvent être considérés comme invariants, la solution de l'équation s'exprime sous forme d'un produit de convolution, ainsi que l'a montré Hayami (VEN TE CHOW, 1959) :

$$Q_x(t) = \int_0^t Q_0(u)\,K_x(t-u)\,du \quad (2)$$

38 avec K, noyau de convolution :

$$K_x(t) = (x/(2t^{3/2}\sqrt{\pi D}))\,\exp[-((x - Ct)/(2\sqrt{Dt}))^2] \quad (3)$$

39 En règle générale, les rivières traitées par l'OCP sont telles que les vitesses de propagation de crues sont très différentes selon le remplissage du lit. Certains aménagements ont donc dû être apportés à la méthode. En particulier les valeurs de C et D, qui ne peuvent être considérées comme constantes, sont recalculées dans chacun des cas. Cela permet d'utiliser un noyau de convolution approprié à chacune des crues traitées (BADER *et al.*, 1988 ; LE BARBÉ et BADER, 1988).

40 — Les modèles auto-régressifs sont utilisés sur les biefs équipés de balises de télétransmission. Les équations d'auto-régression sont déterminées à l'aide d'une

méthode de régression progressive, le « Stepwise » (Draper et Smith, 1981). Elles ont pour forme générale :

$$Q(t_0 + H) = A_0 + A_1\, Q(t_0) + A_2\, Q(t_0 - 1) + \ldots + A_{n+1}\, Q(t_0 - n) \quad (4)$$

41 avec : Q(t) : débit au temps t,

42 t_0 : date origine de la prévision,

43 H : horizon de la prévision (ici 3, 6 ou 12 heures)

44 $A_0,\ldots,A_{n+1}$: coefficients de l'équation.

45 — Les corrélations entre balises et échelles permettent d'affiner l'estimation des débits pour des biefs sur lesquels on ne dispose que d'une lecture hebdomadaire d'échelle limnimétrique.

46 — Les relations empiriques, utilisées en derniers recours, associent les débits de différents cours d'eau dans la détermination du débit d'un bief donné. Ces relations résultent des années d'observation et de la parfaite connaissance du terrain des responsables locaux d'OCP.

47 — Le modèle de tarissement employé en saison sèche, période pendant laquelle les pluies sur la zone traitée sont nulles ou rares (et dans ce cas généralement très faibles), consiste en une fonction exponentielle décroissante dont la formulation est :

$$Q(t_0 + H) = Q(t_0)\exp(-\alpha H) \quad (5)$$

48 avec : Q(t) : débit au temps t,

49 t_0 : date origine de la prévision

50 H : horizon de la prévision (ici 1, 3, 5 ou 8 jours),

51 α : coefficient de tarissement.

52 L'ensemble de ces modèles constitue le cœur de la partie « Prévisions » du logiciel. Le schéma fonctionnel complet en est représenté sur la figure 6.

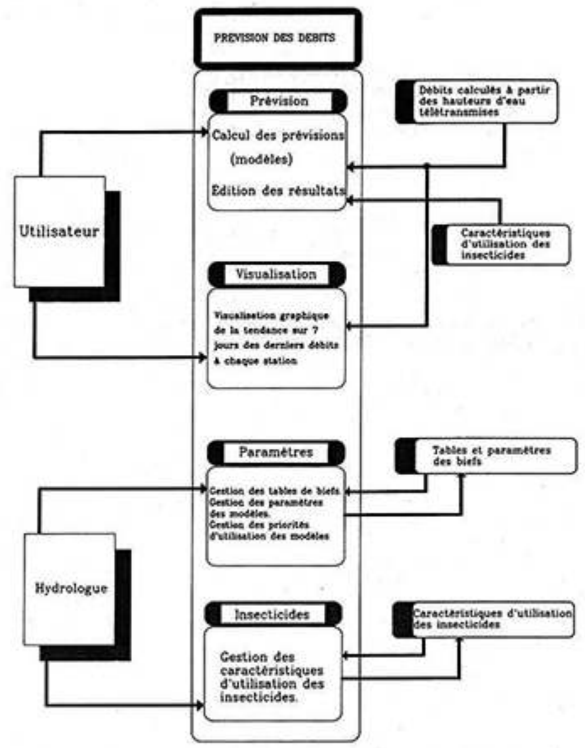

FIG. 6. — Schéma fonctionnel de la partie « Prévisions » du logiciel PERLES.

53 Les valeurs de débit calculées à différents horizons peuvent ensuite être reprises comme variables d'entrées d'un logiciel développé par ailleurs. Celui-ci a pour objectif l'optimisation des circuits de traitement sous l'angle de la gestion des heures de vol et des points de ravitaillement en carburant et en insecticide.

CONCLUSIONS ET PERSPECTIVES

54 Le logiciel PERLES constitue un ensemble très complet. C'est non seulement un outil convivial de prévision des débits et de calcul des doses d'insecticide, mais aussi un gestionnaire de banque de données limnimétriques.

55 Son organisation modulaire (fig. 4, 5 et 6) permet toutes les modifications possibles dont, notamment, l'introduction de modèles de prévision différents si le besoin s'en faisait sentir.

56 L'utilisation de ce logiciel conduit à une importante économie de temps puisque, pour une table de traitement moyenne (soit une dizaine de balises et une soixantaine de biefs), il faut compter moins d'un quart d'heure, du transfert des données de la station de réception à l'édition des résultats.

57 Ce qui se révèle parfaitement compatible avec les impératifs horaires quotidiens de briefing des pilotes et de début des opérations de traitement.

58 Par ailleurs, l'équipement du réseau hydrographique de la zone couverte par OCP, en balises de télétransmission par satellite, a permis de passer d'une gestion hebdomadaire à une gestion qui se fait pratiquement en temps réel.

59 Le couplage de PERLES avec ce système ARGOS a permis d'accroître encore l'efficacité du dispositif en autorisant des prévisions à différentes échéances. On obtient alors une

bonne adéquation entre les doses d'insecticide injectées dans les cours d'eau et les débits propagés. Cet ensemble « plate-forme hydrologique - télétransmission - PERLES » permet ainsi des améliorations importantes au niveau du programme OCP :

- il évite les sous-dosages, générateurs d'échecs, et risquant à terme d'isoler des souches de simulies résistantes aux insecticides utilisés ;
- il évite les sur-dosages importants, inutilement coûteux, et qui peuvent avoir des conséquences nuisibles sur l'environnement ;
- il augmente le taux de réussite et permet donc d'envisager une suspension momentanée des traitements pour certains biefs, autorisant par là l'adoption de stratégies moins coûteuses.

60 Hormis le cas particulier du programme de lutte contre l'onchocercose, la télétransmission par satellite offre de nombreuses perspectives et se présente comme une technique d'avenir en hydrologie. Elle devrait trouver sa pleine utilisation dans le cas de réseaux d'annonces de crues sur de grands bassins versants. Cependant son intérêt dans le cadre de la rationalisation de la gestion d'un réseau hydrométrique national est évident. Les plates-formes hydrologiques sont, en particulier, à même de transmettre certains paramètres propres à leur fonctionnement. Elles réalisent par là une certaine forme d'autosurveillance qui peut réduire considérablement les coûts de fonctionnement des réseaux en diminuant le nombre de tournées d'entretien et en permettant de mieux les cibler (POUYAUD, 1988).

61 Ces avantages rendent la télétransmission par satellite encore plus attractive pour les pays en voie de développement de la zone intertropicale, dans lesquels les voies d'accès aux stations sont parfois impraticables et où, localement, les conditions de suivi peuvent se révéler insuffisantes.

REMERCIEMENTS

62 Les auteurs expriment leurs remerciements à Jean-Claude BADER et Jean-François BOYER pour leur contribution au développement du logiciel PERLES.

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RÉSUMÉS

L'onchocercose, ou cécité des rivières, est une maladie endémique qui cause d'importants ravages en Afrique de l'ouest. Dans le cadre du programme de l'OMS de lutte contre ce fléau (OCP), la télétransmission par satellite est apparue comme un moyen sûr d'obtenir des données fiables concernant les débits des cours d'eau traités. Ces données sont indispensables pour permettre un calcul précis des doses d'insecticides injectés dans les rivières. Une description du matériel et de son protocole d'utilisation précèdent un premier bilan dressé tant en terme de fonctionnement du matériel proprement dit, qu'en terme d'efficacité et d'économies réalisées. On peut ainsi mettre en avant l'amélioration du rendement des traitements et la réduction des coûts de fonctionnement du programme. Un logiciel de prévision des débits à différentes échéances a été développé par l'ORSTOM à la demande de l'OCP. Ce logiciel (PERLES) est décrit au niveau de ses différentes unités fonctionnelles. Son couplage avec le système de télétransmission a permis d'accroître les performances du système. En conclusion, on met en avant l'intérêt des techniques de télétransmission dans le cadre d'une hydrologie opérationnelle (réseaux d'annonces de crues, réseaux hydrométriques).

Onchocerciasis, or river blindness, is an endemic disease which causes great hardship in West Africa. Within the WHO programme to control this disease (OCP), reliable data on river flows have been obtained by using remote satellite transmission. These data are necessary to calculate how much insecticide should be introduced into the rivers. A description of the equipment and its use is followed by an initial report covering the functioning of the equipment, its efficiency and the economies attained. The improvement in the resulting treatment and the reduction in the running costs of the programme are discussed. Software for forecasting river flows over different time intervals was developed by ORSTOM at the request of the OCP.

The different fonctions of this software (PERLES) are described. In conclusion, the advantages of remote transmission techniques for operational hydrology in general are discussed (flood warning Systems, hydrological networks, etc.)

INDEX

Mots-clés : Télétransmission par satellite, Prévisions de débits, Onchocercose, Afrique de l'ouest
Keywords : Satellite transmission, Flow forecasting, Onchocerciasis, West Africa

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Dispositif à gouttières multiples destiné à tester *in situ* la toxicité des insecticides vis-à-vis des invertébrés benthiques

Jean-Jacques Troubat

1. INTRODUCTION

1 Le programme de lutte contre l'Onchocercose humaine en Afrique de l'Ouest vise à stopper la transmission du Nématode *Onchocerca volvulus* par le biais de la destruction des stades préimaginaux du vecteur S. *damnosum.* Le contrôle larvaire du vecteur est réalisé à l'aide d'épandages hebdomadaires de téméphos (Abate), insecticide organophosphoré. Bien qu'actuellement efficace, l'emploi d'un seul pesticide rend fragile un tel programme de lutte. Il est en effet possible qu'à plus ou moins brève échéance, une résistance au téméphos apparaisse chez les larves de Simulies. Dans une telle optique, la recherche de produits de remplacement est nécessaire.

2 Ces produits doivent naturellement être actifs contre *S. damnosum,* mais présenter également une innocuité relative vis-à-vis de la faune non cible.

3 Actuellement la meilleure méthode permettant de mesurer les effets de nouveaux pesticides sur la faune des milieux d'eau courante est celle des gouttières utilisée *in situ* (DEJOUX, 1975). En effet, si les tests effectués en laboratoires dans des milieux confinés donnent généralement des résultats précis et reproductibles, leur interprétation est entachée d'incertitudes du fait de la grande différence entre les conditions de laboratoire et les conditions naturelles. Il devient alors délicat d'extrapoler les résultats obtenus. Par contre, les expérimentations en gouttières sont effectuées dans les rivières et reproduisent dans la mesure du possible les conditions naturelles du milieu. Elles sont cependant sujettes à plus de variations et donc moins reproductibles du fait d'une

certaine hétérogénéité dans la distribution des facteurs biotiques et abiotiques. Malgré cet inconvénient des résultats assez fiables ont déjà été obtenus (DEJOUX et ELOUARD, 1977).

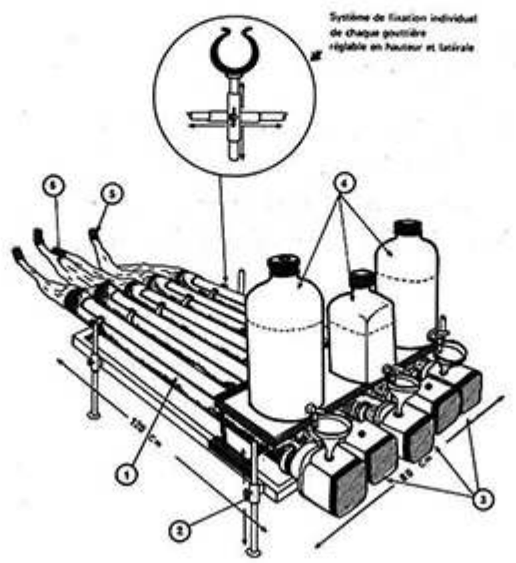

FIG. 1. — Schéma de l'appareil **À** gouttières multiples. 1 : Corps des gouttières (partie expérimentale) où sont disposés les substrats naturels ; 2 : Système de support permettant le réglage en hauteur de l'ensemble du bâti ; 3 : Tamis d'arrêt de la dérive naturelle avec dispositif d'accélération du courant ; 4 : Réservoirs contenant les solutions de pesticide ; 5 : Collecteurs ; 6 : Filets de récolte de la dérive des organismes de gouttières.

PHOTO 1. — Dispositif **À** gouttières multiples en action sur une rivière de la Côte d'Ivoire.

4 Les systèmes actuellement employés, s'ils donnent satisfaction sur un plan de principe, sont souvent lourds ou encombrants. Par ailleurs, l'expérience a montré qu'il était possible de les miniaturiser tout en leur conservant une capacité d'implantation faunistique suffisante et statistiquement utilisable.

2. GOUTTIÈRES MULTIPLES

2.1. Description

5 De conception identique à celle des gouttières uniques, cet appareil regroupe, sur un même châssis métallique, cinq gouttières en tube PVC de 9 cm de diamètre et 1,50 cm de longueur (fig. 1). Chaque unité est fixée au châssis par ses deux extrémités à l'aide d'un dispositif permettant un réglage latéral et en hauteur. De plus, un système de fixation ayant la forme d'une « dame de nage » (médaillon F. 1) autorise également un déplacement longitudinal de chaque tube. Cette mobilité individuelle sur les trois axes de tous les éléments du dispositif rend possible un ajustement très précis de la pente, du débit d'eau et du niveau d'immersion dans la rivière. Cette solution s'avère très pratique lorsque l'appareil est utilisé en zone de rapide où le courant n'est pas homogène sur une grande largeur ; les gouttières reçoivent alors la quantité d'eau nécessaire à leur bon fonctionnement. Un embout en plastique de forme cubique muni d'un tamis est ajusté à la partie amont, permettant le passage de l'eau et empêchant la dérive benthique naturelle de pénétrer dans l'appareil au cours d'expérimentation.

6 A certaines périodes de l'année, l'utilisation de l'appareil ainsi constitué peut être délicate, c'est le cas par exemple de la saison des pluies, quand le niveau des cours d'eau peut varier rapidement de plusieurs dizaines de centimètres, ou bien à l'étiage où la profondeur de l'eau, trop faible, ne permet pas l'immersion du système. Dans ces deux cas et pour pallier ces inconvénients, il est possible de placer l'ensemble des gouttières hors d'eau et de capter cette dernière à l'aide de tuyaux souples plus ou moins longs qui s'adaptent à la place des embouts cubiques. Il est simplement nécessaire dans ce cas d'utiliser une dénivellation de terrain pour obtenir un courant suffisant dans chaque gouttière. Enfin, un filet associé à un collecteur est fixé à la partie aval de chaque élément, assurant la récolte de la dérive provenant des gouttières. Le châssis métallique est supporté par quatre pieds également réglables en hauteur, ce qui donne une bonne assiette à l'ensemble.

2.2. Utilisation

7 Trois principaux types d'expérimentation peuvent être réalisés : le premier sert à étudier *in situ* l'impact sur la faune non cible d'un seul insecticide à des concentrations différentes ; le deuxième permet la comparaison de l'effet toxique de plusieurs insecticides différents employés dans des conditions similaires ; le troisième évalue l'effet du courant sur une faune traumatisée.

2.3. Étude des effets d'un seul insecticide

8 Plusieurs expérimentations de ce type ont été réalisées au laboratoire d'hydrobiologie de Bouaké, le but poursuivi étant d'établir *in situ,* les courbes de sensibilité au téméphos des principales espèces d'invertébrés benthiques ; ou bien d'évaluer par comparaison la quantité réelle d'insecticide épandue lors d'un traitement de routine du programme Onchocercose.

9 Dans de tels cas, l'appareil est mis en place *in situ,* sur les gîtes à S. *damnosum,* puis l'intérieur est garni de substrats naturels, en provenance du gîte, déjà colonisés par des invertébrés benthiques. Une période de stabilisation allant de 10 heures à 24 heures est nécessaire pour que la faune transplantée s'établisse en fonction de ses préférences et des conditions qui lui sont offertes.

10 Au début de l'expérience, les gouttières sont fermées dans leur partie amont par le tamis filtrant et dans leur partie aval par le filet collecteur. Il est préférable de faire débuter l'expérimentation en milieu ou en fin de matinée lorsque le taux de dérive est minimal (ELOUARD et LÉVEQUE, 1977). La chronologie des expériences est désormais classique, les intervalles de temps entre deux récoltes étant d'autant plus courts que l'on est près de l'heure de traitement, ceci afin d'obtenir une plus grande précision dans l'établissement de la courbe de décrochement des organismes testés.

11 Quatre des gouttières seront utilisées pour le test et une sera conservée comme témoin afin d'estimer, en pourcentage, l'intensité de dérive naturelle durant les 24 heures de l'expérimentation. Il sera tenu compte de ce pourcentage pour le calcul des effets de chaque concentration. Il représente le terme correctif dans la formule proposée par DEJOUX (1978).

$$D_P = \sum_{1}^{24} Dc - K_{24}\, N_T$$

D_P = décrochement par effet polluant.

où $\sum_{1}^{24} Dc$ = Somme des décrochements pour chaque intervalle de temps durant 24 heures.

K_{24} = Terme correctif représentant le pourcentage moyen de dérive naturelle en 24 heures, calculé sur témoin.

N_T = Nombre d'organismes testés dans une gouttière

12 Dans la pratique, on peut tester quatre concentrations à la fois avec par exemple dans une gouttière la concentration minimale efficace contre le groupe cible, dans une seconde, une concentration double et dans les deux dernières, des concentrations de respectivement un demi et un tiers.

2.4. Comparaison de plusieurs insecticides

13 Les toxicités de quatre insecticides peuvent être comparées simultanément, entre elles et à un témoin. La gouttière témoin permettant toujours d'estimer l'intensité de la dérive naturelle.

14 L'avantage dans ce cas de l'utilisation des gouttières multiples est de pouvoir tester dans un espace réduit plusieurs insecticides sans qu'il y ait possibilité d'interférence entre les différents toxiques, ceci en un minimum de temps et avec un minimum de manutention ainsi que de très faibles quantités de produit. Ce dernier avantage est très appréciable dans le cas d'une expérimentation d'un composé très toxique.

2.5. Évaluation des effets du courant

15 Grâce au système de réglage individuel en hauteur de chaque gouttière, on peut évaluer l'impact de la force cinétique du courant sur des individus traumatisés par un traitement à une concentration donnée. Les organismes traumatisés par un pesticide (effet de Knock-down) peuvent en effet « récupérer » si le courant ne les arrache pas de leur support.

Dans le cas contraire, les risques sont grands qu'ils se trouvent transportés dans une zone écologiquement non viable ou qu'ils subissent l'action des prédateurs.

3. CONCLUSION

16 L'emploi d'un dispositif à éléments multiples réglables dans les trois axes directionnels représente une amélioration certaine de la technique des gouttières. Il devient alors possible avec un tel dispositif de tester en parallèle et *in situ* la toxicité de plusieurs insecticides ou des concentrations différentes d'un même insecticide sur la faune d'invertébrés benthiques et ce même en période de crue car le principe des tuyaux souples adaptables en amont de chaque gouttière permet de capter l'eau de la rivière tout en ayant l'appareil hors de l'eau.

17 Certes d'un emploi plus complexe en période de hautes eaux, le système s'est avéré très pratique durant la période de décrue ou d'étiage, lorsque les conditions limnimétriques variaient peu. Le réglage individuel fin de chacune des gouttières permet d'obtenir les conditions rhéologiques optimales pour la faune d'invertébrés testés.

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RÉSUMÉS

L'auteur décrit un dispositif expérimental comprenant cinq gouttières réglables solidaires d'un châssis commun et conçu pour tester in situ *la toxicité des insecticides vis-à-vis de la faune invertébrée d'eau* *courante. Après une description détaillée de l'appareil, l'auteur discute de son emploi, de ses avantages et de ses inconvénients.*

A NEW "IN SITU" SYSTEM USING MANY GUTTERS TO TEST INSECTICIDE TOXICITY AGAINST BENTHIC INVERTEBRES

Different types of gutters or through Systems have been utilised in the past 10 years in order to lest the toxicity of new insecticides or new formulations among the non target invertebrate fauna in running waters.

In this work an amelioration of the systems commonly used is proposed, mainly based on a multiplication of the testing units, each one could be independently moved and fixed in the three dimensions.

A full description of the system is given, inconveniences and advantages are discussed.

INDEX

Keywords : Technic, Pollution, Pesticides, Running water, Africa

Mots-clés : Technique, Pollution, Pesticides, Eaux courantes, Afrique

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Joint US-AID/OCCGE/WHO technical-meeting on the feasibility of onchocerciasis control

Joint US-AID/OCCGE/WHO technical-meeting on the feasibility of onchocerciasis control

WORLD HEALTH ORGANIZATION

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2 ORIGINAL: ENGLISH

3 Tunis, 1-8 July 1968

REPORT

4 The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

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Introduction

1 A technical meeting on the feasibility of onchocerçiasis control was convened in Tunis from 1 to 8 July 1968 under the sponsorship of the United States Agency for International Development (US-AID), the "Organisation de Coordination et de Coopération pour la Lutte contre les Grandes Endémies" (OCCGE) and the World Health Organization (WHO). Professor P. G. Janssens was elected Chairman, Dr E. Akwei Vice-Chairman, ànd Dr B. O. L. Duke and Dr M. M. Ovazza were elected Rapporteurs. The meeting was opened by Mr H. Khefacha, Secretary of State for Public Health Affairs of the Government of the Tunisian Republic.

2 The twenty-seven experts gathered represented various disciplines involved in the control of onchocerciasis: public health, tropical hygiene, parasitology, epidemiology, entomology, ophthalmology, medical training, economics, sociology and medical geography. The meeting was serviced by a joint US-AID/OCCGE/WHO secretariat. It was attended by a representative of the International Bank for Reconstruction and Development (IBRD) and by an observer from the "Office de la Recherche Scientifique et Technique Outre-Mer" (ORSTOM) (see list of participants).

3 In 1965 the World Health Organization convened an Expert Committee on Onchocerciasis to provide guidance on the methods of approach to onchocerciasis control best adapted to existing epidemiological conditions.[1] In the three years that have since elapsed, although some advances were made in several fields of research, it was felt that knowledge had progressed but slowly, particularly on the actual geographical distribution of the disease and its pathogenicity.

4 Successful control has been achieved in the last twelve years in some geographically well defined foci, notably in Kenya where the eradication of Simulium neavei was completed in 1956 except for a very small focus on the Uganda border. On account of these encouraging results, a number of new control projects have been started in the last five years. Good results have been obtained but at the price of many difficulties, both technical and financial. It was therefore felt that the time had come to undertake a critical review of the experience gathered in the projects recently carried out.

5 In addition to the technical and financial difficulties encountered, operations in progress have raised another set of problems. Owing to the Limited extent of the areas, under control, these areas, even when successfully treated, remain under the threat of an early

reinvasion by Simulium flies from adjacent areas that have not been protected. This raises the problem of a concerted action between neighbouring countrïes.

6 In view ofthe above, the present meeting was called to advise on the best strategy to be adopted in the present epidemiological circumstances, the conditions under which control should be attempted, the methods to be used, including the most modern techniques, and the requirements (staff, equipment, funds, inter-country co-ordination) to be met.

7 Onchocerciasis is rife in many countries. Obviously, its control cannot be attempted everywhere at the same time. The meeting was therefore asked to point out the areas where the onchocerciasis problem is greatest and where an expanded project could be carried out with the maximum chances of success.

8 In recent years public health administrators and field workers have drawn attention to the devastating economic repercussions of onchocerciasis. Some of the most fertile river valleys in tropical Africa have remained deserted and uncultivated or are progressively abandoned, and it seems that the occurrence of the disease and of the Simulium vectors is a major deterrent to the reclamation of these valleys. Furthermore, the presence of the Simulium vectors, which are dreaded both for the terrible nuisance they cause and as the vectors of onchocerciasis, has also hampered the implementation of large economic development schemes in certain areas. Onchocerciasis has now acquired the status of an "economic" disease and, as such, is of interest to national authorities responsible for economic development planning as well as to international and national organizations providing technical and financial assistance to economic projects planned or carried out in developing countries.

9 A revised strategy of onchocerciasis control, enlarged to include whole river basins, will obviously require financial efforts which must be warranted by the economic benefits they are expected to provide. In order to facilitate this assessment, the experts were asked to define the requirements for a large-scale onchocerciasis control project applicable to the recommended initial project area.

10 The present report is an account of the views expressed and recommendations made on these various questions.

1. REVIEW OF THE PRESENT SITUATION OF ONCHOCERCIASIS

11 Onchocerciasis may be regarded as being in a state of dynamic equilibrium. In the present section the current situation is first reviewed and an attempt is made to foresee some of the changes that may take place in the future.

1.1 Magnitude of the current problem

1.1.1 General situation

12 Onchocerciasis is a parasitic disease widely distributed in tropical Africa and the Americas. In Africa, the disease is known to occur south of the Sahara in a wide belt stretching from west to east. The northernmost boundary of this zone coincides roughly with latitude 15°N and runs from Senegal to Ethiopia. South of the equator the endemic

area extends down to Angola in the west and to Tanzania in the east. Apart from highly endemic areas in the south of the Sudan Republic, small foci have also been reported in the north of that country as well as in Yemen. In the Americas, important foci exist in Mexico, Guatemala and Venezuela. A small focus has recently been discovered in Colombia.

13 It is estimated that approximately 20 million people in the world are infected with Onchocerca volvulus, the majority of whom live in Africa. However, this figure is probably an underestimate and does not fully reflect the importance of the disease. To give a more concrete example, in Upper Volta alone, it is estimated that, out of a population of 4.5 million, 400 000 have onchocerciasis. Of these 400 000, 40 000, or one in ten, suffer from severe eye lesions and many of these are actually blind.

14 A summary of the global distribution of onchocerciasis as known today is presented in Map 1 (Africa) and Map 2 (the Americas). The endemic onchocerciasis areas marked on these maps represent, however, a conservative estimate. The geographical distribution of the disease still requires more precise determination. In many instances the blank spaces in the tropical belt of the African map indicate gaps in our knowledge rather than absence of onchocerciasis. The area in which the vector exists without the disease itself being found is usually more extensive than the endemic area marked on the maps.[2]

15 The greatest impact of onchocerciasis occurs, without doubt, in West Africa between the 8th and 12th northern parallels of latitude, where the prevalence of blindness and other clinical manifestations are extremely high.

1.1.2 Situation in West Africa[3]

16 All the States of West Africa are affected by onchocerciasis: Dahomey, Gambia, Ghana, Guinea, Ivory Coast, Liberia, Mali, Mauritania, Niger, Nigeria, Portuguese Guinea, Senegal, Sierra Leone, Togo and Upper Volta. Moreover, as is generally agreed, it is in West Africa that the most serious foci are located. Nevertheless, in this part of Africa gradations in the severity of the disease may be observed. Thus it is possible to distinguish two distinct types of transmission:

(1) The forest type of onchocerciasis

17 In the forest zones, the foci are extensive and continuous with adjacent foci. However, although individuel infections may be very intense, serious disorders such as blindness are relatively uncommon. It should, however, be noted that the nuisance caused by the vector, which in certain foci and at certain seasons literally swarms (4 000 bites per man per day), may constitute a serious problem for the use of riparian lands by man. This forest type is encountered in the Southern part of West Africa stretching from Cameroon to Guinea.

(2) The savanna type of onchocerciasis

18 Here the foci are restricted to communities either living in close contact with the vector species, or brought into frequent contact with them through the normal activities of their daily life. In these areas, the morbidity rate falls rapidly as one moves away from the rivers.

19 With the second type, in contrast to the first, the individual parasite load is very high. The clinical manifestations are usually severe, with a high rate of blindness.

20 Moreover, a particular feature of certain parts of the savanna zone is that there has been an actual abandonment of villages in the river valleys. This can be considered as the most severe. socio-economic impact produced by onchocerciasis,. For ecological reasons specific to the vector, this all too frequent depopulation effect occurs only in the savanna areas. It is encountered in the northern and central regions of Nigeria, Dahomey, Togo, Ghana, the Ivory Coast and Guinea, and in the southern and central regions of Niger, Upper Volta, Mali and Senegal.

1.2 Factors likely to alter the distribution of the disease

21 The distribution of onchocerciasis is not static. From several parts of Africa evidence exists that the situation has been aggravated by extending and improving agricultural practices through irrigation and open water storage. In many parts of Africa the increasing numbers of dams and causeways, mainly for irrigation purposes, have caused a spread of Simulium vectors, thus extending the area of potential risk of onchocerciasis transmission.

22 On the other hand, the building of dams and the consequent transformation into lakes of stretches of rivers situated above the dams may result, in the elimination of many breeding sites upstream. However, in some cases due to local topographical conditions, new breeding sites appear on tributaries of such lakes.

23 Large-scale movements of population in connexion with development projects may result in the spread of onchocerciasis to new areas.

1.3 Public health importance

24 The most serious consequences of onchocerciasis are impaired vision and blindness. Thus, like trachoma, the disease affects human beings' most valuable and precious faculty, eyesight. The loss of vision not only reduces the working capacity of the population in the endemic areas but has far-reaching repercussions.

25 The relative proportion of ocular manifestations is much higher in onchocerciasis foci than in any other region. Published figures[4] collected by the British Empire Society for the Blind indicate that the number of cases of blindness per 100 000 inhabitants is 250 in Europe, 500 to 1000 where there is endemic trachoma, and 1500 or more in countries where hyperendemic onchocerciasis occurs in some areas (i.e. 1.5%).

26 In some villages of Upper Volta, Ghana and Nigeria the blindness rate reaches about 10%. This corresponds to up to 30 per cent. in the working population and in these areas depopulation is occurring. By comparison, villages free from onchocerciasis but lying in the same geographical region show blindness rates of 0.5 to 1 per cent.

27 The actual situation of blindness in an onchocerciasis endemic area of Northern Ghana, as shown in Map 3, demonstrates the gravity of the problem, especially if one considers that blindness in such hyperendemic areas is often already established in the 20-30 year-old age group.

28 Apart from total blindness of onchocercal origin, various degrees of impairment of vision, which decrease occupational ability to an extent difficult to estimate, must also be taken

into account. Even simple conjunctivitis, because of its lasting nature, is a serious handicap to those affected.

29 Other manifestations of the disease involve primarily the skin and lymphatic system and in their most serious forms can also cause great suffering to the affected individuals. In extreme cases of pruritus and lymphadenopathy, people may be rendered quite incapable of leading a normal life or maintaining normal social relationships.

2. REVIEW OF ONCHOCERCIASIS CONTROL METHODS

30 Control of onchocerciasis may be achieved either by measures designed to control the vector Simulium population or by measures designed to eliminate the microfilarial reservoir from the human population. The latter method is theoretically possible since it is unlikely that any significant animal reservoir exists for Onchocerca volvulus, but it is at present impractical to put it into effect as none of the drugs available is really suitable for use in mass control campaigns. It is greatly to be hoped that further research will be undertaken to find new non-toxic drugs which are active against O. volvulus and which may be used in combination with Simulium control operations to achieve control of onchocerciasis, but for the time being vector control remains the only practical means of reducing transmission on a large scale.

31 Although elimination of the vector may not be necessary in order to stop the reproductive cycle of the parasite, at the present stage of knowledge the maximum degree of control of the vector density should be aimed at. In order to achieve interruption of transmission whenever this seems possible, vector control could be supplemented by measures aimed at reducing the microfilarial reservoir in the human population.

32 The following review refers to new trends and developments that have occurred in control and assessment methods since the Informal Meeting of WHO Advisers on Entomological Needs in Onchocerciasis Control (July 1964)[5] and the meeting of the WHO Expert Committee on Onchocerciasis (June-July 1965).[6]

2.1 Control methods

2.1.1 Vector control

2.1.1.1 Grouhd application of larvicides

33 The objective of larviciding is the elimination of the immature stages of S. damnosum from the rivers of the area under control.

Choice of insecticides

34 DDT has been the insecticide of choice for the past 20 years, and emulsifiable concentrates appear to be the most suitable formulation. Wettable powders, although attractive because of cheapness, have a shorter "carry" than emulsions. Concentration of DDT may vary from 0.01 ppm (p,p'-isonier) to 1.0 ppm per 30 minutes in an inverse relationship to the size of the river. Discharge, velocity and nature of the river also

influence the concentration required. Dieldrin has given good control at concentrations as low as 0.0003 ppm/30 minutes but is not considered suitable because of its high toxicity to man and other vertebrates.

35 Because of the possibility that Simulium larvae may develop resistance to DDT, and because of DDT's persistence and accumulation to texic levels in non-target organisms, field trials have been carried out with a number of other insecticides. Of those tested, methoxychlor, Abate, Dursban, sumithion and carbamyl appear to be the most promising. Methoxychlor is as effective as DDT in killing blackfly larvae when applied in the same time/concentration conditions. It is non-persistent and will not accumulate to toxic levels in fish and other aquatic organisms.

Method of application

36 Simple methods are recommended, the usual method being to let the insecticide drop into the water through a hole in the bottom of a tin.

37 The recommendations as outlined in the report of the WHO Expert Committee on Onchocerciasis (reproduced in Annex 3, section 1) are accepted with the following modifications

> "(6) Dosage be at a rate not exceeding 1.0 ppm and not less than 0.01 ppm maintained over 30 minutes. Discharge can be calculated by measurements of width, average depth and maximum speed of flow in small rivers but in large rivers hydrological data are required."
>
> "(11) The concentration of the insecticide should be calculated on the basis of a theoretical dosing time of 30 minutes. However, the actual dosing time may be reduced to as little as one minute or continued for more than an hour, depending on local circumstances, but the total quantity applied remains the same."

2.1.1.2 Aerial larviciding

38 In large-scale programmes covering a very large area rapid, complete, and effective application of insecticide is of prime importance. These criteria are met with by aerial application techniques.

39 Aerial larviciding for blackfly control has been widely used in heavily forested regions of the United States and Canada with good results for many years. Modern pressure spray equipment was utilized. In Africa, continued aerial spraying was practiced in the Kinshasa area and resulted in the disappearance of Simuliidae for several years. It can be applied in areas otherwise inaccessible or which require extensive road construction. Large areas can be treated quickly allowing more efficient Use of the professional staff. Lower dosages can be resorted to, probably because of better dispersion of the insecticide in the stream, which increases the efficiency of the technique and also reduces the possibility of direct or indirect injury to fish. The dosage is to a great extent automatically regulated by the stream width.

40 Effective dosages in North America have been 15-20% oil solutions of DDT[7] or methoxychlor applied at the rate of one gallon per flight mile in swaths 1/4 mile apart using a wide variety of aircraft with modern spray equipment (e.g. with Piper standard systems, 3-D4 nozzles, 40 psi, flying at 120 mph at tree-top heights). This form of aerial application may be of special value during seasons when rivers are in spate and

inaccessible or, conversely, when very low and divided into a large number of separate breeding areas.

41 Stream treatment by "bombing" from aircraft with cartons containing insecticide may be useful under special circumstances. However, aircraft equipped with conventional insecticide spraying apparatus can do this more accurately and efficiently, and with a more even dispersion of the insecticide, and is therefore to be preferred.

2.1.1.3 Aerial application of adulticides

42 As noted in the WHO Expert Committee Report,[8] DDT aerial spray applied at the rate of 0.2 1b per acre (0.224 kg/hectare) for blackfly control in North America significantly reduces adult fly populations for periods up to ten days. It is a control measure of short duration which quickly reduces adult populations and therefore has some possible use as a supplement to the basic control measure, larviciding.

43 In savanna regions of Africa it may be useful if applied along river margins where the adult blackflies emerge, rest and return to oviposit. It may also be advantageous to form a protection barrier on the periphery of other zones controlled by larviciding.

2.1.1.4 Ground application of adulticides

44 Truck-mounted thermal aerosols are widely used in North America for control of adult blackflies in densely populated areas where there are many access roads. They provide temporary relief from annoyance by killing the flies the insecticide comes in contact with, in very limited areas. 5-8% DDT in oil solutions (fuel oil or heavy aromatic naptha) are commonly used. It is an expensive, non-persistent method of control, probably of little use in onchocerciasis control programmes.

