Mosquito Control: Some Perspectives for Developing Countries

by: Advisory Committee on Technological Innovation
National Academy of Sciences

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A Report of an Ad Hoc Panel of the
Advisory Committee on Technological Innovation
Board on Science and Technology for
International Development
Office of the Foreign Secretary

Con resumen en español
Avec résumé en français

NATIONAL ACADEMY OF SCIENCES
Washington, D.C. • March 1973

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OFFICE OF THE FOREIGN SECRETARY

March 1973

Dr. Joel Bernstein
Assistant Administrator
Bureau for Technical Assistance
Agency for International Development
Department of State
Washington, D.C. 20523

Dear Dr. Bernstein:

Mosquitoes are still the world's number-one vectors of human and animal diseases, and are conspicuous nuisance pests as well, even after massive efforts at eradication or control. Today, we depend almost entirely on synthetic chemical pesticides for protection against mosquitoes. Yet, the appearance of pesticide resistance and adverse ecological effects have diminished our confidence in conventional chemical methods despite their striking success in past decades.

Entomologists agree that mosquito control of the future must combine efficiency with selectivity for a specific target organism. New control methodologies should aim at reducing mosquito breeding sites and using a combination of chemical and biological control methods.

One of our greatest needs is techniques for biological control (in the broadest sense of the term). Existing information, particularly recent findings, justifies a prediction that significant breakthroughs in biological control can be expected within 5 years–given adequate support and sustained research efforts.

Worldwide, current programs do not sufficiently emphasize source reduction and biological control. However, World Health Organization programs, in particular, have been trending in this direction and offer exceptional opportunities for advanced field testing of biological control agents in many parts of the world. The Panel on Perspectives in Mosquito-Control Methods Suitable for Developing Countries has urged me to convey to you a strong recommendation that the United States give support to field evaluations of biological control agents, particularly through existing facilities of international agencies.

Sincerely yours,

Harrison Brown
Foreign Secretary

PANEL ON PERSPECTIVES IN MOSQUITO-CONTROL METHODS SUITABLE FOR DEVELOPING COUNTRIES

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Preface

The Panel on Perspectives in Mosquito-Control Methods Suitable for Developing Countries met in Washington, D.C., three times during 1972 and once in 1973. They were charged with

- Deciding whether there are worthwhile alternatives to conventional pesticides for mosquito control in developing countries;
- Selecting approaches that seem particularly promising;
- Evaluating the state of knowledge and the future potential of the selected approaches; and
- Recommending areas in which further research and development funding could have particular impact.

The panel decided that many approaches to mosquito control other than those in widespread use today are worth considering for future use, and are needed in both developed and developing countries. The growing uncertainty about the overall effects of chemical pesticides and the emerging concept of pest-management technology–combining source reduction, integrated controls, and quantitative analyses of costs and benefits–are current preoccupations that made the study timely.

The panel identified almost fifty topics in biological, chemical, or environmental control of mosquitoes that are not receiving the research attention they warrant. From this master list, the panel chose nine topics that were promising for use in developing countries (though, in general, they are important for developed countries, too). Each topic was evaluated and written up by an individual panelist; each paper was refereed by the other panelists, discussed at subsequent meetings, and modified according to the will of the panel as a whole. This document, therefore, represents a consensus of the panel.

The choice of topics for the final report does not reflect on others; many are equally worthy of attention. Selection was based on technical soundness and future potential for developing countries. **Most of the approaches described in this report are not ready for large-scale application; they are promising control measures that warrant further research, development, and field testing.**

Not a technical handbook, this report aims at arousing interest in some unusual but promising mosquito-control methods that might otherwise be ignored. It is written for administrators or program directors of agencies that fund mosquito-control research and application projects and for scientists working on neighboring topics.

Individuals known by the panelists to be involved in relevant research are listed at the end of each chapter, but these lists are not meant to be exhaustive. The selected readings accompanying each chapter are mainly review articles and papers from readily accessible journals.

No order of priority is implied by the sequence of the chapters. The panel considered assessing the relative merits of the methods in order to assign overall priorities. However, in view of the wide disparities in needs, climate, mosquito species, and diseases of the over 80 developing countries, the panel concluded that priority decisions would be detrimental, particularly in a general report. Local scientists in the developing world, through communication with the "contacts" listed in each chapter, should be far better able to develop priorities appropriate to local requirements.*

The panel is indebted to Mignon Cabanilla, who acted as administrative secretary to the project, and to V. J. Steele, Memorial University of Newfoundland, who designed and produced the cover motif depicting methods described in this report. The manuscript was prepared for publication by Jane Lecht.

