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Biological control of vectors of disease

Sixth report of the
WHO Expert Committee on
Vector Biology and Control

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Geneva, 1-7 December 1981

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BIOLOGICAL CONTROL OF VECTORS OF DISEASE

Sixth Report of the WHO Expert Committee on Vector Biology and Control

1. INTRODUCTION

The WHO Expert Committee on Vector Biology and Control met in Geneva from 1 to 7 December 1981. Dr N. G. Gratz, Director, Division of Vector Biology and Control, opened the meeting on behalf of the Director-General and summarized the current status of vector control programmes. He noted that vectorborne diseases continue to prevail in many Member countries and that the suppression of these diseases is essential to attaining the goal of Health for All by the Year 2000. The development of residual insecticides over the past 40 years provided a relatively simple and inexpensive tool for the control of vectors of disease especially in vast rural areas of the tropics, where diseases such as malaria take a heavy toll in human life and suffering. However, the emergence and spread of insecticide resistance in many species of vectors, concern with environmental pollution brought about by the inappropriate use of insecticides, and the high cost of new types of chemical insecticides make it apparent that vector control can no longer be solely dependent on the use of chemicals.

The Twenty-third World Health Assembly in 1970 recommended the development of alternative methods of vector control (resolution WHA23.33; 1) and action was accordingly initiated. More recently, work on the biological control¹ of vectors has received additional impetus through the promotion of relevant research by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. A significant result of this support has been the development of the bacterium *Bacillus thuringiensis*, serotype H-14, for use as a microbial insecticide. In field trials, formulations of this

¹The term "biological control" as used here is defined as the control of pests, including the vectors of human disease, by the direct or indirect use of natural enemies with or without their metabolites.

bacterium have proved highly effective against larvae of mosquito and blackfly vectors of disease and, at the same time, remarkably safe for nontarget organisms.

In addition to *B. thuringiensis* H-14, the use of larvivorous fish for vector control is receiving renewed attention, and numerous other organisms which attack vectors are being studied in the light of their potential for development as biological control agents.

In view of this situation the Committee was requested to examine critically the place of biological control agents in vector control and the present status of research on these agents, and to discuss the possible constraints on their development and operational use and how such limitations might be overcome. Furthermore, the Committee was asked to advise the Organization on the future plan of action for the development and operational use of biological control agents, bearing in mind the need for simple and economical self-help methods and the concepts of community participation, technical cooperation among developing countries, and primary health care.

2. PLACE OF BIOLOGICAL CONTROL AGENTS IN VECTOR CONTROL

2.1 Main problems faced by vector control programmes

The discovery of residual insecticides revolutionized vector control strategies and largely contributed to the organization of effective and economical programmes for the control of vectorborne diseases. However, in recent years serious obstacles have become apparent. Among these are the technical problems of the development and spread of insecticide resistance and the emergence of refractory vector behaviour.

In its fifth report (2), the WHO Expert Committee on Vector Biology and Control listed 51 anopheline species, 42 culicines and 41 other arthropods of public health or veterinary importance as resistant to one or more insecticides. Many important anopheline vector species have developed multiple resistance to organochlorines such as DDT and dieldrin/HCH, organophosphates and carbamates. Examples include *Anopheles albimanus* in several Latin American countries, *A. sacharovi* in Greece and Turkey, *A. stephensi* in India, Iran, Iraq and Pakistan, and *A. arabiensis* in Africa. More recently, *Simulium soubrense* and *S. sanctipauli* in West Africa have developed resistance

to tephros. So far, tsetse flies are the only major disease vectors in which insecticide resistance has not yet been recorded.

Examples of refractory behaviour are found in *Anopheles farauti* in New Hebrides, Papua New Guinea, and Solomon Islands, and in *A. balabacensis* in South-East Asia. Adults of these species have been observed to show an elusive feeding and resting behaviour which makes the sole use of residual spraying ineffective, as a result of the mosquitos' having insufficient contact with the insecticide deposits.

2.2 Efforts to solve current vector control problems

To overcome vector control problems such as those noted above, a variety of techniques are under development or consideration. Briefly, these include the following:

(1) *Development of alternative insecticides*

The development and use of alternative insecticides is the most immediate answer to the problem of resistance. The WHO programme for the evaluation and testing of new insecticides (3), established in 1960, has tested over 2000 compounds, of which only 10 have been found suitable for operational use as residual insecticides. Thus, the supply of new compounds may not be sufficient to solve the problem.

(2) *Retarding resistance*

Cross-resistance and the emergence of multiple resistance emphasized the need to understand the mechanism of resistance and the judicious use of available insecticides in an effort to retard its development. Techniques include the rotation of insecticides or their application as mixtures.

(3) *Other application techniques*

Where the vector is exophilic, exophagic and peridomestic, the application of insecticides as ultra-low-volume (ULV) aerosols and mists or thermal fogs has been found useful, especially during epidemics of dengue haemorrhagic fever and Japanese encephalitis.

(4) *Genetic control*

Despite intensive efforts, the genetic control of vectors has not proved feasible. However, genetic control aimed at reducing vector populations may have application against riverine tsetse flies.

(5) *Environmental methods*

Environmental management for the purpose of preventing or eliminating vector breeding habitats, and environmental manipulation to prevent or reduce vector breeding through the alteration of habitats, are effective vector control methods. Despite the high initial costs associated with the implementation of environmental methods, the benefits are generally long-term and cost-effective.

(6) *Innovative methods*

These include simple and economical methods which can be used by the community with minimum specialized training. Examples are the inexpensive emergence traps over pit latrines used for *Culex quinquefasciatus* control and the simple conical traps, with or without insecticide impregnation, to control riverine tsetse flies.

(7) *Biological control*

As an example, larvivorous fish have been used in vector control for many years and their role in this respect is now receiving renewed emphasis. New agents—particularly pathogens—are being developed, *Bacillus thuringiensis* H-14 having already reached the stage of operational use (see section 3).

(8) *Integrated methods*

It is becoming increasingly evident that the integration of chemical with biological and/or environmental methods can reduce the present dependence on chemicals. For example, in recent years fish of the genus *Gambusia* have been used on a mass scale in the antimalaria programmes in Afghanistan and Iran in the same areas in which residual insecticides have been employed.

2.3 Role of biological agents in vector control

Natural enemies such as predators, competitors and pathogens play an important role in checking the proliferation of vectors in nature. As far as possible, these natural enemies of vectors must be preserved. In some cases, their impact can be augmented by modifying the environment in their favour. For example, the removal of weeds from aquatic habitats improves the effectiveness of fish as larval predators. The mass production and application of natural enemies is

even more effective, in some instances providing short-term control, and in others (for example, the release of larvivorous fish) leading to long-lasting control. The application of these agents is limited by the species of vector and its habitat, and the relevance and suitability of the procedure in a given situation.

2.4 Relevance of vector ecology and biology to the use of biological control agents

The effective use of biological control methods necessitates an even more detailed knowledge of the ecology and biology of the vector than that required for the application of chemical pesticides, since biological agents are living organisms that interact with the vector and its environment. In many situations, a balance between the vector host and the biological agent is reached and no effective control is achieved; it is therefore necessary to acquire knowledge of the factors that affect the relationship between the control agent and the vector in those particular environments. Furthermore, it is essential to study the factors concerned not only for different geographical regions, but also for different types of habitat, since information relating to one type cannot often be applied to another.