2.1.1.5 Environmental control methods

45 In view of the strict localization of the larval and pupal forms in water-courses or sections of water-courses with adequate speeds of flow, it has been demonstrated that elimination of the indispensable ecological factors renders impossible the development of the vector species. To achieve this, one method is by damming to create reservoirs of sufficient size to eliminate any flow over considerable distances upstream of the dam. The building of these reservoirs will help in the strategy of campaigns by forming protection areas which will reduce the possibility of reinvasion of the controlled areas. Moreover, these lakes, in case of an unexpected interruption of the campaign, could limit the risks of infection in areas recently resettled by non-infected populations.

46 Dam spillways and flood gauges should as far as possible be constructed in such a way as to prevent them forming suitable breeding places for Simulium.

2.1.2 Parasite control

2.1.2.1 Nodulectomy

47 Experience in Guatemala and Mexico has shown that the operation of nodulectomy can be carried out simply and effectively by organized mobile teams, provided they are well equipped and Haye acquired practical experience in this particular form of minor surgery. Regular nodulectomy campaigns have been carried out for the past 25 years, so

that once every 6-12 months all palpable nodules are removed from the population attending. The clinics are popular, attendance is good, and the method is reported to be effective in controlling the high incidence of blindness and of "erisipela de la Costa". In Mexico and Guatemala denodulization has made it possible to reduce the incidence of onchocercal blindness from the 5 to 15% level which prevailed at the beginning of the campaign to the present level of 0.5 to 1%. However, the campaign appears to have brought about little significant reduction in the amount of transmission. Its clinical success in the Central American form of the disease is largely due to the frequency with which palpable nodules are found on the head, leading to early and heavy microfilarial invasion of the eye. Nodulectomy effectively reduces and controls this invasion.

48 The inadequacy of nodulectomy is due to the presence of adult worms which lie deep in the tissues and are impalpable from the surface of the body. One has only to observe the large numbers of microfilariae present in patients who have no palpable nodules to be certain of the existence of these cryptic worms, and worms lying free in the tissues have been reported at operation. In addition, experimental work on chimpanzees has shown that the great majority of the worms in these animals lies deep in the tissues between the muscles and often near the bones and joints (especially around the capsule of the hip joint).

49 As nodulectomy has not been successful, even in Central America, in reducing the microfilarial reservoir in man to a level at which transmission becomes ineffectual, the method can be ruled out as a practical measure to attain this end in Africa. Moreover, as nodules on the head are uncommon in Africa, a reduction in the incidence of blindness is unlikely to be achieved by this means and the operation has never given good results as a control measure.

2.1.2.2 Chemetherapeutic methods

50 The primary aim of a control project is to eliminate the serious eye lesions and other clinical manifestations of the disease. Chemotherapy may play a part in achieving this both by reducing the worm load of an individual to a level at which serious eye lesions no longer occur, and by reducing the microfilarial reservoir in the human population so that treated persons cease to be a source of infection to feeding Simulitdae and transmission is interrupted.

51 While suppressive regimes directed against the microfilariae alone should not be disregarded, provided they can be maintained for the long period necessary before the adult worms die, it is obvious that chemotherapy directed against the adult worms themselves will have a much more prolonged and fundamental action in reducing microfilarial concentrations.

Macrofilaricides

Mel W

52 Until recently this arsenical preparation was thought to hold considerable promise as a macrofi-laricide for mass use by single-shot intramuscular injection. Regrettably, it has since been shown that in a significant proportion of patients fatal arsenical encephalopathies may follow its use, and there is considérable doubt as to the efficacy of

the drug when used in single dose treatments even as high as 10 mg/kg. The use of Mel W in mass campaigns cannot at present be recommended.

Suramin

53 Suramin in adequate dosage (1.0 g weekly to a total of not more than 6.0 g) has proved effective as a macrofilaricide and has also a microfilaricidal action. Severe reactions, including deaths, have been reported when suramin is used (see Annex 3, section 2), and on ethical grounds the drug cannot at present be recommended for mass therapy purely as a means of reducing the reservoir of microfilariae in the human population in an attempt to break transmission. However, where transmission has been reduced by vector control, it may well be justified to treat those persons in a heavily infected population, in whom severe or blinding eye lesions may be anticipated from the effects of their existing load of parasites. Such treatment must be given under medical supervision.

Microfilaricides

Diethylcarbamazine

54 For all practical purposes diethylcarbamazine can be considered as acting against the microfilariae. In mass control schemes it could probably be used with great effect to reduce the microfilarial reservoir in the human population.

55 Treatment consists in giving 50 mg diethylcarbamazine acid citrate once a week until the initial load of microfilariae has been eliminated. This process may take about six to eight weeks, during which time the patient undergoes a series of reactions to the death of microfilariae, declining in severity each week. Thereafter, the weekly dose can be taken with little or no inconvenience and may be continued, if necessary, throughout the life of the adult worms. The life-span of the microfilariae being some 24-30 months, the initial load of microfilariae probably represents about a 12-15 months' supply and, once this has been eliminated, the number reaching the skin each week from the adult worms will thus, in theory, be between l/50th and l/65th of the initial load. The death of this relatively small number each week under diethylcarbamazine can usually be brought about at the expense of an almost insignificant reaction in the patient.

56 Reactions encountered during the early weeks of treatment may be controlled to some extent by simultaneous dosage with antihistamines. However, Mexican workers state that reactions are due to release of serotonin rather than histamine. They have acquired considerable experience of treating reactions with serotonin antagonist drugs. Blind coverage with steroids is not justified in a mass campaign on account of the risks involved with these preparations, but if severe eye reactions are encountered local steroid therapy may be needed.

57 The population to be treated must be warned of the nature and severity of the reaction to be expected following the early doses. It should be explained that this is a result of killing the small worms in their skins, that it is dramatic evidence of the power of the medicine, and that it will be of short duration. Skilful propaganda on these lines may well succeed in overcoming resistance to co-operate.

58 Such a suppressive regime has two great advantages. First, the microfilariae are killed as soon as they arrive in the skin and before they can invade the eye so that its use may serve to halt the progress of eye lesions. Secondly, the patient ceases to be a source of

microfilariae for feeding Simulium and thus a reservoir of transmission. However, the method may be difficult to apply in practice and has never yet been tried on a large scale. If it is considered desirable to use it as an adjunct to a Simulium control programme, it would be wise to conduct a pilot scheme in the first instance in one or two villages (starting with those which are less heavily infected) so as to assess its practicability under field conditions.

2.2 Surveys and assessments

59 Prior to instituting control measures, entomological and epidemiological base-line data should be obtained by means of carefully planned and conducted preliminary surveys. Thereafter, throughout the course of the control campaign, similar surveys should be conducted in order to evaluate the effectiveness of the measures instituted.

2.2.1 Entomological

2.2.1.1 Surveys: Data to be obtained before, during and after treatment

60 (a) Simulium larval survey: This survey should be carried out in all streams throughout the year in the preliminary phase and, during the attack phase in selected points while dosing and after treatment. Quantitative sampling is not practical at present.

61 Careful mapping of all breeding sites is indispensable.

62 (b) Simulium adult survey: Catching female S. damnosum on human bait should be carried out routinely by means of full-day collections made at 1 or 2-week intervals. Such catches must be made at sites inside and outside the treated area and along its margins. Counts should be continued for at least a full year before control is started. The unit is f/m/d (number of flies per man per day). The physiological age of the collected flies should be determined by dissection.

63 (c) Survey of O. volvulus transmission: To obtain information on the level of transmission in any given area the number of O. volvulus infective larvae per fly and the number of infected and infective flies per man per year should be recorded.

2.2.1.2 Evaluation of results

64 (a) Larvae: All S. damnosum larvae should be eliminated by the first treatment and so counts should then be zero.

65 (b) Pupae: The presence and age of pupae after treatment provide supplementary information on the effectiveness of larviciding. Pupae should disappear by the second cycle of treatment.

66 If larvae are not eliminated in the first cycle of treatment, the dosage, or the distance between treatment points, may require adjustment. Should pupae continue to appear, the interval between treatment times should be shortened.

67 (c) Adults: Adult dissections will provide information as follows:

i. Persistence of nulliparous females after treatment indicates that some breeding sites may persist or immigration from untreated areas is occurring.

ii. If control of all breeding sites is complete, with the result that the local fly population has disappeared, an increasing proportion of nullipars indicates a selective migration of young females to the area.

68 (d) O. volvulus transmission: During the campaign, in the absence of invasion from untreated areas, the infection rate will increase temporarily as the average age of surviving females increases.

69 If there is an invasion of flies from outside the control area, the infection rate may decrease because of the lowering of the physiological age of the population.

70 From these data, the number of infective larvae transmitted per man per year can be calculated at different points in the control area. The aim is to reduce transmission at least to levels no higher than those occurring in epidemiologically comparable areas where the disease is present but does not cause disability-in man.

2.2.1.3 Susceptibility of vectors to insectide

71 Decreased susceptibility of Simulium larvae to DDT has been reported in Japan and North America. The possible widespread development of resistance is a factor to be taken into consideration in any large-scale onchocerciasis programme in Africa.

72 A procedure for testing larval resistance has been developed (WHO) and, in modified form, has been extensively used. It can give consistent and homogenous results when carefully used.

73 Susceptibility levels should be routinely checked by discriminating concentrations with modified jar tests in the field and, when detected, followed by more detailed laboratory tests to determine dosage mortality relationships.

2.2.2 Morbidity

2.2.2.1 Objectives

74 The first task is to map the geographical distribution and evaluate the endemicity and severity of onchocerciasis within the country when accurate data are not yet available ("initial prevalence survey").

75 The second task is to record the trend of the disease in relation to control. For this purpose a limited number of indicator areas will be selected ("detailed surveys").

2.2.2.2 Initial prevalence survey

76 This survey should be undertaken to determine the extent of onchocerciasis in a statistically representative number of villages. A qualitative skin snip method as described in the WHO Expert Committee report[9] (see Annex 3, section 3) should be employed, and visual acuity recorded.

2.2.2.3 Detailed surveys of selected indicator areas

77 Along each major river system, areas should be chosen to represent high, medium and low endemic zones (see Annex 3, section 4), care being taken to include communities with high, moderate and low incidence of blindness.

78 The areas chosen should lie in close relation to the entomological assessment sites at which S. damnosum are to be captured (see section 2.2.1.1) so that the effects of the scheme on the amount of transmission can be related to its effects on the disease in the human population.

79 Wherever possible, the entire population of each indicator area should be examined and basic data recorded on each person using the Individual Onchocerciasis Record Card suggested by the WHO Expert Committee (see Annex 3, section 7).

80 The first detailed survey must be conducted immediately before, or at the time, control commences. It should be repeated at 2-3 year intervals, using the same methods and if possible the same personnel.

81 Although in general agreement with the Expert Committee report,[10] the following points are believed to provide important comparative data for the assessment of the success of the campaign.

82 (a) Two approaches might be used in the parasitological assessment of the campaign.

i. Study of selected age groups, by comparing the incidence and intensity of infection in given age and sex groups. The findings should be particularly significant in children;
ii. Follow-up of the progress of the disease in individuals chosen from those examined at the initial survey (cohort study).

83 As there is a time lag between the acquisition of infection and the appearance of microfilariae in the skin detectable by skin snip, it is important that as many cases as possible should be detected in the younger age groups since if they are missed and later show a positive skin snip, they might lead to the erroneous conclusion that infection has been acquired since the initial survey. As a supplementary investigation the Mazzotti test could be employed to identify early infections in the younger age groups. It was reported that this method has proved of greater value in mass surveys than anticipated (see Annex 3, section 5), provided the area is not heavily infected by other filarial diseases.

84 (b) The most important ill effects of the disease are impaired vision and blindness An ophthalmologist, equipped with a mobile slit lamp, must be made available to survey the indicator areas in the control area. He should complete the ophthalmological sections of the Individual Onchocerciasis Record Cards, adding a record of fluffy stromal corneal opacities typical of onchocerciasis and of other causes of impaired vision and blindness.

85 (c) The first survey in the indicator areas will provide data which give a base-line. Subsequent surveys carried out by the same methods will then be comparable.

86 (d) In section 2.1.2.2, treatment is suggested for those persons considered to be under a continuing threat of blindness from their existing worm load. The evolution of the eye lesions in these treated patients should be compared with that in untreated patients also living in the control area.

2.3 Examples of success and failure of control schemes

87 Although the Mayo Kebbi project in Chad could have been successful due to the area being partly isolated, its relative failure may be ascribed to:

a. insufficient co-operation between the various units of the project;
b. the lack of planning (too short preliminary surveys);
c. incomplete assessment of results;

d. indiscriminate use of sophisticated techniques without preliminary study of their applicability under local conditions.

88 At Abuja, Northern Nigeria, where 100 miles (-160 km) of rivers in an area of 1200 square miles (3100 km^2) have been treated annually during the first three months of the wet season since 1957, the S. damnosum biting density has been reduced by over 90% to usually less than one bite per man per hour. In spite of this reduction skin snip surveys show that, although there is an indication that the microfilaria density has been reduced, there is no doubt that transmission is still taking place. The fly density measurements in this scheme were based on weekly 15 minute catches at 43 sites within the controlled area. It is thought that full-day catches might have given a better estimate of the Simulium density, and also provided larger numbers of flies so that age and infectivity dissections would have had greater meaning.

89 The Sikasso focus in Mali has been under treatment for several years and it has been possible, by adapting the treatment to local conditions, by keeping the zone under close surveillance, and by eliminating existing possibilities of reinfestation through the establishment of larvicide barriers, to obtain a considerable reduction in the density of the vector species. Except for one point in the downstream part of the zone where a very limited population of vector females (2% of the pre-campaign population) still persists, it can be considered at present that transmission has practically ceased throughout the zone, as shown by entomological and clinical assessments.

90 Control of S. damnosum in the Victoria Nile in eastern Uganda was first instituted in 1952, when DDT was applied as a larvicide at a rate of 1 ppm/30 min for 10 weekly applications. Eradication was achieved, but reinfestation occurred in 1953. Subsequent treatments in 1956, 1961 and 1964 were necessary to maintain a fly-free state. The picture is one of intermittent eradication for periods of about four years. The source of reinfestation is not known.

91 At this stage, when the control areas are exposed to reinfestation from outside, as in Abuja, Murchison Nile and Sikasso, the campaigns must be continued on a maintenance basis, otherwise the S. damnosum problem will quickly return.

2.4 Problems involved in onchocerciasis control

2.4.1 Problems related to vector control in large-scale projects[11]

92 With large-scale control the problems of transport, staff, and logistics, in general, will be multiplied many times. The problems of collecting data for evaluation will also be formidable. Methods that are satisfactory for small areas of 10 000 square miles (25 000 $km^{2)}$ or less may be unsatisfactory when areas of 50 000 square miles (130 000 $km^{2)}$ or more are to be put under control as too many man hours of professional and auxiliary staff would be required to obtain the necessary data for evaluation. Therefore, modifications of these methods and statistically valid sampling procedures may be necessary.

2.4.1.1 Adaptation of methods proposed

93 Checking of every single breeding site after treatment has commenced, over a large area may prove beyond the capabilities of any team that could be put in the field. Therefore,

(1) sampling of larval populations could possibly be restricted to selected sites after treatment to assess the reduction in larval population following control; (2) dissection of female flies collected at selected sites should be carried out to determine density, physiological age and infectivity within control areas. All flies thus collected should be retained for identification and as large a proportion as possible dissected.

2.4.1.2 Use of aircraft

94 The total area should be subjected to a preliminary aerial reconnaissance to enable the area to be divided into sub-areas which can be covered by a qualified person according to the peculiar topographical difficulties. For example, in savanna areas, it has been found that an entomologist can cover effectively for ground survey and control 100 miles (160 km) of river per year. This represents only a small part of a focal area and will permit a calculation of the number of personnel, transport, etc. to be made at an early stage in the Project.

95 Aircraft should be available throughout the scheme to be used for the following tasks:

a. locating possible breeding sites under all rlverine conditions;
b. observing present tracks and roads and planning others necessary to reach the rivera;
c. transport of the entomologist in charge around the project area to maintain close liaison with the other personnel involved;
d. aerial larviciding, especially in breeding sites otherwise inaccessible;
e. aerial adulticiding, if the necessity arises.

2.4.1.3 Alternative insecticide and insecticide formulations

96 DDT persistence and recycling is a serious problem as this material may accumulate to toxic levels in fish and other organisms. Consequently a scientist should monitor the effects of treatment on fish and other aquatic life.

97 The exclusive use of DDT raises the problem of resistance. Susceptibility levels should be established at the beginning of the programme and their trend should be followed while it is in progress. This will require special facilities.

98 The possible deleterious effect of DDT on the aquatic biotype combined with the possibility that resistance may develop to DDT, make it imperative that satisfactory alternative insecticides be sought for by a screening and field testing programme.

2.4.2 Other problems

99 Sero-immunological methods might be of great help in evaluation but methods suitable for large-scale use under field conditions have yet to be developed.

2.5 Conclusions

100 From the epidemiological and entomological viewpoints, it has been demonstrated that onchocerciasis control is technically possible in Africa. Adequate control of the vector S. damnosum can be achieved using the methods already available, and this alone will result in a very great reduction in transmission.

101 The effects of vector control could be greatly enhanced if this method could be supplemented by chemotherapeutic control designed to reduce the microfilarial reservoir in the human population. However, the drugs at present available all suffer from disadvantages which limit their use on a large scale, and there is urgent need for further research to develop new filaricides active against O. volvulus.

3. FEASIBILITY OF ONCHOCERCIASIS CONTROL

102 While it is technically possible to achieve onchocerciasis control with the methods now available, the question remains as to whether or not it is feasible, i.e. under what set of circumstances is control practicable, justified and advisable. In order to answer this question, a detailed examination of the needs for control and of the related economic, technical, financial and organizational aspects is necessary. Afterwards it can be decided where and how a proposed project can be carried out with the maximum chances of success and benefit to the population concerned.

3.1 General economic considerations

3.1.1 Needs for economic considerations in public health

103 In most tropical countries a dynamic process of development has taken place covering a multitude of fields ineluding public health improvement. However, as the financial and manpower resources of these countries are usually very limited, a sound economic approach to development planning is an essential requirement which applies equally to public health development schemes. Consequently, socio-economic factors have to be considered from the beginning in the planning and programming of health activities. There are, in particular, three points which call for reasonably accurate information on the economic impact of a disease, the cost of its control and an estimation of the control benefits:

a. the establishment of priorities in the planning of health programmes;
b. the procurement of assistance in the financing of specific disease control programmes;
c. the interrelationship of health development with the country's over-all development plan, in particular with projects like water and/or agricultural development schemes which may have a direct bearing on health.

104 The economic effects of controlling different crippling diseases should be taken into account when weighing up the technical reasons for choosing to mount one sort of disease campaign rather than another. Priorities must be established in relation to practicability, costs and probable benefits to the human population. These priorities could well be different from one region to another and from one morbid condition to another.

105 The aim of bringing an economic concept into public health planning is relatively new and there is not much previous experience and knowledge of its mechanisms. It requires the co-ordination of different disciplines which still have to learn each other's way of thinking and language as well as gain the full understanding of each other's problems. A new methodology has to be developed which satisfies all the various scientific elements and which will lead to the information aimed at.

3.1.2 Common conditions of parasitic diseases relevant to assessment of their economic impact

106 Most parasitic diseases have a number of conditions in common which have an influence on the economic aspects and thus justify the separation of this disease group from others in regard to the assessment of its health implications:

the majority of parasitic diseases occur at present in developing countries of the tropics where frequently no state social security organization exists which could provide a sufficient source of reliable data;

they are predominantly diseases affecting rural populations for which social, economic and health information is often difficult to obtain. At the same time it is the rural communities which are involved in agricultural improvement plans;

the clinical manifestations of many parasitic infections are of a chronic nature developing slowly without a dramatic onset. Hence they are frequently overlooked with the result that morbidity data are unreliable sources grossly underestimating the actual situation; the pathological changes of several infections are still under investigation and the establishment of their public health importance is still in progress;

in many instances the parasitic diseases are water-related infections, that is, their degree of transmission is influenced by the proximity of water. Hence, development programmes such as agricultural irrigation, creation, of lakes, etc., will have a bearing on the distribution and level of endemicity of many parasitic diseases; the same applies also to uncontrolled urbanization;

apart from the direct effect of the infection on man, there exists also an indirect effect on the community involving elements such as nutrition, demography, social structure, etc

3.1.3 General considerations of economics in the appraisal of health problems

107 In the context of development planning an appraisal of health problems from an economic point of view is required primarily as a basis for advising on the establishment of priorities within the health budget. It can also be envisaged that a private or international financial organization which receives a request to finance a health programme by loans is likely to interest itself in the narrow economic returns of such a programme, e.g. by means of cost-benefit analysis.

108 It should be emphasized that cost-benefit analysis cannot be applied to all health policy decisions in developing countries. In particular, we are unable to calculate the value of a human life but, nevertheless, we should make a great error by not taking this value into account by considering it as nil, solely because we cannot give a precise answer to the question.

109 It is true that any economic policy implies a precise evaluation of costs, and that we must develop the analysis of costs in the framework of health programmes. The main difficulty lies in the evaluation of nett advantages or benefits.

110 This difficulty does not appear only in health policy. More generally, it concerns a great part of the economic policies of developing countries, in which it is impossible to apply cost-benefit analysis in the way that this technique has been used for the study of specific projects in North America and Western Europe. This is due to two series of factors

i. by their very nature, developing countries have not reached a stage of stable economic organization where present costs and prices of inputs and outputs can be used as terms of reference. Large development projects, for example, inevitably change wage levels and prices throughout a substantial part of the economy of a developing country;

ii. particularly in developing countries, one must consider a number of strategic choices rather than a single project. The nett benefits of any of these projects will depend, to a crucial extent, on complementary and supplementary action on other fronts.

111 When a private commercial firm or financial organization is requested to finance a programme - even a health programme - it is likely to focus its attention, for example with the aid of cost-benefit analysis, on the direct economic returns of the programme. But economists, and particularly development economists, are not absolutely obliged to use narrow quantitative criteria, particularly when this technique is reducing the usefulness of their analysis. Their aim is to present choices between suitable costed alternative projects. Agricultural development, for example, has a high priority rating in most developing countries, and comparisons between various possible uses of natural resources would be useful.

112 In the field of health policy, one should bear in mind that, even if it is impossible to evaluate a human life, an immeasurably positive effect on rural populations might be obtained simply by demonstrating to them that Simulium damnosum can be controlled.

113 Attention must therefore be focused (a) on the practicable policy choices concerning people afflicted with a variety of parasitic diseases, and (b) on obtaining information about areas and costs of research and about partial and specific benefits; e.g. from curing onchocerciasis and preventing blindness due to this disease.

114 The projects could range from relating the costs of research on onchocerciasis control to the question of whether a depopulated valley should be resettled or the development of a programme combinfng several mutually interacting public health measures,

115 This general framework being clarified, it is useful to reaffirm the absolute necessity of gaining better knowledge of different aspects of the economic impact of each health programme. For example, the evaluation of benefits of onchocerciasis control will turn mainly on the increased employability of healthier men. At the same time, however, specific investigation of the economic effects of preventing blindness due to onchocerciasis seems to offer scope for a reasonably precise appraisal.

116 It is however important not to consider individual projects apart from each other, but to keep in mind alternative feasible programmes, and to establish for each programme the time sequence or planning of expenditure. In particular, it is always important to link any health project to general socio-economic development policies, and specially to socioeconomic planning. Onchocerciasis control policy must be a part of general health planning, which in turn must be a part of socio-economic planning. This is equally true in regions where at present no specific development programmes exist, but where it is necessary to control parasitic diseases.

3.2 Criteria to be considered in the selection of an area

117 The principles on which priorities for a control project area should be decided include the following:

1. the area must include foci of high onchocerciasis endemicity with high rates of morbidity, particularly blindness;

2. the presence of the disease must constitute a severe economic drain on the community, particularly by denying use of fertile land in river valleys and by producing local depopulation;
3. there should be evidence from past experience that Simulium control therein can be carried out effectively and at reasonable cost;
4. there should be available a high human population density in nearby zones, which can repopulate and develop any land reclaimed by control operations;
5. there should be economic or development projects envisaged in the area which would benefit from Simulium control;
6. the government or governments of the countries concerned must be anxious to institute control, to co-operate with one another in this respect, and to create conditions favourable to the smooth running of control operations;
7. it is desirable that experience gained in the control area should be likely to prove of practical application to other river systems later on;
8. there should be available basic epidemiological, entomological, topographic, hydrological and sociological data on the area concerned, information which can only be accumulated over some years of careful recording,

118 It is recognized that investigations, carried out for the purpose of collecting information required to assess whether the conditions prevailing in a particular area meet the above criteria, are lengthy and expensive.

3.3 Selection of recommended area

119 In considering where financial-aid for onchocerciasis control projects could best be given with the prospect of producing the greatest return in terms of benefit to the human community, it is profitable to consider all those regions of the world in which the disease is endemic and then to proceed first by a process of elimination, and later by a positive pproach to select a suitable control area.

120 In the context of the present meeting the onchocerciasis areas in America were excluded, because it was believed that some of those organizations which might consider financing onchocerciasis control projects would be to a great extent committed to using their resources on the continent of Africa. However, it was noted that in America, especially in Guatemala, the existing programme for the control of onchocerciasis (Robles' disease), which is based oh nodulectomy, is in urgent need of international assistance so that operations may be extended particularly in the field of entomological control. Technical advisers, particularly an entomologist for a 1-2 year assignment, complementary salaries, ophthalmological equipment and vehicles are needed. The meeting accordingly recommended that the Pan American Health Organization and the office of the Agency for International Development should be asked to give urgent consideration towards supporting and extending the programme of onchocerciasis control in the Americas.

121 The endemic area of onchocerciasis in Africa may, broadly speaking, be divided into two epidemiological zones extending east-west across the continent. There is a forest zone to the south where the disease is widespread but in which the infections, even when intense in dermatological manifestation, are only occasionally associated with blindness; and there is the savanna zone to the north, where, in many localized areas of intense transmission, onchocerciasis is responsible for blindness rates of 10% and more and has caused the inhabitants to desert many fertile river valleys. It is in this savanna zone that

control is most urgently required and it includes foci of blinding onchocerciasis of differing size and importance which are known to exist in Senegal, Mali, Guinea, Upper Volta, Ivory Coast, Ghana, Niger, Dahomey, Nigeria, Cameroon, Chad, the Central African Republic, the Democratic Republic of the Congo and the Sudan Republic.

122 It is considered that the proposed first large-scale control scheme must be carried out under the best conditions obtainable so that it may be given every chance of success and thus prove to be an exemplary precursor to more extensive operations throughout the whole African savanna zone.

123 For a variety of reasons the foci in those countries lying in the eastern half of the savanna zone do not appear at present to offer conditions suitable for a large-scale control programme.

124 The group of foci lying in Senegal, western Mali and Guinea were considered as highly deserving of attention but suffer at present from the disadvantages of seasonal lack of access to the Senegal focus and shortage of basic entomological data in some of the foci which would take several years to collect.

125 There remained then the infamous foci of onchocerciasis situated in the centre of the West African savanna zone and embracing parts of Ghana, Upper Volta, Togo, eastern Mali, Ivory Coast, Dahomey, and Niger; and it is indeed fortunate that choice of these areas should at the same time be supported by many positive arguments. The selected area is outlined on Map 4 and for it a great deal of the basic entomological and epidemiological data necessary to a control programme are already available. The scourge of onchocerciasis shows its sorry effects on the economy of the countries, the suffering population is potentially industrious, active and desirous of progress, and in so far as naturel barriers against reinvasion can be said to exist in.the zone, the area is relatively well protected. In addition, well planned small scale control schemes are already in progress in this zone of evident priority. Two schemes are at present under progress: the first one is sponsored and put into effect by the governments of Ghana, Togo and Upper Volta (with the participation of OCCGE teams in the case of Upper Volta) and with the effective collaboration of WHO.[12] The second one is under the charge of three States members of OCCGE, Mali, Upper Volta and Ivory Coast, with assistance from FAC,[13] FED[14] and ORSTOM, and with WHO's support (for research and development) In fact, a substantial financial effort is already being made by the countries in the recommended area.

126 It was considered that aid for onchocerciasis control could be given with maximum effect to a scheme designed (i) to unite and consolidate the control measures already effective in this area, (ii) to extend the operation so as to cover the whole of headwaters of the Volta system and the tributaries of neighbouring river systems from which reinvasion of S. damnosum may be expected, and (iii) to maintain the scheme for period of 10-15 years so as to permit the Virtual elimination of the parasite O. volvulus from the human population, meanwhile integrating operations with the health services of the countries concerned.

127 The hope was expressed that control operations would later be extended both to the east and to the west of the recommended zone; and that further meetings would be held at a later date to consider means of effecting this.

128 Finally, consideration was given to the existing state of affairs in other zones wherein onchocerciasis is widespread and of severity as a blinding disease but where the basic data and infrastructure necessary for control are not yet available.

129 Among those in West Africa were the foci in Senegal, in the west of Mali and in north Guinea. It was noted that many basic data on the last of these areas have been collected by the joint mission organized by the Governments of Guinea and of the Federal Republic of Germany in 1963-67, but that at present there was a lack of sufficiently trained staff to undertake control. Along with them, the foci in Nigeria, Uganda and Sudan were also considered as areas in which it would be desirable to collect further data so as to interest those institutions, which, in their desire to encourage economic development of Africa, might consider giving further aid to onchocerciasis control.

3.4 Economic considerations in the recommended area

130 There are the usual difficulties in presenting figures from African countries; mainly, in this instance, those of evaluating the proportions of areas and populations that are actually exposed to Simulium bites and onchocerciasis infection. For purposes of collecting information, the recommended zone was arbitrarily divided into six parts, and Table 1 below gives figures many of which, it must be realized, are approximations.

TABLE 1. ECONOMIC DATA (APPROXIMATIONS) IN RELATION TO ONCHOCERCIASIS IN THE RECOMMENDED PROJECT AREA

Subzone	Area (km^2)	Population	Infected with onchocerciasis	Blind	Total uninhabited areas	Uninhabited land capable of cultivation (hectares)
Red & White Volta (Upper Volta)	50 000	800 000	140 000	8 000	1 500 000	300 000
Red & White Volta (Ghana)	15 000	800 000	134 000	7 500	100 000	100 000
Black Volta (Ghana)	25 000	200 000	14 000	280	260 000	260 000
Banfora (Upper Volta)	20 000	145 000	45 000	2 900	700 000	300 000
Sikasso (Mali)	4 000	60 000*	22 000	1 200	30 000	30 000
Korhogo (Ivory Coast)	42 000	820 000	96 800	1 000	500 000	100 000
Black Volta (except Ghana) N. of 10°N	50 000	195 000	47 000	980	750 000	Uncertain
Total	206 000	3 020 000	498 800	21 860	3 840 000	1 090 000

* Includes Sikasso town, 20 000.

131 The various sectors are inhabited by farmers principally raising food crops. These farmers are in the main capable of substantially increasing their productivity provided that the accessibility of the endemic zones, the dissemination of improved agricultural techniques, and the organization of market outlets permit.

132 The zone comprises the worst focus of onchocerciasis in the world, with local blindness rates of up to 15% and all the other ill-effects of onchocerciasis highly prevalent. Blindness is commonest in males over 30 years of age, and 20-25% of these may be blind in a seriously affected village. The labour force is correspondingly depleted. The age-sex population structure shows a marked deficiency in the young male age group. These young men have little incentive to remain at home where fertile land on which to cultivate food and cash crops is denied to them and, in consequence, many of them migrate in search of better economic conditions, e.g. to cocoa farms, mines, etc. of Ashanti or Southern Ivory Coast. As a rule, they return home eventually. However the combined weakening of labour effort resulting from blindness in men in their prime and from migration of young adults can very easily result in a village community starving from simple lack of agricultural manpower.

3.4.1 Estimated costs of onchocerciasis control

133 The cost of onchocerciasis control in the recommended area, based on present expenditures for existihg control services and control operations carried out in the area might reach US$ 2 512 000 a year at its outside limit.[15]

134 Expenditure for the recommended area will be on a substantial scale. However, because control would be more effective when covering a large area and, depending on a wellestablished time-table of operations the total duration of the campaign could be shortened it may be possible to reduce the estimated cost to US$ 2 million or even 1.5 million.

3.4.2 Benefits anticipated from onchocerciasis control

Health

135 In the whole area there are approximately 626 000 persons infected with onchocerciasis, of whom approximately 52 000 are blind. The other effects of heavy onchecerciasis infection, pruritus, debility, probably scrotal elephantiasis, etc., have been described in detail on many occasions. They add up to a great sum of human misery. The first - though not the earliest in time - effect of onchocerciasis control will be the relief of suffering and an increase in the work capacity of the community.

136 The potential increase in communal work capacity can be partially calculated. Blindness falls mostly on adult males over 30 years of age, and one may estimate not too fancifully that the removal of onchocerciasis would in the future for this reason alone make fit for work 20 to 30 thousand men who would otherwise be an economic liability.

137 Secondly, the population structure of onchocerciasis foci (where it is known) shows a marked deficiency in the young male adult groups. For example, in the Upper Volta rural areas the proportion of adult males to females is 100:143 (SEDES[16] report, 1965). In Tumu district, Ghana, the proportion of males to females in the 15-39 years age group is 3:4. In Frafra district, Ghana, there is the even more striking male-female ratio in this age group of 15:26 (Ghana population Census 1960).

138 Admittedly if the young men are emigrating, they are working somewhere. Nevertheless, restoration of well-being in the onchocerciasis areas and the release of farming land at present uninhabitable would offer more inducement to remain at home. One may assume that a normally balanced population structure is a desirable objective.

139 The sum of human inefficiency due to the other effects of onchocerciasis is not calculable without ad hoc investigations that have not yet been undertaken. Anyone with personal experience of living among onchocerciasis sufferers knows well enough that considerable debility is caused.

Training

140 In the course of the project several hundred men will receive training in epidemiological and entomological techniques, carried out in field conditions. These men will constitute a very great contribution to the health infrastructure of the region, and an asset to be reckoned on when campaigns against other mass endemic diseases are planned.

Access to water

141 The savanna zone is traversed by many rivers, but for most of the year is otherwise dry. The mere fact that life can be made possible closer to rivers constitutes an economic benefit in transport of water - either by human power or mechanical means.

Fishing

142 At present virtually the only fishermen in the whole area are immigrants. They prefer to dry their fish and export it towards the coast. Economic development in the region would create a demand for this source of valuable protein.

Protection of labour forces in development projects

143 It is necessary to protect labour forces working on hydro-electric and irrigation projects from Simulium attacks and from onchocerciasis. Key personnel, whose services are essential in the construction of major capital works, have a dread of onchocerciasis which has to be taken into account. In the area of Kainji dam in Nigeria, the cost of Simulium control has averaged $ 70 000 a year.

Psychological effects

144 The control of Simulium and of onchocerciasis will be an unmistakable demonstration to the people of the area (who suffer probably the heaviest load of endemic disease of any community in the world) that amelioration of their conditions of life is indeed possible, thus creating an atmosphere favourable to plans for increasing productivity.

3.4.3 Benefits anticipated from redevelopment of river valleys

145 Table I (see above) shows that in the recommended area the unused land capable of being put to agricultural production amounts to 1 090 000 hectares. Care has been taken not to make this figure grossly optimistic. For example in Upper Volta 300 000 hectares of unused land could be developed for pastoral use.

146 A general indication of the additional farm revenue obtainable in Upper Volta, taken as a whole, can be gained from the following table.

Type of farming	Annual revenue/hectare (US$)
1. Traditional subsistence farming on plateaux	16
2. Traditional subsistence farming in valleys	24
3. Traditional supervised (co-operative) farming operations	32
4. Modern mixed farming schemes: (a) dry cultivation (b) irrigated	 56 240

147 In Northern and Upper Ghana it appears that the productive value of land is somewhat higher:

Potential gross value per hectare of rain-fed crops, Ghana

Crop	Annual revenue/hectare (US$)
Guinea corn and millet	96
Maize	96
Ground nuts (shelled)	100
Rice (in grain)	80
Cow peas	40
Tobacco (dry leaf)	240
Vegetables	450

148 It is stated that these yields could be doubled by irrigation.

149 Tentative calculations can be made of the gross annual income at different yields per hectare. It must be assumed that each additional hectare under active cultivation by traditional methods requires the work of two adults. The table below provides an indication of alternative combinations of manpower and yields per hectare which would provide sufficient revenue to offset the upper estimated annual cost of Simulium control, i.e. $ 2.5 million a year.