*In April 1973, soon after the first printing of this report, a "Conference on the Safety of Biological Agents for Arthropod Control" was held in Atlanta, Georgia, under World Health Organization auspices. An informal report on the conference includes further details on cautions for using biological agents in mosquito control. It is available from the Vector Biology and Control Division, World Health Organization, Geneva, Switzerland.

Contents

CHAPTER I

Introduction and Summary

Mosquito-borne diseases afflict both developing and developed nations. In much of the world the ancient scourges of mankind—malaria, filariasis, yellow fever, and dengue—continue to limit progress.

In certain countries, eradication programs have been dramatically effective, but in other areas eradication is not feasible for the foreseeable future, mainly because of technical problems and mosquito resistance to chemical insecticides. *Control* is a more realistic goal than eradication, but new techniques must be added to our arsenal of controls before further progress can be expected.

In number of victims, malaria is possibly the most prevalent disease of mankind. About 400 million people live in areas where malaria is still highly endemic; it is estimated that at least 100 million cases occur annually and result in 1 million deaths.*

Filariasis, although somewhat more limited in distribution than malaria, is a common disease in tropical areas, affecting more than 250 million people. In recent years, this disease has increased alarmingly because spreading urbanization has led to an increase in breeding areas for vector mosquitoes—i.e., mosquitoes that transmit disease-producing organisms (pathogens).

Yellow fever is one of the most virulent diseases of man, though an effective vaccine is available. This viral disease is limited now to Central and South America and some parts of Africa. Its urban form is transmitted by the "domestic" mosquito *Aedes aegypti*. One difficulty in attempting control is that alternate hosts (such as monkeys) harbor the disease, and from them it is transmitted to man by forest mosquitoes.

*L. J. Bruce-Chwatt, 1971, Bull. WHO. 44:419.

the factors that make the habitat* favorable for the mosquito. A concerted effort at reducing mosquito-breeding places ("source-reduction programs) can often be the most immediate route to disease control. In the pre-DDT era of the 1930's, *A. aegypti* was eradicated from Brazil by large-scale use of manpower in a program of strict legal restraints and elimination of water in domestic containers. In Panama, the reduction of mosquito-breeding sites, also through a massive use of labor to drain pools of water that form during the wet season, has substantially reduced populations of the malaria vector *Anopheles albimanus.* For many years this program has been so successful that only small-scale use of chemical pesticides has been necessary.

The panel strongly advocates that the first step in any mosquito- and disease-control project should be an ecological investigation to determine if a minor environmental change, or minor change associated with man's agriculture or living patterns, may eliminate the source of mosquitoes and/or disease.

Although manipulating the environment for mosquito control has many desirable features, it may result in new problems. Draining and filling marshes may eliminate fish and wildlife habitats—an example that indicates the need to assess the costs, benefits, and risks of each environmental manipulation before starting any control program.

In some instances, source-reduction programs will not eliminate the sources of mosquitoes or disease, so various combinations of biological and chemical methods are needed as supplementary controls. In any situation, however, the information gathered for mosquito-source reduction is a prerequisite to formulating appropriate control programs.

The success of all mosquito-control programs—designed to rely on source reduction, chemicals, or biological controls—depends on a continuing regimen of surveillance and follow-up action, because the environment and mosquito populations are subject to frequent change. For example, a single release of natural enemies seldom keeps the mosquito population down for long. Surveillance is always a necessary adjunct to control.

Successful anti-mosquito programs today must depend on source reduction and combinations of biological and chemical methods (integrated control); future mosquito-control programs, in the panel's opinion, should employ a systems approach (pest-management technology). A systems approach would consist of

- *Quantitative analysis of the costs and benefits of the program to man and his environment;*
- *Source reduction; and*
- *Integrated control.*

*The place where a plant or animal species naturally lives and grows.

Such complex control programs require professional biologists experienced in ecology, entomology, parasitology, and genetics. In addition, the scientific team should have specialists in economics and systems-management techniques and, in some cases, agronomy, horticulture, agricultural engineering, and soil science.

To plan any systems-management program for mosquito control, prior information is needed on mosquito ecology, disease epidemiology, and the spectrum of environmental manipulations and/or alternative control techniques that might be combined in a particular environment.

ALTERNATIVE CONTROL METHODS

This report deals with some alternative control techniques and considers their potential and their limitations. To summarize, the following is a brief listing of these techniques and how they work.