2.5 Role of biological control agents in integrated vector control programmes

Integrated control of vector species has drawn considerable support from integrated pest management (IPM) strategy in agriculture. The highly specific activity of a biological control agent such as *Bacillus thuringiensis* H-14 permits it to be used alone to reduce vector densities below the critical level of disease transmission. However, the greatest potential of these agents lies in their ability to augment the effect of natural enemies or in their use in conjunction with agricultural practices or even in combination with limited amounts of chemical pesticides. The methodology applied is likely to vary with different vector species or even with the same species in different habitats. The cost-effectiveness of such an approach will depend on the circumstances and objectives of the control strategy.

The use of more than one biological control agent for the suppression of a vector species may prove feasible and should be encouraged wherever possible, since it may lead to an optimum level of vector suppression. An example of this approach in its simplest form would be the combination of *B. thuringiensis* H-14 with larvivorous fish in

large bodies of water. *B. thuringiensis* H-14 applied at intervals as required will act as a short-term agent, while fish will serve as a long-term agent keeping populations in check for extended periods.

The type of fish to be used in a combined operation may vary from one situation to another. For example, fish such as *Oryzias latipes* can be used in permanent pools, while others, such as *Nothobranchius* spp., could be used in temporary pools. Where possible, local fish species should be used to minimize the effects on nontarget species.

Mosquito breeding associated with crop irrigation is a major cause of disease transmission. The use of chemical pesticides for the control of agricultural pests and mosquitos has contributed to widespread resistance in both. It is obvious that an integrated control strategy should be the main priority in the case of mosquito breeding in agricultural ecosystems. This can be approached in a variety of ways depending on circumstances. For example, an obvious choice is the use of the most appropriate formulation of *B. thuringiensis* H-14 and periodic introductions of suitable larvivorous fish. However, it must be emphasized that such an approach should also make the best possible use of all available natural regulating factors to achieve optimum results, while at the same time doing least damage to the environment. An additional advantage of such a programme is that the inclusion of fish provides a tangible return in the form of an extra source of protein and ensures community participation in resource management.

Little is known about the possible use of integrated vector control (IVC) measures in controlling vectors such as triatomines, tsetse flies and phlebotomines. The significant suppression of populations of these vectors and the consequent reduction of disease incidence may ultimately depend on the development of IVC strategies. The parasitoid complexes of these species are perhaps more important in regulating population densities than is the case with aquatic vector groups.

2.6 Community participation in the use of biological control agents

It was agreed that individuals and communities could play the following useful roles in the utilization of biological control agents in vector control programmes:

(1) persuading the public, by means of education, to accept the use of biological control agents as safe and effective methods of vector control; and

(2) monitoring the intensity of vector breeding and vector densities (individuals could be trained to keep simple records of their findings and to submit regular reports to the appropriate authorities).

Developing countries should receive encouragement and support in acquiring the relevant expertise and facilities for research into the biological control of vectors, and also in establishing suitable installations for the production of certain biological agents to satisfy local needs as well as for export to other countries. However, the Committee noted that most communities were not technically prepared to employ biological agents for vector control, apart from the culture and use of fish for mosquito control in special situations. At this stage, the production of such agents as *B. thuringiensis* H-14 should not be undertaken at the community level, in view of the expertise and technology needed to ensure high quality and safety.

2.7 Technical cooperation among developing countries (TCDC)

This concept was recognized as offering great promise for the development and use of biological control agents. Under its terms of reference, developing countries could share their experience, train personnel and second workers to undertake specific activities in one or another of the participating countries. These activities should be encouraged, as should the transfer of technology from developed countries.

3. PRESENT STATUS OF RESEARCH AND DEVELOPMENT

The balance of nature depends to a large extent on the regulation of population densities by parasitoids, predators, competitors, parasites, and pathogens. Biological control, in essence, should be the manipulation of this natural regulatory system to the advantage of man.

In considering the possibility of biological control, it must be emphasized that most natural regulatory systems are dependent on, among other factors, the seasonal build-up of populations of natural enemies. Control practices which have a nonselective impact—for example, the use of broad-spectrum insecticides—often cause severe disruptions in the balance of nature. These disruptions can lead to abnormally high densities of pest or vector populations. In the elabor-

ation of vector control practices, it is therefore of the utmost importance to conserve as far as possible the natural enemies which act to suppress the build-up of vector populations. The development of carefully designed vector control programmes which follow this principle will greatly enhance our ability to employ biological control agents effectively.

The scheme for screening and evaluating the efficacy, mammalian safety and environmental impact of biological agents for the control of vectors of human disease was recommended by the WHO Expert Committee on Insecticides in its twenty-first report (4). This has been revised, and in addition a plan has been developed for the evaluation of pathogens, parasitoids, predators and competitors (Annex 1).

3.1 Most promising biological control agents for use against vectors

Among the many agents under development, the Committee noted that the following have the greatest potential for vector control. Safety studies carried out on agents which are insect pathogens have proved their exceptionally high margin of safety for mammals and other nontarget organisms.

— *Bacillus thuringiensis* H-14. A formulation of this bacterium has recently become operational against the blackfly vectors of onchocerciasis and awaits large-scale field trials against mosquitos. Although it has potential for use against a wide range of mosquitos and blackflies, it does not recycle in the environment at levels high enough to provide any significant residual activity.

— *Bacillus sphaericus*. This bacterium has a potential similar to that of *B. thuringiensis* H-14, but against a smaller range of vectors. There is evidence that it can recycle in polluted habitats rich in organic matter, thus providing significant levels of mosquito control for extended periods. Small-scale field trials are under way to assess its potential in different aquatic habitats.

— Fungi. *Culicinomyces clavosporus*, *Lagenidium giganteum*, *Tolyposcladium cylindrosporium* and several species of *Coelomomyces* are being investigated and are expected to have potential against mosquito larvae in certain situations.

— Nematodes. Several species of mermithid nematodes, including *Romanomermis culicivorax*, *R. iyengari* and *Octomyomermis mus-*

pratti, have potential for use in specific situations against mosquito larvae.

— Fish. In general, the use of indigenous rather than introduced fish should be encouraged. *Oreochromis spilurus spilurus* is to be used operationally against *Anopheles arabiensis* in Somalia, and holds promise for use in semi-arid habitats elsewhere. Other examples of fish with considerable promise include members of the families Cyprinodontidae (e.g., *Aphanius* spp., *Aplocheilus* spp., and *Oryzias* spp.), Hemirhamphidae, Anabantidae, and Cichlidae (including *Tilapia* spp.).

— *Gambusia* spp. are operational in some areas, but may destroy local fish and therefore should not be introduced into new areas without careful study of the ecology and the fish fauna. Similarly, the use of other exotic fish is to be avoided, with the notable exception of annual fish such as *Nothobranchius* spp. (see section 3.7.1). These fish are useful against vectors breeding in temporary water-bodies, since they are unable to invade permanent waters owing to their specialized breeding habits.