Estimate of annual gross revenue (US$ millions) according to different combinations of yields and hectares under active cultivation

No. of hectares (thousands)	Yield per hectare (US$)			
	40	60	80	100
20		1.2	1.6	2.0
30	1.2	1.8	2.4	3.0
40	1.6	2.4	3.2	4.0
50	2.0	3.0	4.0	5.0
60	2.4	3.6	4.8	6.0

150 It can be seen that 60 000 men would need to produce between $ 80 and $ 100 a year by traditional agriculture to offset the cost of Simulium control, whereas at yields of $ 40 a hectare, more than 120 000 men would need to be employed.

3.4.4 Conclusions

151 While the costs of Simulium control for the whole area may seem considerable, nevertheless the benefits to be expected both in human and material improvement are sufficiently great to encourage the belief that a campaign for the control of Simulium would be worth while from an economic standpoint and further justified since population increases rapidly in areas close to onchocerciasis foci.

152 It is still necessary, however, to verify and complete certain sources of information both about estimated costs and, more especially, concerning the potential benefits to be obtained in different parts of the recommended area.

153 At the present time the only dossier which exists, although it is not complete, concerns the Volta Basin and its adjacent areas.

154 The dramatic improvement in human well-being that will follow the control of Simulium is not open to doubt. Nevertheless, it must be emphasized that any onchocerciasis control activity that is not accompanied by organized economic development of the reclaimed lands (including better farming methods to increase cash revenues) will fail to enable the countries benefiting therefrom to take charge of the maintenance of control after the initial phases. The associated economic studies must therefore pave the way for the beneficiary countries to participate in, and later assume in full, the financing of the campaign. It is essential that international organizations mainly concerned with agricultural development in tropical countries should be consulted and their participation sought in programmes that will be associated with the control of onchocerciasis.

155 It is of course a sine qua non that the first move in the search for sources of financial aid to carry out widespread and costly control measures should come from the government or governments of the countries concerned.

4. REQUIREMENTS FOR RECOMMENDED AREA AND PLANNING OF PROJECT

4.1 General principles

156 A mass campaign against onchocerciasis should aim at the eradication of this endemic disease. Although this may prove technically difficult, it is imperative that a degree of control such as to render impossible any serious - especially ocular - complications should be attained. It should make possible resumption by the riparian populations of a life that is normal in every respect, and even resettlement in the area if the scourge of onchocerciasis has driven them away from it.

157 A reduction in vector density such as has been obtained by certain larviciding campaigns already in operation is, by itself, capable of lowering the transmission potential of the vector Simulium population to a point where severe eye complications - impaired vision and blindness become very rare.

158 In practice, there will be no difference in the methods used to achieve eradication and maximum control of the vector. The different phases to be planned and the resources to be brought to bear are the same in both cases.

159 Even in an extensive regional-type focus, if the natural or artificial barriers are effective, if insecticiding activities have been properly carried out and evaluated throughout the treated zone, it should be possible to attain a satisfactory end result with the virtual elimination of transmission. This may be obtained either by elimination of the vector (e.g. S. neavei in Kenya) or by complete control in all breeding sites (e.g. Victoria Nile or Sikasso area), or even by slow attrition as in the Budongo area (Uganda).

160 The operational phases of a campaign against onchocerciasis will be in some ways similar to the well-mapped-out phases of a campaign against malaria:

1. Preparatory phase;
2. Attack phase;
3. Maintenance phase.

161 Adequate evaluation must be made throughout the campaign.

Preparatory phase

162 Its duration should be at least three years and it should provide for the following:

163 (1) An adequate survey of human onchocerciasis prevalence should be made by existing health personnel using standard methods as recommended by WHO (see section 2.2.2). If the survey cannot be carried out by existing health services, teams specially trained for the purpose should be formed. Standard maps should be drawn up with conventional signs showing the degrees of infection at the district level (districts coloured or marked in black and white according to the prevalence and severity of the disease, as far as possible). These maps should be kept up to date as accurately as possible (using medical record cards) in every hyper-endemic focus. Experience has shown that national major endemic disease control services are the most suitable bodies for drawing up such exhaustive inventories, for which they generally have the necessary experience and equipment.

164 In hyper-endemic foci a full-scale ophthalmological survey by a specialist is indispensable before undertaking larviciding.

165 (2) Collection of all necessary data on topography, climatology, hydrology and entomology (see section 2.2.1).

166 (3 Advantage should be taken of this period for recruitment of staff, for their specialized theoretical and practical training, and for their installation. This applies to necessary staff at all levels.

Attack phase

167 This should follow immediately the preparatory phase. Control methods (e.g. insecticide treatment, damming of rivers, etc.) found effective in the preparatory phase, will be applied according to the methodology developed during that phase. Technical responsibility will be exercised by the chief entomologist, as the specialist responsible for the antivector campaign, on the understanding that all the necessary logistic means have been placed at his disposal in due time together with adequate facilities for maintenance treatment.

168 In an extensive regional type project it is impossible to specify precisely in advance the duration of the attack phase. Flexibility must be allowed for in regard to certain procedures and even to the duration of the attack phase, for experience has already shown that. Imponderables - in particular anormal rainfall, unforeseen difficulties in vector surveys both at the larval and adult stages, etc. - nearly, always arise.

Maintenance phase

169 The duration of this phase will be long if eradication of the vector proves difficult to achieve. Normally the maintenance phase will be the responsibility - with or without outside assistance - of the beneficiary States that have participated in the regional campaign. Its annual cost should be much lower than that of the preceding phases. Under the responsibility of the States, it can be taken in charge by the national staff trained during the mass campaign.

4.2 Time-table and phasing in the recommended area

170 The time-table is deduced from the preceding general principles applied to the local conditions. The recommended area contains not only foci where the vector population is present all the year round but also areas where for part of the dry season it is not present. In the sequence of operations the permanent breeding foci, and especially those in which breeding persists during the dry season, must be attacked first.

171 When funds, personnel and equipment are available, the following programme could be followed:

1st Year:	(1)	Control in area of AFRO-0131 project;[17]
	(2)	extension of control to Comoe-Leraba area (FED project);[18]
	(3)	continuation of control in Sikasso and Korhogo area (FED project).

2nd Year:	(1)	Continuation of 1st Year's programme;
	(2)	last surveys and planning of control in Black Volta/Bougouri-Ba area.
3rd Year:	(1)	Continuation of 2nd Year's programme;
	(2)	Initial control of Black Volta/Bougouri-Ba area (FED project and AFRO-0131, Ghana).
4th Year:	(1)	Continuation of 3rd Year'sprogramme;
	(2)	review of contact zone east of AFRO-0131 project area (Niger and Northern Dahomey foci).
5th Year:	(1)	Continuation of preceding programme;
	(2)	initial control of eastern contact zone (Niger and Northern Dahomey foci).
6th Year:	(1)	Continuation of 5th Year's programme;
	(2)	evaluation of results.
7th Year)		
8th Year)	As 6th Year.	
9th Year)		
10th Year:	(a)	Dependent on results;
	(b)	possibilities of national services adopting the responsibilities for maintenance service.

172 Note: Concerning the eastern zone, which comprises the western Niger foci and the northern Dahomean focus, control will be possible only after the permanent breeding sites of Ghana and Togo have been treated (see first paragraph of section 4.2). Moreover, the complete survey and collection of data have yet to be achieved.

173 The timing of the hoped-for extension of operations outside the initial control area into Guinea, Senegal and other countries will be dependent on the rapidity with which basic information is provided from these zones and it cannot be stipulated in advance (see requirements for operational phase, section 2.2).

4.3 Organization and staffing

174 For operational reasons the following organization is proposed in the recommended area:

4.3.1 Headquarters

4.3.1.1 Functions of headquarters

175 The planning, direction, co-ordination and administration of the campaign, as well as the training of staff devolve upon headquarters. Planning must be based on required entomological and epidemiological data collected during the initial field surveys. Co-ordination is needed between onchocerciasis control services and also between them and all services or agencies interested in rural, industrial and economic promotion in the area. This is especially true in the case of all the water-development projects.

176 Specific functions of headquarters include: logistics, accounting, cost-analysis, collection and analysis of data, exchange of information, assessment of the campaign.

4.3.1.2 Headquarters staff

177 The staff at headquarters consists of:

- the Project epidemiologist and the Project entomologist responsible for the total onchocerciasis control operations;
- a consultant ophthalmologist who will assist, at all phases of the project in the planning of epidemiological investigations on morbidity (see 2.2.2) and assess survey data on prevalence and severity of onchocercal eye lesions; consultants for special technical fields as required by epidemiological circumstances, as well as consultants during the staff training period;
- to each the Project epidemiologist and the Project entomologist is attached a junior to provide inter alia for liaison periods;
- a finance administrator (and necessary staff);
- a vector control engineer;
- a hydrological engineer.

178 Headquarters needs also common services for the operation of the project, such as:

- mechanics, aircraft personnel (subject to the development and approval of aerial spraying techniques, some of the field teams would be replaced by aerial units, with resulting changes in organization), a statistician, draughtsmen, secretarial and clerical staff;
- a central laboratory (parasitology and entomology). At least two technicians are required, one of whom must be a technician in chemistry.

4.3.1.3 Operational organization

179 For surveys and control operations, field units should be organized as follows.

4.3.2 Entomological units

180 Vector surveys to collect data before, during and after treatment (see 2.2.1) and control operations are carried out by entomological units according to the project plan.

181 Each unit is superVised by an entomologist. At the unit's base, the entomologist is assisted by the following staff; an entomological assistant, an administrative assistant to handle routine work such as finance and supplies, a secretary, two drivers and six general labourers.

182 A unit would comprise two to four teams. Each team would consist of: one field officer, three or four field assistants, five drivers, 12-18 labourers.

183 The number of teams that could normally be included in an entomological unit would depend not only on the area and rivers concerned, but also on the quality and reliability of the individual field officers and their supervisors.

184 Each field team could command a stretch of river 100 miles (160 km) in length making the unit responsible for a minimum of 400 miles (640 km). Such a unit is capable of dealing with approximately 30 000 km^2.

185 An entomological unit would be responsible for the pre-control surveys and assessment in its area. Once larviciding is begun the same unit wouid carry out both the dosing of the rivers and the assessment techniques.

186 The entomologist in charge of a unit ought to be authorized to concentrate at any moment as many of his teams on a given part of his area as the situation requires.

187 Whenever possible the entomologists in charge of units should be relieved of routine administrative duties.

188 Every six or eight entomological units will need to be organized into groups, headed by the most skilled and experienced entomologist of the group. A group would be responsible for the supply of the units below it and also form the base for heavy, specialized equipment, e.g. aircraft, radio links, road building equipment, etc.

189 Entomological groups should be loosely connected so that exchange of equipment and supplies and information can take place. Maximum liaison between groups is essential.

4.3.3 Epidemiological unit

190 The epidemiological unit is composed of two teams, each team comprising: one medical officer epidemiologist trained in basic modern epidemiological methodology with parasitological background; two male nurses; two technicians; two microscopists; two drivers and one labourer.

191 The epidemiological unit is responsible for carrying out epidemiological work as required under the plan of operations established by headquarters, i.e. an initial prevalence survey and detailed surveys in indicator areas (see section 2.2.2).

192 The initial survey will have to investigate a large number of communities in the country. If this cannot be conducted by existing services, two teams will be organized during the first year to determine the prevalence of onchocerciasis and blindness by investigating clusters of 200 persons and covering 2% of the population.

193 When Simulium control commences, the teams will conduct the detailed surveys in selected indicator areas. An indicator area should be inhabited by approximately 250-500 persons, whom it will probably take about one week to examine.

194 There will be an interval of two to three years between detailed surveys, during which the teams may be used to undertake chemotherapy for onchocerciasis under ophthalmic supervision. Apart from assisting in the planning of surveys and assessing ophthalmological data, the consultant ophthalmologist attached to headquarters (section 4.3.1.2) will join the teams for survey work and for providing ophthalmic treatment as required.

195 Although some progress has been made in the nomenclature and classification of eye lesions due to onchocerciasis, there are still differences of opinion between specialists in this field. The meeting therefore agreed that it was important that the services of the

same ophthalmologist should be obtained for both the initial and all follow-up detailed surveys.

196 The following work schedule for epidemiological investigation carried out under the Project plan is suggested:

- one year for the initial survey by two teams (200 persons per day during 20 days a month, which equals 48 000 persons per year per team, i.e. approximately 100 000 persons in one year if two teams are at work;
- hall a year for the first detailed survey by the epidemiological unit covering one indicator area of 250-500 persons per week, i.e. 18 indicator areas;
- 15 year follow-up programme:
- re-survey of indicator areas at two to three year intervals;
- onchocerciasis sample surveys as in the initial prevalence survey covering those parts of the project area not previously surveyed;
- treatment under ophthalmic supervision of individual patients who risk becoming blind from their existing load of onchocercal infection (see section 2.1.2.2).

4.3.4 Training

197 There is a world-wide scarcity of all types of trained personnel required for parasitic diseases and especially in the field of onchocerciasis. Specialized training in Simulium/ onchocerciasis control will be needed at all levels. It is highly important that training candidates should possess an aptitude for field work, a willingness for hard work and a readiness to put up with rough conditions. Whenever possible, preference should be given to personnel with previous experience of vector-borne disease control. As far as possible, training should be arranged at institutions within the area of the control project and preference should be given to locally-recruited staff provided that they have the necessary qualifications; however, in areas where qualified candidates are not available in sufficient numbers, recruitment will have to be made from a much wider field of candidates.

198 Training should be organized on the following principles:

1. Only two categories of staff should be considered for advanced training:
   i. professional staff;
   ii. technicians/field officers.
2. Priority should be given exclusively to training to meet the requirements of the project.
3. The training courses should be given at the Project headquarters and be in the charge of those responsible for the project (entomologiste, epidemiologists, etc.). The best training for Simulium control is acquired in the field.
4. The training period for professional staff will last three months and for technicians six months, the time in each case being divided equally between laboratory and field.
5. The training of the personnel will be in direct relationship to their respective responsibilities.
6. For entrance into the project and training course, the basic qualifications considered desirable are as follows:
   a. Epidemiologist: Medical graduate with special training in epidemiology or an entomologist with knowledge of parasitology and epidemiological methodology;
   b. Entomologist: Graduate with basic knowledge of entomology and parasitology, preferably with Masters degree or equivalent;

c. <u>Technician/field officer</u>: Minimum: middle school graduate or state certificated (male) nurse with specialized training in entomology; possessing attributes essential for field work.

7. Training of all other staff will be carried out locally.

4.4 Transport, equipment and supplies

4.4.1 Transport

199 Three types of cars and trucks are necessary:

a. long-wheel based four-wheel-drive station wagon and/or pick-up;
b. lorry - one-and-a-half to three tons;
c. car suitable for travelling the trunk roads of the area.

200 For an entomological unit, the following number of vehicles would be required at the unit's base: (a) one car; (b) two all-wheel-drive station wagons or pick-ups; (c) two lorries; (d) eight bicycles; (e) one boat with one or two outboard motors.

201 Each team would require: (a) four all-wheel-drive station wagons; (b) two lorries; (c) bicycles; (d) two boats with three outboard motors.

202 This takes into account necessary replacement while maintenance and repairs are being carried out. The boats should be of lightweight construction but robust and portable.

203 The number of planes required for the project will be directly related to the size of the area and the problems it poses. The campaign must have a full-time pilot-engineer and a mechanic to fly and maintain each project aircraft.

204 Full and adequate repair facilities as well as spare parts for the engines of all motorboats and vehicles on the project must be furnished to provide against break-down, especially once control operations have started.

205 The vehicles, boats, outboard motors, etc. should be depreciated over two years in view of the high mileage and rough terrain covered by entomological teams.

206 Each epidemiological team will require three Land Rovers or similar vehicles.

4.4.2 Equipment

207 <u>Laboratory equipment</u> - The headquarters laboratory must be fully equipped with the necessary optic apparatus, etc. and spare parts for replacement and the general needs of the units.

208 Each professional or field officer should have at least one dissecting microscope and one binocular microscope (with immersion lens) plus all the necessary laboratory equipment and supplies (this must include all the material necessary for catching files etc. in the field).

209 <u>Field equipment</u>:

- Adequate camping equipment;
- hydrological and topographical equipment;
- ophthalmic equipment;
- simple equipment for radio-communication between units and teams would be. highly desirable.

210 Office equipment - For headquarters, units and teams.

4.4.3 Supplies

211 Laboratory supplies; insecticides; drugs for the treatment of ophthalmic lesions; drugs for mass chemotherapy whenever effective, non-toxic compounds are available.

4.5 Existing services (in the recommended area)

212 In the member countries of OCCGE there exist public health organizations known as the Services des grandes endémies which are responsible for the control of endemic diseases. These organizations are staffed by auxiliaries supervised by local medical officers where available. In addition these countries have the support of French doctors and entomologists of the OCCGE. At the Centre Muraz at Bobo-Dioulasso (OCCGE) the Onchocerciasis Section specializes in long-term research on the biology, ecology and distribution of the vector (S. damnosum) as well as in epidemiological studies of onchocerciasis in the member States of OCCGE. This section maintains or has maintained at various times, sub-stations at Ouagadougou and Garango (Upper Volta, Korhogo (Ivory Coast), Bougouni (Mali), Natitingou and Parakou (Dahomey. The Onchocerciasis Section is directed by a medical entomologist from ORSTOM, who is assisted by two other ORSTOM entomologists (at Bobo-Dioulasso and at Ouagadougou), by several ORSTOM technicians (at Korhogo and Bobo-Dioulasso) and by a technical assistant of OCCGE (at Bougouni). In addition, this section is responsible for the organization, direction and execution of the FED/OCCGE campaign against the vector of onchocerciasis. A certain number of specialized personnel (technicians from ORSTOM and from FAC) also work within the framework of this section. Dahomey and Mali both have their own entomologist.

213 In Ghana the Division of Epidemiology at the Ministry of Health is responsible for the control of communicable diseases. Apart from the headquarters units there is a Medical Officer of Health responsible for communicable diseases control in each region. The headquarters organization has a vector control unit from which entomological work is organized. This is at present under the supervision of a senior research entomologist from thé Ghana Academy of Sciences. Routine survey work is undertaken by auxiliaries of the Rural Health Service who form part of the regional health organizations. The Volta River Authority has its own health organization and there is an entomologist in this organization, who works on Simulium control. He has his own staff of auxiliaries. The Institute of Aquatic Biology at the Academy of Sciences also collects information about Simulium during its routine work. The work of all these organizations is co-ordinated by the medical officer in charge of the Epidemiological Division through a Committee.

214 The WHO project AFRO-0131, staffed by an ophthalmologist/epidemiologist and an entomologist, and based in Ghana, is assisting the governments of Ghana, Upper Volta and Togo in the preparation of a plan for an inter-country onchocerciasis control project in the area of the White and Red Voltas (Map 4)[19]

215 The existing services for countries outside the recommended areas are described in a separate document[20] along with the distribution of onchocerciasis in those countries.

5. RESEARCH NEEDS

216 The recommendations of the WHO Expert Committee on Onchocerciasis[21] (see Annex 3, section 6) on the subject of research needs in onchocerciasis were endorsed, and it was suggested that the following items should be added:

i. The clinical manifestations in the younger age groups up to five years should be carefully investigated;
ii. The possible role of the parasite in producing an arrested development (pseudo-nanism), and its impact on general health should be studied;
iii. Reliable and more specific immuno-serological methods for detecting onchocerciasis under field conditions should be sought;
iv. More effective, non-cumulative, cheaper insecticides should be developed as well as formulations to improve their dispersion and carry;
v. Accurate information on the role of Simulium biting density as a determining factor in the abandonment of fertile land should be collected;
vi. The antifilarial action, pharmacology, and toxicology of Mel W require study. Special attention should be paid to the mechanisms producing encephalopathy and to ways and means of avoiding it.[22] For instance, further research should be carried out on the use of Mel W in association with antihistaminic drugs or possible detoxicants in an attempt to find a dosage schedule which may be less toxic and more effective against the adult worms of O. volvulus and free from frequent, serious risks of encephalopathy;
vii. Every encouragement should be given to the development of new drugs effective against O. volvulus with especial emphasis on the need for non-toxic macrofilaricidal preparations.

6. RECOMMENDATIONS

217 The meeting made the following recommendations:

218 1. Co-ordination will be necessary between the governments of the countries within boundaries of which the recommended area lies, in order to synchronize and strengthen anyapplication for financial assistance which may be made to outside sources; once financial resources are assured for the continuation and extension of existing control operations, it would be desirable that the responsible public health officers and financial controllers of the countries concerned should meet to agree on ways and means for ensuring effective co-operation among themselves so as to attain their mutually desired objective of controlling onchocerciasis with the utmost expedition.

219 2. Whereas the magnitude of the recommended programme places it beyond the present fiscal and manpower resources of the countries involved, and whereas the nature of onchocerciasis control excludes any effective action on a piece-by-piece basis, the programme must be accepted in its entirety, therefore the attention of the three sponsors of the meeting should be directed to this fact and they should be urged to pursue energetically the procurement of the needed resources from whatever sources possible, be they national or international, bilateral or philanthropic. Contributions of funds will afford greatest flexibility and freedom of action but provision of personnel, especially those of most specialized qualifications, is highly desirable and even necessary.

220 3. All current operations for onchocerciasis and Simulium control within the recommended control scheme area, and elsewhere, should be encouraged, maintained and, where possible, extended.

221 4. Training Centres for entomologists and technicians to be employed on Simulium control in the recommended area and in adjacent zones should be organized without delay.

222 5. An international study mission should be formed at an early date, with the following brief:

i. to assemble and analyse all the existing information relevant to a technical and economic study of onchocerciasis control and regional development;
ii. to provide a more detailed assessment of the costs and benefits expected from the proposed programme of onchocerciasis control in the recommended area, especially in combined regional rural development projects including health, agricultural and industrial activities.

223 It is recommended that a suitable team for the mission would comprise two experts in onchocerciasis, an agricultural development expert, an economist and a socio-demographer. The team should be bilingual French/English. A working programme is provided in Annex 2.

224 6. Although an appreciable amount of information has been gathered in recent years, knowledge of the geographical distribution of onchocerciasis and its vectors should be completed as soon as possible and exact quantitative epidemiological data collected throughout the endemic area on a uniform basis. In particular, every assistance should be given to promote the training and provision of personnel to collect such data as rapidly as possible in the onchocerciasis zones into which it is hoped to extend the present recommended control operation.

225 7. The control project should be utilized as much as possible to encourage field research. Full use should be made in this respect of the research institutes existing in the countries in the recommended project area.

7. CONCLUSIONS

226 Control of the disease onchocerciasis depends on the control of Onchocerca volvulus transmission and at present this depends largely on controlling the vector Simulium damnosum by means of larvicides. Much information is now available indicating that control of onchocerciasis in the savanna zone of West Africa is feasible, and it is considered that the time is now ripe to undertake a large-scale Simulium control scheme for the purpose of reducing the prevalence and intensity of onchocerciasis among the people living in this zone. Such control would stop the further occurrence of onchocercal blindness, which under present conditions is such a frequent sequel to the infection, having dire socio-economic repercussions on the afflicted communities and obliging them to abandon much fertile land in river valleys.

227 For the reasons given in section 3, the area centred on the head-waters of the Volta River and contiguous river basins is recommended as the one which should receive priority for a large-scale control project. Operations in this area could, if successful, be extended later to embrace neighbouring onchocerciasis foci in the African savanna zone.

228 As it is likely to be beyond the means of the governments concerned to put the proposed scheme into operation, considerable financial aid will have to be sought from outside sources.

NOTES

1. Wld Hlth Org. techn. Rep. Ser., 1966, 335.

2. A detailed description of the onchocerciasis situation country by country is given in a separate document (in preparation).

3. For geographers, the division between West and Central Africa is approximately the valley of the Cross River in Southern Nigeria. Reference to other onchocerciasis areas in Africa is also made in section 3.3 of the present report.

4. Greenslade, C. (1956) Incidence and causes of blindness, London, British Empire Society for the Blind, p. 5.

5. Wld Hlth Org. techn. Rep. Ser., 1966, 335, pp. 51-78

6. Wld Hlth Org. techn. Rep. Ser., 1966, 335, pp. 15-24, 28-45

7. Solvent: Heavy aromatic naptha with fuel oil added to give a specific gravity of about 0.995 and with 0.5% Triton X-161 added as a surfactant to aid initial penetration.

8. Wld Hlth Org. techn. Rep. Ser., 1966, 335, 31

9. Wld Hlth Org. techn. Rep. Ser., 1966, 335, pp. 16-24.

10. Wld Hlth Org. techn. Rep. Ser., 1966, 335, pp. 16-24.

11. A review of "Research problems involved in vector control methods" is appended (Annex 1)

12. WHO Regional Office for Africa project AFRO 0131 carried out by an Onchocerciasis Advisory Team, financed under the United Nations Development Programme (Technical Assistance).

13. Fonds d'Aide et de Coopération du Gouvernement de la République française.

14. Fonds européen de Développement, du Marché commun.

15. Detailed costs will be provided after the first short-term survey referred to in Annex 2.

16. Société d'Etudes pour le Développement économique et social, Paris.

17. WHO Rgional Office for Africa project AFRO 0131 carried out by an Onchocerciasis Advisory Team, financed under the United Nations Development Programme (Technical Assistance).

18. Fond européen de Développement, du Marché commun.

19. Other services provided by WHO under regional programmes of assistance to countries for operational surveys and control operations or under the headquarters research programme covering the whole field of onchocerciasis (including research projects supported in the recommended area) are not referred to in this report as they do not specifically apply to the proposed control project.

20. In preparation.

21. Wld Hlth Org. techn. Rep. Ser., 1966, 335, 45-48.

22. WHO Expert Committee on Filariasis (Wuchereria and Brugia infections) (1967) Second report, Wld Hlth Org. techn. Rep. Ser., 359, 25, 39.

Annex 1. Research problems involved in vector control methods[1]

1. INTRODUCTION

1 The purpose of the meeting was to review the present situation of onchocerciasis control, to discuss its feasibility in large-scale projects and to indicate the immediate steps that need to be taken in the future.[2] Viewed in this light, the most important research requirements are those which could help: simplify present techniques, lower their cost, increase the efficacy of control campaigns in coming years and, finally, allow the treatment both of larger areas and of foci where present control methods are but little effective due to local conditions.

2 WHO asked a number of experts[3] for their opinions on these research requirements, and the intent of the present annex is to relate main points expressed in their answers.

3 As far as possible, the experts are quoted in their own words unless more than one proposed the same study, in which case their common opinion is summarized. This exposé is divided into two parts: (a) urgent research aiming to perfect present techniques of control and methods for assessing their effects; (b) longer-term research aiming to develop new techniques and increase present knowledge.

4 Both parts are sub-divided in the same way, except for certain subjects which are not included in the second part.

5 Concerning the general feasibility of onchocerciasis control, it should be recalled that eradication of S. neavei has been achieved in some foci of Kenya and that control of S. damnosum is proving to be a success at least in one area in Mali and in two others in the Ivory Coast (OCCGE reports). The following opinions may also be quoted: "In some areas control is economically practicable, and is being, or will be, carried out. Experience gained may enable control to be extended to large areas where the problem is quite untouched" D. J. Lewis. "There is, however, no reason why control schemes should not be undertaken in the near future. Indeed, the only way to acquire the necessary experience is in the field; research can proceed at the same time and in the same areas, as well as in adjacent, uncontrolled areas" (McMahon). "Briefly it has been shown that insecticide

applied to breeding waters can lead to perfect control and even eradication. What better situation can possibly exist than where the larvae are confined to a breeding-place whose very natural attributes lead to spreading and mixing of the insecticide so that it needs only to be dosed in by very simple methods here and there" (de Meillon). This last author recommends at the same time the establishment of pilot zones where techniques and knowledge could be perfected during the campaigns themselves.

2. URGENT RESEARCH AIMING TO PERFECT PRESENT TECHNIQUES OF CONTROL AND METHODS FOR ASSESSING THEIR EFFECTS

2.1 Pre-adult stages

6 In Africa all present schemes and projects are based on the use of larvicides. The reports on these campaigns seem to show that few of the difficulties encountered are due to a lack of precise knowledge of the ecology of pre-adult stages, at least as far as S. damnosum is concerned. At the same time, however, a majority of the experts consulted is of the opinion that research in such a direction might lead to better control with present means and to the possibility of extending the campaigns to areas which seem for the moment difficult to treat.

2.1.1 Eggs

7 Since they are known to be unaffected by all available larvicides, research on them will mostly be discussed in section 3.1. To perfect present dosing techniques, however, Burton proposes to study: "...to what extent are eggs laid above the water-line on overhanging grass or leaves, or on the bank, and kept viable by the spray? Do such egg-masses become desiccated by the sun? How many viable larvae result from this position?".

2.1.2 Larvae

8 Several experts insist on the importance of studying migration during the larval stages and the influence it may have on control measures (Burton, Duke, Hocking, Le Berre. McCrae, Marr, Noamesi, Ovazza). For most, the main point to determine is over what distance the larvae are able to drift downstream to then re-attach themselves. Hocking, however, requests that studies be made on their ability to creep upstream or across the rivers. For his part, McCrae would like more knowledge on the possibility of larvae of the S. neavei group to drift downstream in the first stage, and afterwards to change host (i.e. the crab). Duke stresses yet another point: the possible difference in location between larvae and pupae sites and the ovipositing sites. Lewis, Marr and Noamesi wish that more precise data be acquired on the length of larval life in different conditions. Le Berre and most of the others admit that for S. damnosum a larval cycle lasts about eight days, but can be shorter under exceptional conditions; and Lewis points out that there is need of "further knowledge of minimum periods".

9 To obtain better results in larviciding campaigns Fallis, Hocking, and Jamnback ask for a study on the size of the particles which compose the food of S. damnosum larvae.

10 Fallis proposes to investigate: "the water velocities that cause larvae to release from substrate". This may have a direct bearing on the measures which can be taken as far as artificial breeding sites such as dams are concerned. The same expert proposes research which would attempt to throw light on the existence of biological rhythms as this might lead to the possibility of determining the hours of the day when larvae might prove to be more susceptible to insecticide.

2.2 Adult flies

11 This section refers to studies concerning the biology and ecology of the adult fly. All research related to recent knowledge on the existence of a S. damnosum complex, and its possible consequences on ecology and transmission will be discussed in section 2.3.

12 A general preoccupation amongst the experts is the lack of knowledge regarding the migration, dispersion and, generally speaking, the flight of females (Burton, Duke, Fallis, Le Berre, Lewis, McCrae, Noamesi, Ovazza).

13 Burton proposes the utilization of gold-dust marking to study both dispersal and possible aestivation of adult flies. Noamesi underlines the importance of this study and draws attention to the fact that research on the subject is in progress. For Lewis it is necessary to study such questions in different climatic conditions. In this connexion, Duke's remark is of interest: "A great deal more information is needed on the dispersai and flight range of S. damnosum in different environments. The lack of knowledge here is not a reason for postponing attempts at control, indeed it may well be that from such attempts we shall learn much of what we want to know about these insects' dispersal. Nevertheless without further knowledge on the subject it may be difficult to prevent reinvasion of treated areas. Studies of the decline of biting density at different distances from known breeding sites can give at best only approximate results on dispersal which are liable to grave errors. Large-scale critical experiments with marked flies are really needed, but a prerequisite for these is the development of reliable techniques for marking and recapture. In connexion with the use of coloured dyes and dusting powder it should be remembered that at certain times a considerable number of wild-caught parous S. damnosum show ovaries tinted with a pinkish-purple colour and that this colour may sometimes spread to all the organs of the body."

14 (Page 44 manquant)

15 right into the forest.". Ovazza also attempts the following explanation to account for the differences between Le Berre's findings and those of Duke: "It should merely be observed that Le Berre was able to study the same 'variety' in different environments, as confirmed by Lewis' and Duke's above-mentioned map, whilst the two latter authors were led by circumstances to study two populations which they consider to be different. The intervention of biological factors inherent to both these populations consequently made it impossible for Lewis and Duke to put in evidence the influence of extrinsic factors." Ovazza therefore advocates that the taxonomical status of these two "varieties" be more precisely defined; a study be made of their hybridization potentialities; more precise information be given on their distribution (here Ovazza's opinion joins that of Lewis and Duke).

2.4 Insecticides and other methods of control

16 All experts insist that studies to improve control techniques should receive first attention The following improvements are mainly needed: a longer effective carry of larvicide, development of a compound with a shorter persistence than DDT, dosing techniques providing a better dispersion of the larvicide in streams. Several experts emphasize that substitute insecticides should now be available in case vector strains develop resistance to the insecticides used. On these points, Jamnback's views are quoted:

> "(a) Particularly interesting are larvicides such as carbamates that are fairly soluble in water (40 ppm) and so have some potential for remaining effective for long distances downstream, especially through areas of slow water. Stomach poisons such as GS 10133 that would be selective for filter feeders in streams also require further investigations as do non-persistent organo-phosphorous compounds such as sumithion and Dursban that have shown promise in laboratory testing.
> "(b) Formulations, especially finely particulate, low-density dusts or wettable powders that would be ingested or concentrated by the blackfly larvae when applied in low concentrations to the water with resulting high selectivity in killing blackfly larvae should be investigated. Another possibility might be blocks of carbamyl placed in streams that would gradually dissolve over a period of several months providing control over a long period.
> "(c) While the drip can method of application is simple and effective in applying emulsions which tend to disperse themselves in streams, it is less effective with formulations that tend to settle in the can, as is the case with suspensions. Solutions are emitted in large droplets which are less effective than fine droplets. A simple apparatus to disperse these formulations should be developed."

17 In addition, all experts agree there is need for field tests permitting continuous observations to be made on the susceptibility level of larvae.

18 A number of experts also suggest that studies be carried out on the following: size of the particles ingested by the larvae (see 2.1.2); behaviour of the larvicide in flowing water (Burton, Le Berre, McCrae, Ovazza); the precise maximum depth at which larvae are found, in relation to that reached by the larvicide (Burton, Le Berre); possible variations in larval susceptibility according to extrinsic and intrinsic factors (Burton, Le Berre, Hocking).

19 A study concerning the fate of larvae once they leave their substrate under the effect of sub-lethal doses is also proposed, but opinions differ on this subject: Hocking, Le Berre and Marr believe that the larvae die because according to Hocking, they are exposed longer to the larvicide, and also because, in the natural conditions of the Sudan savanna during a dry-season campaign, they are unable to find another attachment point (Le Berre, Marr). Hocking proposes that a releasing agent be found; Marr asks for a larvicide which would have mostly a knock-down effect. While sharing also the opinion that once detached the larvae will remain in longer contact with the larvicide, Muirhead-Thompson seems to fear that part of them at least will be able to survive. He is the only expert to propose the development of techniques requiring the application of very small doses over much longer periods.

20 Concerning artificial breeding sites, Fallis (as already mentioned in 2.1.2) wishes to study the feasibility of eliminating the larvae in such sites by "flushing", requiring the use of water velocities higher than those which the larvae are able to withstand. Le Berre draws attention to the fact that large dams will concentrate breeding in one point, viz: the

discharge canal. He therefore asks for studies regarding definitive construction modifications which would prove unfavourable to the species; these studies should be carried out whenever hydrological projects permit it.

2.5 Methods of study

21 This section refers to research proposed with a view to improving methods in order to meet ecological and epidemiological needs as well as to provide a better assessment of results.

22 For the most part, the experts agree as to the choice of goals to be set, but they sometimes differ as to the selection of methods to be used. Regarding the dispersal of adult flies and the assessment of the effect of campaigns on the female fly population, several experts request the development of better trapping techniques: Davies thinks that an effective trapping device is necessary for two reasons: (1) after a campaign capture on human bait provides few data because of the small number of remaining female flies; (2) he finds this method inaccurate. Jamnback, also, has not much faith in the human bait method. McCrae, though he admits that the traps he tried did not give him the expected results, finds that capture by seated immobile-people is unnatural.

23 It is to be noted that these objections were already raised at the time of the Informal Meeting of WHO Advisers (July 1964). The opinions expressed by other experts are either opposed or, at any rate, less radical.

24 On this subject, Le Berre's remarks: "Its (capture on human bait) advantages: it allows the capture of female flies at a very precise time of their life, and knowledge of the physiological age of the females captured makes it possible to follow the campaign's progress with great precision; ... when the adult fly population is greatly reduced, the human collector method provides excellent results. Its inconveniences: these arise from the collector himself." Concerning the use of traps, the same author underlines that none up to now has given valid results in Africa with S. damnosum. Another obstacle, to his mind, is the fact that trap collections must be sorted out in order to separate females of S. damnosum from a high number of other insects. A third point is that a trap will take females at different stages of their physiological cycle and results will be very difficult to analyse. His conclusion is that capture by trapping techniques does not present an advantage for the assessment of campaign results but these techniques must all the same be perfected as they are useful for studying the biology of adults and perhaps as a complementary method for assessing campaign results.