LARVIVOROUS FISH

In pools, ponds, marshes, etc., various minnow-sized fish have controlled mosquito breeding by eating mosquito eggs, larvae, and pupae.

INVERTEBRATE PREDATORS

Prominent among these predators is a group of large, nonbiting mosquitoes (*Toxorhynchites*) whose larvae devour the larvae of other mosquito species that are disease vectors. The predatory mosquito larvae may be especially effective in reducing pest-mosquito production in tree holes and other containers such as tires, tin cans, household water jars, and flower vases.

GENETIC CONTROL

Mosquitoes with altered genetic material may be used to control their own species. For example, when strains of mosquitoes with chromosomal translocation mate with normal individuals, reproduction is partially inhibited. Also, sterile males can be liberated to mate with normal females, thereby reducing the population of succeeding generations.

PARASITIC NEMATODES

Several species of parasitic nematodes (roundworms), notably mermithids, attack certain mosquitoes in the larval stage. These minute roundworms can be mass-produced, and field trials are under way to determine their potential for mosquito control.

PARASITIC PROTOZOANS

In nature, microsporidans, a group of spore-forming protozoans, are common pathogens of mosquitoes; if their numbers can be increased or their effectiveness improved, they may contribute even more to mosquito control.

PARASITIC FUNGI

Many fungi, especially members of the genus *Coelomomyces*, kill a wide variety of mosquito larvae in a broad range of aquatic environments.

PATHOGENIC BACTERIA

A spore-forming bacterium *Bacillus thuringiensis* (strain BA-068) is highly lethal to certain mosquito larvae. It can be mass-reared and applied to aquatic habitats.

JUVENILE HORMONE MIMICS (INSECT GROWTH REGULATORS)

Insect juvenile-hormone mimics hinder and derange the normal development of immature mosquito stages. Applying low doses of these compounds to aquatic habitats can prevent the mosquito from completing its life cycle.

LARVICIDAL PLANTS

Various larvicidal plants, including the rooted-algae of the family Characeae, can under certain environmental conditions prevent mosquito breeding by exuding a toxin into the water. Also, certain seeds that exude mucilage may drown larvae that become stuck to them.

OTHER ALTERNATIVES

The control methods just listed were given special attention by the panel because of their general potential for use in mosquito-control programs. However, other methods may actually be more effective for a particular mosquito or a particular natural or socioeconomic environment. For example:

Competitive displacement, in which one species is replaced by another, can control disease. For example, if a harmless mosquito can be made to dominate the space or food resources of a harmful vector species, it can force the latter's extinction. Such a displacement can be encouraged either by introducing a highly competitive, nonvector mosquito species or by altering the environment to favor a harmless species already present.

Hydra, invertebrate predators that inhabit fresh water and capture by stinging and lassoing a variety of small animals, including mosquito larvae. In shallow water, dense hydra populations sometimes occur attached to vegetation, stones, and other subsurface objects.

Pheromones, chemicals produced in one individual that elicit a behavioral or physiological response in another individual of the same species. Currently, there is great interest in sex attractant pheromones for control of many agricultural pests. Individuals of one sex are lured into a trap baited with chemicals resembling pheromones of the other, or pheromones are released in the pest habitat to prevent convergence of the sexes for mating (the confusion method). Recently, a sex pheromone has been extracted from female mosquitoes. Another mosquito pheromone, produced by glands associated with the reproductive organs of males, renders females refractory to subsequent insemination. In the laboratory, this material has been extracted and applied to virgin females; treated females are then prevented from ever producing offspring.

Oviposition attractants. Work undertaken in the South Pacific 50 years ago suggested that a better knowledge of the factors attracting mosquitoes to sites characteristically used by their species to lay eggs (oviposit) might be applied to mosquito control. For example, synthetic attractants could be used to lure the female to a trap or to a site where the eggs could be prevented from hatching. Recent work has filled in some details, but considerable gaps remain. Concentrated research in this area might open up pathways toward new, highly specific controls.

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*Out of print, but the panel suggests it be reprinted.

CHAPTER II

Larvivorous Fish

As biological mosquito-control agents, larvivorous fish—especially the minnow *Gambusia affinis*—have been singularly successful. Given the proper conditions, they can destroy large numbers of mosquitoes. Larvivorous fish (i.e., those that feed on immature stages of mosquitoes) perhaps lend themselves better than any other biological agent to immediate use in developing countries to augment control of pool-inhabiting mosquitoes.