— Predacious mosquitos. Species of *Toxorhynchites* (e.g., *Toxorhynchites amboinensis*), after having been introduced into certain isolated islands, have exerted a considerable suppressive effect on vector mosquitos breeding in confined spaces, such as holes in trees.

— Snail competitors. The snails *Marisa cornuarietis* and *Thiara granifera* are among several promising competitors of aquatic vector snails. They have, for example, eliminated populations of *Biomphalaria* from several habitats in Puerto Rico and Saint Lucia respectively.

3.2 Bacteria

All the bacteria considered for vector control form spores and potent, stable toxins. Consequently they can be stored for long periods even at ambient tropical temperatures. They can be formulated as suspensions, wetttable powders and dusts for easy application with conventional equipment normally used for chemical pesticides.

Mass production is readily accomplished in typical fermentation installations or sterilized liquid or solid media. Many other bacteria can grow rapidly on these media, and contamination must therefore be prevented during the whole fermentation period. The media must be continuously and vigorously aerated by sterile air.

The toxins are potent larvicidal gut poisons. They take effect only when ingested by larvae, as there is no contact action. They attack a wide range of aquatic dipteran vectors against which they can be effectively applied inundatively. However, they must be so formulated as not to sink away from the feeding zones of vector larvae, a feature that still needs improvement. Eggs, prepupae, pupae and adults are not affected.

The bacteria are harmless to nontarget fauna and so do not lead to a resurgence of increased vector populations consequent upon the suppression of natural enemies. Being tolerant of a wide range of environmental conditions, such as high temperature and salinity, as well as of most chemical pesticides, the bacteria can be used in virtually all habitats where larviciding is practicable against vector species. Since they are highly safe for man they may be applied to domestic potable water supplies and in the presence of food crops, even up to harvest time (5).

These bacteria fall into two groups: *Bacillus thuringiensis* and *B. sphaericus*.

3.2.1 *Bacillus thuringiensis* H-14

The production of *B. thuringiensis* to control agricultural and forest pests is an expanding industry. At a very conservative estimate, 12 000 tonnes had been used up to 1980, much of it on food crops. The absence of reports of harm to man bears testimony to the safety of the use of this species. A new variety—serotype H-14—particularly active against mosquito and blackfly larvae, is now industrially available. It has been extensively tested against nontarget fauna. It is most active against *Aedes*, *Culex* and *Psorophora* spp., and slightly less so against *Anopheles*, although this difference may be an artefact of the bioassays used to measure potency. It is in operational use against blackfly larvae in the Ivory Coast and is shortly to be field-tested on a large scale against mosquitos.

The toxin crystal is formed alongside the spore. Larval enzymes digest the crystal, releasing the toxin within seconds of ingestion, and larvae are killed within hours of ingesting a lethal dose. The bacteria do not persist longer than a few days and regular treatments are necessary during the vector breeding season. However, since natural enemies are unharmed, these frequently build up to suppress new infestations, thus allowing the period between treatments to be increased.

According to available evidence, there is no cross-resistance with chemical insecticides, nor is there any indication of the development of resistance to *B. thuringiensis* H-14 itself.

Genes coding for the toxic protein have recently been transferred to other bacteria. It may be possible to develop more active toxins in different varieties and serotypes.

3.2.2 *Bacillus sphaericus*

The toxin of this species is located in the spore coat. It is more active than *B. thuringiensis* H-14 against certain mosquito species and most active against culicine larvae. Activity against culicines and anophelines varies greatly with species. It is not active against blackfly larvae. *B. sphaericus* can increase saprophytically in some polluted waters (unlike *B. thuringiensis* H-14, which is more effective in clear water), including settling tanks, which follow the turbulent aeration tanks in sewage treatment installations. This could lead to continuous mosquito control. The bacteria are killed in the chlorination tanks, but the toxin is unharmed and affects any larvae occurring downstream of these tanks. More research is required on the water habitats in which *B. sphaericus* can effectively recycle, since this ability will give the species an advantage over *B. thuringiensis*.

Small experimental batches have been tested in the field. Safety testing has been completed, and *B. sphaericus* is considered harmless to mammals.

3.3 Fungi

Many different types of fungi are considered to have vector control potential. They belong to the fungal classes Chytridiomycetes, Oomycetes, Zygomycetes, and Deuteromycetes (Fungi Imperfecti), the classes being based on differing life-cycles.

3.3.1 *Chytridiomycetes*: *Coelomomyces* and *Coelomycidium*

Fungi of the genus *Coelomomyces* are obligate parasites most commonly reported from mosquito larvae. They have complex life-cycles in which an alternate crustacean host, either a copepod or an ostracod, is required to complete the life-cycle. Although most species have been reported from mosquitos, a few are known to attack

chironomid larvae. The results of recent field observations and laboratory trials indicate that some species of *Coelomomyces* have broad host ranges, with a few species being capable of attacking mosquitos of different genera. There is considerable interest in the development and evaluation of *Coelomomyces* for mosquito control because several species of this genus are known to be capable of causing epizootics in larval populations, resulting in mortalities of more than 90%. Moreover, once established in a habitat, the fungus often recycles for several years, producing moderate to high levels of larval control. Studies in the USSR have shown that *Coelomomyces iliensis* can be transferred to new habitats if the intermediate copepod host is present, and that its establishment often results in effective suppression of *Culex modestus*.

Being obligate parasites that require both a crustacean and a mosquito host for development, most species of *Coelomomyces* have proved difficult to study and evaluate. Nevertheless, as highly specific obligate parasites which seek out mosquito larvae, they may prove extremely valuable for vector suppression in the future.

Coelomycidium consists of a complex of species known to attack blackflies in many parts of the world. This fungus selectively invades and kills blackfly larvae. However, little is known about its biology, since attempts to transmit it in the laboratory have been unsuccessful.

3.3.2 Oomycetes: *Lagenidium giganteum*

Oomycete fungi are saprophytic organisms, many of which have a preference for chitinous substrates. Several isolates of one species, *Lagenidium giganteum*, have been reported to cause high larval mosquito mortalities in nature. As a result of recent studies, it is known that *L. giganteum* is capable of parasitizing larvae of most species of *Aedes* and *Culex* against which it has been tested, but anophelines have often proved difficult to infect. The fungus produces zoospores which encyst on heavily sclerotized regions of the cuticle or other suitable substrates. Those which encyst on larvae invade the host within a few hours and colonize it over a period of a few days, eventually causing its death. Subsequently, progeny zoospores are produced. This fungus is considered to have substantial potential for development as a vector control agent because in some habitats it produces larval mortalities that average 90% during the spring and early summer. Furthermore, unlike *Coelomomyces*, it can be grown on artificial media, and can maintain itself in a habitat without the pres-

ence of a host. Experimental field trials, however, have not yielded consistent results and additional trials are under way.