25 Marr considers that capture on human baits is still the basic method; nevertheless, he is in favour of the development of better traps which, although possibly less effective, would be more accurate.

26 De Meillon underlines that main interest lies in the degree of residual transmission after a campaign. Therefore, capture on human bait is necessary as it affords information on just those flies which are implicated in transmission.

27 Opinions also widely differ as to what capture methods should be studied and as to the advantages of those presently in use or being developed. Burton proposes trying sugar-baited traps, blackflies being nectar feeders and consequently likely to be attracted by sugar. Davies favours a trap which would offer bait more attractive than human bait, would be able to work continuously and keep its collection for three to seven days. Fallis

wants to promote catching with visual attractants + CO_2 and carried out tests with such a trap in Ghana where these are presently being continued by Noamesi. Jamnback suggests experimenting the sticky trap designed by West. McCrae, as mentioned above, has tried several types of traps, most of which proved disappointing; however, he has had interesting results with a modified Fredden's trap allowing the use of human bait and of bait-animals, even large-sized ones; he also asks that a combination of amino-acids + hormones + CO_2 be tried as attractant.

28 As already mentioned, many authors ask that the drifting possibilities of larvae be studied (2.1.2) and also that the effects of larvicides on them be closely followed during the campaigns. With regard to these two related subjects of study, certain methods are proposed. Fallis suggests the destruction of existing supports at a given point of the river and replacing them by green strips of plastic or mops of string, for instance. De Meillon's technique is somewhat similar, but he would not destroy the existing supports and merely put floating pieces of wood a week before larviciding. Larvae would attach themselves on these floats allowing an assessment of their susceptibility. As a reminder, it must be mentioned that, in Ghana, Burton and Noamesi used plastic tubes closed by gauze in which they placed larvae with a naturel support such as grass or leaves. To study the drift of larvae, Hocking recalls that white plastic cones trailed in the water have given good results in Canada. For carrying out sensitivity tests in the field, Jamnback speaks of a technique in which the larvae would be tested against the concentration used in plastic bags, these bags being afterwards placed in the river during the time of observation.

29 Some experts have already expressed doubts as to the efficacy of marking and recapture techniques for studying dispersal of the adult fly (2.2). Nevertheless, Duke believes that their development ought to be promoted. Fallis, if it were first possible to improve quantitative methods of sampling which would provide large samples of adults and larvae, would prefer marking with radio-isotopes. Gold-dusting gave good results in the studies carried out by Noamesi.

30 Lewis and McCrae would like research to continue on a method which would permit complete laboratory rearing of S. damnosum. Both believe that the main point of interest resides in the feasibility of forced mating; and McCrae recalls that McMahon has an effective technique of membrane feeding. Muirhead-Thompson is also pursuing research in the same direction.

31 McMahon believes that the effects of reinvasion on the one hand, and possible dry-season survival on the other, ought to be assessed by campaigns in a completely isolated S. damnosum focus. For this purpose, he suggests the island of Fernando Po.

2.6 Operational problems

32 De Meillon points to the necessity of improving present methods for reaching rivers to which access is required as well as of obtaining better results with the larvicides actually in use.

33 With the same aim in view, Duke, Jamnback, Le Berre and Marr insist on the various possible uses of aircraft, be it for preliminary surveys, for larviciding in certain conditions, or for providing greater transport facilities, mainly for the entomologist in charge. They consider it urgent that the limits and difficulties of larviciding by aircraft be estimated and that this technique be adapted to conditions in Africa.

34 Lewis, McCrae and McMahon suggest that the possibility to effect control by long-term application of larvicides - a method used in the Budongo forest against S. neavei - be studied for use in other limited foci.

35 Hocking requests the development of simple techniques of flow measurement in order to ensure correct dosing.

2.7 Methods of assessing results

36 All the experts recognize that the present techniques of entomological evaluation are sound, at least in their principle. But Duke and Jamnback underline that assessment based only on an entomological evaluation of the reduction of the vector population does not, at present, give a true idea of the actual degree of reduction in transmission. Davies believes that persisting transmission in the Abuja area, where very few simuliids are still to be found, signifies that transmission can be maintained with a very low number of female simuliids. According to these experts, a particular effort should be made to increase present knowledge on that question.

37 From a practical point of view, those experts who, for entomological surveys, prefer the use of traps to human bait as sampling techniques, also give preference to trapping techniques for the assessment of results.

3. LONGER-TERM RESEARCH AIMING TO DEVELOP NEW TECHNIQUES AND INCREASE PRESENT KNOWLEDGE

38 The aims of long-term research are not the same in West Africa as in East Africa. In the first instance, the experts are interested in studies that will in the long run, not only improve but also, most probably, radically change present methods which nevertheless could be put into immediate practice. In East Africa, owing to the existence of numerous "varieties" of the damnosum complex, some of which probably sympatric, improvement of fundamental knowledge of the vector is a prerequisite to successful control.

3.1 Pre-adult stages

39 Burton asks for detailed studies on the mechanisms by which eggs and pupae develop resistance to insecticides.

40 Burton, Fallis and Hocking consider that physiological and ecological research on larvae must be promoted, particularly on:

- mechanisms and stimuli governing the looping movements and the use of silk for attachment and drifting;
- feeding and rate of growth;
- possibility of competition in the breeding sites between different Simulium species;
- animal environment in the breeding places, particularly with regard to other arthropods and possible predators on the different stages of blackflies.

3.2 Adults

41 Two types of research are proposed:

42 The first aims at improving present knowledge of the ecology and behaviour of adults, particularly of the female, e.g. stimuli involved in oviposition, attractants, fat-body formation and utilization, physiological influence of nectar feeding, different aspects of flight and dispersal (Burton, Fallis, Hocking, McCrae).

43 The second would consist in a detailed study of the bio-ecological differences that may exist between the "varieties" in the "damnosum complex" and the species in the "neavei group", e.g. anthropophily and zoophily, possible autogeny of some damnosum "varieties", transmission power, behaviour and ecological reaction to the seasonal disappearance of breeding sites, annual cycle, biting cycle (Fallis, Hocking, McCrae).

3.3 Microtaxonomy

44 Hocking, Lewis and McCrae insist on the need for more accurate knowledge regarding the composition of the "damnosum complex", the morphology, genetics and degree of speciation of its "varieties".

3.4 Methods of control

45 Attention is drawn to the biological, ecological or even integrated methods of control that could be used if present knowledge were improved and enriched. Burton asks that the following possibilities be studied:

- chemical and mechanical techniques of destruction of eggs and pupae;
- ways of making breeding sites unacceptable for oviposition;
- use of attractants in constructing new traps for the destruction of adults.

46 Duke proposes a detailed study of the different members of the "damnosum complex", aiming at the introduction of non-anthropophilic "varieties" in onchocerciasis foci.

47 Fallis, like Burton, advocates killing traps and suggests also a study of possible repellents to interrupt transmission.

3.5 Methods of study

48 Several experts express the need, already mentioned in section 2.5 for a study of the technical possibilities to promote complete laboratory rearing of flies and subsequently a study of cross-mating between the different forms of the "damnosum complex".

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NOTES

1. Prepared for the meeting by Dr M. Ovazza, Office de la Recherche scientifique et technique outre-mer (ORSTOM), Paris, France, WHO Consultant.

2. The meeting was, in part, a follow-up of the Informal Meeting of WHO Advisers on Entomological Needs in Onchocerciasis Control, July 1964, Wld Hlth Org. techn. Rep. Ser., 1966, 335, 51.

3. List of contributors appended.

Annex 2. Work programme of the international study mission

1 In accordance with recommendation 6.5 of the present report, it is proposed that an international study mission be formed with the following terms of reference: (1) to present, in the shortest time possible, an information dossier on the area recommended as the one to receive priority in an inter-country onchocerciasis control programme, on the understanding that this programme may be later extended, by successive stages, to embrace neighbouring onchocerciasis foci in the African savanna zone; (2) to undertake, subsequently, a study on the socio-economic aspects of onchocerciasis control in relation to regional development. The purpose of these studies and the programme for carrying them out are specified below.

2 Accredited to the Governments of the seven countries concerned in the initial onchocerciasis project, i.e. Dahomey, Ghana, Ivory Coast, Mali, Niger, Togo and Upper Volta, the international study mission must have available all the existing documents as well as be able to make any contact it considers necessary in order to obtain authoritative information and opinions.

1. INFORMATION DOSSIER ON THE INITIAL AREA RECOMMENDED

3 To compile this dossier, the mission will be required. to assemble and analyse all available information on the items listed below which are indispensable to prepare a plan of operations and, in a more general way, to work out a-global strategy of onchocerciasis control covering the entire recommended area.

4 1.1 Review of the studies carried out

a. Health situation in the project area, in particular the collection of local statistics pertaining to water-related diseases. Review of health resources and equipment. As regards onchocerciasis, the distribution, importance and evolution of the disease, and knowledge of the breeding-places found.
b. Hydrological and climatological data.

c. Topographical data (existing maps, lay-out of the road network, location of villages, schools, sanitation posts, etc.; boundaries, dates, scales, etc., of the aerial photos, etc.).

d. Soil studies (soil surveys, suitability for crops, etc.).

e. Demographic and sociological data: the size, distribution and evolution of the population both on the plateaux and in the valleys; sociological studies already carried out or in progress on the groups affected by the development of the valleys; development activities in progress and supervisory staff on the spot, etc.

f. Development potential of the various regions in the recommended area. Inventory and description of all the national, bilateral and/or international development projects at local or national level that are in progress or planned in the recommended area. Their global incidence, both in respect of benefits and expenditures, on the enlarged onchocerciasis control project (operations and engineering works of common use to onchocerciasis control and development plans, changes made in the location of communities, etc.).

5 On the basis of the existing documents and in accordance with the purpose of its study, the mission will have to determine the gaps in the said documents and define very precisely what measures need to be taken in order to fill them as well as the cost these measures would entail.

6 Furthermore, this documentation will contain not only a summary of the projects being worked out in the various spheres but also an indication of the priority given to them by the responsible authorities. It will also list the existing means and resources which could be utilized for the project.

7 1.2 Onchocerciasis control

a. Resources in men, equipment and money at present being used for onchocerciasis control. Extension of the operations that are planned in present programmes, and their financing. Means and resources likely to be made available to the project.

b. Preparation of a draft plan of operations for the project and of a cost estimate by year and by type of expenditure, including, if possible, the cost of maintenance operations after completion of the actual control campaign.

8 1.3 Exploratory study on expected benefits to be derived from the implementation of the project, based, in particular, on the examples provided by areas where control operations have been followed by development activities.

9 1.4 Preparation of the study on the socio-economic aspects of onchocerciasis control in relation to regional development: definition of the parameters to be used and determination of the regions best suited for the carrying out of this study.

2. STUDY ON THE SOCIO-ECONOMIC ASPECTS OF ONCHOCERCIASIS CONTROL IN RELATION TO REGIONAL DEVELOPMENT

10 The main-object of this long-term study is to analyse the interrelationships that exist between economic development and disease control campaigns. In order to evaluate these interrelationships, such a study demands that a methodology be developed which could then be perfected and tested in parallel with the onchocerciasis control campaign.

Annex 3. Who expert committee on onchocerciasis extracts from the second report[a]

1. PROCEDURES FOR FIELD LARVICIDING

1 "The group recommends that:

1. Simulium-control campaigns be directed by a qualified entomologist, if possible one with previous experience of Simulium control.
2. Control measures should be directed against the larvae.[1]
3. A thorough survey should be made for all actual and potential breeding sites for a whole year prior to treatment. This study should include an aerial survey to spot possible breeding sites and access routes.
4. Length of larval life in the control area should be determined.
5. DDT as an emulsifiable concentrate should be used and the dosage calculated and expressed as the number of parts of p, p'-isomer per million parts of water.
6. Dosage be at a rate not exceeding 1.0 ppm and not less than 0.1 ppm maintained over 30 minutes. In the absence of hydrological data, discharge can be calculated by measurements of width, depth and speed of flow of the river (for the last, a simple float method is sufficiently accurate).
7. The season chosen for larviciding should be determined from results of pre-treatment observations on vector population dynamics, fluctuations in river discharge, State of access roads at different times of the year, etc,
8. Selection of dosage points must be made on basis of pre-treatment observations and on distance over which larvicide is fully effective. In a new control area, effective distance will not be known initially, and experience elsewhere may be used to decide distance apart of dosing points. Within control areas, the position of the dosing points may need modification-as a result of direct observation on effectiveness of the first and subsequent larvicidings; and it is-recommended that, in any control programme, continuous observations be made to assess the effective carry of larvicide, the dosing points being moved as need be.

9. The dosings at each point should be made every seventh day, unless local observations on length of larval life indicate that less-frequent applications can be made safely.
10. The initial treatment should be maintained for at least six weeks.
11. The emulsifiable concentrate should be diluted to at least 1/3 with water and run into the main flow of the river over the 30-minute dosing time, using simple ad hoc devices.

2. SURAMIN

2 "Suramin (used at a dosage similar to that described under individual treatment) has been tried in mass campaigns on several occasions but, in retrospect, it has never been a success, not on account of failure of its filaricidal properties, but because its use requires very careful medical supervision, regular examination of the urine, intravenous injection, and assembly of the treated population at weekly intervals for about two months. Also, the occurrence of serious toxic side-effects (some of which are undoubtedly associated with the death of the parasites) are too frequent to justify its general use on a large scale. It should be noted that recent experiments have shown that the toxic reactions encountered with suramin cannot be related to unsatisfactory quality of the drug used. Preparations supplied by three different manufacturers were found to be equally stable chemically, equally effective in killing macro-and micro-filariae and equally prone to produce albuminuria and other toxic reactions.

3 The use of suramin on any considerable scale can perhaps only be envisaged at present in certain small savannah villages in Africa in which treatment is urgently needed on account of a very high incidence of blindness. In such circumstances, the drug may be recommended for individual treatments on a village scale, under careful, whole-time medical supervision."

3. SKIN SNIPS

4 "It is generally agreed that skin snips are better for detecting microfilariae than is scarification of the skin.

5 Skin snips are normally taken by the insertion of a needle into the skin to elevate a cone of skin, which is then cut off with a razor blade or a sharp-bladed scalpel. The area snipped should be approximately 3 mm in diameter. Antiseptic precautions should be taken.

6 The portion of tissue removed should-be placed on a microscope slide in a drop of saline or water, covered with a coverslip, and allowed to stand for 30 minutes before being examined. If desired, the specimen may be teased and allowed to stand 10-15 minutes before examination without a coverslip.

7 Examination is made with a magnification of not more than x 100. If it is desired to stain the preparation, it should be allowed to dry thoroughly, and for this purpose, preliminary covering with a coverslip is-undesirable. The specimen should then be fixed by immersion in methanol for 30 seconds and stained with Mayer’s haemalum or with Giemsa’s stain.

8 In prevalence surveys, at least two snips should be taken from each person; this number represents a compromise between accuracy and acceptability in a population. The snips

should be taken from the sites most likely to be heavily infected. In Africa and Venezuela, these are the buttocks and the lower legs; in Mexico and Guatemala, they are the shoulders and buttocks. In areas where there is doubt as to the best sites to use, preliminary investigations should be carried out, taking skin snips at multiple sites.

9 In a properly taken skin snip, contamination with blood-circulating microfilariae is very rare. In some areas of Africa, infections with Dipetalonema streptocerca will be encountered but, with a little experience, the microfilariae of this species can readily be distinguished from those of O. volvulus, both in wet–and in stained preparations. In cases where there is doubt as to the identification of microfilariae in wet preparations, those from skin snips should be stained. Likewise, if difficulty is encountered in examining wet films in regions of very low humidity, the preparations should also be examined after staining."

4. INDICES OF ENDEMICITY AND MORBIDITY

10 "The Committee decided that three levels of endemicity should be established, on the basis of the percentage prevalence of O. volvulus microfilariae in the skins of the population sampled, namely: high endemicity, 67% and more; medium endemicity, 34%-66%; and low endemicity, 33% or less.

11 In areas where the prevalence is less than 5%, microfilarial carriers may well have acquired their infections elsewhere, and the disease should not be regarded as endemic unless clear proof is obtained that transmission is occurring on the spot (i.e., finding of infected Simulium or of infections in persons who have never left the area)."

5. MAZZOTTI TEST

12 "This test has been much used as a diagnostic aid in the field, but it is, of course, of qualitative value. It should be remembered that, once it has been used, definite diagnosis and quantitative assessment become impossible for an appreciably long period of time. There is also a considerable subjective element in its interpretation."

6. RESEARCH NEEDS

13 "The problems on which research is needed are almost unlimited; the ones mentioned here are chiefly those that have an important bearing on the control of the disease.

4.1 Vectors

4.1.1 Biology of adults

14 As current sampling methods used in evaluation are based on one aspect of the vector's activities, namely, its biting contact with the human host, it is recommended that additional methods of trapping or collection currently used in Europe and North America-should be explored and developed with regard to the vector Simulium. Promising methods include the use of mechanical traps, light traps, sticky traps, etc. Further studies on Visual attractants and on Chemical attractants should also be pursued.

Special methods or-combinations.of methods may have to be developed in order to find out more about the resting places and concentration sites of engorged and gravid adult females.

15 A wider range of sampling techniques is essential for a fuller understanding of vector biology, flight range, survival, etc. and would be particularly helpful, for example, in providing unbiased samples of engorged females for blood-meal identification. More accurate knowledge about blood-feeding activities of vectors on hosts other than man is required.

16 Methods for establishing self-perpetuating colonies of the vector Simulium are urgently needed. Research on this important aspect is being vigorously pursued in several centres at present, and should be encouraged.

4.1.2 Ecology of breeding places

17 Long-term studies on the ecology of breeding places, particularly with regard to factors determining the suitability or otherwise of different types of stream for vector species, should be encouraged. Such studies would also assist in evaluating the general biological effect of control (larvicide) measures on associated fauna and microflora of the habitat.

18 "There is need for more accurate information about the length of larval life in different habitats and in different areas. A bioassay method, already developed in one country for studying this question, might be tested with advantage under other conditions. This technique has an added advantage in that it can be used to study the effect of larvicidal treatment on larvae under natural conditions.

19 The question of larval drift, migration and re-attachment has been studied in the European and North American species. There is need for similar investigations on vector species in Africa and in Latin America.

20 Further studies on oviposition habits under various conditions, e.g., with regard to different types of substrate and different-types of spillway, should be encouraged. Nothing is as yet known about the ovipositing habits or ovipositing sites of the S. neavei group.

4.1.3 Insecticides and other methods of control

21 The evaluation and testing of new Simulium larvicides has recently been incorporated in the long-established WHO-coordinated programme for evaluating and testing of new insecticides in general. Continued research on these lines is to be encouraged, together with the further improvement of techniques for testing new Simulium larvicides and the development of a standard susceptibility test. There is need for further work on the evaluation and testing of recommended insecticides in relation to changes in flora and fauna and to their accumulation in animals used as food.

22 The evaluation and testing of repellents and attractants requires more investigation particularly in relation to future possibilities in the use of chemosterilants as a supplementary control measure against adult Simulium vector species.

4.2 Epidemiology

23 The contrasting epidemiology of the disease in the Americas and in different climatic zones of Africa should be studied so as to provide dues to some of the-paradoxes of onchocerciasis. Indices of endemicity should also be determined.

24 Further development of epidemiological methods for assessing the results of-control schemes is desirable, and further studies on the variations of microfilarial numbers in the skin from day to day and hour to hour are needed.

4.3 Clinical and ophthalmological investigations

25 Longitudinal studies should be made on patients with onchocerciasis to determine the order of appearance of clinical signs and the effects on life expectancy and health status. The pathology of onchocerciasis is still poorly understood, partly because the patients studied suffer from or are exposed to several other diseases concomitantly. The State of nutrition in relation to the appearance and intensity of onchocercal infection must be studied, for example, by investigating vitamin A and carotenoid blood levels and minimum light thresholds. Attempts should be made to correlate the localization of nodules with ocular symptomatology and to investigate the effects of denodulization on the relief of certain ocular lesions. The use of intravital staining techniques, and possibly also of radioactive tagging, were mentioned as means by which the movements of microfilariae in the body and in the eye might be traced. The whole question of the etiology and pathogenesis of posterior-segment lesions requires further study and might include observations as to whether their progress is halted by treatment.

4.4 Chemotherapy

26 Improved macrofilaricidal drugs are required. Present drugs need further investigation for possible chemoprophylactic action. The differences in the reactions to drugs of patients with onchocerciasis and patients with trypanosomiasis needs elucidation. For chemotherapeutic control purposes, more knowledge is needed on the minimum dosage of diethylcarbamazine that will clear the skin of microfilariae, and much further work must be done to determine how Mel W may best be employed as a single-dose macrofilaricide in the field.

4.5 Immunology

27 Studies on the isolation, purification and Chemical characterization of specific antigens from various stages of the parasite, as well as the essential components, should be encouraged. Those antigens that come in contact with the host would probably serve as the best possible testing and immunizing agents. It is essential for the standardization of the antigen to know the biochemical constitution and the dose response. For the complement fixation test, as well as other bio-immunological tests, it is important to ascertain both the specificity and the sensitivity of the antigen since, in long-standing infection, the test may become negative although microfilariae may remain in the skin. The use of exo-antigens rather than somatic antigens and their comparable immunogenic

activity should be investigated. In order to compare results, a standard complement fixation test should be used uniformly in several areas where large-scale surveys are being conducted. A correlation between the results obtained by intradermal tests and complement fixation tests should be sought. The use of fluorescent-antibody techniques should be studied, and the relationship of immune reactions to eye lesions observed in onchocerciasis should be investigated.

4.6 Helminthology

28 Studies on possible differences between strains of O. volvulus from different areas should be encouraged. The use of the chimpanzee as a laboratory host for the infection opens the way for many basic studies on the parasite in the vertebrate host, and possibly later for studies on chemotherapy.

29 There is an urgent need for a laboratory culture of some species of Onchocerca that can be maintained by cyclical transmission in an alternative, more easily obtainable definitive host, and further efforts should also be made to culture the parasite in vitro. In the meantime, much could be learned from a study of species such as O. cervicalis of the horse, O. gutturosa of the cow and O. flexuosa of the deer, onchocercae that are prevalent in Europe. It has recently been shown that the microfilariae of O. guttorosa will survive in the skin of rats for several weeks. This finding provides an opportunity for assessing diagnostic techniques, for testing microfilaricidal drugs, and for studying various aspects of the pathology of onchocerciasis.

30 The possibility of a natural animal reservoir host for O. volvulus must be borne in mind, and basic research into the physiology of filarial worms should be encouraged."

7. INDIVIDUAL ONCHOCERCIASIS RECORD CARD

COUNTRY: ....

DISTRICT: ....

VILLAGE: ....

NAME: ....

NAME OF HEAD OF FAMILY: ....

AGE: .... SEX: ....

SERIAL NUMBER: ....

DATE OF INITIAL SURVEY: ....

1st follow-up ....

2nd follow-up ....

3rd follow-up ....

OCCUPATION: ....

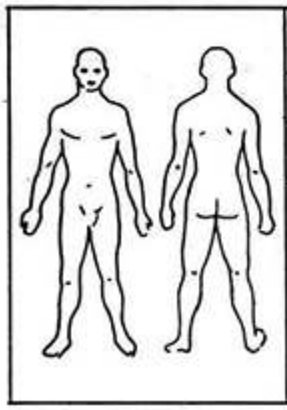
RESIDENT IN THE AREA SINCE: Birth
OR:

BIOPSIES: Method used:

Site	Initial survey		First follow-up		Second follow-up		Third follow-up	
	Result *	m.f. density	Result *	m.f. density	Result *	m.f. density	Result *	m.f. density
Shoulder								
Buttock								
Lower legs								
....								

* Indicate whether positive or negative.

NODULES:



	Initial survey	First follow-up	Second follow-up	Third follow-up
Examined **	Yes/No	Yes/No	Yes/No	Yes/No
Site of nodules	Number of nodules			
Cephalic region				
Thoracic region				
Upper limbs				
Lumb. and abdom. regions				
Lower limbs				

** Delete where inapplicable.

NOTES

1. The possibility of adult control as distinct from larval control is stressed (Vargas), particularly by measures against the older parous flies (Davies). The ovipositing female may represent a vulnerable phase for attack, and more knowledge about behaviour, concentration sites, etc., at this stage is needed (Davies)."

ENDNOTES

a. Wld Hlth Org. techn. Rep. Ser., 1966, 335.

Effects of permethrin as *Simulium* larvicide on non-target aquatic fauna in an African river

L. Yaméogo, E.K. Abban, J.-M. Elouard, K. Traoré and D. Calamari

1 Received 1 June 1992; accepted 3 January 1993

Introduction

2 The use of insecticides against the larvae of *Simulium* has been the most successful method for the control of river blindness in the WHO (World Health Organization) Onchocerciasis Control Programme (OCP) in West Africa. Since the commencement of insecticide treatment in 1974, temephos, an organophosphate, has been the main insecticide used in OCP. Other insecticides used include chlorphoxim and the biological control agent *Bacillus thuringiensis* var. *israelensis* (*B.t.* H-14). This agent is used during the low water period (October to June) while the Chemical insecticides are applied from June/July to October/November.

3 The ecological effects of these treatments have been studied extensively and three review papers have been published (Lévêque *et al.,* 1988, Yaméogo *et al.,* 1988; Lévêque, 1989), providing an evaluation of ten years of larviciding and monitoring. The conclusions were the following: “The results obtained after many years of treatment lead us to assume that the larvicide employed had little effect on the non-target fauna. Although the first application of temephos and chlorphoxim had a fairly strong impact on invertebrate communities in the short term, it would seem that these situations disappear fairly quickly after a year or less of successive applications”.

4 Unfortunately, after several years of treatment, resistance to temephos (Guillet *et al.,* 1980) and chlorphoxim (Kurtak *et al.,* 1982) has been reported in some of the cytospecies of the *Simulium damnosum* complex. As a resuit of this, a number of carbamates and pyrethroids have been selected as alternatives, based on the toxicity against the vector, the relative harmlessness to the non-target fauna and also the type of formulation, the

cost of utilization, mammalian toxicity and other considerations. Among the candidate larvicides, permethrin was selected in 1984 for potential operational use. A preliminary assessment of its impact on aquatic fauna in tropical conditions was made through acute toxicity tests on fish (Yaméogo *et al.,* 1991) and by means of semi-field experiments in mini-gutters (Yaméogo *et al.,* 1992). These experiments led to the conclusion that a pilot scale field trial was necessary before the insecticide could be used operationally.

5 This paper is based on data collected during an *operational* control effort in an operational programme, hence the lack of repetition (low number of drift nets, Surber samples) and the difficulties encountered in completing the sampling programme. The first objective of the programme is to control the disease, so the aquatic monitoring is mainly carried out to detect important changes in the communities that could in the long run affect aquatic resources available to the human populations. The analyses in this paper have therefore been made with this in view.

Materials and methods

Description of the area

6 The pilot operational trials were conducted on the Sassandra river in Côte d'Ivoire, one of the seven countries (Bénin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger and Togo) in the initial OCP area (Fig. 1) which covered about 654 000 km^2 in West Africa. Since 1987/1988, the programme has been extended to four other countries (Guinea, Guinea Bissau, Senegal and Sierre Leone). The total area is now about 1 300 000 km^2, but more than 80% of the initial OCP area is no longer being treated so the total length of rivers treated in the whole area is between 20 000 km and 25 000 km.

7 The Sassandra has been under treatment since March 1979. It is a river which can reach flow rates of the order of 900 $m^3 s^{-1}$ at the height of its spate between August and November. During the operational trial conducted in 1984, however, flow rates fluctuated between 60 and 600 $m^3 s^{-1}$ (Fig. 2). The length of the section treated was 235 km. The permethrin dose used during the 15-week (August to November) trial was 0.045 1 of 20% emulsifiable concentrate (EC) formulation per m^3. The effective carry of the product had been estimated at 5-10 km at the time of the operation. Therefore, based on the discharge and the number of potential *Simulium* breeding sites, the number of spraying points was between 27 and 38 per week. About 20-100 kg of active ingredient of permethrin were used weekly on the Sassandra.

8 For the purposes of the study, three monitoring stations on the treated section of the river and a control site on a main tributary (Feredougouba or Bagbe river) were selected for the monitoring of non-target aquatic fauna (Fig. 3). The most downstream monitoring station was completely drowned and inaccessible for invertebrate monitoring after the first nine weeks of larviciding. Furthermore, only drift samples could be taken regularly at the other two stations because of the hydrological conditions.

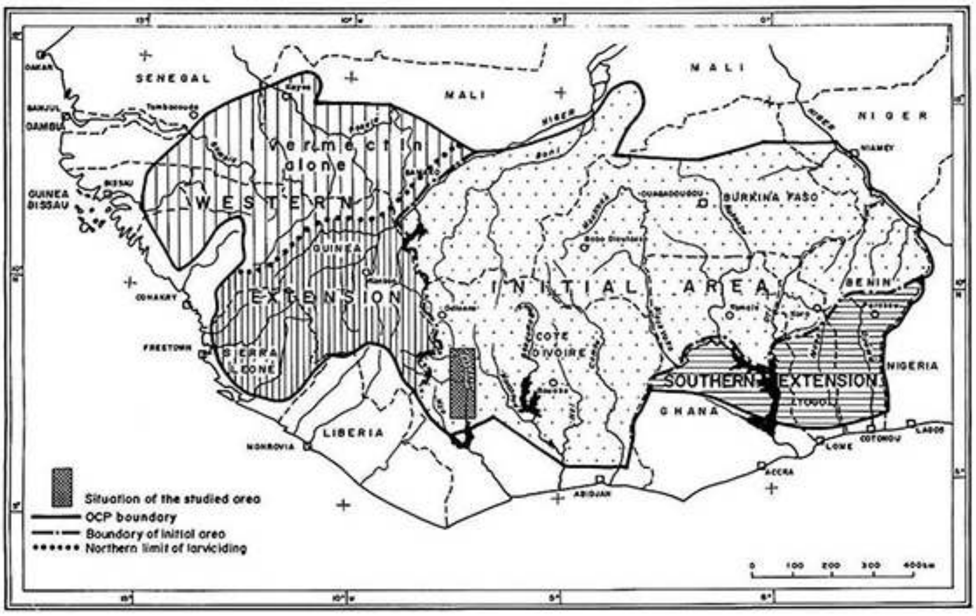


Fig. 1. The Onchocerciasis Control Programme in West Africa, showing the zone studied.

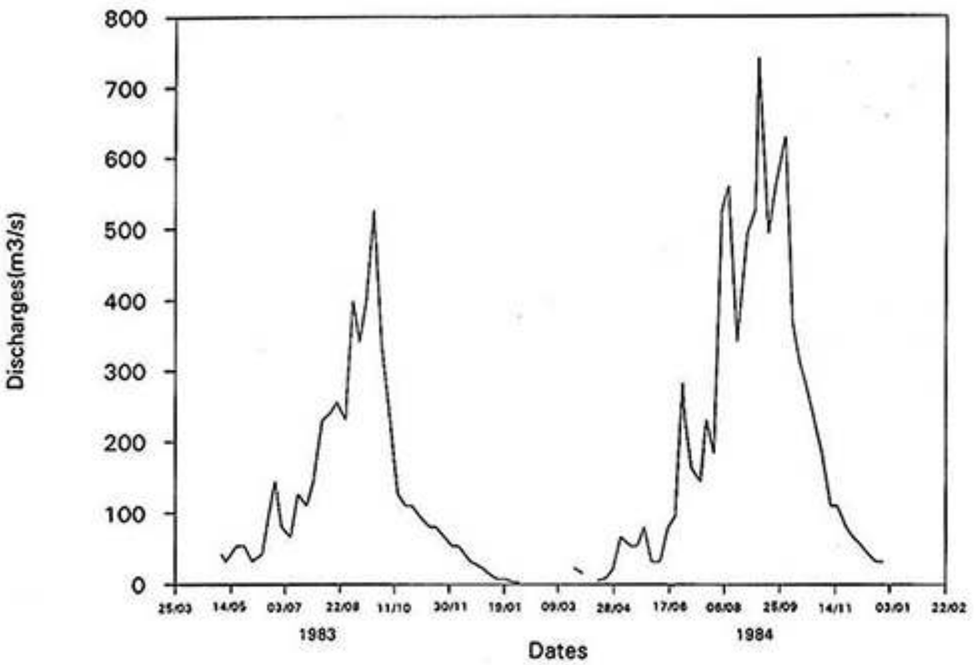


Fig. 2. Trend of the discharges of the Sassandra at Sémien from 06/05/83 to 28/12/84.

Invertebrate and fish monitoring methodology

9 *Invertebrate.* A battery of two nets (diameter, 12 cm) was used to estimate the abundance of drifting insects, which fluctuates, as in temperate zones, according to a circadian rhythm. This is low during the daytime and maximal one to two hours after sunset, and then the value decreases during the rest of the night (Elouard and Lévêque, 1977). Day drift, which is regarded as passive, is considered a reflection of the morbidity and mortality of the organisms. On the other hand, night drift, which is supposed to be mainly voluntary, reflects an active period. The measurement of drift during these two phases

therefore reveals environmental differences. Schematically, a large day drift reflects an effect of external factors on the invertebrates. By contrast, a high night drift reflects an increase in densities of benthic organisms (Dejoux, 1977; Dejoux and Elouard, 1977; Statzner *et al.,* 1985a,b, 1987). It is also reasonable to assume that drift increases with current speed. Therefore, most analyses were standardized by dividing by the filtered water volume the number of individuals captured in that water volume. The ratio is called the 'drift index' (DI), and expresses the number of individuals captured per m^3 of water filtered by the nets.

10 The quantitative evaluation of the benthic fauna of rock substrates was undertaken using a modified Surber sampler which covers an area of 625 cm^2. The samples were taken in shallow riffles where *Simulium damnosum* breed. These are the areas which are subjected to direct and maximum impact of the larvicides. Therefore, the data collected give a direct indication of the effects of treatments, especially when pre-treatment data are available. Five replicates were normally used as the optimum number to give a reliable indication of variability without denuding the area. For each sampling date, the mean number of individuals per Surber and the 95% confidence limits were calculated, using the method described by Elliott and Décamps (1973) for small samples taken from a contagious distribution. For drift as well as for benthos, samples were taken 24 h before and 24 h after the weekly larviciding, when possible. The sampling frequency was reduced to once a month after the cessation of the operational trial.

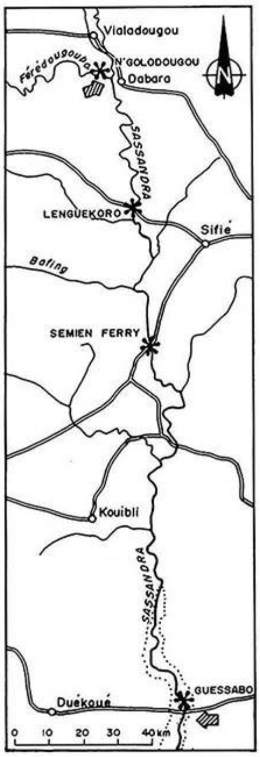


Fig. 3. Location of the hydrobiological monitoring sites (*) according to accessibility and possibilities for the application of different monitoring techniques. The arrows show the limits of the section treated with permethrin.

11 *Fish.* To estimate changes in species composition of catch and catch per unit effort of fishing (CPUE) in relation to the Chemical application, 60 h fishings were undertaken at experimental and control stations in connection with the first and eighth larviciding. The 60 h fishing at experimental stations consisted of three 12 h samples taken immediately

before larviciding (from 18.00h two days before larviciding to 06.00h the day of larviciding) and two 12 h samples taken later (from 06.00h the day of larviciding to 06.00h the day after). At these stations, samples were taken between 0.5 and 1.0 km downstream of the larvicide application points. Samples obtained between 06.00h and 18.00h were classified ‘day’ as against ‘night’ samples obtained between 18.00h and 06.00h.

12 A sample consisted of fish caught in a battery of gill nets, made up of seven pairs of different mesh sizes of nets (12.5, 15.0, 17.5, 20.0, 25.0, 30.0 and 40.0 mm mesh sizes). Each net had a surface area of 50 m^2 and CPUE was estimated by the formula $N \times 100/(l \times 2n \times d)$ and $W \times 100/(l \times 2n \times d)$, where N is number of fish caught, W is the weight of fish caught in grams, l is the length of net in metres, d is the depth of immersion in metres, and n is the number of ‘nights’ or ‘days’ of fishing (Lévêque *et al.,* 1988; Lévêque, 1989; Dejoux, 1980).

