IDRC-006e

Preventing Onchocerciasis through Blackfly Control

A proposal for Afro-Canadian research into the feasibility of using mermithid parasites as biological agents in the control of disease-transmitting blackflies

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ABSTRACT: Human onchocerciasis, which produces disability and/or blindness among millions of Africans, is caused by a filarioid worm transmitted by blackflies. Control of the blackfly has been attempted mainly with chemical pesticides, but supplementary biological methods of greater environmental acceptability are needed. Mermithids, which parasitize blackflies, are widely regarded as the leading candidates: in North America more than 95% mortality occurs in some populations of blackflies parasitized by certain genera of mermithids. A collaborative project is outlined between the Organisation de Coordination et de Coopération pour la Lutte contre les Grandes Endémies, which covers eight francophone West African countries, and Memorial University of Newfoundland, Canada, for investigation of the feasibility of using mermithids as blackfly control agents in Africa. The project, which would take five years, involves studies of the biology and ecology, and methods of mass production of mermithids, as well as field trials. It also includes a training component for African students.

RÉSUMÉ: L'onchocercose humaine, maladie provoquant un affaiblissement de la vue, voire la cécité, chez des millions d'Africains, a comme agent causal un ver microfilaire transmis par la mouche noire. Les tentatives de lutte contre celle-ci ont jusqu'à présent comporté l'emploi d'insecticides chimiques, mais il est nécessaire de mettre au point des méthodes complémentaires de lutte biologique moins dangereuses pour le milieu naturel. De l'avis général, ce sont les mermithides, parasites des mouches noires, qui présentent le plus de chances de succès. En Amérique du Nord, les mouches noires parasitées par certains genres de mermithides, présentent un taux de mortalité dépassant quelquefois 95%. La présente étude concerne un projet à être exécuté en collaboration entre l'Organisation de Coordination et de Coopération pour la lutte contre les grandes endémies, qui réunit huit pays francophones d'Afrique de l'Ouest, et l'Université Memorial, de Terre-Neuve au Canada. Le programme sera orienté vers la recherche des possibilités d'utilisation des mermithides en tant qu'agents de lutte contre la mouche noire en Afrique. Les travaux dureront en principe cinq ans et comporteront des études sur la biologie des mermithides et leur production à grande échelle, aussi bien que des essais sur le terrain. Le projet comporte également une partie "formation" au bénéfice d'étudiants africains.

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INTRODUCTION

In January, 1972, a scientific advisory group met at Memorial University of Newfoundland (MUN), St. John's, Canada, to discuss the feasibility of providing a means of biological control of species of African and North American blackflies involved in the transmission of the disease, human onchocerciasis. In its extreme form this disease causes "river blindness," and is one of the most important scourges in Africa.

The group consisted of 13 participants invited by the International Development Research Centre and eight observers (see Appendix). Included were members of the MUN Centre for Environmental Biology, the World Health Organization, the National Research Council of Canada and the IDRC.

The group produced this report on research needs of a program of biological control, and outlined such a program for possible funding by the IDRC in collaboration with the Organisation de Coordination et de Coopération pour la Lutte contre les Grandes Endémies (OCCGE) and MUN. A long-term project based on these recommendations has since been funded by IDRC. The Mission Entomologique of the Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM) will be involved through inputs from senior research personnel.

THE PROBLEM

Simulium damnosum in Africa

Several species of tropical African and American blackflies of the genus *Simulium* transmit *Onchocerca volvulus*. This filarioid worm causes the important disease human onchocerciasis, which in its extreme form brings about "river blindness." The main African blackflies involved comprise *Simulium neavei* and the as yet taxonomically confused species-complex collectively called "*Simulium damnosum*". This complex is widely distributed in West and Central African river systems, with important East African foci too.

The advisory group focussed its attention upon *S. damnosum* and West Africa, where this species is the only vector.

For many years, *Simulium damnosum* was regarded as a rather uniform species, apart from the existence of a non-manbiting variant in East Africa. In West Africa, however, there is a gradual, visible transition from a forest form to a savanna form. The former tends to be larger and darker, and has a deeper fore basitarsus with higher scale-crest. In the forest zone, there is some transition from east to west. Cytotaxonomic studies of larvae suggest that *S. damnosum* is a complex of species. Forest and savanna forms evidently differ physiologically, for they are associated with different variants of *Onchocerca volvulus*.