3.3.3 *Zygomycetes*: Entomophthora

The fungi in this group that are of interest for vector control belong to the genus *Entomophthora*, of which several members have been reported from vector species, in some cases from larvae, but more typically from adults. It is their ability to kill and spread among adults under conditions of high humidity which indicates that they may eventually be of some use in vector control. However, although they can be produced on artificial media, the conidia—the fungal stage active against larvae—often do not germinate readily, and *Entomophthora* species are currently considered to possess little potential for vector control.

3.3.4 *Deuteromycetes* (*Fungi Imperfecti*)

Three species of imperfect fungi are being evaluated as vector control agents: *Metarhizium anisopliae*, *Culicinomyces clavosporus* and *Tolyposcladium cylindrosporum*. When grown on natural substrates or artificial media, these fungi produce conidia. Most trials have been carried out against mosquito larvae.

When sprayed on the surface of water in which mosquitos are breeding, conidia of *M. anisopliae* eventually stick to the larval siphon valves, germinate, and kill the larvae, apparently by suffocation. Several field trials have been conducted with conidia of *M. anisopliae* against larvae of *Culex* and *Anopheles*, but the results were considered unsatisfactory, since spray rates as high as 3 kg/ha did not result in any significant level of control. Furthermore, the fungus does not recycle effectively in aquatic habitats and at the present time does not appear to possess much potential for vector control.

Conidia of *C. clavosporus* are club-shaped, miscible with water, and covered with a mucilagenous substance which apparently allows them to stick to the chitinous lining of the pharynx of mosquito larvae. The fungus invades larvae after sticking to the pharynx, killing them within a few days and producing more conidia. Conidia of this species can be produced on liquid artificial media in submerged culture, harvested and applied to habitats as aqueous suspensions. Preliminary tests indicate that *C. clavosporus* has a broad mosquito host range and is more effective in the field than is *M. anisopliae*. However,

current application procedures and rates are excessive and need to be greatly modified before this fungus can be considered operational.

T. cylindrosporium is adapted to aquatic habitats; it has only recently been isolated from mosquito larvae. Preliminary studies indicate that this fungus is as effective against mosquito larvae as *C. clavosporus* and has a higher salt tolerance, possibly making it suitable for use against larvae which breed in brackish waters.

3.4 Protozoa

Of the protozoans, which represent a large group of diverse organisms ranging from the ciliates to the sporozoans, those considered to have vector control potential belong primarily to the microsporidia. On the basis of their life-cycles, these protozoa can be classified as either monomorphic or dimorphic microsporidia.

3.4.1 *Monomorphic microsporidia*

The monomorphic microsporidia are protozoans with relatively simple life-cycles in which only a single spore type is produced. Those with vector control potential include *Nosema algerae*, *Vavraia culicis*, and schistosome microsporidia. *N. algerae* is primarily a parasite of anopheline larvae and adults, whereas *V. culicis* is a parasite of culicines. Laboratory studies have shown that both species can be mass-produced in lepidopterous larvae and that the resultant spores maintain their infectivity for mosquitos. Field trials with *N. algerae* have demonstrated that significant reductions of vector populations can be achieved only with spore application rates considered too high to be economically practicable. Furthermore, *N. algerae* has shown little ability to recycle at rates high enough to yield the necessary levels of control in subsequent generations. However, other studies have shown that although mortality rates for larvae and adults may not be high, as long as adults are infected, their fecundity and ability to transmit malaria are reduced.

3.4.2 *Dimorphic microsporidia*

Dimorphic microsporidia, which, as far as their presence in vectors is concerned, have been reported only from mosquitos, have two different developmental cycles. In adult females, these microsporidia form bacilliform spores which eventually result in the infection of eggs.

All male larvae hatching from infected eggs develop patent infections and die. During sporogony in male larvae, spores of a different type from those produced in females are formed via a separate developmental cycle—hence the term dimorphic microsporidia. In female larvae which develop from infected eggs, the infection remains benign. The microsporidia continue development into the adult stage, when they complete the cycle by invading another egg batch. Unlike the monomorphic microsporidia, spores of the dimorphic types cannot be transmitted *per os*, and the infection appears to be maintained in mosquito populations by vertical transmission. Although no trans-ovarian cycles in the microsporidia attacking blackflies have been confirmed, most of the organisms concerned are thought to be of the dimorphic type. The present paucity of knowledge prevents an estimation of their potential.

3.5 Nematodes

All nematodes at present being considered for development as biological control agents against mosquitos and blackflies belong to the family Mermithidae. These nematodes are obligate parasites which must undergo part of their development within a living host. They are probably able to recycle better than most of the pathogens described above. Three species are currently receiving study and evaluation: *Romanomermis culicivorax*, *R. iyengari* and *Octomyomermis muspratti*.

Of the above nematodes, *R. culicivorax* has received the most study. Adults of this species lay eggs in the mud or detritus of mosquito habitats. After hatching, preparasitic nematodes seek out and invade mosquito larvae. After growing within the larva for approximately seven days, the nematode emerges, killing the larva by exiting through the cuticle. After emergence, the nematode drops to the bottom of the habitat, moults, mates, and the females begin laying eggs. Small field trials have demonstrated that *R. culicivorax* can reduce larval populations by 70–90%.

R. culicivorax has a broad mosquito host range and can survive in a variety of habitats. Relatively simple techniques have been developed for its mass production. However, field tests thus far indicate that although single applications of this nematode may result in recycling, the degree of long-term mosquito control is limited, and the proportion of the population that continues to be infected is generally

less than 25–30%. Further tests, especially on the repeated applications of this agent to various habitats, are needed.

R. iyengari is a nematode very similar to *R. culicivorax*. Unlike the latter, it was originally isolated from larvae collected in tropical regions and therefore might be of greater use in the tropics. *In vivo* techniques for its mass production have recently been developed, but field trials have not yet been undertaken.

O. muspratti was originally isolated from mosquitos breeding in tree-holes. It has a greater tolerance to alkalinity, salinity and pollutants than *R. culicivorax* and *R. iyengari* and is thought to possess considerable potential for mosquito control. Another attribute is its ability to invade late-instar larvae and develop in their adult stage, providing a natural mechanism for its dissemination. At present, the main field of inquiry with regard to this species consists in determining the conditions required to obtain a synchronous hatch of eggs.

3.6 Viruses

Most of the viruses attacking vectors fall into the well-known groups of viruses pathogenic to insects, including nuclear polyhedrosis viruses, cytoplasmic polyhedrosis viruses, and iridoviruses. In comparison with agricultural pests, from which more than 600 viruses have been isolated, relatively few insect viruses (only a few dozen) have been isolated from vectors of human diseases. Of these, the most promising are the nuclear polyhedrosis viruses. These attack the midgut epithelium of larvae, causing an acute disease which results in death in about 48 hours. So far, they have been isolated only from mosquito larvae. Cytoplasmic polyhedrosis viruses have been reported from blackfly larvae as well as from mosquitos. In most cases this type of virus, which also attacks the midgut epithelium, produces a chronic disease from which larvae can often recover, making its potential for vector control questionable at this time. Iridoviruses have also been reported from mosquito and blackfly larvae. These viruses attack a wide range of tissues, and although they are highly lethal to their hosts they are not very infectious.