13 Catches by small-mesh nets provide information on small-sized fish including juveniles. When they are great, recruitment can be considered satisfactory or normal. Low catches of this category of fish could signify either poor reproduction of the species or an effect of external factors on spawning or juveniles. A decrease in the catches of big-mesh nets is also of concern since it indicates potential impact on mature fish.

14 Generally, natural, seasonal and interannual fluctuations are observed in multifilament gill-net catches. In the rainy season, for example, catches fall not only because of the difficulty of placing nets under good conditions due to water velocity but also because often nets catch fish on only some part of the water depth. They then increase with the decrease in water level. Furthermore, from year to year, catches can vary according to the hydrology and other abiotic and anthropic factors.

15 Another factor studied during this trial was the coefficient of condition of the fish (K), or simply the ‘condition factor’, calculated from a formula of the type: $K = W \times 10^5/L^{3,}$ where W is the weight of fish in grams and L is the standard length of fish in mm. It allows an evaluation of the evolution of ‘fatness’ or general ‘well-being’.

Results and discussion

Invertebrate fauna

16 *Impact studies.* Before the first permethrin treatment of the Sassandra, the mean daytime drift index was relatively high (11.3) at Guessabo, the furthest station below the section of the watercourse studied. The day-drift intensity was low at Sémien Ferry and Lenguekoro where the values obtained ranged between 0 and 2, thereby falling within the range of values generally recorded on untreated watercourses (0.2-4) as stressed by Dejoux (1978) and Yaméogo *et al.* (1988). The night drift was also high at Guessabo (30.3 individuals per m^3 of filtered water) compared with that measured at Sémien Ferry and Lenguekoro. Despite these differences between the stations, the general trend of the drift index (Fig. 4) was comparable for all the stations and quite close to that described by Elouard and Lévêque (1977). Low during the daytime, the drift index increased after 18.00h, reached its maximum around 20.00h and decreased again up to 04.00h. The young stages of Baetidae, Caenidae and the Chironomini were encountered almost regularly in the pre-larviciding samples. By contrast, the Hydropsychidae, Philopotamidae, Tanytarsini,

Orthocladiinae, Ephemeridae, Euthyplociidae, Prosopistomatidae and Oligoneuriidae were rare in this drift at Sémien Ferry, Lenguekoro and Guessabo (Okakro).

17 After the first permethrin treatment, the drift intensity increased significantly (Fig. 5ad), by a factor ranging between 500 and 1,300 depending on the monitoring station and its location in relation to the larvicide's spraying point.

18 The drift was composed mainly of Ephemeroptera (Baetidae and especially Caenidae). Many other taxonomic groups were also present in the post-larviciding drift. The Ephemeroptera (e.g., Leptophlebiidae, Euthyplociidae, Ephemeridae and Heptageniidae), which were practically absent in the 24 h pre-larviciding drift samples, were collected in great quantities after the passage of permethrin. The same applies to the Trichoptera (Hydroptilidae, Ecnomidae and Leptoceridae). These observations were made at all the monitoring stations in the treated zone.

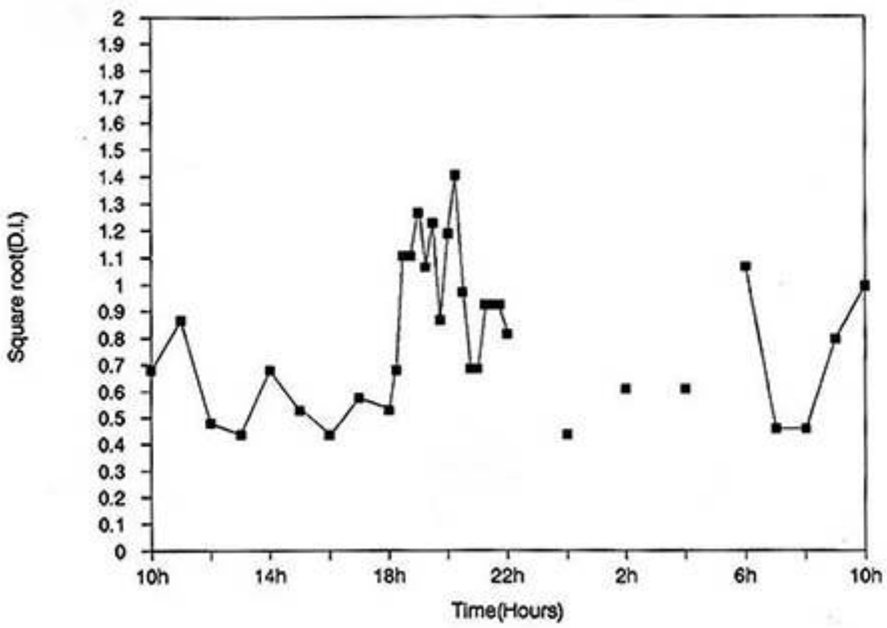


Fig. 4. Trend of the pre-larvicidal total fauna drift indices at Sémien Ferry for a 24 h period.

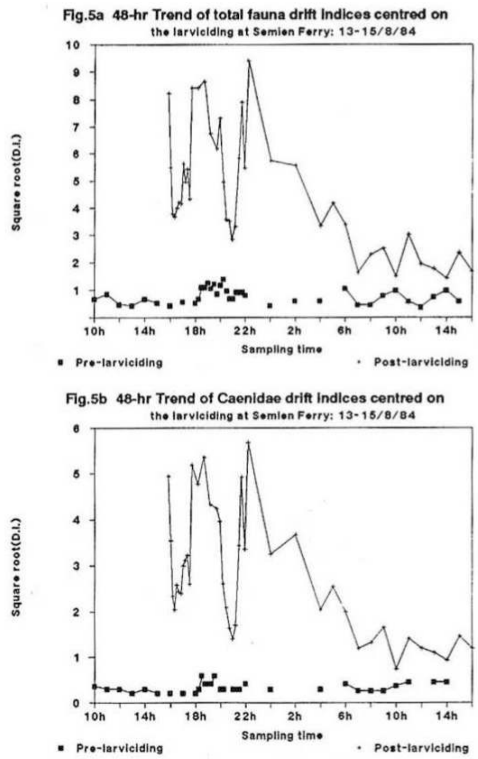


Fig. 5. The 48 h trends of the drift indices centred on the larviciding at Sémien Ferry and at Lenguekoro: (a) total fauna at Sémien Ferry, (b) Caenidae at Sémien Ferry, (c) total fauna at Lenguekoro, and (d) Caenidae at Languekoro, for the period 13-15/8/84.

19 Of interest was the appearance after the larviciding of two drift peaks with high values within an interval of 2-5 h (Fig. 5a,c), depending on the site considered. In some of the cases (Sémien Ferry and Lenguekoro), the second peak was higher than the first one, but they were all influenced by the Caenidae. It should be noted that the larviciding was carried out after 15.00h and that the carry of the product (i.e., the distance over which it is effective), estimated at 5-10 km, was found to be between 10 km and 15 km. The night-time drift index rose from 31 to a maximum of 8,041 for the first peak and 1,040 for the second one at Guessabo. At Lenguekoro, it increased from an average of 9 to maxima of 2,977 and 5,488 for the first and second peaks, respectively, while at Sémien Ferry it rose from 1.9 to 68 and then 89.

Fig.5c 48-hr Trend of total fauna drift Indices centred on the larvicidlng at Lenguekoro: 13-15/8/84

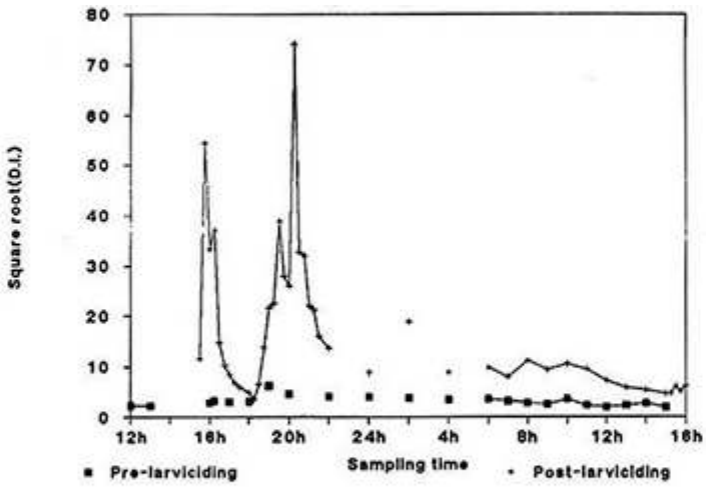
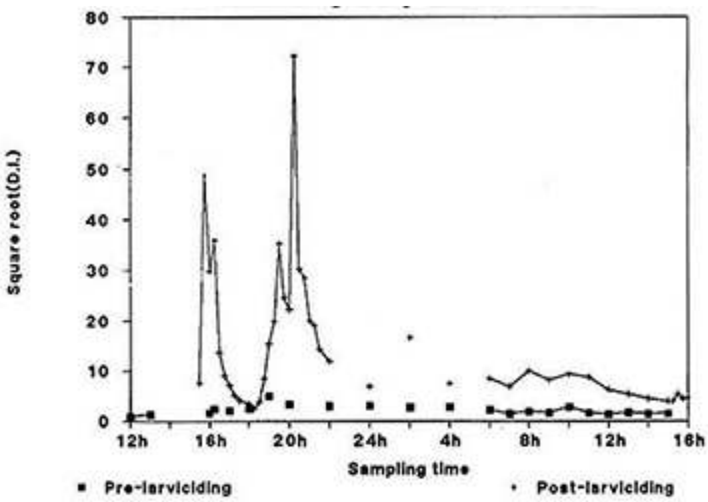


Fig.5d 48-hr Trend of Caenidae drift Indices centred on the larvicidlng at Lenguekoro: 13-15/8/84



20 Permethrin caused a considerable increase in the drift intensity of most of the taxa. The catastrophic drift due to the pesticide seemed to have been increased by the nighttime biological-activity drift. The second peak could be related to the impact of a second wave of permethrin (the product's carry having been underestimated) or to the drift of organisms from sites upstream or to a combination of the two factors. The acute toxicity of permethrin confirms what has been described by many authors for different pyrethroids vis-à-vis aquatic fauna in general and invertebrates in particular (Leahey, 1985a). The intensity of the drift caused by the second permethrin spraying reduced after the first week of larviciding. Moreover, the drift was increasingly composed of young stages, with the exception of the Elmidae (Coleoptera) and Chironomidae (Diptera), and the Ephemeroptera were almost absent.

21 To evaluate the impact of the 15-week use of permethrin on the aquatic invertebrate populations under high-water conditions, the trend in the abundance of the fauna in place was assessed by considering the night-time drift (which indicates the density of the fauna in place). This night-time 'biological activity' drift measured for the total fauna the day before each spraying, (i.e., 6 days after larviciding) increased slowly after the

decrease recorded before the second and third spraying cycles (Fig. 6). The rise of the curve of the total fauna was due essentially to the capture of Elmidae, Hydrae and Oligochaeta. The Ephemeroptera presented very low indices, almost nil during the permethrin larviciding period, reflecting their low abundance in the environment. Only the Chironomidae presented relatively stable indices and were considered less affected.

22 The faunistic composition of the drifts collected before each larviciding remained more or less constant but differed from that observed before the first spraying. Thus, the Chironomidae constituted the greater part of the drift, the Baetidae, the Caenidae (Ephemeroptera) as well as the Trichoptera were rare and the Leptophlebiidae, the Tricorythidae and the Euthyplociidae (Ephemeroptera) were sampled only very rarely.

23 The organisms collected during the operational trial were in general young larval stages from either old eggs hatching later or new eggs deposited by surviving adults or those from tributaries of other river basins not treated with permethrin. The recovery of aquatic invertebrate stocks under such conditions is therefore difficult.

24 During the flood subsidence, the qualitative collections as well as the Surber sampling made at Lenguekoro and Sémien Ferry confirmed the rarity of the Ephemeroptera and the predominance of the Chironomidae (especially Chironomini) in the river (Fig. 7c). *Recolonization study.* Quantitative estimation of benthos was impossible during the highwater period, but Surber samples were taken during the flood subsidence. A survey carried out at the end of November 1984, only a few days after larviciding was stopped, made it possible to observe that at the starting point and at the end of the stretch treated the fauna were more diverse than at Lenguekoro and Sémien Ferry. The great number of potential breeding sites between these stations necessitated larviciding at fairly short intervals, which led to a considerable weekly impact on the aquatic invertebrates. At the time the operational trials were stopped, the densities of the saxicolous fauna at Sémien Ferry (the only station having pre-larviciding data) were low, compared with those for the same period in 1983 and to those of the control site (Fig. 7a-c), with a change in the structure of the community. Signs of recolonization were visible in December 1984 (Fig. 7d) only a month after the cessation of the trials. The Chironomidae (Chironomini and Tanytarsini) were still dominant, representing 91% of the fauna (Table 1), followed by the Hydropsychidae (Trichoptera) whereas, in December of the previous year, the Chironomidae represented only 45% of the aquatic invertebrates followed by the Simuliidae, the Leptoceridae and the Baetidae. The density and the structure of the populations were close to those of the same period in the previous year after 3-4 months' suspension of any larviciding, but pollution from a sugar factory, which occurred in April/May 1985, interrupted the recolonization process. This type of pollution, which was severely felt by the Chironomidae, seems to have had a reduced effect on Ephemeroptera while Trichoptera were not affected by it (Fig. 7e,f). The density of the organisms was 136 and 175.4 individuals per Surber in April and May, respectively, as against 512 in January. It should be noted that the water became greenish due to a strong influence of algae density in it.

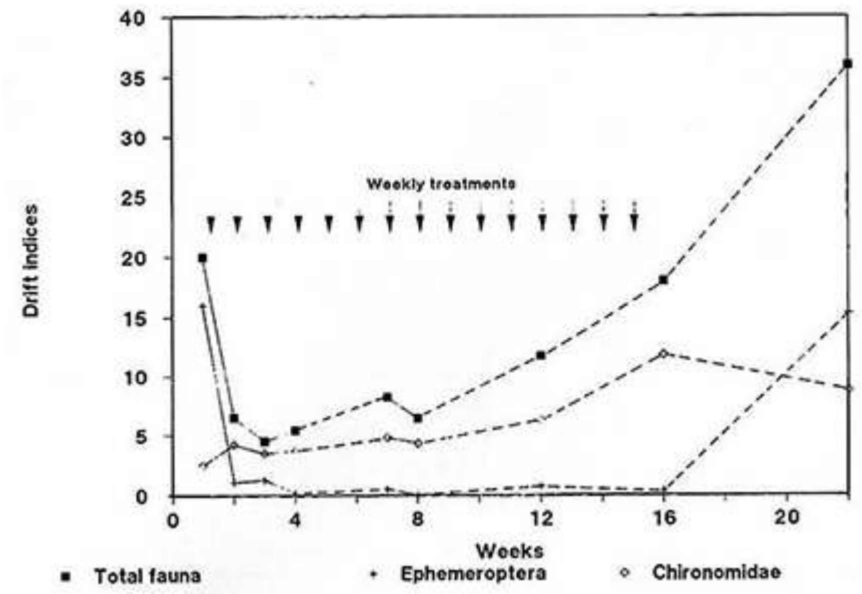
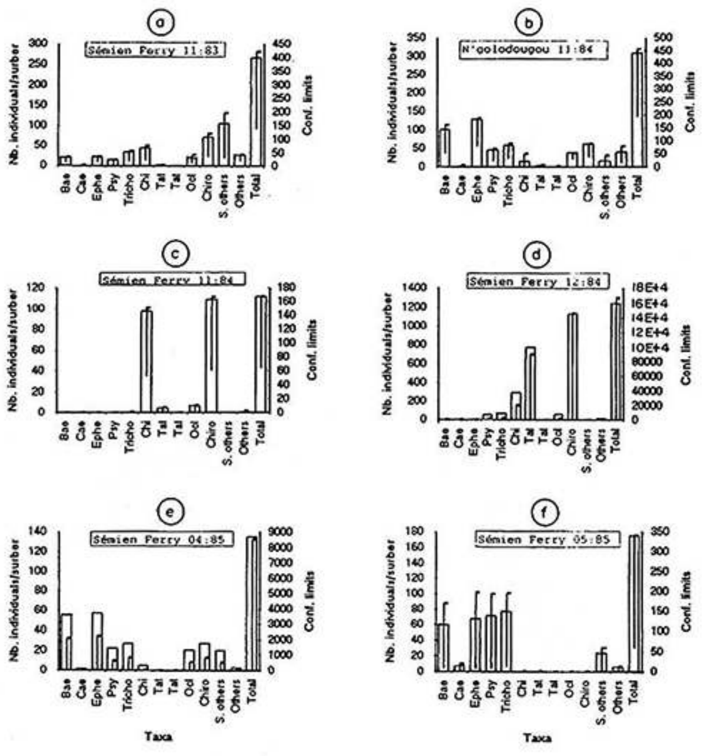


Fig. 6. Trend of the mean night-time drift indices measured the day before spraying at Lenguekoro.

25 Unlike the Sémien ferry station, where the Ephemeroptera were absent from the Surber samples at the time the larviciding was stopped, this taxonomic group represented, during the same period, nearly 50% of the saxicolous fauna at N'Golodougou on the control station. The composition of the fauna was quite similar at the two stations in January 1985 (Table 1), only two months after the trial was stopped.

Fish fauna

26 *Variations in the catches per unit effort (CPUEs).* A series of experimental catches was made at the different monitoring stations established for that purpose. Generally, Fig. 8a-c shows that reduced catches were recorded within the first week of permethrin application, as would be expected with continuous removal of fish from an area. By estimating population size by removal (Bower and Zar, 1977), it emerges that the decreases recorded are lower than those expected, except for the fish caught with 30 and 40 mm meshes. Decreases in CPUEs were observed during the trial period in the treated zone as well as in the control (Fig. 9) which were related to the discharge of the rivers. However, the fall in catches in the control zone was less than that in the treated section.



Abreviations
Bae: Baetidae
Cae: Caenidae
Ephe: Ephemeroptera (Total)
Psy: Hydropsychidae
Tricho: Trichoptera (Total)
Chi: Chironomini
Tat: Tanytarsini
Tap: Tanypodinae
Ocl: Orthocladiinae
Chiro: Chironomidae (Total)
S.others: Simulium others
Others: Other taxa

Fig. 7. Density (per 625 cm^2) of the main taxa sampled before and after the permethrin trial on the Sassandra river in Côte d'Ivoire. Thin vertical bars indicate 95% confidence limits after a log (x + 1) transformation.

Table 1. Relative abundance and mean number of individuals per Surber collected at Sémien Ferry and N'Golodougou at the end of the trials and 2 months later.

	Sémien				N'Golodougou	
	12/83	11/84	12/84	01/85	11/84	01/85
Baetidae	3.1	0	0.5	3	32.8	15.7
Caenidae	0.1	0	0.04	0.04	0.9	0.5
EPHEMEROPTERA	4.5	0	0.5	3.1	41.5	23.9
Hydropsychidae	0.5	0	4.6	5.1	14.9	7.2
TRICHOPTERA	6.3	0.4	5.9	22.7	18.9	14.8
Chironomini	23.1	87.6	23.7	41.7	5.4	11.1
Tanytarsini	2.2	4.3	63.4	0.6	1.3	3.3
Tanypodinae	0.9	0	0.08	3.4	0.5	1.1
Orthocladiinae	18.5	6.5	5.2	15.2	13	29.8
CHIRONOMIDAE	44.8	98.4	91.4	60.8	20.2	45.3
OTHERS	44.5	1.3	2.2	13.4	19.2	16
Mean number	(595.6)	(111.2)	(1239.6)	(511.8)	(312)	(424.2)

27 After the larviciding was stopped (at the flood subsidence), the CPUE values recorded rose on all the stations. The trend of the catches of the main species made at Sémien (Fig. 10a,b) shows that the values recorded since the beginning of the permethrin larviciding of the Sassandra are (for the most part) greater than those for the same periods in 1983. The drought of 1982 and 1983, although not favourable for recruitment, did not interfere markedly with the use of permethrin. The return of better hydrological conditions led to an improvement in most of the catches despite the use of permethrin which, therefore, did not hamper the seasonal succession of catches.

28 A comparison of species in samples at experimental and control stations before the first and eighth applications of permethrin showed that ‘new’ species in catches immediately before the eighth treatment were generally the same. Furthermore, the total number of species caught was quite stable during the trial. All the ‘new’ species caught except *Malapterurus electricus* and *Polypterus endlicheri* had been considered as fast migrating species by Baldry *et al.* (1981). This fact and their occurrence as ‘new’ species at the control station allowed their presence later at the treated sections of the river to be dissociated from the effect of the Chemical to a large extent. Although decreases in catches were recorded, the number of species did not vary fundamentally. It is therefore apparent that the ‘stress’ induced by the weekly permethrin applications has not caused the fish to flee from the treated zone. No mortality was recorded.

29 The spraying was carried out during the spate. It is generally admitted that catches made during the low-water period (post-larviciding period in our case) are better than those of the spate period. While permethrin had an effect on the catches, it was masked by dilution. The evolution of catches in treated zones generally follows the seasonal cycle, with an increase in catches during the low-water period and a decrease during the spate.

30 *Variations in the coefficients of condition.* Table 2 gives the coefficients of condition of the main species caught at Sémien. The results recorded before the first spraying as well as

during and after the weekly larviciding do not show any notable differences. It appears that the larviciding had no detectable effect on condition.

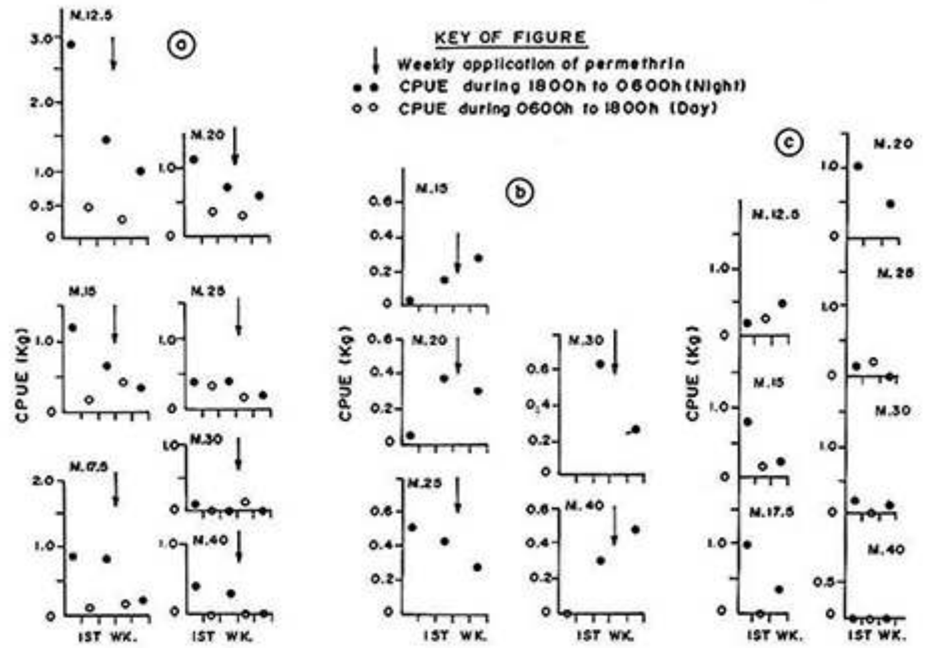


Fig. 8. Trend in CPUEs of individual sizes of net (*M*) during the first week of permethrin application at (a) Lenguekoro, (b) Guessabo and (c) N'Golodougou.

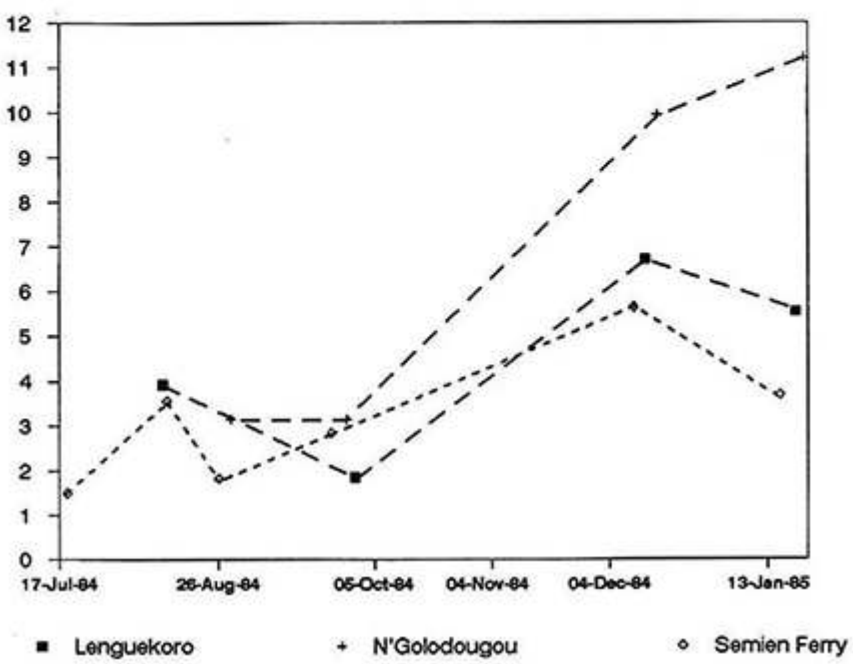


Fig. 9. Time trend in total CPUEs in some monitoring stations during the trial.

Table 2. Mean 'condition' (K) of main fish species caught at Sémien Ferry before and during the permethrin trial.[a]

			$K \pm SD$ (Nb)		
Species	15/05/84 (BT)	12/08/84 (BT)	26/08/84 (DT)	24/09/84 (DT)	10/12/84 (AT)
Brycinus nurse	–	2.231 ± 0.33 (30)	2.316 ± 0.29 (170)	2.258 ± 0.28 (61)	2.373 ± 0.35 (26)
Eutropius mandibularis	1.309 ± 0.48 (5)	1.106 ± 0.05 (9)	–	1.030 ± 0.16 (7)	1.137 ± 0.12 (14)
Chrysichthys maurus	2.232 ± 0.14 (5)	1.896 ± 0.27 (29)	1.994 ± 0.25 (69)	1.813 ± 0.28 (29)	1.798 ± 0.13 (16)
Synodontis punctifer	1.907 ± 0.20 (5)	1.971 ± 0.26 (13)	–	1.895 ± 0.15 (6)	2.160 ± 0.19 (16)
Synodontis bastiani	–	1.673 ± 0.15 (5)	2.051 ± 0.31 (8)	1.587 ± 0.11 (25)	2.076 ± 0.17 (14)

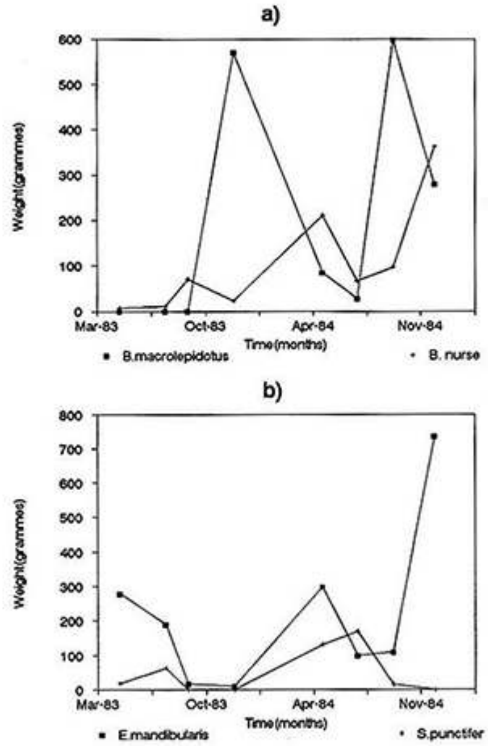


Fig. 10. Trend of catches of the main species made at Sémien from May 1983 to December 1984.

Conclusion

31 In the short term, the entomic fauna were very much affected by the use of permethrin as a larvicide. The impact was not reduced by the high discharge, and recurred with more or less the same degree week after week. Only the Chironomini (Diptera) were tolerant.

32 After 15 weekly larviciding cycles, permethrin led to changes in the composition of the lotic invertebrate populations of the Sassandra. Nonetheless, the existence of an upstream reach and untreated tributaries facilitated recolonization which was already quite marked only a month after the cessation of the trials. Four months later, after the replacement of permethrin by Teknar, the reconstitution of the fauna was almost complete but pollution independent of OCP activities interfered with the colonization of the benthic fauna.

33 As regards the fish fauna, no direct mortalities attributable to permethrin were observed. Under natural conditions the dose at the spraying points could be 10-20 times the operational dose (0.015 mg l^{-1} x 10 min), therefore a level of concentration of 0.150-0.300 mg l^{-1} could be present for a few minutes while the 24 h LC_{50} is 0.040 mg l^{-1} (Yaméogo *et al.* , 1991). However, exposure to high concentrations during the spate period will always be short, since the fish can flee from the larvicide wave and bioavailability is reduced by sorption on suspended solids. In fact, due to the high log K_{oc}, (5.9), permethrin has an elevated affinity for suspended solids present at high level during the flood, and it is well known that toxicity is greatly reduced under such conditions (Hill, 1985). No bioaccumulation could be expected despite the high potential for partitioning (log K_{ow} 6.1), as permethrin is quickly metabolized and excreted by many organisms including fish (Leahey, 1985b).

34 In the medium term, the product did not seem to affect gill-net catches. Taken as a whole, the decrease observed during the spate followed the seasonal evolution with increase in catches during the low-water period. As regards the coefficients of condition of the main species no incidence of the larviciding on the fatness and therefore on the feeding of the fish was observed. The relatively high toxicity of permethrin on invertebrates has been confirmed by different laboratory tests. However, medium-term trials under field conditions show that under certain conditions the effects are reduced and recolonization takes place quite rapidly among the most affected organisms. This reversible impact of permethrin at high water and, particularly, its efficacy on blackfly species resistant to the organophosphorus compounds and the relatively low cost of its use (US $16 per kilometre of river treated between 100 and 150 m^3 s^{-1} as against US $20 for Abate) have led to its selection as a replacement for Abate. However, any use of this product should be subject to close monitoring in addition to rigorous control of the larviciding conditions; also, the number of treatment cycles should be limited.

Acknowledgements

35 This work was fully financed by the WHO Onchocerciasis Control Programme. We are grateful to the Programme Director, Dr E.M. Samba, and the Chief of the Vector Control Unit, Dr D. Quillévéré, who accepted the publication of this document. We thank the OCP Ecological Group also for having inspired and supported the preparation of this article.

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ENDNOTES

a. SD, standard deviation; Nb, number of individuals; BT, before the trial; DT, during the trial; AT, after the trial.

ABSTRACTS

The side-effects of permethrin (20% EC) as a *Simulium* larvicide on aquatic invertebrates and fish was studied under operational vector control conditions to contribute to the evaluation of the product for its possible adoption by the Onchocerciasis Control Programme in West Africa. After 15 weekly applications of the formulation at 0.045 litre per m^3 of water discharge, drift samples virtually contained no Ephemeroptera. Saxicolous fauna density and proportional diversity were also affected. However, both drift and saxicolous fauna recovered to almost pretreatment levels a month after treatment was terminated. Fish showed some evidence of stress but remained in the active treated zone to make trends of catches in the area comparable with those of the control station. The treatment did not resuit in fish mortalities, and the condition of fish before and after the experimental period was significantly unchanged. Thus, operational use of permethrin by the Programme would not be expected to have permanent adverse effects on the non-target fauna.

INDEX

Keywords: permethrin, blackfly control, fish, invertebrates, non-target fauna, West Africa

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Typology of susceptibilities of aquatic insect larvae to different larvicides in a tropical environment

L. Yaméogo, J.-M. Elouard and M. Simier

INTRODUCTION

1 The strategy adopted against Simulium damnosum complex, which transmits human onchocerciasis (Philippon, 1977), by the Onchocerciasis Control Programme (OCP) was the use of larviddes since 1974. Because of the short larval life span of the blackfly, the larviciding was done weekly in fast-flowing river stretches where the vectors' larvae developed. For many years temephos, an organophosphorus insecticide, in a 20% emulsifiable concentrate formulation, was the only product used until the appearance in 1980 of resistance to it (Guillet et al., 1980). By 1982 resistance to chlorphoxim (another organophosphorus compound) had also developed in certain forest cytotypes of the vector (Kurtak et al., 1982). These findings led to the acceleration of a screening programme in a search for possible alternative larvicides.

2 The selection of insecticides is based mainly on their efficacy against the vector (Kurtak, 1986) but also on their toxicity as regards the non-target fauna (Yaméogo et al., 1988, Lévêque, 1989). More than some sixty products from different Chemical families have been tested but less than half of these larvicides have been the subject of studies on the non-target fauna, particularly insects.

3 Among the different techniques for studying the short-term impact of larvicides on non-target benthic fauna, that of gutters (Dejoux, 1975 and 1980; Troubat, 1981; Yaméogo, 1984) is usually employed. However, the general comparison of test results has proved difficult just as the general estimation of the toxicity of the Chemicals. Two major reasons account for these difficulties. First, the environmental conditions under which tests are conducted vary considerably from one experiment to another. Secondly, there is a differential susceptibility of the taxa to different insecticides. Besides, from a theoretical

and maybe a predictive point of view, it will also be interesting to know whether products belonging to the same Chemical family (organophosphorus, carbamates, organochlorine, pyrethroid and growth regulator compounds, bio-insecticides, etc.) have the same toxicity for the same taxa.

4 All these tests, carried out on the non-target fauna as part of the selection of larvicides, have therefore been analysed and the main results are discussed in this document.

MATERIALS AND METHODS

5 The results, presented and discussed in this paper were obtained during tests conducted using the technique of multi-gutters (Troubat, 1981). A gutter is a plastic experimental apparatus modelled to represent a reduced stretch of river (Fig. 1) with, at one end, a stop screen for natural drift and, at the other end, a net for collection of drift of organisms taken from the river with the substrates (stones, sand, dead leaves and wood, etc.) to colonize the gutter.

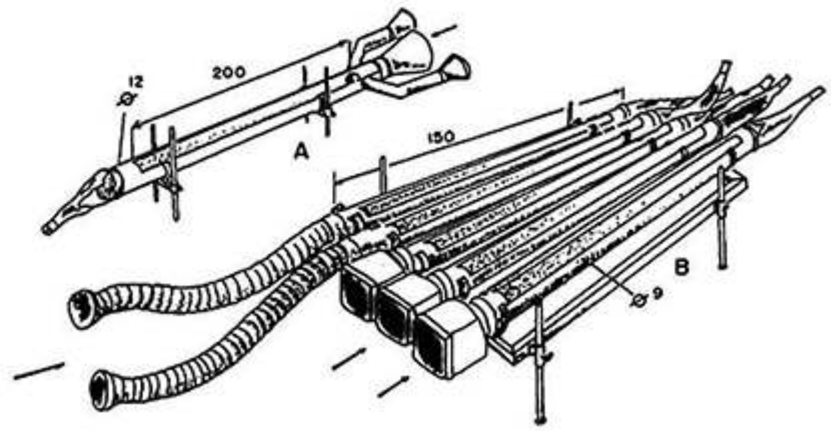


Fig. 1. Diagram of two types of gutter (simple A and multiple B) used in situ for the study of the impact of insecticides on benthic fauna.

6 The System is installed in rapids' zones of a river which is not very deep, in such a way that the water runs right through them; the water level reaching about half of their section. Drums containing the pesticide solutions to be tested can be placed at the upstream end of the gutters. Thus, different concentrations of a given pesticide or different pesticides can be tested while maintaining a control gutter.

7 Drifting fauna is sampled periodically (every 30 min. for 4 hours, then every hour for 20 hours) for a 24-hr period. Then, the remaining organisms in the gutters are collected separately, fixed in alcohol and labelled (as all the other samples) to be studied in laboratory.

8 It is therefore possible to compare the impact of different larvicides or different concentrations of a larvicide using the detached organisms or those remaining in the gutters.

9 The organisms used in the different tests were identified to the family level for all the aquatic insects with the exception of the Chironomidae which were separated to the subfamily or tribe level. Furthermore, only the taxa presenting a wide geographic distribution and which were relatively abundant in almost all the tests (Hydropsychidae,

Baetidae, Caenidae, Chironomini, Tanytarsini, Orthocladiinac, Tanypodinae) have been taken into account.