In the many West African human populations where the incidence of onchocerciasis is often more than 50%, one-third of those affected have impaired vision; up to 20% are permanently blind. Besides the obvious and major health significance of onchocerciasis to the developing countries of West Africa, there is a very real economic hardship imposed by the disease. The highest incidence being among adults, many of these are lost to the work force. Some become a complete burden to the society to which they belong – so great a burden in certain instances that extensive fertile and potentially productive areas now lie abandoned (10% of the total area of the savanna regions of West Africa).

The need for *S. damnosum* control to limit the transmission of onchocerciasis to the utmost is self-evident. To date, control has been affected through the periodic introduction of DDT into river systems to kill aquatic stages of the vector. Field trials are already in progress, under other auspices, to test the feasibility of substituting chemical pesticides of less environmental persistence than DDT. Candidate substitutes include such compounds as methoxychlor, Abate and Methyl-Dursban.

Nevertheless in view of the possibility of insecticide resistance and danger to non-target organisms by chemical larvicides, there is pressing need for new, supplementary and alternative control measures, including biological ones, for use in future integrated control programs of greater environmental acceptability.

Invertebrate pathogens available for consideration in this context include an irridescent virus, fungi (e.g. *Coelomycidium* spp.), microsporidan protozoa (e.g. *Pleistophora* spp., *Thelohania* spp.) and mermithid nematodes of several genera. The group concentrated its attention on mermithids, which are (a) widely regarded as the leading candidate invertebrate pathogens for use against blackflies; (b) already quite well known as regards certain aspects of their taxonomy, occurrence and incidence; (c) generally lethal to those *Simulium* species that are parasitized; and (d) on occasion capable of causing the elimination (even if only temporary) of specific blackflies from particular foci – this has been demonstrated under natural conditions.

The most persuasive reasons for this concentration, however, were that (a) much has already been learnt about the occurrence of mermithid infection in certain West African *S. damnosum* populations; (b) these parasites are known to be naturally absent from other such West African populations; and (c) of two species of mermithids known from, and under study in Newfoundland blackflies, one (*Mesomermis flumenalis*) has rather a wide host and geographical range. This species thus merits consideration for intensive laboratory and field investigations with a view to experimental field introductions into unparasitized West African *S. damnosum* populations in carefully selected natural situations.

Mermithid Nematodes

In the family Mermithidae, about 50 genera have been described, most of them more or less inadequately. These roundworms are primarily obligate parasites of the larval, pupal and adult stages of 15 different orders of insects. More rarely, they occur in some other invertebrates. In insects, the mermithid parasites are found inside the body cavity (usually in the abdomen and thorax), where they obtain their nourishment from the haemolymph.

In many instances, the proper identification of mermithids has been handicapped by the lack of availability of adults. Most collections are restricted to larvae, since it is this stage that is found as a parasite in the insect host. Adults may be obtained fairly easily by allowing the larvae to mature and emerge naturally from the host into soil or water. Postparasitic larvae may be induced to moult into the adult stage by placing them on moist sand for periods of from one day to two weeks.

Mermithids as Blackfly Parasites

In North America, it is possible to find more than 95% mortalities in some populations of blackflies parasitized by certain genera of mermithids; other genera do not affect so great a percentage of the population. Percentage parasitism is a true indication of imminent mortality or at least sterility. Some genera of mermithids kill simuliids before they pupate; with others, mortality is delayed until the adult stage of the blackfly. Death is usually caused by inanition or by the loss of body fluids through the large hole caused by the postparasitic larva as it leaves the insect.

Although the life cycles of mermithids in blackflies are incompletely known, the mermithids that parasitize aquatic Diptera appear to have basically the same life cycle, as follows:

Eggs hatch to give rise to the infective, preparasitic larva. The route of entry into the blackfly larva is unknown. Having penetrated into this host, the parasite grows in the haemocoele, typically in the abdomen. The postparasitic larva moults once or twice, depending on the species, to give rise to an adult. After mating the females oviposit. The postparasitic periods of the life cycle vary in length from a few days to several months.