While problems of insufficient infectivity or virulence confront the development of viruses as biological control agents, the most serious obstacle to their development and use is that, since they are highly specific obligate pathogens, there is at present no efficient method for their mass production.

3.7 Potential of parasitoids, predators, and competitors

The associations of various parasitoids, predators, and competitors with different groups of vectors are typically quite specific. It is therefore relevant to review the potential of these diverse agents in relation to different groups of vectors.

3.7.1 Mosquitos

For mosquito vectors of disease, fish are one of the most important natural regulatory agents. They are very efficient and there is relatively little expenditure involved in their introduction into a particular biotope. Indigenous fish should be used for this purpose, and exotic species should be introduced only when it is considered ecologically acceptable. However, annual and seasonal fish such as *Nothobranchius* spp. can be introduced into new areas, since they cannot infest permanent water systems and have a singular potential for controlling mosquito breeding in temporary waters. Indigenous fish are better adapted to local conditions and are also easily and economically established, with the help of the community, near vector breeding habitats. Phytophagous fish in their young stages may feed on mosquito larvae and as adults will clear vegetation, thereby exposing mosquito larvae to attack by larvivorous fish and other predators. The use of fish can prove to be a simple and economical measure in an integrated control programme.

The main criteria for the selection of fish as larvivores are a marked preference for mosquito larvae over alternative sources of food, small adult size (less than 6 cm long), which makes for easier colonization and access to shallow depths, rapid maturation and high fecundity, a high degree of tolerance to salinity and pollution, and harmlessness to nontarget fauna in the aquatic ecosystem. *Oryzias latipes*, for example, shows many of these favourable attributes and can withstand a wide range of climatic and ecological conditions.

The most promising fish species belong to genera of the family Cyprinodontidae—for example, *Aphanius*, *Valencia* (Mediterranean region and western Asia), *Aplocheilus*, *Oryzias* (southern Asia), *Epiplatys*, *Aphyosemion*, *Roloffia* (West Africa), *Nothobranchius*, *Pachypanchax* (East Africa), *Rivulus*, *Fundulus*, *Cynolebias* and *Cyprinodon* (the Americas). Viviparous fishes of the families Poeciliidae and Goodeidae can be applied in the areas of their original distribution. However, one of the species—namely, *Gambusia af-*

finis—belonging to the family Poeciliidae must be viewed with caution. *Gambusia* has a harmful effect on indigenous fish species and therefore its introduction into new areas should not be attempted without adequate ecological studies. During a WHO Informal Consultation on the Use of Fish for Mosquito Control (Geneva, 1981),¹ the participants compiled a global list of potential and known larvivorous fishes.

The predatory mosquito *Toxorhynchites* spp. deserves special mention. The adults of this group are nonbiting, but rather feed on nectar and pollen, thus aiding pollination. The female oviposits in small bodies of water, such as those formed in tree-holes, where mosquito larvae are present. *Toxorhynchites* larvae feed voraciously on other mosquito larvae. There are approximately 70 species of this beneficial mosquito genus, reported mostly from the Far East. Some species have been found to be particularly effective on small islands, where the numbers of container-breeding mosquitos have been markedly reduced, following a simple inoculative release that has led to the establishment of *Toxorhynchites*.

There are also a number of aquatic insects, such as members of the Dytiscidae, Odonata, and Notonectidae, that have shown themselves to be useful predators of mosquito larvae.

No effective parasitoids of mosquitos are known.

3.7.2 Blackflies

Parasitoids of blackflies are unknown. There are, however, a number of predators, but little information exists on their effect on blackfly larval populations.

3.7.3 Tsetse flies

The adult flies are not known to have efficient natural enemies. However, a large number of parasitoids attacking pupae have been recorded. These include Mutillidae (three species) and Bombyliidae (*Exhyalanthrax* spp.), which are being considered for use in biological control.

3.7.4 Triatomine bugs

Several natural enemies attack all developmental stages of these vectors. The adults are attacked by spiders, ants, and predatory reduviid bugs. The egg parasites deserve special mention. *Telenomus fariai* occurs throughout the neotropics. *Gyron triatoma* attacks the

¹ Unpublished document WHO/VBC/82.838; TDR/BCV/ICMC/81.3.

eggs of *Triatoma rubrofasciata* in Asia, but its effects on the bug population are variable. For persistent control of triatomine bugs the parasitoid must have alternative hosts. *Telenomus costalimai* is an example—it also attacks pentastomid and lepidopteran eggs. More work is needed to identify other promising species.

3.7.5 *Phlebotomines*

The adult insects are active fliers and the immature stages occur in various habitats, which are usually difficult to locate. This probably accounts for the paucity of information about parasitoids, predators and competitors of the sandflies.

3.7.6 *Molluscs*

Snail intermediate hosts have a large number of natural enemies, which include predatory/parasitoid sciomyzids, certain trematode parasites, several competitive/predatory snails and malacophagous/phytophagous fish.

The snail species *Thiara granifera* and *Marisa cornuarietis* are probably the most promising potential biocontrol agents against schistosome-bearing snail species. Both have effectively suppressed populations of intermediate snail hosts in field situations in the Caribbean and, more recently, in the United Republic of Tanzania. In addition, several other competitor species of snails are known to exist in various parts of the world, including, for example, *Helisoma duryi*, *Melanopsis* spp. and *Potamopyrgus jenkinsi*. The efficiency of these snail competitors deserves to be evaluated under representative field conditions; they must, however, be shown to have no seriously adverse environmental impact.

Some species of fish may be useful as snail-control agents, being either directly malacophagous (e.g., *Astronotus* sp.) or phytophagous (e.g., the grass carp, *Ctenopharyngodon idella*), the latter suppressing snail infestation by feeding on the aquatic vegetation.

3.8 Cost-effectiveness considerations

3.8.1 *General*

The concept of cost-benefit of disease control programmes is generally accepted as being extremely difficult to quantify. Determi-

nation of cost-effectiveness tends to be more feasible, as both terms of the ratio can be expressed numerically, provided clear-cut objectives have been set.

3.8.2 *Estimation of costs*

The costs of biological control may be divided into three components—namely, production, distribution, and evaluation. Great variation in these components in different areas means that estimates of cost-effectiveness cannot be extrapolated from one region to another. Therefore each situation must be treated on its own merits.

Production costs: These will vary with the particular biological agent or agents involved and may depend on climate. In cooler climates intensive cultivation of the agents may be required at the beginning of the transmission season, while in tropical areas needs can often be met merely by harvesting and transfer from natural habitats. In certain cases, the density of biological agents, once introduced, may be maintained by recycling so that production costs are reduced or eliminated.

Distribution costs: These will also depend to a large extent on the ability of the introduced agents to maintain their numbers at an effective density. Many agents will tend to distribute themselves in the environment naturally.

Evaluation costs: Evaluation is a key function in the use of biological agents and should not be limited for reasons of economy. The more thorough the evaluation the more effective the use of the regulator can become through feedback and operational adjustment.