10 The percentage of detachment of the organisms in the gutters, considered as a percentage of mortality after correction by the Abbott formula (Finney, 1962) taking the natural mortality in the control gutter into consideration, is the value indicating the toxicity level of the larvicides. Reciprocal averaging analysis (correspondence analysis) were applied to these corrected data by using the "BIOMECO" programme of the Biometrics Group of CEPE-CNRS. Montpellier (France). This method of analysis, introduced by Foucart (1978) and Benzecri (cf. Benzecri & Benzecri, 1986), possesses a good descriptive power as regards tables of positive numbers (without these being tables of probabilities or frequencies). It should also allow a good description of the long-term effects on non-target aquatic insects of the use of pesticides against blackflies (Elouard & Jestin, 1982; Elouard & Fairhurst, 1990; Fairhurst & Curtis, 1988) and of the biotypology of running waters (Culp & Davies, 1980; Dakki, 1985).

11 To answer the different questions posed in the introduction, the analyses covered, firstly, the fauna that colonized the gutters before treatment (tested fauna) and then the fauna that remained in the gutters 24 hours after the larviciding was taken into consideration.

RESULTS AND DISCUSSIONS

1. Typology of the tested fauna

12 Analysis of the basic communities that colonized the gutters before treatment and tested using the same protocol at different periods of the year is illustrated in Figure 2. The examination of this factorial plane 1 x 2, whose first axis (Fl) explains 44% of the variance (inertia) out of a total of 79% for the plane, calls for two remarks:

- the F1 axis is based on the contrast of the flood-subsidence structure of macroinvertebrate communities at Koperagui (October), influenced by the Tricorythidae, with the others (Amou-Oblo and spate period at Koperagui);
- as regards the second axis (F2), it contrasts the spate period at Koperagui (July-August). influenced by the Tanytarsini and Hydropsychidae, with that of the flood-subsidence at Koperagui and the rise in water level at Amou-Oblo influenced by the other taxa with the exception of the Tanypodinae which did not contribute much to this axis.

13 The typologies described above are therefore related mainly to the hydrological seasons.

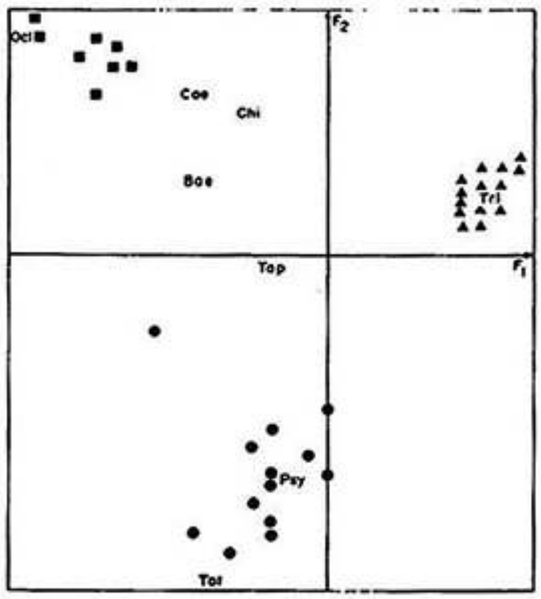


Fig 2. Typology of fauna in place in gutters during tests conducted under different conditions.
Abreviations
Bae: Bactidae
Cae: Caenidae
Tri: Tricorythidae
Chi: Chironomini
Tat: Tanytarsini:
Tap: Tanypodinae
Ocl: Orthocladiinae
Psy: Hydropsychidae
Legend
● Spate at Koperagui
▲ Flood-subsidence at Koperagui
■ Beginning of rise in water level at Amou-Oblo

2. Topology of drift in the control gutters and in the gutters treated

14 In order to reveal the impact of the Programme's operational larvicides on the drift of the organisms, we used the data on the tests carried out at one site but during different hydrological periods and therefore as has been brought out in the previous chapter, with different benthic community structures. The structures of the drift in the gutters treated with operational larvicides and in the control gutters are represented by factorial plane F_1 x F_2 (Fig. 3). This plane shows different typologies according to the insecticides. The presence of Controls makes it possible to apprehend the extern of the changes caused by the insecticides compared to a "natural" drift. The percentages of inertia of the first two axes of the correspondence analysis arc 39 and 35%. i.e., 74% for the factorial plane.

15 Groups corresponding to each of the larvicides tested and to the control gutters are well individualized on the factorial plane. Besides, it is observed that on the F_2 axis the Controls project close to temephos and opposite the toxic insecticides (chlorphoxim, permethrin and carbosulfan).

16 It indicates, on the one hand, that a different structure in the basic gutter communities does not change the structure reflecting the toxicity of the products. Some reservations are however necessary if the relative abundance of certain taxa is too low in certain tests. On the other hand, this analysis confirms the fact that temephos is a not-very-harmful

insecticide compared to the other three tested at the same site using the same methodology.

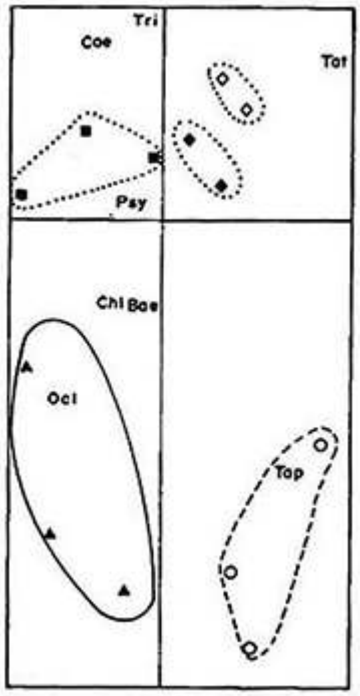
3. Typology of remaining fauna (in gutter)

17 The remaining fauna is that which has escaped from the impact of the larvicides. A comparison of the community structures obtained in this way with the basic ones of the gutters, will make it possible to understand eventual modifications caused by the insecticides. Besides, the typologies of the remaining fauna should be quite close to those of the saxicolous fauna of the watercourses treated with the antiblackfly larvicides; the gutter being considered as a miniature watercourse.

18 The objective of this study is to correlate the short-term toxicity of larvicides in gutter Systems with community structures in treated rivers and, later on to establish a model for forecasting the long-term impact of the larvicides on the saxicolous fauna on the basis of their short-term toxicity. Analysis of the typology of the remaining fauna in the gutters seems therefore to be more appropriate than that of the mortality in gutters.

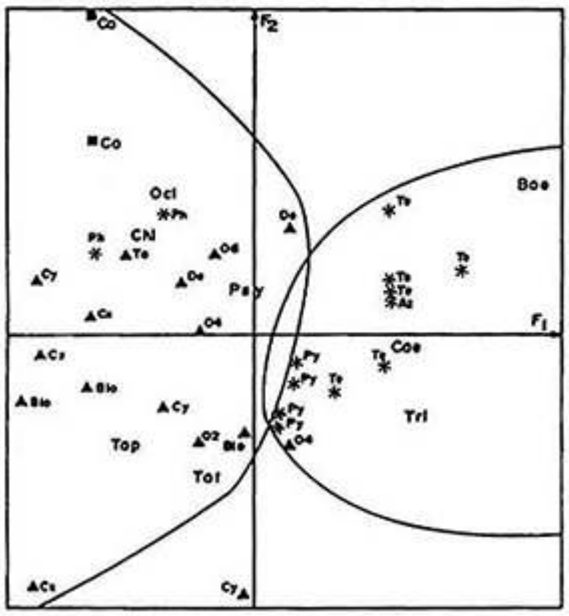
19 As seen earlier, the typologies of the fauna in place in the gutters before treatment are related to the hydrological seasons (Fig.2). A correspondence analysis has been made for the fauna remaining in these same gutters 24 hours after treatment (Fig.4). This factorial plane 1 x 2 explains 61% of the inertia. The first axis contrasts the Chironomidae, which are characteristic of the gutters treated with the relatively toxic insecticides (carbosulfan, pyrethroids and chlorphoxim), with the Ephemeroptera which are associated with the most selective doses or larvicides. The second axis contrasts the Chironomini and Orthocladiinae with the Tanytarsini and Tanypodinae. It reflects, therefore, the selectivity of the products as regards the Chironomidae while the F1 axis classifies the larvicides according to their general toxicity to the fauna as a whole. It will be noted that

the Hydropsychidae, which present a medium susceptibility to most of the larvicides (Table I). do not contribute much to the axes.



Abreviations
Bae: Bactidae
Cae: Caenidae
Tri: Tricorythidae
Psy: Hydropsychidae
Chi: Chironomini
Tat: Tanytarsini
Tap: Tanypodinae
Ocl: Orthocladiinae
Legend
▲ Abate
♦ Chlorphoxim
■ Permethrin
◊ Carbosulfan
○ Control
Fig. 3. Comparison between the typology of fauna that drifted from the gutters treated and from the control during tests conducted at the same site using the same protocol and

20 The typologies revealed seem to be stable and do not depend on the basic structure of the gutter communities. They suggest that taxa that are not much affected by an insecticide should remain in the watercourses treated with it, while the population of the susceptible taxa would decrease. However, the complexity of the biological phenomena calls for caution because the immediate impact observed during an isolated treatment could be affected in the long run by factors such as trophic and spatial competition, habituation or resistance or other forms of adaptation, the duration of the larval development cycles, etc.



Abreviations
Az: Azamethifos
Bio: Bioresmethrin
Ca: Carbosulfan
Ct: Permethrin
Cy: Cyphenothrin
De: Deltamethrin
Etf: Etbofenprox
O2: OMS 3002
O4: OMS 3040
O6: OMS 3036
Py: Pyraclofos
Ph: Chlorphoxim
Ta: Talstar
Te: Temephos

Fig. 4. Typology of fauna remaining in multi-gutters after the action of different insecticides tested by OCP using the same protocol.

Abreviations Legend
Bae: Baetidae
Cae: Caenidae
Tri: Tricorythidae
Chi: Chironomini
Tat: Tanytarsini
Tap: Tanypodinae
Ocl: Orthocladiinae
Psy: Hydropsychidae

Legend
▲ Pyrethroids
■ Carbamates
* Organophosphorus compounds

CONCLUSIONS

21 The correspondence analyses applied to the multi-gutter test data show that the impact of the larvicides is greater than the effect of the test period. Besides, although this type of analysis is solely descriptive, it gives a good idea of the toxicity of the larvicides on the main components of the biotic environment. The typologies recorded for the fauna remaining in the gutters contrast the selective insecticides, which are associated with the

Ephemeroptera (susceptible organisms), with the relatively toxic insecticides associated with the ubiquitous organisms.

22 These typologies do not depend on the insecticide family because some of the pyrethroids present a greater toxicity to the Chironomini (cyphenothrin), and others to the Tanytarsini (deltamethrin, ethofenprox). On the other hand, a certain classification of the toxicities of the products according to insecticide families is observed. Thus, generally speaking, the organophosphorus compounds are less toxic than the pyrethroids which are effective against the onchocerciasis vector.

Table I: Susceptibility of different taxa to various insecticides tested in multi-gutters.

Insecticide/Taxa	Tricorythidae	Baetidae	Chironomini	Tanypodinae	Tanytarsini
Temephos	+	++	++	++	++
Chlorphoxim	+++	++++	++	++	++
Pyraclofos	+	++++	++	+	+
Azamethiphos	+	+	++	++	+
Carbosulfan	++++	++++	++	++	++++
Cyphenothrin	++	++++	++++	+	+
OMS 3034	++	++++	+	++	+
OMS 3036	+++	++++	+++	+++	+++
Coopex	+++	++++	++	+	++
Biphenthrin (Talstar)	+++	+++	+	+	++
Deltamethrin	+++	+++	+	+++	+
OMS 3002	+	++++	+	+	+
Bioresmethrin	+++	++++	++	++	+
Ethofenprox (OMS 3002 de 86)	+++	++++	+	++	+++

Insecticide/Taxa	Orthocladiinae	Hydropsychidae	Caenidae	Faune Totale
Temephos	++	+	++	+
Chlorphoxim	++	++	+++	++
Pyraclofos	++	++	+	++
Azamethiphos	+	+	+++	++
Carbosulfan	++	++	+++	++++
Cyphenothrin	+++	++	+++	+++
OMS 3034	++	++	++	++
OMS 3036	++	++	+++	+++
Coopex	+++	++	++++	+++
Biphenthrin (Talstar)	++	++	++++	+++
Deltamethrin	++	+	++++	++
OMS 3002	++	++	++++	++
Bioresmethrin	++	++	+++	++
Ethofenprox	+	+++	++++	+++

+ Detachment less than 30% (low susceptibility)
+ + Detachment between 30 and 60% (moderate susceptibility)
+ + + Detachment between 60 and 80% (high susceptibility)
+ + + + Detachment more than 80% (very high susceptibility)

23 Finally, the typologies revealed in this way and analysed, taking into account those established after the long-term use of pesticides in lotic environments (Elouard et al. 1990), should make it possible to develop a model for the prediction of the long-term impact of the larvicides.

ACKNOWLEDGEMENTS

24 This work, financed entirely by WHO/OCP. was carried out in the field with the technical collaboration of the staff of the OCP Environmental Monitoring Unit (particularly Mr B. Coulibaly and Mr M. Bihoum) and consultants, including Mr B. Wahle, to whom we are grateful. We are also indebted to the Programme Director (Dr E.M. Samba), the Chief of

the Vector Control Unit of the Programme (Dr D. Quillévéré), and the members of the Ecological Group of the Programme who encouraged the publication of this paper.

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ABSTRACTS

Multi-gutter tests are used by the Onchocerciasis Control Programme in West Africa to screen antiblackfly larvicides with reference to non-target aquatic insect larvae. Because the tests are not always conducted under the same environmental conditions, direct comparison of results by the usual methods of data analysis presents some difficulties. The application of reciprocal averaging (correspondence analysis) to data from tests carried out using the same protocol indicates that the test periods have no incidence on the impact in gutter of the larvicides. The analysis also makes it possible to classify the insecticide families according to their degree of general toxicity on fauna. In addition there is no evidence of a uniform effect produced by each insecticide family on the principal taxa.

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Short-term toxicity of pyraclofos used as a blackfly larvicide on non-target aquatic fauna in a tropical environment

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INTRODUCTION

2 Human onchocerciasis (or "river blindness") is a filarial disease transmitted in West Africa by the female of the blackfly, Simulium damnosum s.l. (Diptera: Simuliidae), whose larval instars breed in swiftflowing portions of rivers. It is a disease which causes skin reactions and eventually severe ocular lesions followed by blindness. For many years, it was a major public health problem and an obstacle to the socioeconomic development of the fertile valleys in many West African countries.

3 In 1974, an Onchocerciasis Control Programme (OCP) was launched in seven of these States, under the auspices of the World Health Organization (WHO), the United Nations Development Programme (UNDP), the Food and Agriculture Organization of the United Nations (FAO), and the World Bank, and with the financial support of many donor countries and institutions, to control the disease.

4 Because of the absence of an effective drug for mass treatment of infected humans, it was decided to apply larvicides on the fast-flowing sections of the watercourses where the aquatic larval stages of the vector develop. The very short larval life of the blackfly led to the choosing of weekly larvicide applications to eliminate transmission of the filarial worm by the blackflies. Temephos, an organophosphorus insecticide, in a 20% emulsifiable concentrate formulation, was the only product used until the development in 1980 of a biological resistance in the flies to it (Guillet et. al, 1980). By 1982 resistance to

chlorphoxim (another organophosphorus compound) had also developed in certain forest cytotypes of the vector (Kurtak et. al. 1982).

5 These findings led to the large-scale use of Bacillus thuringiensis H-14 (Kurtak, 1986) and to the acceleration of a screening programme in a search for possible alternative larvicides. This acceleration became all the more necessary and was strongly recommended by the Ecological Group (an independent advisory body of the Programme) because of a planned extension into four additional countries. With this in mind, permethrin and carbosulfan were selected among many candidate blackfly larvicides (Yaméogo et. al, 1988). Recently, pyraclofos has proved to be an effective insecticide against the blackfly larvae. This paper discusses the results of tests carried out to evaluate the toxicity of pyraclofos for the non-target aquatic fauna as part of the screening of candidate blackfly larvicides undertaken in OCP.

CHARACTERISTICS OF THE PRODUCT

6 Pyraclofos (ISO draft), with the Chemical name of (RS)-(0-l-(4-chlorophenyl)-pyrazol-4-yl-O-ethyl S-propyl phosphorothioate), is an organophosphorus compound with a molecular weight of 360.8 (Kono, 1988). It is soluble in water up to 30 ppm at 20°C, and soluble also in alcohol and acetone. Its density is 1.271 (28°C). In water at pH 7 pyraclofos can be hydrolysed with a Chemical half-life in 700h and 1900h respectively at temperatures of 25 and 37° C. It biodegrades at a fast rate, especially in eutrophic waters. Hydrolysis also occurs at basic pH level. Bio-accumulation is unlikely to occur (as with other organophosphorus insecticides), mainly because of metabolic degradation. The formulation used in the trials was an emulsifiable concentrate with 50% of active ingredient, pale yellow in colour. It is an insecticide/acaricide that acts by contact and ingestion. The dose used for the control of S. damnosum s.l. larvae in the Programme area is 0.12 litres of formulation per m^3/s of river discharge (0.1 mg x L^{-1} for 10 min or 60 mg x L^{-1}.s).

MATERIALS AND METHODS

7 The toxicity study was carried out in the laboratory and in a river using the standard OCP protocol: acute toxicity in tanks for fish (Yaméogo, 1980), gutter tests, sampling of benthic insect drift (Dejoux, 1975 et 1980; Troubat, 1981; Yaméogo, 1984; Yaméogo et. al, 1988) pre- and post-larviciding qualitative observations.

1. Gutter tests

8 A gutter is a plastic experimental apparatus modelled to represent a reduced stretch of river with, at the upstream end, a stop screen for natural drift in the river and, at the downstream end, a net for the collection in the gutter of the drift of organisms taken from the river with the substrates (stones, sand, dead leaves and twigs, etc.) to colonize the gutter. The System is installed in rapids' zones of a river which is not very deep, in such a way that the water runs through the gutters; the water level reaching about half of their section. Drums containing the pesticide solutions to be tested can be placed at the

upstream part of the gutters. Thus, different concentrations of a given pesticide or different pesticides can be tested while maintaining a control gutter.

9 Drifting fauna is sampled periodically, (every 30 min. for 4 hours, then every hour for 20 hours) for a 24-hr period, then, the remaining organisms in the gutters are collected separately, fixed in alcohol and labelled (watercourse, locality, type of sampling, date, time), like all the other samples, to be studied in laboratory. It is therefore possible to compare simultaneously the impact of different larvicides or different concentrations of a larvicide using the detached organisms and/or those remaining in the gutters.

10 In addition to a direct comparison between the percentages of detachment of the organisms tested, factorial correspondence analysis (Benzécri, 1983; Benzécri & Benzécri, 1986) was applied to data collected from several gutter tests with carbosulfan (a carbamate compound), permethrin (a pyrethroid), temephos, chlorphoxim and pyraclofos (organophosphorus compounds) using the "BIOMECO" programme of the Biometrics Group of CEPE-CNRS, Montpellier (France). This method of analysis introduced by Foucart (1978), possesses a good descriptive power as regards tables of positive numbers (without these being tables of probabilities or frequencies). It allows an easy descriptive generalization of the impact of blackfly pesticides on non-target benthic insects (Elouard & Jestin, 1982) and of the biotypology of running waters (Culp & Davies, 1980; Dakki, 1985).

2. Benthic insect drift sampling

11 A battery of two nets (ϕ 12 cm) was used to estimate the abundance, in the river, of drifting insects, which fluctuates, as in temperate zones, according to a circadian rhythm (Elouard et Lévêque, 1977). The drift is low during the daytime and maximal during the night-time. Daytime drift is considered as a reflection of the morbidity and mortality of the organisms; it is regarded as passive. On the contrary, night-time drift reflects an active period, a voluntary drift of the organisais, which reflects the density of benthic organisms (Statzer et. al. 1985 et 1987) Since the abundance of benthic insects in the drift is supposed to increase with current speed and other environmental factors (Statzer et. al. 1984), different factors (pH, conductivity, turbidity, human disturbance, etc.) are measured, and the calculated "drift index" is used for the analysis instead of the raw number of organisms collected; the "drift index" is the number of organisms collected per cubic metre of filtrated water. Because of the high number of organisms sampled after larvicide sprayings, drift is collected for 2 min. only, every 30 min, from 6 a.m. to 10 p.m., then every hour. "Drift index" is generally spcaking ten to twenty times higher during night-time than daytime in untreated rivers. Daytime "drift index" increases when a river becomes treated (Yaméogo et. al. 1988).

3. Tank tests on fish species under laboratory conditions

12 Acute toxicity tests are performcd in 10-litre glass tanks containing 10 fish each, using the technique with periodic replacement of solutions (Ward & Parrish, 1983). The insecticide solution is changed every 12 hours to avoid the effect of degradation of the insecticide. The water temperatures were 27 ± 2°C. The end point is death. The lethal concentrations are calculated according to probit analysis (Finney, 1952).

13 The test concemed mainly Chrysichthys nigrodigitatus Lacépède, 1803 (Bagridae) and Pollimyrus isidori Valenciennes, 1846 (Mormyridae) two fish species quite common in the study area. C. nigrodipitatus is known in the Gambia, Senegal, Niger and Volta basins and in most coastal rivers (Lévêque & Paugy, 1984). The maximum standard length is 475 mm but the average length of the individiuals tested was 65 mm. The species feeds mainly on insect larvae (Chironomidae) but also on small mollusca, zooplankton and Hemiptera (Lévêque et. al. 1988). P. isidori is a small species well known in the Niger, Gambia, Senegal and Volta basins and in coastal rivers (Lévêque & Paugy, 1984). It feeds on insect larvae and on zooplankton (Lévêque et. al. 1988). The average length of the individuals tested is about 47 mm and the maximum standard length of the species is 83 mm.

4. Immediate river toxicity evaluation

14 Drift of fish juveniles is collected using a large net (45 x 50 cm and 3 mm of mesh) set up for at least a 48-hr period of time, 24 hrs before treatment and 24 hrs after. The net is placed in a flowing section of the river at 8 a.m., removed at 4 p.m., emptied and replaced immediately after for the whole night up to 8 a.m. the next day. The same protocol is applied for the remaining 24 hrs. The samples collected are preserved in formaldehyde solution and analysed in laboratory. The impact of the larvicide on the fish is made by comparing pre-and post-treatment catches.

5. Taxonomie level

15 The level of identification is the genus and even species for the Hydropsychidae (Trichoptera), Baetidae and Tricorythidae (Epheme-roptera). Chironomidae are identified at tribe or subfamily levels while most of the other benthic insects arc identified at the family level because of lack of detailed information. Fish are normally identified at the species level but for juveniles the identification is confined to family or order.

16 The organisms retained for the tests are those representative of the study area and which adapt themselves well to the working conditions (Dejoux et. al. 1983; Lévêque et Paugy, 1984).

6. Location of field trial sites

17 For the operational trial conducted on the White Bandama in Côte d'Ivoire in 1989, several sampling sites were set up (Fig. 1).

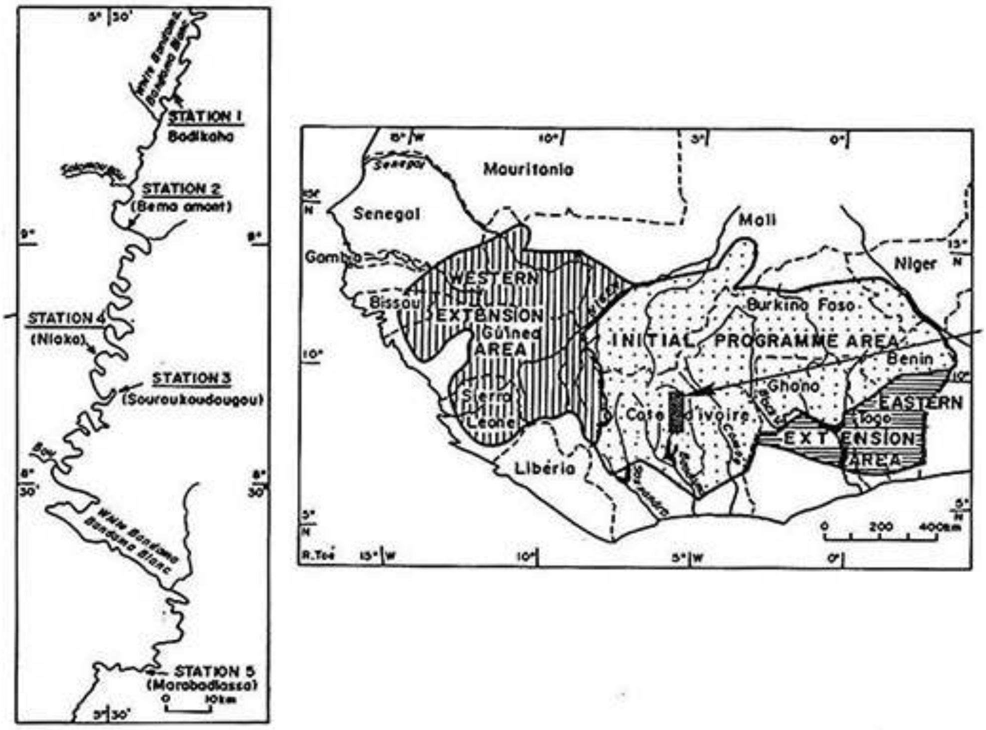

Fig. 1. Location of the operational trial river and the monitoring site in the Onchocerciasis Control Programme in West Africa.

18 The total length of the river is about 800 km. The upper half of the watercourse concerned with the trial is in a wooded savanna area. During the operational trial (high-water season), the depth of the stretch of the river studied was between one and 15 metres while the length was about 250 km. The discharge during the first pyraclofos treatment cycle was maximal and around 450 $m^3 \times S^{-1}$ (Fig.2).

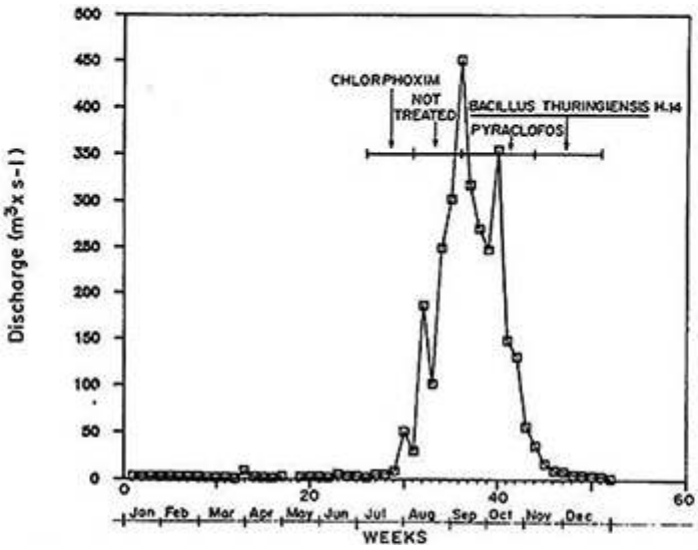

Fig. 2. Weekly discharge of the White Bandama at station 4 from January to December 1989.

19 The sampling point at Station No.2 was situated at some 300 m downstream from the nearest spraying point. It was selected to study the direct and maximum impact of the product on the invertebrate fauna. Station No.3 was located at about 5 km below the spraying point to study the carry of the product. Station No.4 was set up to follow the

impact of pyraclofos on fish juveniles. It is also a regular monitoring site for the "Aquatic Monitoring Unit" of the Programme.

RESULTS AND DISCUSSION

1. Gutter tests in the field

20 The experiment was carried out on the Sassandra river, in Côte d'ivoire, at the same site, with pyraclofos at 0.1 mg of active ingredient xL^{-1} for 10 min., in comparison with temephos at 0.1 mg (a.i) x L^{-1} for 10 min. and chlorphoxim at 0.05 mg (a.i.) x L^{-1} for 10 min in the presence of an untreated gutter (see materials and methods for details on the techniques).

1.1 Control

21 Drift was very low (5% detachment of the total fauna) compared to that usually obtained in this type of experiment (10 to 20% detachment). However, Amphipsyche senegalensis. (Trichoptera: Hydropsychidae) the Simuliidae and Chironomini (Diptera: Chironomidae) presented a percentage of detachment greater than that of the total fauna in the gutter, but it did not exceed 15%.

1.2 Pyraclofos

22 The percentage of detachment of the total fauna is around 35%, 24 hours after treatment. Most of the organisms tested presented percentages of detachment less than 40% except Simuliidae, Centroptilum, Baetis and Pseudopannota bertrandi. The detachment of Trichoptera is very low (7%). That of Chironomidae is higher with a mean detachment value of nearly 25%.

1.3 Chlorphoxim

23 The total percentage of drift due to chlorphoxim was almost 59%, a value which is within the range of the data reported in Yaméogo et. al 1988. Among the non-target organisms, it was the Ephemeroptera (mainly Centroptilum and Baetis but Pseudopannota bertrandi and Tricorythus also) which were affected most (almost 85% detachment). The effect on the Trichoptera as a whole was moderate but Amphipsyche senegalensis seems to be more susceptible (55% detachment) than the other species tested. The Chironomidae (Diptera) are also not very susceptible to chlorphoxim even though, just as for the other taxonomic groups, this product induced the highest detachment.

1.4 Temephos

24 The total detachment of the fauna was about 17%, a value which is within the range of values usually obtained with this pesticide at the dosage of 0.1 mg x L^{-1} for 10 min. Apart from the Simuliidae which presented a detachment of more than 98% and two ephemeropteran taxa (Centroptilum and Baetis) which were affected up to 85%, most of the other taxa seemed to be little perturbed with a detachment which was less than 20%.

1.5 Comparative impact of the three larvicides

25 Since the tests were carried out on the same day and under the same conditions, with a faunal composition similar from one gutter to the other, it is possible to compare directly the results obtained with the different insecticides used (Fig.3).

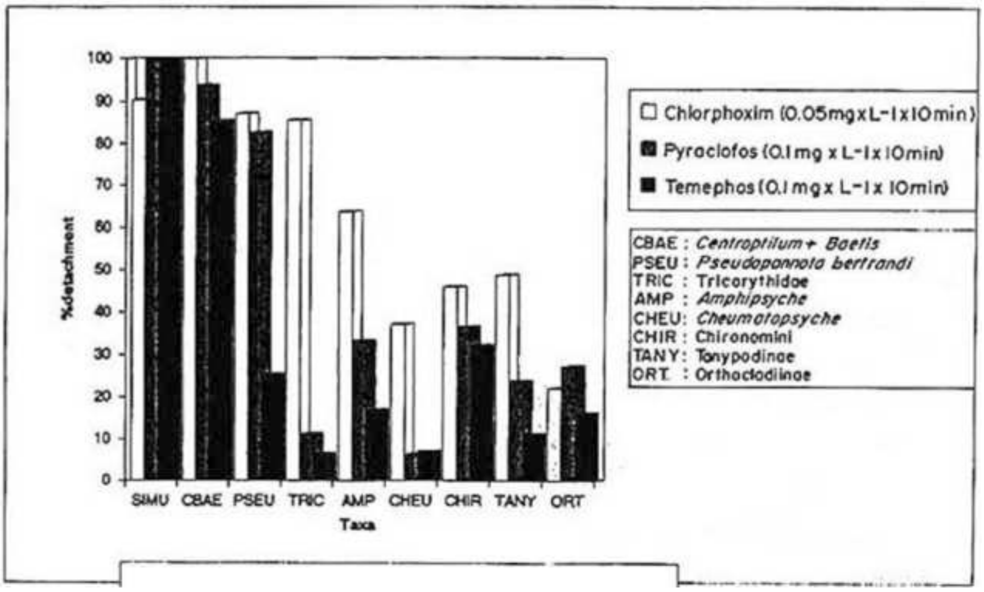

Fig.3: Percentage of detachment of different taxa after the application of the three larvicides tested

26 While at the operational doses Simuliidae, Centroptilum and Baetis were the organisms affected most by the organophosphorus compounds mentioned above (Fig. 3) the classification of the other taxa in terms of their susceptibility varies from one larvicide to another among the organophosphorus compounds. On the other hand, a regrouping of the organisms into Ephemeroptera, Trichoptera, Chironomidae, Simuliidae gives the following classification according to their susceptibility: Trichoptera < Chironomidae < Ephemeroptera < Simuliidae

27 Finally, the diagram on the percentage of detachment of the principal taxa reveals two distinct groups, of organisms for pyraclofos and temephos:

- Simuliidae, Centroptilum and Baetis, on the one hand, which are very susceptible (detachment of more than 80%) and could be affected most in the long terni by the weekly spraying of these larvicides;
- the Chironomidae, Cheumatopsyche. (Trichoptera), Tricorythus (Ephemeroptera) and Amphipsyche. (Trichoptera), on the other hand, which do not appear to be very susceptible to the effects of pyraclofos and temephos (detachment of less than 40%) and should therefore be able to withstand the impact of the larvicide applications if other phenomena do not corne into play.

28 For chlorphoxim, the most susceptible taxa were Tricorythus with Centroptilum, Baetis and Pseudopannota bertrandi. Cheumatopsyche falcifera, C. digitata and the Tanytarsini presented an average susceptibility while Amphipsyche senegalensis is more susceptible than this group of organisms but less than the previous one.

29 A factorial correspondence analysis of data collected from gutter tests with carbosulfan, permethrin, temephos, chlorphoxim and pyraclofos was made. Figure 4 shows a clear separation on the second axis (F2) between the most selective larvicides (temephos and

pyraclofos) on one side, and permethrin, carbosulfan and chlorphoxim on the other side. The inertia percentages of the first two axes (F_1 and F_2) of the plane are 37% and 27%, i.e., a total of 64% for this factorial plane $F_1 \times F_2$.

30 The Tanypodinae seem to be particularly affected by temephos, on the First axis (Fl), as against the Caenidae and Tricorythidae which are influenced by carbosulfan, chlorphoxim and permethrin. Among the other Chironomidae, Chironomini and Orthocladiinae do not seem much affected by these larvicides.

31 It emerges, therefore, from this experiment that all the organisms do not completely present the same reaction to insecticides belonging to the same Chemical family formulations. Furthermore, generally speaking, a marked impact of the larvicides should be expected on the Ephemeroptera in the long term. However, the monitoring, which has been going on for some fifteen years now in the watercourses treated in the Programme area (Yaméogo et. al, 1988), shows a rarefication of the Tricorythidae and certain Baetidae species in the worst cases. Contrary to all expectations, a marked presence of Pseudopannota bertrandi has been observed in certain hydrobiological monitoring stations (Ano, 1989), due to its short larval life-span (Wuillot, 1990) which allows emergence to occur between two treatment cycles, so that recolonization can take place.

2. Impact of an operational treatment on the insects

32 The results presented in this section arc those recorded during the operational trial of pyraclofos on the White Bandama during the high-water period; the operational trial in OCP is the final step of screening before adopting a new insecticide as operational. It covers hundreds of kilometres of a river using a helicopter and operating as in a real larviciding campaign. Drift sampling with double nets and qualitative observations were the only techniques under which the experiment was conducted.

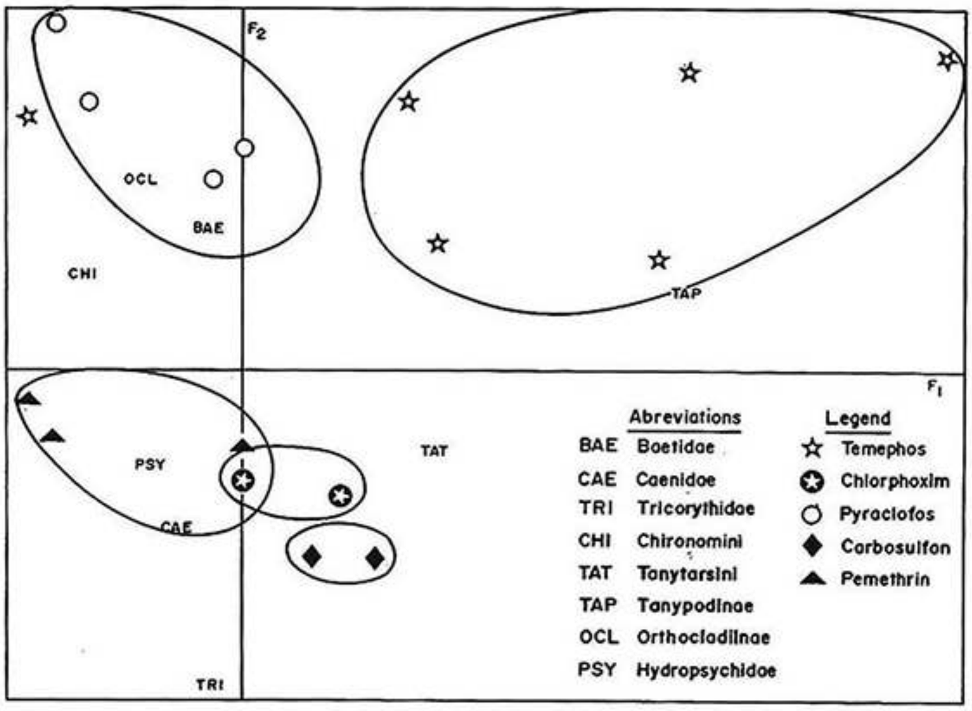

Fig. 4. Typology of the mortality percentages (corrected according to Abbott formula) of the main taxonomic groups in gutters treated with five blackfly larvicides (plane 1 - 2).