Occasionally, in those species of parasites that normally emerge from the larval host, the parasite is carried over into the adult stage from which it later emerges. The factors influencing survival to the adult stage of infected hosts are incompletely known.

Variants of this life cycle are found, depending on the species of worm. These differences include the duration of the parasitic period, the free-living habits of the adults and their longevity, sexual maturation, number of generations per year or season, host range, and ecological requirements of the adults.

The specific effects of various environmental parameters, such as temperature, oxygen tension, salinity, ionic concentration, and photoperiod, on the several stages of the life cycle, are with few exceptions unknown.

Three species of mermithids are known as parasites of simuliids in Canada and the U.S.A.:

1. *Mesomermis flumenalis* from several species of simuliids in Newfoundland and Ontario,
2. *Gastromermis viridis* from *Simulium vittatum* in Manitoba, Ontario and Wisconsin,
3. *Isomermis wisconsinensis* from *S. venustum* in Newfoundland and *S. vittatum* in Wisconsin.

Preliminary studies on *M. flumenalis* and *I. wisconsinensis* have been initiated at MUN.

Parasitism of *S. damnosum* by mermithids has been observed in six countries of West Africa (Senegal, Mali, Upper Volta, Ivory Coast, Togo and Dahomey). These parasites have not been identified and their effect on the blackfly population has not yet been quantitatively assessed.

Pilot Projects with Mermithids

The mosquito parasite *Reesimermis nielseni* (formerly known as *Romanomermis* sp.) has been developed by the Lake Charles laboratory of the United States Department of Agriculture as a biological control agent for larvae of culicines and anophelines. Very large numbers of preparasitics can be produced in the laboratory in *Culex pipiens fatigans (quinquefasciatus)* for inoculation into mosquito larval habitats. In Louisiana, 50-64% parasitism of *Anopheles crucians* and certain culicines was induced by treatment of ten sites and the levels of parasitism subsequently established have reached 49%. In California, approximately 50-80% control of *Anopheles freeborni* was achieved in rice fields. In Taiwan, inoculations at the rate of one preparasitic *Reesimermis* per millilitre resulted in 95% parasitism of *C.p. fatigans* in ricefield water. Further work with *Reesimermis* in Bangkok and elsewhere is planned within the WHO program on Alternative Methods of Vector Control.

Possible Health Hazards

As with chemical pesticides, it is obviously necessary to examine all possible hazards to the health of non-target organisms that may be posed by biological control agents.

A thorough search of the literature has revealed six reports of alleged larval mermithid infections in man during the past century. It should be noted that these reports are incomplete.

Foci of mermithid infection in Wisconsin blackflies showed no extension into other forms of life. In African regions where mermithid infections of Simuliidae and other vectors occur, infection of human populations has not been reported.

INVESTIGATIONS REQUIRED

The objectives of the program, as defining the research needs, are as follows:

1. The investigation of the feasibility of manipulating the mermithid parasite(s) of *S. damnosum* that are indigenous to West Africa;
2. The development of protocols for the manipulation of mermithid parasites of blackflies in the field and in the laboratory;
3. The feasibility of introducing exotic mermithids, possibly from North America, into West Africa for the control of *S. damnosum*.

It was the opinion of the scientific group that some of the information that might be required for the manipulation of mermithid parasites in West Africa could be obtained from studies of North American species of Mermithidae, whether or not it was the eventual decision to utilize a North American species of mermithid as a biological control agent of *S. damnosum* in West Africa.

Studies to be Undertaken in West Africa

The advisory group made the following recommendations:

Life Cycles of *Simulium* Hosts:

Studies of the life cycle of *S. damnosum*, especially of the immature stages, should be continued. The investigations of *S. damnosum* should involve studies of phenology, rates of growth, habitat features and larval drift. The intention should be to obtain as many quantitative data on all aspects of the life cycle as is reasonably feasible. These studies should be correlated with similar studies of the mermithid parasite(s).

Rearing Program for Simuliids:

Further studies should be undertaken to improve the methods of rearing simuliids from egg to adult. These investigations are deemed necessary in order to provide a mechanism for the study of host-parasite relationships.

Survey of Mermithid Parasites:

The survey for potentially useful mermithid parasites of blackflies in Africa is far from complete and it should be continued.