3.8.3 *Estimation of effectiveness*

One may encounter serious difficulties when attempting to measure the effectiveness of the use of an agent in terms of the final objective. As biological agents may mostly be used in integrated control programmes in combination with other methods of disease control—notably chemotherapy, chemoprophylaxis, and the use of chemical insecticides—the specific impact of the agent may be difficult to quantify in epidemiological terms. The position is somewhat easier if the measure of effectiveness is confined to the intermediate target (the vector species).

Attempts should be made prior to the launching of a control programme or in parallel with an existing programme to design the use

of a biological agent for maximum impact under specific local conditions and then demonstrate the actual effect obtained on the target vector species and, where possible, the effect on disease transmission. Comparison may then be made with other control methods.

4. COMMON FACTORS IN THE BIOLOGICAL CONTROL OF AGRICULTURAL PESTS AND DISEASE VECTORS

Considerable success has been achieved in the biological control of pests in agriculture. Populations of these pests need to be controlled to a point below the threshold of economic importance. The agents include many entomophagous predators and parasitoids, several nematodes, *Bacillus popilliae*, *Bacillus thuringiensis* (3 varieties), 3 registered baculoviruses (and some 10 others showing promise), 3 registered fungi (and several others showing promise), and 1 registered microsporidian. In addition, measures to encourage certain birds and to avoid harm to complexes of beneficial fauna have given valuable results. Some agents have been successfully introduced into virgin areas, while others, following commercial production, have been spread inundatively in existing habitats.

At present, a few species of pathogen are operational or show promise against both agricultural pests and disease vectors. However, in all instances different varieties or strains are involved and formulations with different characteristics are used. Thus no common agents are known, although this does not rule out the possibility of finding some in the future. Some strains for use against both agricultural pests and disease vectors can possibly be produced through genetic manipulation.

It is important to note, however, that the same industrial installations can profitably be used for producing the different species and varieties of bacteria. Technology and installations developed for the production of agricultural strains of *B. thuringiensis* enabled the serotype H-14 to be used operationally only 5 years after its initial discovery. Common installations and technical expertise could also be pertinent in the case of a number of fungal pathogens.

Campaigns for the control of agricultural pests and disease vectors can mutually benefit from efforts to preserve fauna operating against each type of organism. Thus, terrestrial predators may consume both agricultural pests and some terrestrial vectors of disease. Likewise, aquatic predators may capture some agricultural pests that fall into

water. However, at present neither category involves predators worth manipulating for a dual purpose, except in the case of certain fish, which are also a source of protein. Nevertheless, it is important to coordinate agricultural pest and disease vector control operations to the greatest extent possible in order to preserve fauna that are beneficial to both types of campaign.

5. FACTORS AFFECTING THE DEVELOPMENT OF BIOLOGICAL CONTROL AGENTS

5.1 Review of the evaluation scheme

During the past 10 years research on the development of biological agents for vector control has been intensified the world over. This heightened activity, which has resulted in substantial progress, is largely due to increased funds made available through the aforementioned Special Programme for Research and Training in Tropical Diseases.

There is a definite need to continue and expedite the search for new biological agents and to evaluate them carefully. Schemes have been developed for both laboratory and field evaluations of promising biological control agents but, so far, a large proportion of the work has been conducted in the developed countries, despite great efforts to transfer the necessary expertise to developing countries. The screening and evaluation and—even more important—the field evaluations should be conducted in areas in which vectorborne diseases prevail. There is an urgent need for the large-scale evaluation of agents which offer the highest potential for controlling important vector species. *Bacillus thuringiensis* H-14 is the most promising control agent developed to date. This is an example of an agent for which large-scale evaluation against both mosquitos and blackflies is needed in endemic areas; the Committee noted and strongly supported the action taken by WHO in this respect.

Larvivorious fish have been used for mosquito control since the beginning of the century. However, this endeavour has largely involved *Gambusia* and, to a lesser extent, *Poecilia* and *Tilapia*, and there has not always been an adequate evaluation of their epidemiological or ecological impact. Too little priority has been given to the

development of indigenous larvivoracious fish species, in preference to exotic species, and to adequate field evaluation in endemic areas. In many cases, the mass production of fish in breeding-ponds is relatively simple once the methods of fish culture and production are established. A local community may develop an interest in fish production as a source of food and participate in distribution and in clearing the water of algae and aquatic weeds to help predation.

As part of the evaluation scheme, a series of vector species should be designated as candidate target organisms to ensure that screening covers an adequate host spectrum.

It is essential that new biological agents should be evaluated with regard to their compatibility with other control measures that are being used in the same habitat. Greater coordination between control efforts being made against vector species and those being used against agricultural pests is needed. Such coordination will not only allow for the more effective use of agents but will also be important in reducing the potential of insecticide resistance and the detrimental effect on natural enemies. In some cases in which chemical treatments are essential for controlling agricultural pests it may be possible to develop resistant strains of biological control agents—e.g., fish. This concept is currently being applied in agriculture.

It is essential to consider the combined effects of various biological control agents in the reduction of vector populations. Agents that have very temporary effects may be utilized initially in order to gain time for other agents, which are relatively ineffective in low numbers, to build up sufficient densities so that they can play a role in the natural regulation of vector populations. For example, *B. thuringiensis* H-14 is effective against mosquito larvae, but it does not recycle and has only short-term residual activity; however, this agent is harmless to the various components of the predator complex. Applications of *B. thuringiensis* H-14 early in the season can control mosquito breeding without detriment to population increases of fish and larvivoracious arthropods. These predators can then continue to attain densities which would provide long-term control. Conversely, early-season applications of nonselective pesticides may provide temporary mosquito control but will also invariably impair the development of the predator complex, which would necessitate further chemical applications. Strategies that limit the use of promising new agents to one or a few applications per season will also necessarily delay the onset of resistance to these agents, since the overall selection pressure will be reduced.

5.2 Problems of specifications, standardization and quality control

To ensure quality control, it is important that adequate specifications should be developed for commercial products containing biologically active agents. In order to measure insecticidal activity, bioassay procedures must be used. These should be improved and standardized; it is essential that detailed methodology should be clearly described in order to allow for comparisons of results, particularly between different laboratories. In the case of *B. thuringiensis* H-14, a good correlation between standardized bioassay results and field performance data is not to be expected because of the influence of differences in formulations.

In some cases in which a given control agent can be produced by relatively simple procedures—e.g., the fermentation of *B. thuringiensis* H-14—national production may be cost-efficient. For example, in China raw materials for fermenting *B. thuringiensis* H-14 are locally available and relatively inexpensive. The importation of bacterial insecticides would result in considerable purchase and transport costs. Locally produced *B. thuringiensis* H-14 has shown operational efficacy, indicating the feasibility of local production.

It is absolutely essential that an effective quality control programme should be applied to every production batch in the manufacture of any bacterial or fungal agents. This will ensure efficacy and guarantee the safety of the end-product.

5.3 Research and training needs

5.3.1 Research needs

The development of new biological agents should consider strategies to circumvent the important problem of insecticide resistance. The fifth report of the WHO Expert Committee on Vector Biology and Control (2) lists five main approaches to combat resistance—namely, the use of alternative chemicals, dosage management and the control of gene dominance, the mosaic application of chemicals, the use of insecticides in mixtures, rotations and optimum sequences, and the adoption of integrated control. In two of these approaches, biological control agents could possibly play an important role: (1) in serving as alternatives to chemicals, and (2) in integrated control strategies. Research in this area is critical, since a number of vector species already show multiple insecticide resistance.