2.1 Impact on drift near the spraying point (station 2)

33 Before treatment, the trend of the drift index curve was of the classic type (Elouard & Lévêque, 1977; Yaméogo, 1980); low during the day, the drift increased at night to reach 33 individuals per m3 of filtered water, and then decreased regularly (Fig.5a).

34 After treatment, the drift collected for 4 hours was comparable to that of the previous day at the same time. The drift index increased only a short time before sunset, then fell to merge with the eve's biological-activity drift. This increase which occurred only few hours after the application of the product was not due to a delayed effect nor did it correspond to the night drift. It could be due to the larviciding made at 13.4 km upstream, ten minutes before that made at 300 m from the sampling point. Twice greater than the pretreatment night drift, the peak occurred two hours earlier and was influenced by the Chironomidae while the Ephemeroptera made up the greater part of the pre-treatment drift (Fig.5b).

35 On the whole, the drift remained low at this station, marked by the abundance of early larval stages and the almost complete absence of surface Hemiptera in the collections.

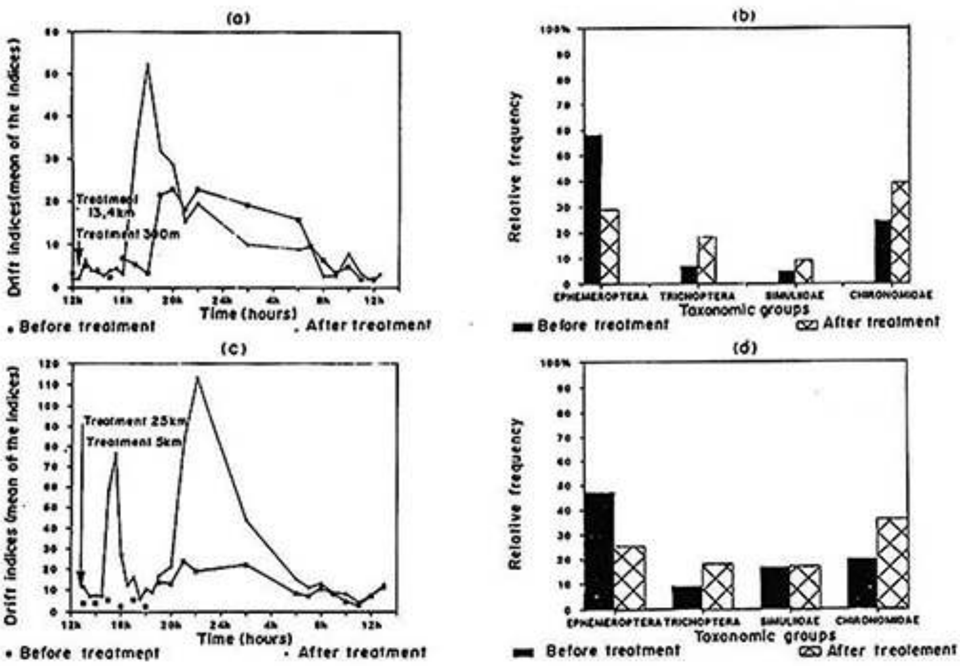

Fig. 5. Trend in drift of non-target benthic fauna at station 2 (a) and station 3 (c). Relative frequencies of main taxonomic groups in the drift at station 2 (b) and station 3 (d). 13-15/09/89.

2.2 Impact on drift at 5 km downstream spraving point (station 3)

36 Just as for the previous station, the pre-treatment drift was of the classic type. It was however more diversified here, indicating different mesological conditions.

37 Since the nearest spraying point upstream was located at 5 km from the sampling area, the drift intensity increase occurred 1 hr 30 min. after treatment, and was characterized by the abondance of Orthocladiinae. Of greater magnitude than that recorded at station 2, the drift intensity subsequently decreased but another peak occurred in the night with the peak recorded around 2200h (Fig.5c). The Orthocladiinae, Simuliidae, Baetidae (

Centroptilum and Baetis), and Hydropsychidae (Aethaloptera) presented drift curves having more or less the same appearance. The Caenidae and Leptophlebiidae did not seem to have been particularly affected by the treatment. After treatment, the relative composition of the communities underwent a change similar to that of the previous station; the Chironomidae became the most represented, followed by the Ephemeroptera, the Trichoptera and the Simuliidae (Fig.5d).

38 The drift here was greater than at 300 m from the spraying point and the second peak recorded around 2100h could be due to a combination of several factors including the natural night drift, probably intensified by a weakening of the organisms, and the effects of a second pyraclofos wave from the treatment point located sorae 20 km upstream.

3. Impact of pyraclofos on the fish fauna

3.1 Acute toxicity test

39 The medium lethal concentrations calculated according to the probit analysis (Finney, 1952) for different exposure times are not very different for the two fish species tested. However, looking at the slopes of the mortality curves (Table I), it appears that the longer the exposure time, the more small increases in dose resuit in considerable P. isidori mortalities while C. nigrodigitatus becomes less affected by slight dosage increases.

Table I: Median lethal concentrations (LC_{50}) of pyraclofos (TIA-230) for two tropical fish species.

	Median lethal concentrations -µg/l (Confidence limits) Slopes		
	24 hr	48 hr	72 hr
	170	70	40
Pollimyrus isidori	(149-184)	(41-87)	(3-66)
	5.27	3.71	2.95
Chrysichthys nigrodigitatus	150	78	68
	(113-632)	(53-95)	(48-82)
	2.66	3.41	4.45

40 Apart from the 24-hr LC_{50}, which are higher than the highest dosage used in onchocerciasis vector control (100 μg x L^{-1} for 10 min.), the other data are between 40 and 100 μg x L^{-1}.

41 Compared to other data obtained with permethrin, carbosulfan and cyphenothrin by different authors, particularly Yaméogo et al. 1991, one may note that pyraclofos has the highest 24-hr LC_{50} (150 μg/1 against 40 μg x L^{-1}, 82 μg x L^{-1} and 15 μg x L^{-1} respectively), which means that it is less toxic than the others. But taking into consideration the operational doses, it appears that the 24-hr LC_{50} of pyraclofos is doser to its highest operational dose (O.D.) than the 24-hr LC_{50} of permethrin and its O.D. Besides, the 24-hr LC_{50} of cyphenothrin is equal to its O.D. and the difference between the 24-hr LC_{50} and the O.D. of carbosulfan is somewhat comparable to that recorded with pyraclofos. It is therefore considered that when used for Simulium control, cyphenothrin presents the

highest risk for fish; the risk of permethrin is the lowest, while that of carbosulfan and pyraclofos is medium.

3.2 Immediate river toxicity

42 Direct observations made during the larviciding of pyraclofos in a river at the dose of 0.1 mg x L^{-1} for 10 min did not reveal any fish mortality even though the dose at the spraying point was almost ten times this dose.

43 Collections were made with drift nets both before and after the pyraclofos sprayings. The results do not show an increase in the drift intensity but a slight change in the relative composition of the communities (Fig.6). Schilbe mystus (Schilbeidae) dominated in the night drift but the proportion of Characidae increased in the post-treatment night drift.

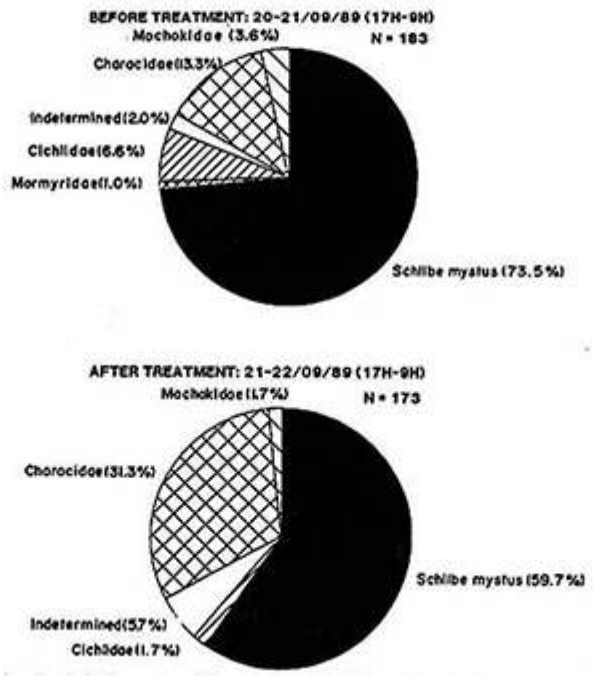

Fig. 6. Relative composition of juvenile fishes in the night drift before and after pyraclofos spraying at station 4.

44 On the other hand, the day drift, solely composed of Characidae, was not influenced by the pyraclofos application. This product does not seem therefore to have a direct impact on fish in rivers. The posttreatment decrease in the drift intensity and the increase in the proportion of Characidae are the main facts which should be mentioned.

CONCLUSION

45 From the above results, it can be seen that the product presents at the operational dose, a toxicity on the aquatic entomofauna which is between that of temephos and that of chlorphoxim. It acts particularly on Centroptilum, Baetis and Pseudopannota bertrandi which detached by more than 80% while the other nontarget organisms presented an average drift of 40%.

46 While in the laboratory pyraclofos presents, for fish, a relatively high toxicity which increases with exposure time, this has not been confirmed by the results of the river

tests. No fish mortality was recorded and the slight behavioural change observed for Characidae (a particularly fragile fish) was transient. This change did not resuit in an increase in the drift (in number) of Characidae. It should be recalled that in rivers dilution occurs quickly and the fish can flee and avoid areas of high concentrations of the larvicide (Abban & Samman, 1980).

47 Short-term toxicity tests are the first stages of the hazard assessement of a candidate antiblackfly larvicide. Since the results presented in this document show that the short-term impact of pyraclofos on the non-target aquatic fauna is not drastic, it has been decided to undertake a large-scale experiment to assess its medium and long-term effects on the aquatic environment. The results will be published when completed.

ACKNOWLEDGEMENTS

48 This work was fully supported financially by WHO/OCP, and the Programme Director, Dr E. M. Samba, agreed that the results should be published. We are therefore indebted to him.

49 Many persons contributed to the realization of this work. We would like to thank, in particular, Mr J. Wuillot of the University of Lyon, Mr Fanfodé Kondé of the University of Kankan and Mr B. Coulibaly, Mr L. Bakoné, Mr B. Dolbézanga and Mr S. Bakayoko of OCP who participated in the data collection during the operational trial of pyraclofos. The fish specimens were fished by Mr Simpore who gave us a highly appreciated help in the laboratory too.

50 Finally, we are grateful to Mr T. Mills who undertook the literary revision of the document, and to all those (drivers and technicians) who, in one way or the other, also contributed to the carrying out of these tests.

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ABSTRACTS

Among the many larvicides tested for the control of Simulium damnosum s.l. larvae, the vector of human onchocerciasis in West Africa, pyraclofos proved to be 100% effective at 100 μg x L^{-1} for 10 min in river, with a cany of 20 km at 100m^3 xsec^{-1}. Tests were then performed both in laboratory and field conditions to evaluate its toxicity on the non-target aquatic fauna. In experimental short-term gutter tests, the detachment of the total benthic insects was 35% al 100 μg x L^{-1} for 10 min against 17% for temephos at the same dose and 59% for chlorphoxim at 50 μg x L^{-1} for 10 min. Centroptilum, Baetis and Pseudopannota bertrandi were the most affected organisms. The treatment of a river resulted in a considerable detachment of the same taxonomic groups, plus Orthocladiinae. On the other hand, investigations conducted in tanks showed that the 24-hr LC_{50} for Chrysichthys nigrodigitatus is 150 μg x L^{-1} and that for Pollimyrus isidori 170 μg x L^{-1}, values which are not very different from the operational dose of the larvicide (100 µg xL^{-1} for 10 min.). Nevertheless, in a river, no fish mortality was recorded. Based on fish LC_{50} and drift of benthic insects, pyraclofos at 100 μg x L^{-1} was judged to be less toxic to aquatic fauna in the short term than permethrin and carbosulfan.

INDEX

Keywords: pyraclofos, simuliidae, bioassay, factorial correspondence analysis, blackfly larvicide, West Africa, aquatic fauna

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Risk assessment of etofenprox (vectron®) on non-target aquatic fauna compared with other pesticides used as *Simulium* larvicide in a tropical environment

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EDITOR'S NOTE

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1. Introduction

2 The weekly destruction for 14 years of the aquatic stages of *Simulium damnosum* s.l. is the principal strategy used since 1974 by the Onchocerciasis Control Programme in West Africa (OCP); the objective being to eliminate onchocerciasis as a disease of public health importance as well as an obstacle to socio-economic development.

3 Several activities have been undertaken to minimize the environmental effect of such a Programme and they have been recently reviewed by Calamari et al. (1998). The present paper describes the toxicological studies performed in order to make a risk assessment of etofenprox. For several years, temephos or Abate®, an organophosphorus insecticide formulated with 20% of active material, was the only product used till the appearance in 1980 of a resistance in the vector *S. damnosum* (Guillet et al., 1980). Chlorphoxim, another organophosphate and *Bacillus thuringiensis* ser. H-14 were then introduced, but rapidly, a

resistance to chlorphoxim was developped by the forest cytotype of the vector (Kurtak et al., 1982). The Programme was then obliged to give priority to the search for new larvicides or new formulations of larvicides. Among the numerous insecticides tested (Yaméogo et al., 1988, 1992), only pyraclofos, which is equally an organophosphate, permethrin (a pyrethroïd) and carbosulfan (a carbamate) were retained for use under well-established conditions in relation to their level of toxicity. Toxicological studies and assessments for pyraclofos (Yaméogo et al., 1993b) and permethrin (Yaméogo et al., 1993a) have been published elsewhere. Pyraclofos treatment was to be carried out at discharges higher than 15 $m^3 s^{-1}$ and, permethrin and carbosulfan spraying concerned discharges higher than 70 $m^3 s^{-1}$ for a maximum of six cycles in the year for the same stretch of river. Therefore, six insecticides could be used in rotation considering the risk of resistance, the operational costs and the effects on the aquatic environment. However, between 15 and 70 $m^3 s^{-1}$, the only usable larvicides, taking into consideration the cost/efficiency ratio, were the organophosphates (temephos, phoxim and pyraclofos), then increasing the risks of resistance of the simulids to these products. It therefore became necessary to continue the research with the view of identifying a non-organophosphorus insecticide that could be used on demand between 15 and 70 $m^3 s^{-1}$.

4 It is within this context that the bibliographic search permitted the selection of etofenprox or Vectron®, a pseudopyrethroid described as having weak effects on fish, for an evaluation of its toxicity on the non-target aquatic fauna in the field conditions of the Programme. Some tests were then undertaken on fish and invertebrates in laboratory as well as in the field and under conditions of an operational Programme. This document gives the results obtained during tests conducted in a short-term period.

2. Material and methods

2.1. Characteristics of the product

5 Chemical name Ether 3-phenoxybenzylic 2-(4-eth-oxyphenylic)-2-methylpropylic, with popular name (or ISO) etofenprox, the product is a pseudopyrethroid with a molecular formula $C_{25}H_{28}O_3$, with a density of 1.157/23°C and a molecular weight of 376.5 (Udagawa, 1986). It is soluble in a series of ordinary solvents at 25°C and almost insoluble in water (<1 ppb). Its aspect is solid white crystalline; it is without any characteristic odour and is stable in some acids and alkalines. It is an active insecticide through ingestion and contact, having a very low toxicity in mammals and fish (Mitsui Toatsu Chemicals Inc., 1986). Its partition coefficient (log *P*) in water/*n*-octanol is 7.05, its half-life in soil is 6 days and the vapour pressure is $2.4x10^{-4}$ mm Hg/100°C. The formulation used in the zone of the Programme against the larvae of the *S. damnosum* s.l. is an emulsifiable concentrate with 20% of active ingredients/litre.

6 Other Chemicals tested for comparison were: permethrin (3-phenoxybenzyl-(1 *RS*)-*cis, trans* 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate); pyraclofos (*RS*)-[*O*-1(4-chlorophenyl)-pyrazol-4-yl-*O*-ethyl-*S*-propylphosphorothioate]; OMS 3050 1,1,1,-trifluoro 2-(4-ethoxy-phenyl) 3-(3-(4-chlorophenoxy) benzyloxy) propane. The formulations of the products were diluted taking into account the percentage of active ingredient.

2.2. Gutter tests

7 This method makes it possible to compare simultaneously in field conditions the impact of different larvicides or different concentrations of a larvicide. The system is composed of a certain number of plastic experimental apparatus modelled to represent reduced stretches of rivers (gutters) and is directly alimented with river water. The gutter (Troubat, 1981) is equipped, at the upstream end, with a stop screen for natural drift and, at the downstream end, a net for the collection of the drift of organisms taken from the river with the substrates (stones, sand, dead leaves and twigs, etc.) to colonise the gutter. The results were expressed for the total fauna and for the principal taxa present in the gutters as percentage of detachment per insecticide tested. To appreciate the effect of the above-mentioned insecticides on the main species in the gutters, treatments were made every 2 h with increasing doses in two parallel gutters (for each larvicide) having comparable discharges. The drift was collected every 30 min and the results expressed in number of taxa affected and in percentage of detachment for each of the taxa studied.

2.3. Tank test on fishes and shrimps under laboratory conditions

8 The acute toxicity tests are performed in 200 1 tanks. The solutions were 150 l for each tank and were oxygenated. The technical products were diluted taking into account the percentage of active ingredient and the results were expressed for each time, e.g., concentration of active principle (mg l^{-1}). Ten to fifteen individuals of fish or 20 shrimps were put into each tank, knowing that 1 g of fish should be reared in 1 l of water. For each of the products, five different doses were prepared and three replicates were done. The larvicide solutions were prepared 1 h before the start of the trials. The tests were performed using the static technique (Ward and Parrish, 1983). The water temperature were 25±1°C. The end point is death. The lethal concentrations are calculated according to probit analysis. The behaviour of the fishes in the tanks was observed and reported three times a day.

9 *Caridina africana* as regards shrimps and two species of fish from the family of Cichlidae (*Oreochromis niloticus* and *Tilapia zillii*) were the species tested. They are the most common species encountered in the watercourses of the Programme area. *C. africana* (Atyidae) is one of the most widespread shrimps species in intertropical Africa. It is a small shrimps (10-30 mm) encountered in small and large rivers, lakes, streams, ponds and pools. *O. niloticus* Linné, 1758 is well known from many river basins in Africa (Senegal, Gambia, Volta, Niger, Benoue and Tchad basins). *T. zillii* Boulenger, 1901 is more widespread. In addition to the above-mentioned basins, it is encountered in the Ogun, Oshun, Comoé, Mé, Bandama, Boubo and Sassandra river basins in West Africa. Elsewhere in Africa, *T. zillii* is known from Oubangui, Uélé, and Ituri (Zaïre), lake Mobutu, the Nile, lake Turkana and from Jourdain basin. These species are of commercial importance in the Programme area and therefore, were considered for testing as an impact on them should be prejudiciable to the activity and life of people in the area. The medium length of the individuals tested was 6.98±1.52 cm for *O. niloticus* and 7.18±2.5 cm for *T. zillii.*

2.4. Shrimps monitoring methodology in field conditions

10 A test was performed at the operational dose on the Leraba river at 19 $m^3 s^{-1}$ to assess the impact of etofenprox on shrimps. An operational dose is the concentration of insecticide that will kill *Simulium* larvae with a carry of 6 km at 8 $m^3 s^{-1}$ and of 13 km at 100 $m^3 s^{-1}$ in high flow conditions and it is usually expressed in mg l^{-1} 10 min. Drifting shrimps (*C. africana* Kingsley 1882) were sampled using a large net (45 cm x 50 cm and 3 mm of mesh) set up for at least a 48 h period of time, 24 h before and 24 h after treatment. The current velocity within the net was measured at least twice a day. The net is placed in a flowing section of the river and emptied every 4 h. The individuals collected just after treatment were kept under observation in cages placed upstream in the untreated section in order to monitor their fate in uncontaminated water. The impact of the larvicide on the shrimps is evaluated therefore by comparing the difference between pre¬ and post-treatment catches for the same stretch.

2.5. Taxonomic level

11 The level of identification for insects is the genus and even species for the Hydropsychidae (Trichoptera), Baetidae and Tricorythidae (Ephemeroptera). Chironomidae are identified at the tribe or subfamily levels while most of the other benthic insects are identified at the family level because of lack of detailed taxonomic information on the larvae. Fish and shrimps are normally identified at species level but for the juveniles or individuals in a bad State, the identification is confined to family or order levels. The organisms retained for these tests are those representative of the study area and which adapt themselves well to the working conditions (Dejoux et al., 1983; Durand and Lévêque, 1980; Lévêque et al., 1990).

3. Results and discussions

12 The main objective of the tests is to help take a decision on the possible introduction of etofenprox in the larvicides' rotational scheme of the Programme. The acute toxicity tests as well as the field trials were therefore conducted bearing in mind this objective.

3.1. Gutter tests in the field

13 The experiment was carried out on the Oti river, in Togo, at the same time, with etofenprox in comparison with permethrin, OMS 3050 and pyraclofos in the presence of an untreated gutter (see materials and methods for details on the techniques). The percentages of detachment presented below (Table 1) are those corrected using the Abbot formula.

Table 1. Detachment (%) in gutters of some taxonomic groups of invertebrate during the test conducted on the Oti river in Togo with operational doses

	Etofenprox (0.03 mg l^{-1} 10 min)	Pyraclofos (0.1 mg l^{-1} 10 min)	OMS 3050 (0.025 mg l^{-1} 10 min)	Permethrin (0.015 mg l^{-1} 10 min)	Control
Non-target fauna	47	43	56	91	19.5
Ephemerotera	61	54	66	97	45.9
Trichoptera	24	15	51	90	7.1
Simuliidae	56	91	31	90	54.6
Chironomini	3	45	10	52	
Tanypodinae	13	23	17	80	4.9
Orthocladiinae	38	68	13	69	12.5
Chironomidae	13	34	15	70	4.2

3.1.1. Control

14 Fig. 1 shows the trend and the percentage of detachment for the total fauna. In the control, the detachment is equal to 27% when considering the drift of simuliids but, it becomes less than 20% (when taking into account the non-target fauna alone) which is considered as acceptable in experimental conditions. The drift is dominated by the Baetidae (*Centroptilum* spp., *Baetis* spp., *Ophelmatostoma kimminsi* and *Pseudopannota bertrandi*).

3.1.2. Etofenprox

15 The real detachment of the non-target fauna due only to the use of this product at the operational dose (0.03 mg^{-1} 10 min) is around 27% taking into account the 20% of the natural drift, 24 h after treatment. Ephemeroptera and Simuliidae have a percentage of detachment (61% and 56%, respectively), higher than that of the non-target total fauna (Table 1). Among the Ephemeroptera, *Neurocaenis* spp. from the Tricorythidae are the most affected, and it is important to note that the Ephemeroptera present a detachment higher than that of the target, the simuliids.

16 During the acute toxicity test conducted with five increasing doses of etofenprox (0.015, 0.03, 0.06, 0.15, and 0.3 mg l^{-1} 10 min), the number of taxa entering into the drift did not increase much with the dosages (23 as against 24 at the highest dose). Furthermore, all the taxa affected entered the drift within 30 min of the etofenprox treatment and then the number decreased regularly (Fig. 2).

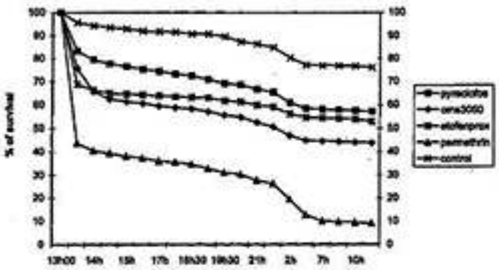

Fig. 1. Comparison of the detachment of the total fauna in gutter caused by different larvicides.

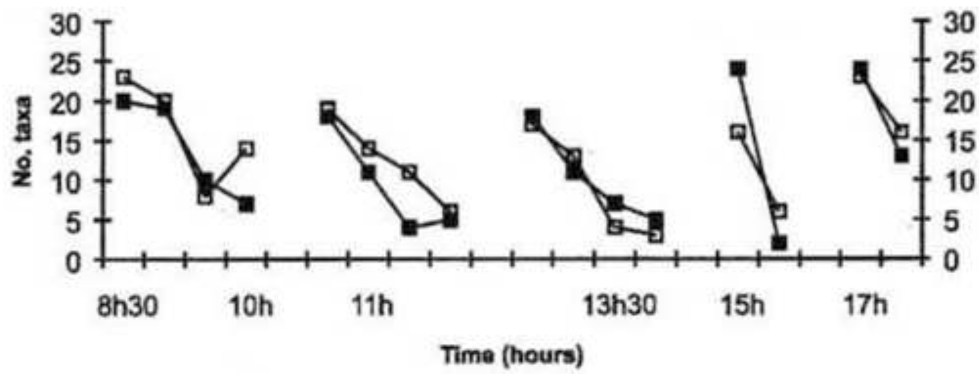

Fig. 2. Trend of the number of taxa collected in the drift after treatment of two parallel gutters with increased doses of etofenprox: 0.015; 0.03; 0.06; 0.15; 0.3 mg l^{-1} 10 min.

17 Instead of the graduai increase in the number of taxa after the dose increase described by Yasuno et al. (1981) for temephos, a decrease was rather recorded at doses 0.03 and 0.06 mg l^{-1} 10 min. Before a new increase occurred at 0.15 mg1^{-1}10 min, i.e., at five times the operational dose. For most of the families, the greatest detachments occurred at the dose of 0.03 mg l^{-1} 10 min (0.06l m^3 s^{-1}) as shown in Fig. 3. An increase in the dose does not necessarily cause a higher detachment.

3.1.3. Permethrin

18 More than 70% of the non-target fauna is affected and here again, the detachment of the Ephemeroptera (Caenidae, Baetidae but especially *P. bertrandi, Neurocaenis* spp.), is the highest. The less affected organisms are belonging to Diptera from the family of Chironomidae with a detachment of 66%. Even the Trichoptera which present usually a moderate susceptibility to most of the larvicides (Yaméogo et al., 1992), were highly affected (83% of detachment).

19 The treatment made at doses 0.015, 0.03, 0,075 and 0.15 mg l^{-1} 10 min did not lead to an increase in the number of taxa in the drift as compare to that made at 0.0075 mg l^{-1} 10 min, i.e., half the operational dose (Fig. 4).

20 The Simuliidae, Caenidae and Tricorythidae experienced their greatest drift at half the operational dose. As regards the Chironomidae, permethrin caused the greatest drift at the dose of 0.075 mg l^{-1} 10 min (five times the operational dose) while for the Philopotamidae 0.03 was the most harmful dose (Fig. 5). With regard to the Hydropsychidae, the greatest drift was at 0.015 mg l^{-1} 10 min (operational dose).

3.1.4. OMS 3050

21 At the operational dose (0.025 mg l^{-1} 10 min), the detachment of the non-target fauna is about 36%, with the Ephemeroptera being the most affected organisms (66% of detachment).

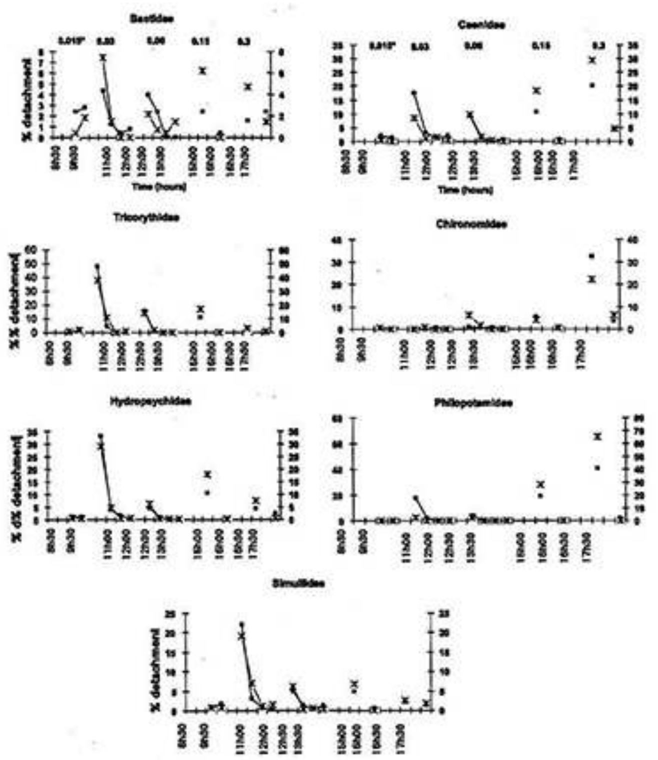

Fig. 3. Toxicity of etofenprox on the principal benthic invertebrate families tested in two parallel gutters (*Dose in mg l^{-1} 10 min).

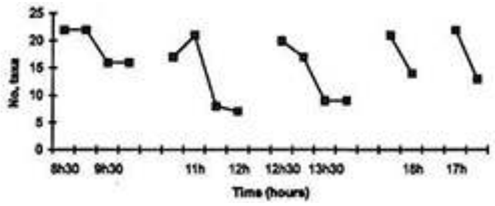

Fig. 4. Trend of number of taxa collected in drift after treatment in a gutter with increasing doses of permethrin: 0.0075; 0.015; 0.03; 0.075; 0.15 mg l^{-1} 10 min.

22 The Chironomidae are the less affected one (15% of detachment). The toxicity of the product on the main species considered in this study (*C. falcifera, C. copiosa, C digitata, A. senegalensis, Neurocaenis* sp. and *P. bertrandi*) is higher than that of etofenprox and permethrin.

3.1.5. Pyraclofos

23 The detachment of the non-target fauna due to the effect of pyraclofos alone is close to 23% at the operational dose (0.1 mg l^{-1} 10 min). Apart from the Simuliidae which are highly affected (more than 90% of detachment), more than 50% of the Ephemeroptera become detached 24 h after treatment. Only 8% of the Trichoptera are affected, and the Chironomidae present 30% of detachment.

24 Increasing doses (0.04, 0.1, 0.2, 0.5 and 1 mg l^{-1} 10 min) applied induced at 0.1 mg l^{-1} 10 min, a drift of maximum number of taxa (Fig. 6) then, at 1 mg l^{-1} 10 min, i.e., 10 times the operational dose.

25 At 0.2 and 0.5 mg l^{-1} 10 min, the maximum number of taxa present in the drift was 20 as against 23 at 0.1 mg l^{-1} 10 min. In considering the principal families present in the gutters

treated, Simuliidae and Baetidae had a considerable detachment right from the treatment at half the operational dose (Fig. 7). Despite the dose increases, the detachment percentage was low at 0.1 and 0.2 mg l^{-1} 10 min. Before increasing at 0.5 and 1 mg l^{-1} 10 min. As regards the Hydropsychidae and Philopotamidae (Trichoptera), the dose increases led to drift in creases up to 0.2 mg l^{-1} 10 min (twice the operational dose) at which the detachment was maximal.

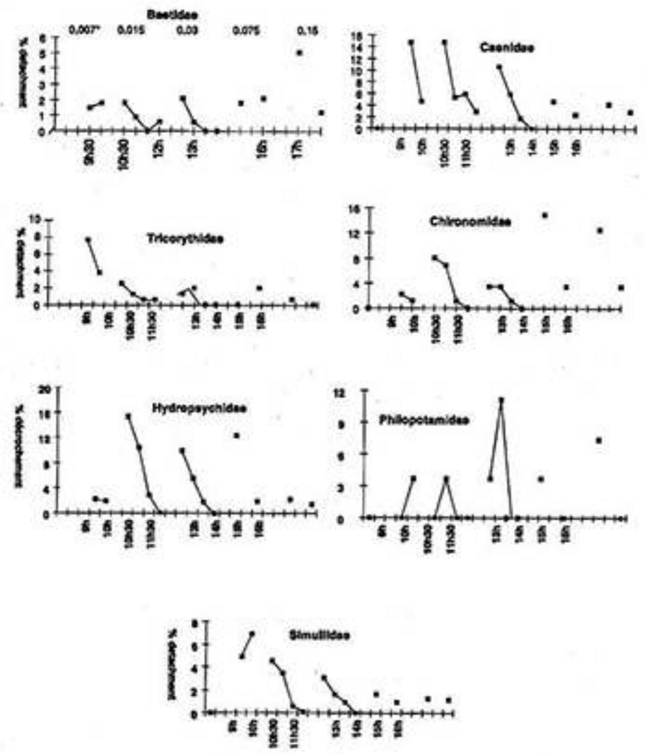

Fig. 5. Toxicity of permethrin on the principal benthic invertebrate families tested in two parallel gutters (*Dose in mg l^{-1} 10 min).

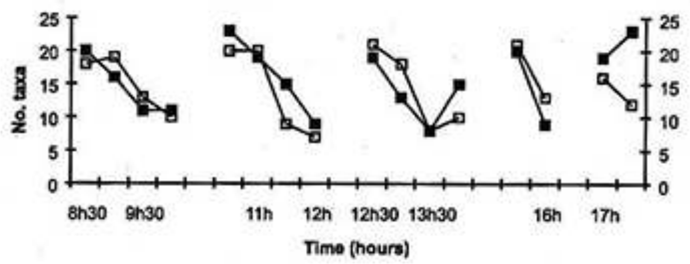

Fig. 6. Trend over time of the number of taxa collected in the drift after treatment of the two parallel gutters with increased doses of pyraclofos: 0.05; 0.1; 0.2; 0.5; 1 mg l^{-1} 10 min.

26 For the Chironomidae (Diptera), the maximum drift was recorded at the operational dose while for the Caenidae and the Tricorythidae the greatest drifts corresponded to the highest doses. All the benthic invertebrate families react in the same way to pyraclofos. While the Simuliidae and the Baetidae became detached at the dose of 0.05 mg l^{-1} 10 min, the Tricorythidae and Caenidae were affected mainly from 0.5 mg l^{-1} 10 min onwards, i.e., a susceptibility that is 10 times less.

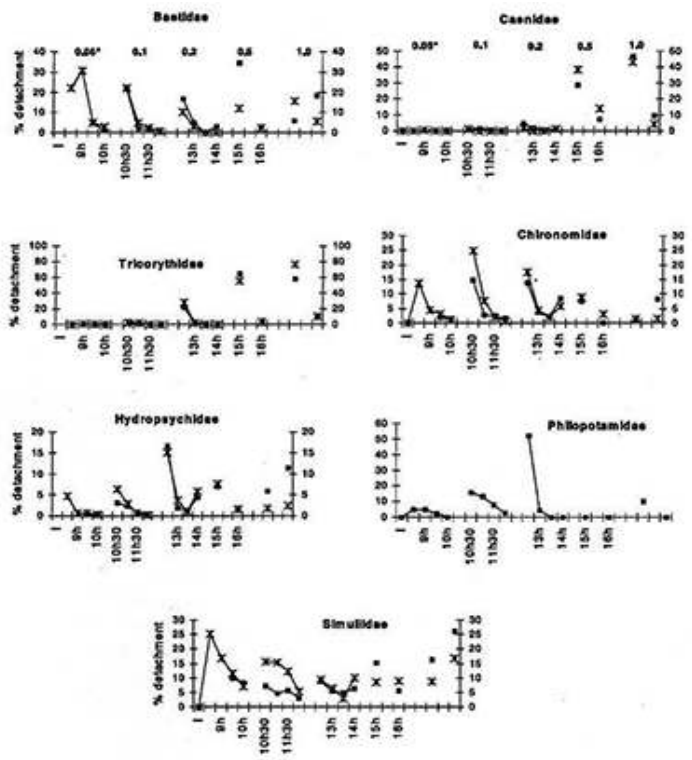

Fig. 7. Toxicity of pyraclofos on the principal benthic invertebrate families tested in two parallel gutters (*Dose in mg l^{-1} 10 min).

27 As regards the non-target aquatic insects populations as a whole, the short-term impact of the operational dose of etofenprox is not significantly different from that of pyraclofos at 0.1 mg l^{-1} 10 min using Wilcoxon *T*-test. By contrast, it is significantly less than that of permethrin.

3.2. Acute toxicity tests in tank on fish

28 The results are reported in Table 2 together with confidence limits and slopes for two of the larvicides (permethrin 20% EC and etofenprox 30% EC) on the two species tested (*O. niloticus* and *T. zillii*). No mortality was recorded in the control tanks and the behaviour of the individual was normal up to the end of the experiment.