Controlled experiments should be undertaken to determine whether the absence of mermithid nematodes from some habitats can be attributed to certain gross chemical or physical features of those habitats. These investigations should take the form of controlled exposure of postparasitic larvae and adult nematodes to stream conditions. Identifications of African mermithids by competent authorities should not only continue but be accelerated. Attempts should be made to acquire some local expertise on mermithid taxonomy and biology.

Life Cycles of Mermithids:

Detailed investigations of the life cycles of the indigenous parasite(s) should be undertaken. These studies should include investigations of the mode of infection, rates of growth, host ranges, number of generations per year, pathogenicity and relation to the habitat.

Host Resistance:

Research should be undertaken to investigate the possible reactions of blackflies to their nematode parasites. Host defence reactions have been reported in mosquitoes, and a similar phenomenon may occur in simuliids. These investigations might be conducted in association with studies of the host ranges of the parasites.

Physical and Chemical Influences:

Studies of the ecological tolerances of various stages of the parasites should be undertaken with emphasis on environmental conditions likely to be experienced in West African simuliid habitats. Tests should be made of the susceptibility levels of the parasite to insecticides that may be used in *Simulium* control, such as Methyl-Dursban, Abate and methoxychlor.

Ecological Effect:

Investigations of the susceptibilities of various other stream organisms to the parasites should be undertaken to attempt to evaluate, in part, the ecological impact of such biotic agents, including the effects of reducing the population of the target species. In addition, possible effects on birds and mammals should be studied.

Studies of the Host:

Careful study should be continued of the cytologically recognisable forms of *S. damnosum* and if possible, any differing ecology and life cycles of these forms should be elucidated.

Complementary Studies to be Undertaken in Canada

The advisory group recommended that studies of the biology and ecology of mermithid nematode parasites of blackflies in Canada be vigorously pursued with the hope of providing information that could aid the program to manipulate mermithids in Africa. These studies should be centered at the Memorial University of Newfoundland, and should include, if necessary, assessment of the feasibility of introducing non-indigenous mermithids into West Africa.

Mass Culture *in Vitro*:

Investigation of the feasibility of *in vitro* cultivation of mermithid parasites of simuliids is of high priority. It is recommended that these studies be undertaken in North America rather than in West Africa in order to reduce the costs of setting up laboratories equipped to investigate the initial phases of this problem. The initial investigations, which should be designed to test the suitability for mermithids of culture methods already established for other nematodes, need not necessarily be confined to species of aquatic mermithids actually parasitizing blackflies.

CULTURE AND MASS PRODUCTION

Choice of Culture Method

The collaborative program outlined in this report depends upon the availability of suitable quantities of mermithids, specifically species naturally parasitizing, or capable of parasitizing, *Simulium damnosum*. Various techniques will be applied to obtain infective stages of mermithids:

(a) *In vivo* methods, using either the natural hosts under field or laboratory conditions, or alternate hosts in laboratory culture.

(b) *In vitro* methods, dealing specifically with natural mermithids of the target blackfly, or a model that may be readily applied to *Simulium damnosum*.

One way of attacking this problem is to begin by applying various known culture techniques to preparasitics of locally available mermithids. This could be done with field collections using mermithids from *S. damnosum*. Included in these studies could be eggs of mermithids other than those indigenous to West Africa.

Wherever possible the relevant work should be done in West Africa. MUN investigators concerned with this problem should have the opportunity of seeing actual West African material frequently. At least some of those concerned should spend adequate time in both the MUN laboratory facility and the field project area in West Africa. The natural host should be available in the laboratory when investigations into *in vitro* culture begin.

Culture *in Vivo*

Using field collected material, the object is to make blackfly larvae and the appropriate mermithid(s) available in the respective laboratories in West Africa and Newfoundland. Successful accomplishment of this phase will be essential in order to provide the needed stage(s) in the mermithid life cycle for field release studies and also for *in vitro* studies. These *in vivo* studies could be patterned initially after the *in vivo* system now in existence for the mosquito mermithid, *Reesimermis nielseni*.

Culture *in Vitro*

The successful culture of mermithids *in vitro* would furnish the key to scaling-up through the substitution of materials suitable for mass propagation. All known culture techniques would be assessed, in order to determine whether it is feasible to proceed further without developing new culture methods.