Improved formulations of *B. thuringiensis* H-14 for controlling different vector species under different ecological conditions need to be developed. Formulations with different contents of active ingredients are required for different operational purposes. Research is needed on the production of formulations in the area of operations. For example, granular formulations are sometimes necessary in order to penetrate canopies of vegetation, but usually the active-ingredient content of granules is low (5% or less) in order to allow enough mass to provide adequate coverage of the area to be treated. The carrier for granular formulations, such as sand, clay or agricultural by-products, can usually be found locally, which reduces shipping costs.

Another problem in developing new agents is to define optimum storage conditions in order to maximize effectiveness. For example, it is known that *B. thuringiensis* H-14 begins to decompose much more readily when powder formulations contain more than 5% moisture.

Additional research is required on promising agents. The potential for the recycling of *B. sphaericus* strain 1593 in various habitats has not yet been defined, and the feasibility of producing *Romanomermis culicivorax* at peripheral centres should be assessed.

Finally, in order to expedite integrated vector control, methodologies must be developed to monitor the population densities of target as well as beneficial organisms by operational personnel.

5.3.2 Training needs

One of the key factors limiting the isolation, identification, development and use of biological agents for the control of vector species is the lack of trained personnel in endemic areas. Protocols need to be developed for determining the potentials of various pathogens, and collaborating centres in which visiting scientists could assist in carrying out the specified procedures and in training local biologists should be designated in endemic areas.

An understanding of the ecology of vector species is even more important for biological control strategies than for the relatively simpler techniques of chemical control. Consequently, local entomologists require more training in all aspects of the biology and ecology of the prevalent vectors.

Operational personnel must understand how a biological control agent functions. For example, the immature stages of mosquitos,

which do not feed, will not be affected by *B. thuringiensis* H-14, and the timing of operational treatments will be crucial for successful control.

5.4 Information dissemination and coordination of research

5.4.1 Information dissemination

The Committee noted the substantial efforts made by WHO to disseminate information on the development of biological control agents, particularly to individual scientists and institutions in developing countries whose access to the relevant literature may not be easy.

5.4.2 Coordination of research

WHO is playing an active and productive role in promoting and coordinating worldwide research on biological control agents and their development for disease vector control. However, institutions in many developing countries lack the necessary expertise and facilities to participate effectively in the global effort. There is an urgent need to rectify the situation through the establishment of collaborating centres in the developing countries, some of which could eventually become regional centres for the development, field testing and operational utilization of biological control agents. Efforts should be accelerated to secure the participation of individual scientists in those countries by generating interest and strengthening expertise in the subject. Meanwhile, the delays and obstacles confronting such workers could be alleviated by establishing a procedure for supplying the materials in question from existing reference centres in the developed countries.

6. GUIDELINES FOR FUTURE ACTION

Having examined previous guidelines (4, 6-9) in the light of the more copious information available today, the Committee recommended the following course of action, designed to harness biological control agents as rapidly as possible in the fight against vectorborne diseases. Usually it will contribute to the integrated control of vectors, which may, if necessary, include the use of chemicals.

Research and development should be encouraged and supported in countries in which vectorborne diseases are endemic. Wherever local expertise and facilities are lacking, they should be created and supported. This would be the best way to circumvent serious prevailing obstacles, including, in some cases, the hesitation of governments to allow the importation of such organisms as bacteria and fungi.

It must be clearly recognized that some vectors, or vector-habitat combinations, are not amenable to biological control and that, as far as efficiency and cost-effectiveness are concerned, some control agents offer only limited potential, or none at all. Available knowledge about the control of target vector species must be examined to determine which vectors are the most amenable to control by biological agents. Among the selected vector species, priority should be assigned on the basis of the greatest urgency in terms of human disease and factors such as insecticide resistance. The most promising biological control agents should then be selected and progressively tested in selected sites.

Control agents fall into two broad categories:

- (1) Those, such as *B. thuringiensis* H-14, which affect a wide range of target vector species.
- (2) Those with particular local promise, such as a number of indigenous fish and competitor snails, or with habitat limitations, such as some fungi.

A large proportion of facilities should be devoted to developing the best agents in these two categories. Simultaneously, some effort should be directed to developing candidate agents lower on the priority scale and some attention given to opportunistic projects to provide agents with potential for future development. Considerable effort should be devoted to the search for new agents and new isolates of the present promising agents. An efficient screening system is required to determine which are the best to form a second generation of agents with improved efficacy.

During the development of biological control agents, information must be communicated to institutions in endemic areas to ensure the early utilization of advances made.

At appropriate points in the sequence of developing an agent, its safety to man and nontarget organisms, as well as other aspects of environmental impact, should be ascertained. A suitable scheme for screening and evaluating the efficacy, safety and environmental impact of biological agents for the control of disease vectors is shown in Annex 1.

The logical sequence and time schedule of operations can be illustrated by the example of the development of *B. thuringiensis* H-14 for vector control:

- 1976: Discovery; laboratory assessment very encouraging and initial safety tests completed.
- 1977: Small-scale field trials against mosquitos and blackflies confirm potential in endemic areas.
- 1979-80: Safety tests completed. Pilot commercial production commenced. Standardization and bioassay procedures investigated. Larger field tests carried out. Formulation research intensified. Steps taken to create collaborating centres in developing countries.
- 1981-82: Large-scale tests carried out in endemic areas. Efforts initiated to secure local production in endemic areas. Search for new isolates intensified and their screening rationalized.
- 1983-85: Large-scale testing increasingly handed over to developing countries, with emphasis on local products. Research concentrated on investigating problem situations and developing new strains and species. Superior new strains made available to all manufacturers.
- 1980-85: Information distribution and training of personnel in endemic areas progressively intensified.

7. CONCLUSIONS AND RECOMMENDATIONS

1. Having noted the emergence of insecticide resistance, refractory vector behaviour and other constraints on the use of chemicals alone for disease vector control, and having examined the various efforts being made to circumvent the problem by seeking alternative methods, the Committee agreed that biological control agents play a useful role in naturally regulating vector populations and could be utilized effectively as a component in integrated vector control programmes.

It endorsed the work being done in this field and recognized the relatively rapid progress made in developing biological control agents by the World Health Organization and the scientific community at large. The Committee recommended the expansion of the search for and evaluation of organisms with vector control potential, particularly in the tropical endemic regions of Africa, South America and Asia. These regions, which remain virtually unexplored for biological control agents (other than fish), are precisely the areas that are most in need of them, owing to the widespread occurrence of vectorborne diseases.

2. Recognizing that natural enemies play a role in checking the proliferation of vectors, the Committee stressed the importance of avoiding their destruction and of the need for research aimed at

evolving simple economical ways of augmenting their impact on vector populations to a point at which disease transmission could be reduced or arrested. This would involve a detailed study of the ecology and biology of the vector and its natural enemies so as to identify the factors governing the established balance of nature and to modify them in favour of the natural enemies.