3.2.1. Permethrin

29 The 24 h LC50 for *O. niloticus* is relatively low (0.040 mg l^{-1}) and quite close to that obtained by Yaméogo et al. (1991) with *Pollimyrus isidori* using static System with periodic replacement of the solution. The same applies to the 48 h LC50. For *T. zillii,* the lethal concentrations for 24 and 48 h exposure time (0.075 and 0.049 mg l^{-1}, respectively) are almost twice that of *O. niloticus.*

3.2.2. Etofenprox

30 The fishes introduced into the etofenprox solution are a bit more active than those in the control tanks. At the highest doses, some of the individuals demonstrated disorderly

movements a few hours after the start of the trial followed by death. Some others corne periodically to the surface of the solution, the mouth open.

31 The 24 h LC50 for the two species tested is at least 60 times that obtained with permethrin. As regards the employaient safety margin, it is 400-800 times the operational dose for *O. niloticus,* and 200-400 times the operational dose for *T. zillii.* This species is therefore more susceptible to etofenprox than *O. niloticus* when the opposite is observed with permethrin. Very little data is available on acute toxicity of etofenprox. Kariya et al. (1982), testing the 20% EC formulation of etofenprox on carps at a lower temperature (20° C), reported a medium tolerance limit of 44 mg l^{-1} for an exposure time of 24 h. At higher temperature in a tropical environment, in comparison to the results of our test on two african characidae species, etofenprox seems to be safer.

Table 2. Median lethal concentration LC50 mg l^{-1} for two tropical fish species and two Chemical larvicides

	O. niloticus		*T. zillii*	
	24 h	48 h	24 h	48 h
Permethrin	0.040	0.027	0.075	0.049
	0.034–0.049[a]	0.024–0.032	0.056–0.12	0.037–0.060
	3.77[b]	6.32	1.87	2.1
Etofenprox	8.4	8.2	5.0	–
	8–12[a]	7–9.7	4.7–7.0	–
	9.1[b]	8.44	9.2	–

[a] Confidence limits.
[b] Slopes.

3.3. Acute toxicity tests in tanks on shrimps (C. africana)

32 For an exposure time of 2 h in etofenprox solution, the mortalities recorded in tanks made possible the calculation of the following lethal doses:

33 LC50-2 h: 0.18 mg l^{-1} 10 min,

34 LC95-2 h: 0.88 mg l^{-1} 10 min.

35 Six times the operational dose which is 0.03 mg l^{-1} 10 min, and longer exposure time are therefore necessary to cause 50% mortality of this species of shrimps, and 30 times this same dose for 95% mortality. With permethrin, the LC50-2 h is 0.036 mg l^{-1} 10 min (twice the operational dose) and the LC95-2 h is 0.1 mg l^{-1} 10 min, i.e. seven times the operational dose.

3.4. Short-term toxicity tests in river on shrimps

36 The catches in the control area were higher than in the treated section. The maximum drift was recorded at night, between 20h00 and 24h00, while the minimum occurred between 12h00 and 16h00. It was only after the treatment at the operational dose (at 16h00) that a difference was observed between the drift in the two zones. The comparative trend of the drift of *Caridina* (before and after treatment) in the treated section as well as in the control one (Fig. 8(A) and (B)), indicates a similarity of drift in these sections. It was mainly after the treatment (at 16h00) that a difference was recorded between the drift in the two zones. The individuals collected were of small sizes (<1 cm) and it should be noted that none of them was dead 24h00 after the treatment.

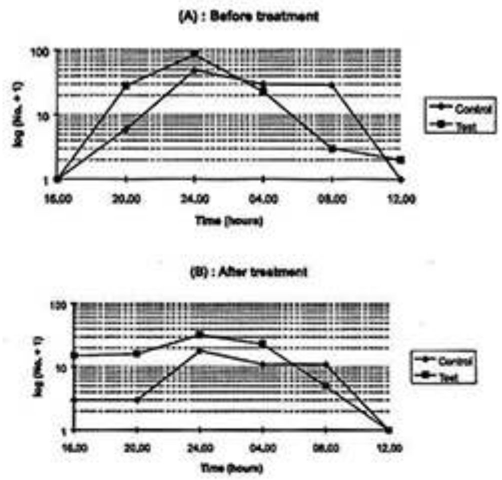

Fig. 8. Comparative trend of the drift of caridina in the two zones before (A) and after (B) treatment with etofenprox at Leraba-gare on the Leraba from 21 to 23 October 1993.

37 There is therefore a short-lasting impact of etofenprox on the young *Caridina* at a discharge of 19 m^3 s^{-1}. Taking into account the usual susceptibility of these organisms to pyrethroids, this resuit could be considered as exceptional.

4. Conclusion

38 Although etofenprox is a pseudopyrethroid, its impact at the operational dose on the benthic insects taken as a whole is quite comparable to that of pyraclofos and far below that of permethrin.

39 Almost all the taxa present in the gutters experienced an impact of etofenprox like permethrin at half the operational doses with the result that an increase in the number of taxa in the drift was witnessed with the increases in the insecticide doses in the systems, contrary to what had been reported by some authors for temephos.

40 The level of changes to be expected if etofenprox is used in river was estimated using the index proposed by Elouard and Simier (1990) which showed a good correlation between the values observed in the field and those calculated (theoretical) from the gutter test data. The relationship that had been found by the authors from three insecticides (temephos, *B.t.* H-14 and chlorphoxim) used at the operational dose is

41 $Y = 0.089x$ 4-1.86 with $r = 1$.

42 By including pyraclofos, we obtained the following relationship:

43 $Y = 0.083x + 1.98$ with $r = 0.99$.

44 Since the index was established on the basis of eight taxa (Baetidae, Caenidae, Tricorythidae, Hydropsychidae, Chironomini, Tanytarsini, Orthocladiinae and Tanypodinae), we calculated the index for etofenprox and permethrin by using the detachments of the same taxa in gutter. The values obtained for etofenprox (4.7) is comparable to that observed for pyraclofos (4.7) and quite different from that of permethrin (7.5). It could therefore be expected that the changes which the use of etofenprox would cause on the benthic communities would be of the same proportion as those brought about by pyraclofos.

45 The level of acute toxicity of etofenprox on fish, and even on shrimps, is far less than that of permethrin. Furthermore, no direct mortality of fish or shrimps was observed in river at low discharge at operational dose even if a short-lasting increase of shrimps in the drift was observed. It is also reported (Mitsui Toatsu Chemicals Inc., 1986; Udagawa, 1988), that administered to rats, the product is excreted into the faeces and urine rapidly. Etofenprox was therefore considered as a serious candidate to fill the operational gap of non-organophosphate insecticide at discharges between 15 and 70 $m^3 s^{-1}$.

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ABSTRACTS

Within the rotational scheme developed by the Programme to fight the resistance of *Simulium damnosum* to Chemical larvicides, there was an operational gap at discharges between 5 and 70 m ^{3} s^{-1} for the treatment of rivers where resistance to organophosphates was present. The use of permethrin and carbosulfan was precluded because of risk of environmental impact and, *Bacillus thuringiensis* ser. H-14 treatments were not envisageable due to cost and logistics constraints. Among the possible complementary groups of larvicides tested, the pseudo-pyrethroids, held promise, because of a mode of action similar to that of pyrethroids, but along with a usually lower toxicity for fish. Etofenprox, one of the pseudo-pyrethroids tested, shows a global detachment of non-target insects in 24 h close to that of pyraclofos, an organo-phosphorus compound (27 against 23%). In laboratory conditions, six times the operational dose which is 0.03 mg l^{-1} 10 min, is needed to cause 50% mortality of *Caridina* sp. (a small shrimps species) and 30 times this same dose for 95% mortality. For fish species, a safety margin of 400-800 times the operational dose is observed for *Oreochromis niloticus* and 200-400 times for *Tilapia zillii*.

INDEX

Keywords: Blackfly-larvicide, Etofenprox, Shrimps, Fish, Insects, Non-target fauna, Onchocerciasis

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Design of *Onchocerca* DNA probes based upon analysis of a repeated sequence family

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EDITOR'S NOTE

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1 *Abbreviations:* SSC, saline sodium citrate; SDS, sodium lauryl sulfate; PCR, polymerase chain reaction; ATP, annual transmission potential; TE, Tris/EDTA buffer; OVS2 *Onchocerca volvulus* specific oligonucleotide probe; OCH *Onchocerca ochengi* specific oligonucleotide probe.

2 *Note:* Nucleotide sequence data reported in this paper have been submitted to the GenBank™ data base with the accession numbers L04875 through L04898.

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Introduction

4 *Onchocerca volvulus,* the filarial parasite which is the causative agent of river blindness, is transmitted by blackflies of the *Simulium damnosum* species complex [1]. Although *O. volvulus* is an obligate human parasite, most of the other members of the genus *Onchocerca* are parasites of ungulates [2]. In West Africa, several species of *Onchocerca* exist which are parasites of the endemic ungulate species. One of these animal parasites, *O. ochengi,* has particular relevance to ongoing efforts to control onchocerciasis. The Onchocerciasis Control Programme (OCP), an international effort to eliminate onchocerciasis as a public health problem, monitors the effectiveness of its efforts by measurement of the annual

transmission potential (ATP). Calculation of the ATP involves estimation of the number of parous flies found to be carrying *O. volvulus* infective larvae in a given area. However, *O. ochengi* is transmitted by the same members of the *S. damnosum* complex that transmit *O. volvulus* [3], and the infective stages of the two species are difficult or impossible to distinguish morphologically [3,4]. The presence of *O. ochengi* can thus adversely affect calculation of the ATP in areas where both *O. volvulus* and *O. ochengi* are endemic. The ability to characterize individual infective larvae using DNA probes that hybridize specifically to *O. volvulus* has the potential of overcoming this problem. However, the practical use of *O. volvulus* specific probes would be enhanced by the development of a DNA probe specific for *O. ochengi,* as it would allow unambiguous positive identification of infective larvae of both species.

5 In the past several years, DNA probes have been isolated which demonstrate varying degrees of specificity for *Onchocerca* parasites [5-9]. Some of these probes are specific for the genus *Onchocerca,* while others are specific for *O. volvulus,* or for distinct strains of *O. volvulus.* All of these probes contain specific members of a tandemly repeated DNA sequence family with a unit length of 150 bp found in the *Onchocerca* genome. This family has been designated O-150. DNA sequence analysis of the O-150 family demonstrated that individual examples of O-150 tended to group into distinct clusters. Within each of these clusters, the individual repeats were found to be identical, or nearly identical (ref. 10 and P. Zimmerman, Ph.D thesis). This finding is consistent with the hypothesis that such repeated sequences are subject to mechanisms of concerted evolution [11].

6 The fact that variation within such repeated sequence families is constrained suggests the possibility of a rational approach to the design of additional DNA probes with defined specificities. To accomplish this, the repeat population in question could be amplified using the polymerase chain reaction (PCR) and the composition of the amplified population analyzed by DNA sequence analysis. Comparison of DNA sequence data from different parasite populations might then be used to design oligonucleotides that were specific to a given parasite population. As a test of this method, sequences of the O-150 family from the forest and savanna strains of *O. volvulus* and from *O. ochengi* have been compared. The sequence analysis provides an explanation of the observed specificities of previously identified *O. volvulus* species and strain specific DNA probes. The sequence data have been utilized to design an oligonucleotide probe which hybridizes specifically to *O. ochengi* PCR products, as well as an oligonucleotide that recognizes PCR products from both forest and savanna strains of *O. volvulus,* but not from *O. ochengi.* Since repeated noncoding DNA sequences appear to be a common feature of eucaryotic genomes, this method should be applicable to the rational design of DNA probes for the diagnosis of other parasitic infections.

Materials and Methods

7 *Parasite materials.* Onchocercomata were surgically removed from infected humans *(O. volvulus)* and cattle *(O. ochengi)* and preserved in isopropanol. Adult parasites were surgically removed from the surrounding host tissue and DNA purified as previously described [12]. Individual infective larvae were isolated from infected *Simulium damnosum s.l.* and treated to release their DNA as previously described [13]. Herring sperm DNA (1 μg) was added to these preparations as a carrier and the parasite DNA further purified by

adsorption to a glass slurry, following the manufacturer's protocol (Bio 101, La Jolla, CA). The DNA was eluted from the glass slurry into 100 μl of TE.

8 *Polymerase chain reaction, cloning of PCR amplification products and DNA sequence analysis.* The repeated sequence family O-150 was amplified from 10 ng samples of purified adult stage genomic DNA, or from 25 μl of the purified larval DNA preparations, as previously described [13]. The primers utilized in the PCR were derived from the most conserved region of the O-150 repeat, as judged by a comparison of the sequence of the 17 known examples of the repeat determined prior to the experiments described in this manuscript [13]. The primers were synthesized with sufficient degeneracy so that all known examples of the O-150 repeat were represented in the primer population. Furthermore, the primers were synthesized to contain synthetic *Hind*III sites, to facilitate subsequent sequence analysis. Analysis of the previously characterized members of the O-150 family [5-9] demonstrated that no known member contained a *Hind*III site, nor were there any sequences found within the known examples of the O-150 family which could be easily mutated to form a *Hind*III site. Finally, genomic Southern blot analysis suggested that *Hind* III sites were rare or absent in the O-150 array [6]. The sequence of the primers used in the PCR were 5′ CCCAAGCTTGATTYTTCCGRCGAAXARCGC 3′ and 5′ CCCAAGCTTGCXRTRTAAATXTGXAAATTC 3′, where R = A or G, Y = C or T and X = A,G,C or T. PCR amplification of the O-150 repeat family was carried out as previously described [13]. The amplification products were treated with *Hind*III, and multimers of the 150-bp monomeric unit were separated by electrophoresis on a 10% polyacrylamide gel. Monomers from each sample were electroeluted from polyacrylamide gel slices using the Elutrap System (Schleicher and Schuell, Keene, NH). The purified monomers were cloned into the single stranded bacteriophage vector M13mp19 and the DNA sequence of individual monomers determined using standard procedures [14]. Clustering of sequences was done visually following transformation of the sequence data into geometric symbols, as previously described [10] and by maximum parsimony methods [15]. Both methods produced essentially identical clustering.

9 *Oligonucleotide hybridization.* PCR products, prepared as described above, were separated on a 2% agarose gel and transferred to a nylon membrane (Hybond N, Amersham, Arlington Heights, IL). The transferred DNA was immobilized by UV crosslinking (Stratalinker, Stratagene, La Jolla Ca.) and the blot prehybridized in a solution containing 5 x SSC/ 20 mM sodium phosphate, pH 7.0/ 10 x Denhardt's (0.2% Ficoll 400/ 0.2% polyvinylpyrrolidone/ 0,2% bovine serum albumin)/ 7% SDS/ 100 μg ml^{-1} herring sperm DNA.

10 Oligonucleotides were labeled using either [^{32}P-γ]ATP and polynucleotide kinase, following standard protocols [16], or with digoxigenin using the Genius 3 labeling kit (Boehringer Mannheim, Indianapolis, IN), following the manufacturer's protocol. Control experiments indicated that the two labeling methods were equivalent in specificity and sensitivity. Labeled oligonucleotides were added to the blot in the prehybridization buffer described above supplemented with dextran sulfate to a final concentration of 10% and hybridization allowed to continue overnight. Blots were washed once for 30 min in a solution containing 3 x SSC, 10 mM sodium phosphate (pH 7.0), 10 x Denhardt's solution and 5% SDS, followed by a second 30 minute wash in 1 x SSC and 1%SDS. For blots probed with OVS2, hybridization was carried out at 42°C and washing was carried out at 50°C. For blots probed with OCH hybridization and washing were carried out at 53°C. Signals on blots hybridized with radioactively labeled probes were visualized by autoradiography.

Positive signals on blots hybridized with the digoxigenin labeled probes were developed according to the manufacturer's instructions.

Results

11 As a first step in the rational development of new oligonucleotide probes, it was necessary to determine the DNA sequence of a large number of PCR amplification products derived from the O-150 family. To obtain a PCR product population that was as representative of the O-150 family as possible, the primer populations utilized in the PCR were degenerate and the annealing temperature used was low enough to allow extension from imperfectly matched primers. For this reason, sequence data collected from the regions from which the primers were derived could not be considered reliable. Thus, analysis of the sequence data were restricted to the 106 nucleotides of the O-150 sequence located between the primer sites.

12 One potential difficulty in this approach was the possibility that PCR induced mutations in the product population might complicate the interpretation of the data. To assess the frequency that PCR induced errors occurred in this System, PCR products derived from the plasmid pOVS134 were analyzed. pOVS134 is a clone derived from *O. volvulus* genomic DNA that contains 12 tandemly linked monomers of the O-150 family [8]. PCR amplifications were carried out with pOVS134 as an experimental template, using conditions identical to those utilized to amplify the O-150 family directly from *O. volvulus* genomic DNA. The amplification products produced from pOVS134 were then cloned into M13mp19, as described in Materials and Methods. Twenty clones were selected at random and their DNA sequence determined. When the sequence of these clones were compared to that of pOVS134, only 1 transition mutation was found to have occurred. Thus, the PCR induced mutation rate per site in this System could be estimated at approximately 4×10^{-4} . This is approximately two orders of magnitude less than variation seen between individual repeat units (see Fig. 1). It was therefore concluded that PCR generated

artifacts would not significantly affect the process of DNA sequence analysis for the purpose of oligonucleotide probe development.

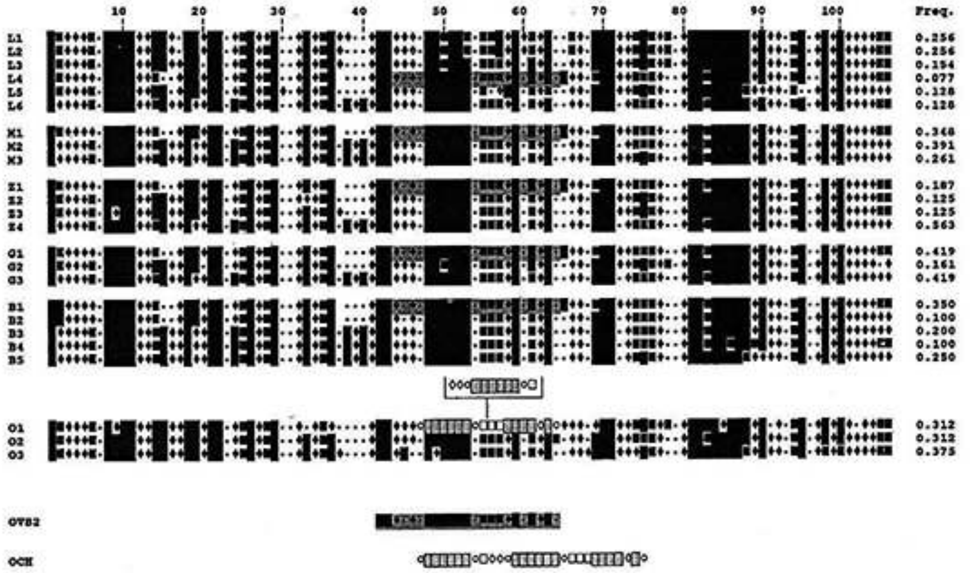

Fig. 1. Consensus sequences for the O-150 clusters present in *O. volvulus* and *O. ochengi.* The sequence data shown represent the 106 nucleotides of the O-150 family located between the PCR primer sites. Clustering of individual O-150 monomers was accomplished as described in Materials and Methods. Consensus sequences were determined by choosing the modal nucleotide at each position. L1-L6, cluster consensus sequences for the clusters found in *O. volvulus* from Liberia (rainforest strain), M1-M3, consensus sequences for the clusters identified in Mali *O. volvulus* (savanna strain), Z1-Z4, cluster consensus sequences identified in *O. volvulus* from Zaire (savanna strain), G1-G3, cluster consensus sequences from Guatemalan *O. volvulus,* B1-B5, cluster consensus sequences from Brazilian *O. volvulus* and O1-O3, cluster consensus sequences found in *O. ochengi.* Freq., the relative proportion that individual sequences belonging to a given cluster are represented in the O-150 population of a given isolate as a whole. OVS2, the sequence of the *O. volvulus* specific oligonucleotide OVS2 and OCH, the sequence of the *O. ochengi* specific oligonucleotide OCH. Cluster consensus sequences identical to OVS2 are indicated by shading; the cluster consensus sequence identical to OCH is indicated by open symbols. Rectangles;, A, ■, G, ●, C and ♦, T.

13 Examination of the raw DNA sequence data derived from the O-150 family suggested that variability within the family was constrained (ref. 10, and P. Zimmerman, Ph.D thesis). Thus, the individual monomers present in each isolate could be arranged into 3-6 clusters. Within these clusters, the sequence of individual monomers were identical or nearly identical. Consensus sequences for the clusters found in 5 different geographic isolates of *O. volvulus* and *O. ochengi* are shown in Fig. 1. The consensus sequences were derived from a total of 145 individual examples of O-150. Examination of this data revealed that differences exist between *O. volvulus* and *O. ochengi* and between the forest and savanna strains of *O. volvulus.* For example, the L1 through L3 clusters are found in the Liberian rainforest isolate of *O. volvulus,* but not in any other *O. volvulus* isolate, or in *O. ochengi.* Comparison of this sequence to the previously identified DNA probes demonstrated that the *O. volvulus* forest strain specific DNA probe pFS-1 [6] contained a repeat which was most homologous to the L3 cluster and was closely related to the L1 and L2 clusters. The specificity of pFS-1 is thus likely to be due to its homology with repeats that are members of these forest specific clusters, which together comprise 66% of the O-150 family present in the Liberian rainforest isolate. In a similar fashion, the *O. volvulus* specific probe pOVS134 [8] contained sequences similar to the L6, M2, M3, Z4, G3 and B3-5 clusters, but did not contain sequences similar to the clusters found in *O. ochengi.*

14 The sequence data presented in Fig. 1 thus provided an explanation for the specificities of pFS-1 and pOVS134, 2 DNA probes isolated by conventional screening methods. Unfortunately, it is not possible to utilize pOVS134 to characterize PCR products of O-150, since it contains sequences homologous to the primers used in the PCR. However, the data presented above provides information necessary to develop new oligonucleotide probes that could be used to classify O-150 derived PCR products. For example, the data presented in Fig. 1 identified a sequence family common to all 6 isolates of *O. volvulus.* This is represented by the L4, Ml, Z1, G1 and B1 clusters shown in Fig. 1. The consensus sequences of these clusters were identical, with the exception of the B1 cluster consensus sequence, which differed from the others by a single G to A transition at position 2. Furthermore, this cluster was not found in either *O. ochengi* (Fig. 1) or *Onchocerca gibsoni* (data not shown). In a similar manner, the O1 family of *O. ochengi* contained an insertion of 11 bp located between bases 53 and 54 (Fig. 1). This insertion was not found in any of the *O. volvulus* sequences. Based upon this analysis, 2 different oligonucleotides were constructed, as indicated in Fig. 1. The oligonucleotide OVS2, consisting of nucleotides spanning positions 42-64 of the *O. volvulus* conserved cluster, was predicted to hybridize to all isolates of *O. volvulus,* but not to *O. ochengi.* Similarly, the oligonucleotide designated OCH, which was derived from the sequence of nucleotides 47-64 in the 01 cluster and which contained the 11bp insertion specific to this cluster, was predicted to be specific for *O. ochengi.*

15 To test the specificity of these probes, the oligonucleotides were labeled as described in Materials and Methods and hybridized to PCR products from 3 standard isolates of the forest strain of *O. volvulus,* 3 standard isolates of the savanna strain of *O. volvulus* and 4 *O. ochengi* isolates. The oligonucleotide OVS2 was found to hybridize specifically to all of the standard *O. volvulus* isolates, but to none of the *O. ochengi* isolates (Fig. 2B, lanes labeled C, VF and VS). In contrast, the oligonucleotide OCH hybridized to the *O. ochengi* PCR products, but not to the PCR products derived from the *O. volvulus* standard isolates (Fig. 2C, lanes labeled C, VF and VS).

16 In a recent study testing the ability of *O. volvulus* strain specific DNA probes to predict the pathogenic potential of different parasite populations, it was found that a small proportion of the samples tested hybridized to neither the forest strain specific probe pFS-1, nor to the savanna strain specific probe pSS-1BT [12]. This suggested that these isolates contained a repeat population distinct from that found in the parasites which were used to develop the previously identified probes. To test the ability of the rationally designed oligonucleotide OVS2 to detect these samples, PCR products from 6 of the double negative isolates were hybridized with OVS2. PCR products from all of these isolates hybridized to OVS2 (Fig. 2B, lanes labeled VU). In contrast, none of the isolates were detected by the *O. ochengi* oligonucleotide OCH (Fig. 2C, lanes labeled VU). Similar experiments have demonstrated that all of the *O. volvulus* isolates previously found to be negative with both strain specific probes were recognized by OVS2 (data not shown).

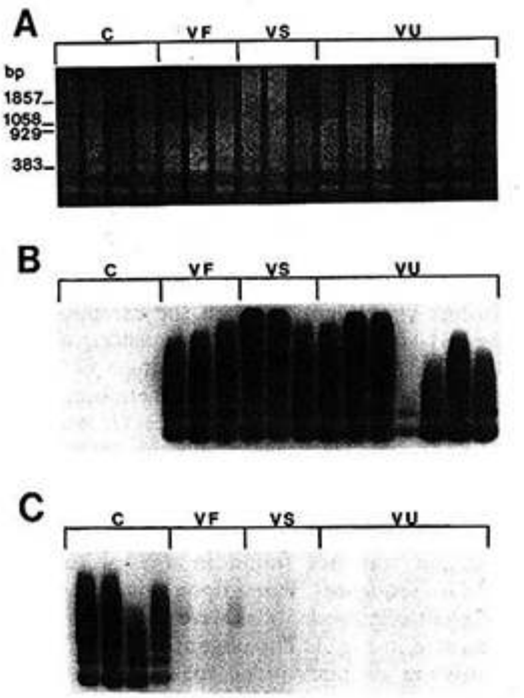

Fig. 2. Test of the specificity of the rationally designed oligonucleotides OVS2 and OCH. PCR products, prepared as described in Materials and Methods, were separated on a 2% agarose gel and used to prepare a Southern blot. (A) Ethidium bromide staining pattern of the gel; (B) blot hybridized with OVS2 and (C) blot hybridized with OCH. In each panel, lanes labeled C, PCR products from *O. ochengi,* VF, PCR products from *O. volvulus* rainforest strain isolates, VS, *O. volvulus* PCR products from savanna strain isolates and VU, *O. volvulus* PCR products from isolates that did not hybridize to either strain specific DNA probe. Isolates were classified based on the 2 step classification procedure previously described [12]. Control reactions without added parasite DNA resulted in no detectable product, either by ethidium bromide staining, or by hybridization to either probe (data not shown).

17 As an initial test of the practical utility of these newly designed oligonucleotide probes, the OVS2 and OCH oligonucleotides were used to characterize infective larvae dissected from *Simulium damnosum s.l.* captured in the Banafing IV River basin in Mali and in the Bandama Blanc basin in northern Côte d'Ivoire. These waterways had been under intensive vector control for over a decade, resulting in the interruption of *O. volvulus* transmission. In 1990, vector control was terminated in this region of the control area and, as expected, *S. damnosum* reappeared at breeding sites (1992 Report of the Joint Program Committee of the Onchocerciasis Control Programme in West Africa, Document JPC13.2, World Health Organization). Subsequent surveillance of fly populations along the Banifing IV and Bandama Blanc identified several parous flies carrying *Onchocerca* infective larvae, resulting in a calculated annual transmission potential far in excess of what was predicted by the current prevalence of *O. volvulus* infection in these areas. Because of this fact, larvaciding was resumed at these foci (1992 Report of the Joint Program Committee of the Onchocerciasis Control Programme in West Africa, Document JPC13.2, World Health Organization). However, the disparity in the epidemiological assessment and the calculated ATP presented the possibility that the infected flies were in fact carrying larvae of *O. ochengi.* To test this hypothesis, infective larvae isolated from wild caught flies collected from the Banifing IV and Bandama Blanc basins were subjected to analysis using the DNA probes. To obtain larvae for this study, a total of 500 flies were dissected. Of these 500 flies, 350 were found to be parous. In the parous group, 13 infected flies were found, which together contained a total of 23 larvae. From the 13 infected flies

collected, amplification of the O-150 repeat family was successfully obtained from 10 samples. The 10 PCR positive samples were then hybridized with the OVS2 and OCH oligonucleotides. None of the 10 samples tested hybridized to the *O. volvulus* specific oligonucleotide probe OVS2 (Fig. 3A). In contrast, 7 of these samples hybridized to OCH, (Fig. 3B, lanes 2,3,5,7,8,10 and 11) while 3 samples hybridized weakly or not at all to the *O. ochengi* specific oligonucleotide (Fig. 3B, lanes 4,6 and 9). The results of this study thus suggested that none of the larvae collected from this focus were *O. volvulus* and that the majority were *O. ochengi.* As a resuit of these findings, larviciding has been suspended in these waterways (1992 Report of the Joint Program Committee of the Onchocerciasis Control Programme in West Africa, Document JPC13.2, World Health Organization).

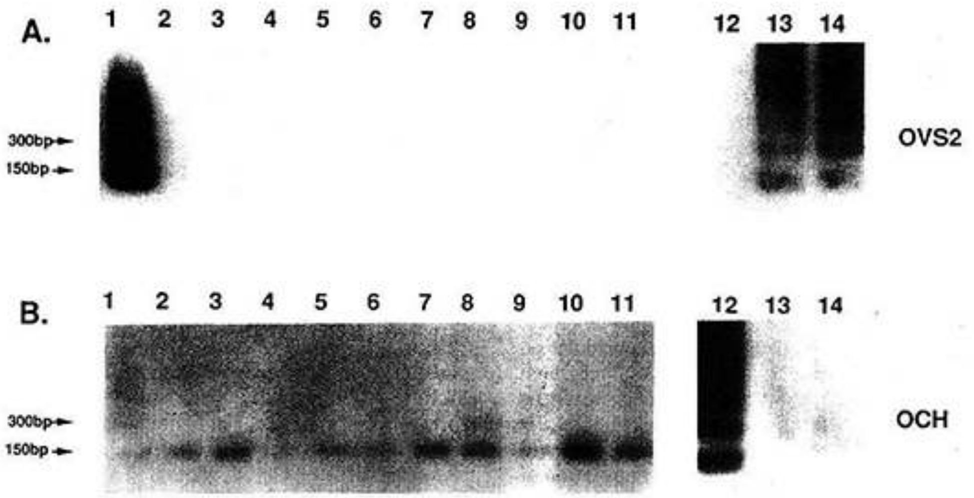

Fig. 3. Hybridization of Infective Larvae from individual wild caught *Simulium damnosum s.l.* from the Banifing IV and Bandama Blanc river basins. Preparation of larvae, amplification by the PCR and hybridization to the species specific oligonucleotides were as described in Materials and Methods. (A) Blot hybridized with OVS2 and (B) Blot hybridized with OCH. In each panel, lane 1, PCR positive control (10 ng pOVSl34 as template DNA), lanes 2-11, PCR products from infective larvae dissected from individual infected flies from the Banifing IV and Bandama Blanc river basins, lane 12, *O. ochengi* standard isolate, lane 13, *O. volvulus* rainforest strain standard isolate and lane 14, *O. volvulus* savanna strain standard isolate. Control reactions without added parasite DNA gave no detectable product (data not shown).

Discussion

18 Highly repeated DNA sequence families are a common feature of eucaryotic genomes and such sequence families appear to evolve at a more rapid rate than do single copy, noncoding sequences [17]. Such sequences have been utilized to develop DNA probes specific for a variety of parasitic organisms [18-22]. In general, these probes have been identified using conventional techniques, such as differential screening of libraries with labeled total genomic DNA. The results presented above demonstrate that it is possible to rationally design oligonucleotide probes with a desired specificity, based upon such repeated sequence families. This process involves amplification of a given repeated sequence family using PCR, followed by examination of the composition of the PCR amplified product population by DNA sequence analysis. Differences found between the PCR product populations arising from different parasite strains or species may then be exploited in the design of oligonucleotides with defined specificities.

19 For this method of probe design to be effective, the PCR products sampled by DNA sequencing must yield an accurate picture of the composition of the PCR product population as a whole. If variation within a repeat population was unconstrained, the population of amplified products would have a very high complexity. If this were the case, it would be necessary to determine the DNA sequence of a very large number of PCR products in order to obtain an accurate picture of the population of PCR amplified products. Fortunately, it is likely that the variation within such sequences is in fact under some constraint. For example, in the case of the O-150 family of *Onchocerca,* defined clusters of sequences are found to exist. Between 3 and 6 such clusters exist in each isolate of *Onchocerca* examined to date. Within these clusters, the individual sequences are identical, or nearly identical. Such clustering appears to be a common feature of repeated sequence families, having been noted in the Alu and minisatellite repeat families of humans [23] as well as in the C repeats of rabbits [24] and in the CR1 repeated sequence families in birds [25]. It is thought that this clustering arises through the action of mechanisms of concerted evolution, which act to homogenize the individual members of the sequence family within a given genome [11]. Because of this constraint, it is possible to gain an accurate picture of the composition of a given repeat population by analyzing a relatively limited number of repeat units. This information can then be used to design oligonucleotides with the required specificities.

20 It should be noted that the DNA sequence data used in the rational design process will usually be based upon a limited number of parasite isolates. This is due to the fact that performing such a detailed analysis on a large number of parasite isolates would be technically prohibitive. In this sense, a rational design strategy is similar to the approach of differentially screening genomic libraries with labeled total genomic DNA. In the latter case, the initial analysis is limited by the number of parasite isolates used to prepare the genomic DNA used to construct and screen the libraries. In both cases, it is therefore necessary to test additional parasite isolates not used in the initial development of the probes, in order to obtain an accurate estimate of their sensitivity and specificity.

21 The *O. volvulus* specific oligonucleotide developed in the present study provides a useful addition to the previously identified species specific probes [7,8]. For example, the plasmid probe pOVS134 contains 12 complete examples of the O-150 repeat. Therefore it contains sequences homologous to those used to prime the PCR amplification of the O-150 family. Thus, pOVS134 cannot be used to classify parasite isolates following PCR amplification of the O-150 family, a procedure that is necessary in our hands in order to reliably classify individual L3 by DNA probe analysis [13]. However, probably the more important of the oligonucleotides developed in the current study is OCH, which appears to be specific for *O. ochengi.* Since *O. volvulus* and *O. ochengi* are co-endemic in the control area of the OCP, are transmitted by the same species of *S. damnosum s.l.* and are often morphologically indistinguishable, *O. ochengi* presents a challenge to the accurate estimation of the ATP for *O. volvulus.* This problem is likely to become more acute as active vector control is phased out within the OCP control area. In such areas, it is expected that the blackfly population will rapidly increase and the presence of *O. ochengi* in cattle found in these areas will lead to a concomitant increase in the transmission of *O. ochengi.* The results presented above demonstrate that the species speeific oligonucleotides, when used in conjunction with a general scheme to amplify the O-150 family by PCR, are capable of classifying individual infective larvae dissected from *S. damnosum s.l..* The ability to determine if a given focus of infected flies is carrying *O.*

volvulus or *O. ochengi* will thus have value in efficiently allocating scarce resources during the final years of the OCP's operation, as well as in the years following the end of large scale vector control efforts in the OCP area.

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ABSTRACTS

Repeated DNA sequences have been instrumental in the development of DNA probes for many different parasites. Isolation of such DNA probes has generally been accomplished by differential screening of genomic libraries with total genomic DNA preparations. In the current work, a rational design strategy is presented for the development of oligonucleotide probes based upon repeated sequence families. A repeated sequence family present in the genome of *Onchocerca* parasites, designated O-150, has been amplified from various samples of genomic DNA using PCR. DNA sequence analysis of the resulting PCR products demonstrated that the sequences may be arranged into clusters within which the individual sequences are identical or nearly identical. Differences among the cluster consensus sequences have been exploited to explain the specificities of previously isolated O-150 based probes and to develop two new oligonucleotide probes. One of these probes hybridizes specifically to *Onchocerca volvulus* O-150 PCR products, while the second hybridizes specifically to O-150 PCR products from the closely related bovine parasite *O. ochengi.* These oligonucleotide probes have been used to characterize *Onchocerca* infective larvae isolated from wild caught infected flies in West Africa. Because repeated sequence families are a common feature of most genomes, including those of parasites, this method should be applicable to the rational design of oligonucleotide probes for other parasitic infections.

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Keywords: Onchocerca, DNA probe, Repeated sequence, Polymerase chain reaction, Epidemiology

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