It is desirable to proceed at once from eggs to determine whether known techniques can carry development to fertile adults. It may be desirable to concentrate the culture medium by the introduction of nutrients in colloidal form. Solid matrices such as glass wool may be necessary to provide support for the mating of nematodes. It is anticipated that different stages may require different environments. Also known, stabilized associated microflora may be needed. Other possible complications may develop; e.g. laboratory cultures may not necessarily result in infective worms, since they may lack certain accompanying microorganisms that may be necessary to enhance the pathogenicity of the mermithid.

Some ancillary techniques that can be used after conventional culture procedures have been tried are the following:

1. The investigation of media based on insect haemolymph such as those employed for the culture of parasitic wasps of the genera *Itoplectis* or *Pteromalus*, and under trial for fungi of the genus *Coelomomyces*.
2. Full consideration of the great body of relevant information now existing on culture of an altogether unrelated group, the trypanosomatid parasites of insects, in exploring the possible range of media.
3. The use of cell culture systems, both insect and mammalian. If the axenic gnotobiotic system is not successful, then one would go to a monoxenic system with deliberate use of insect tissue cultures, or established mammalian cell lines, with a preference for the latter since a greater amount of information is already available for them.

Parallel to development of a medium with a suitable chemical composition are considerations of its physical nature and the composition of the gas phase.

As the program develops, it will be important to pay constant attention to the scaling-up possibilities. One imponderable concerns the likely time-lag between achievement of laboratory successes and their translation to the field in West Africa. The group, however, expresses the opinion that a two-year lag is a reasonable expectation. Throughout the study, close contact will be kept with current developments concerning related host-parasite and life cycle studies.

PROJECT TIMETABLE

It is anticipated that at least five years will be required for completion of this project and assessment of the feasibility of mermithids as a routine biological control agent against *Simulium damnosum.*

In the initial phase of the MUN-based component of the program, at least two and possibly three species of mermithids should be investigated to (a) obtain infective stage preparasitic larvae from Newfoundland (and as feasible, elsewhere in North America); and (b) attempt to raise at least one species of mermithid in culture through a complete life cycle.

During this same period in Year 1, related activities should be commenced in West Africa, to (a) survey mermithid occurrence, incidence and ecology with respect to blackfly habitats, in a designated intensive study area (and beyond that area as opportunities afford); and (b) if acceptable with respect to quarantine regulations, to send *Simulium damnosum* egg masses and perhaps gravid females to Canada, as well as West African mermithid material.

The next phase (possibly the second half of Year 1) should see the commencement of transfers of North American blackfly mermithids to West Africa under close quarantine conditions, and the inception of laboratory studies at MUN involving the exposure of various stages of *S. damnosum* (kept closely quarantined in a suitable controlled environment facility) to North American and West African mermithids. The transfers mentioned should be carried out by consultants travelling between the West African and Canadian groups.

Years 2 - 5 would involve the continuation and expansion of these activities with greater and greater emphasis upon

- mass cultivation procedures for appropriate mermithid nematodes;
- the study and elucidation of ecological problems relevant to the field application of such mermithids in West Africa;
- the testing in West Africa of procedures for the effective field use of these parasites;
- the extension of such procedures to other areas where *Simulium damnosum* and onchocerciasis are endemic;
- the monitoring of these field trials to evaluate any successes or failures achieved in reducing the incidence of the vector of onchocerciasis;
- the incorporation of any such biological control procedure found to be of use in these contexts, into increasingly selective integrated control methodologies calling for the joint use of biological, chemical and other types of procedures.

LIAISON WITH THE WORLD HEALTH ORGANIZATION

The proposed project falls within the interests of WHO, not only with respect to its general program on Alternative Methods of Vector Control but also as regards the responsibilities of the WHO Regional Office for Africa for activities to control onchocerciasis. Therefore, it is hoped that throughout the progress of this project liaison will be maintained with the WHO International Reference Centre for Vector Control located at Bobo-Dioulasso, Upper Volta, and the African Regional Office project on onchocerciasis based at Bolgatanga, Ghana (AFRO-2201).

APPENDIX

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