The Committee realized that the effective use of biological control agents in integrated vector control programmes would be comparatively more difficult than the use of chemical insecticides and recommended that increased emphasis should be given to studies on:

- (a) the behaviour and bionomics of the vector and the natural enemy complex;
- (b) the recycling potential of selected parasites and pathogens, with special reference to defining the habitats favourable to their recycling, which would provide long-term vector control; and
- (c) the development of an effective strategy for integrating the use of biological control agents either sequentially or in combination with other vector control measures.

3. Recognizing the potential of predators, especially fish, in the simple and economical control of mosquitos and other vectors, such as snails, the possibility of their being used at the peripheral level through participation of members of the community with the minimum of specialized training, and the collateral benefit of the fish serving as a source of nutrient, the Committee recommended that efforts should be intensified to evaluate and use indigenous larvivorous fish for vector control. Realizing the recent advances made in the biological control of the snail intermediate hosts of schistosomiasis and the fact that the snails are restricted to freshwater habitats, the Committee advocated the encouragement of applied research on competitor snails and phytophagous/malacophagous fish in tropical countries for their ultimate use in the biological control of snails. Noting that more information is needed on biological control agents of tsetse flies, triatomines, phlebotomines and Simuliidae, the Committee suggested that greater efforts should be made to promote research in this area.

4. The Committee appreciated the great potential of the mass production of suitable biological control agents and their introduction into vector breeding habitats. It endorsed studies leading to the

utilization of a combination of agents in conjunction with other methods in integrated vector control programmes. Having reviewed the present stage of development of such biological control agents and identified the most promising among them, the Committee was encouraged by the performance of *B. thuringiensis* H-14 in helping to solve the problem posed by insecticide resistance among the black-fly vectors of onchocerciasis, as well as its potential against some mosquito vectors. Noting that industries in developing countries could develop the technology for the mass production of this agent at low cost utilizing locally available materials and giving due emphasis to the quality control of each batch produced in the interests of safety and efficacy, the Committee recommended the following procedures:

- (a) facilitate action already initiated to demonstrate in large-scale trials the impact of the agent on mosquito-borne diseases;
- (b) continue the development of improved formulations;
- (c) screen, develop and evaluate new strains in the field;
- (d) encourage the production of the agent in developing countries;
- (e) explore the possibility of improving the suitability of the agent for vector control through genetic technology;
- (f) continue the study of the chemistry of the crystal toxin;
- (g) investigate the potential of *B. thuringiensis* H-14 in operational use to prevent or retard resistance to chemical insecticides;
- (h) intensify studies on *B. sphaericus*; and
- (i) make efforts to isolate and evaluate other, more effective, species and strains of bacteria.

5. To ensure a critical and accurate evaluation of newly discovered and existing candidate biological control agents, the Committee recommended the development of standardized procedures for laboratory bioassays¹ and field evaluation guidelines. In assessing efficacy, the cost and ease of mass production must be considered in relation to the likely method of use. For example, with agents that have little or no ability to recycle and produce significant long-term levels of vector control, such as *B. thuringiensis* and some imperfect fungi, cost-effectiveness should be determined in the light of the need for repeated applications.

6. The Committee stressed that the development and use of biological control agents will require specialized knowledge and hence

¹ See, for example: "Bioassay protocol for *Bacillus thuringiensis* H-14 preparations" (10, Annex 4).

the additional training of research workers, vector control personnel and even lay members of the community. To facilitate this training, the Committee recommended:

- (a) the development of regional centres for training in research on and the use of biological control agents, these centres also serving as repositories for biological control agents to be exchanged among interested countries;
- (b) the periodic holding of regional and local workshops to demonstrate the use of biological control agents;
- (c) the preparation of illustrated manuals for the mass production and use of biological control agents;
- (d) application of the principle of technical cooperation among developing countries (TCDC), together with the transfer of technology from developed countries;
- (e) the development of participatory programmes at the community level, where appropriate, to initiate or enhance the use of biological control agents; and
- (f) further efforts to disseminate relevant information as widely as possible to collaborating centres and individual scientists in developing countries.

7. Realizing that vector resistance in some agricultural ecosystems, such as rice- and cotton-growing areas, is due largely to the use of insecticides to control agricultural pests, the Committee recommended closer collaboration among organizations of the United Nations system and other bodies in the formulation of programmes to preserve the natural enemies of vectors and reduce vector control problems. Efforts should be made to secure economy by producing different agents of the same species, or similar organisms, wherever possible in the same industrial installations.

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* A limited number of copies is available, on request, to professionally interested persons, from the Division of Vector Biology and Control, World Health Organization, 1211 Geneva 27, Switzerland.

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Annex 1

**WHO SCHEME FOR SCREENING AND EVALUATING THE EFFICACY,
SAFETY AND ENVIRONMENTAL IMPACT OF BIOLOGICAL AGENTS FOR THE CONTROL OF DISEASE VECTORS**

Stage I Search and laboratory operations	Stage II Experimental laboratory operations	Stage III Preliminary field trials	Stage IV Pilot production	Stage V Large-scale field trials
PATHOGENS^a				
A. Identification and characterization.	A. Mammalian infectivity tests to ensure safety to laboratory and field personnel.	A. Strictly regulated tests under WHO supervision to determine efficacy against disease vectors under natural conditions.	A. Pilot production and more detailed tests on mammalian infectivity/safety.	A. Production for large-scale trials with quality control and preliminary standardization of the products.
B. Assessment against selected target vectors.	B. Preliminary assessment against certain non-target species in the laboratory.		B. Detailed laboratory and field studies on non-target fauna, especially that in habitats where Stage V trials may be conducted.	B. Large-scale trials and entomological assessment.
C. Preliminary evaluation of ease of rearing in quantity.			C. Studies on stability of suitable formulations and delivery system.	

PARASITOIDS, PREDATORS AND COMPETITORS^b

<p>A. Observations on presence in field. Preliminary identification and dispatch to a reference or identification centre.</p>	<p>A. Recording both biological and environmental effects in actual breeding places.</p>	<p>A. Trials in small-scale natural habitats of important vectors.</p>	<p>A. Pilot production by culture or by collection from field.</p>	<p>A. Production for large-scale trials with quality control and preliminary standardization of the product.</p>
<p>B. Observations on biological effectiveness and on environmental effects.</p>	<p>B. Preparation of a clear statement of desirable characteristics and constraints as a guide to field trials.</p>	<p>B. Moderate-scale trials and impact on nontarget organisms.</p>	<p>B. Large-scale trials and entomological assessment.</p>	
<p>C. Production of experimental quantities in the laboratory or by collection from field.</p>	<p>C. Observations on nontarget organisms.</p>	<p>C. Quantitative assessment of biological effectiveness.</p>		

^a Safety testing will be designed to meet the requirements of the countries involved in the research and development.

^b The agent may be applied at any stage beyond II B according to its potential and the habitats in which it is to be released, particular importance being attached to environmental impact if the species is exotic. Most countries have regulations for the introduction and quarantining of exotic beneficial organisms, which should be followed.

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