Gambusia have been investigated and used for mosquito control since the early 1900's. Originating in the United States, stocks of *G. affinis* were sent in 1905 to the Hawaiian Islands, where they were credited with reducing *Culex pipiens fatigans** populations; in 1913 to the Philippines, and from there to various parts of Southeast Asia; into Spain and Italy in the 1920's, where they were reported to give excellent anopheline control; and thence to North Africa and parts of Europe. After the 1930's, use of larvivorous fish as mosquito-control agents diminished for several reasons. Many *Gambusia* had been introduced into habitats unsuitable for these fish, or that supported few mosquitoes. DDT and other synthetic compounds initially gave such spectacular results that older methods were often de-emphasized.

What is the method for using this control agent?

Gravid females (pregnant with young or eggs) are collected with nets or traps from sites where they are well established, and moved in plastic bags or aerated tanks to sites selected for release. In suitable habitats (pools, ditches, borrow pits) fish reproduce to the population-carrying capacity of

*In compliance with international usage, *Culex pipiens fatigans* is used throughout this document rather than *Culex pipiens quinquefasciatus*, preferred in the United States.

the environment within weeks or months. Gravid live-bearers, such as *Gambusia*, may be released without males.

How is it suitable for developing countries?

Fish are easily cultured on a small scale (mass-rearing techniques remain to be worked out) and distributed with minimal apparatus or facilities. Unlike other methods discussed in this report, technical aspects of production and dissemination are fairly well understood.

Once breeding stock is obtained, the fish can be reared in any developing country by local manpower using local resources. Unlike chemical insecticides, this method requires little or no foreign currency or imports. Larvivorous fish are particularly appropriate for controlling mosquitoes in rice paddies and small water impoundments.

In many developing countries heavy rainfall produces short-lived puddles and ponds in which most fish cannot long survive. In some of these, the so-called annual fish that lay desiccation-resistant eggs (for example, *Nothobranchius* and *Cynolebias*), might be particularly appropriate. Annual fishes are adapted to countries with extended dry spells during which water impoundments dry out, to be refilled only during the rainy season.

Their small size and hardy nature allow mosquito-eating fish to be transported and handled with ease. For example, *Gambusia* have been dropped into waterways from airplanes and helicopters from altitudes up to 100 feet. Almost no mortality of *Gambusia* was reported. They can tolerate marked rises in water temperature during transportation.

Because fish are easily seen and their mode of action is readily understood, this method, with minimum teaching, could be taken up by the general public in developing countries.

What are its advantages compared with other control methods?

Fish are suitable for controlling mosquito strains resistant to chemical insecticides.

Gambusia have other advantages for mosquito control:

- Their small size (usually less than 5 cm) allows them to penetrate easily most sites of pool-inhabiting mosquito larvae.
- They feed heavily on mosquito larvae and pupae when these are available; they are diverse feeders, capable of persisting at high densities when mosquito larvae are absent.
- They multiply rapidly; under favorable conditions, a single female produces an average of 200–300 young per season.

- Being live bearers, *Gambusia* require no special oviposition (egg-laying) site.
- They tolerate wide ranges of temperature and salinity, as well as moderate sewage pollution.
- They may be used effectively in combination with other control techniques, such as bacterial pesticides, nematodes, and some chemical pesticides.

What are its limitations compared with other control methods?

Mosquito fish have definite limitations. For example, they seldom inhabit two important larval sites—small containers and highly polluted water. In temporary water sites, repeated introductions of *Gambusia* will be required.

Mosquito-eating fish can harm beneficial organisms (e.g., other fish or insect predators) by eating their eggs and young or by superior competition for food. Their release carries the potential to reduce or eliminate other species valuable to man.

Larvivorous fish may be preyed upon by larger fish. Their vulnerability to fungi and other pathogens may keep their populations in check.

Where larvivorous fish are harvested and eaten, their populations could be reduced to a level inadequate for mosquito control.

Mosquito fish may prefer food other than the mosquito larvae. In many situations, mosquito-larvae production far outruns the increase in fish population that would be necessary for control.

What is the stage of development of this control agent?

The use of mosquito fish under tropical conditions is well documented. Fish are now used in many areas and are available in limited quantities.

Fish should not be introduced into any habitat and neglected; once well established, they thrive, but habitats change through aging, erosion, plant growth, pollution, and man-made alterations, all of which may kill or interfere with the fish.

What are the areas in which further research and development could have particular impact?

Efficient mass-culture methods to supply *Gambusia* in large quantities on a continuing basis need to be developed for areas where fish can be useful supplements to mosquito-control programs.

The total ecological impact of larvivorous fish on other organisms must be carefully studied before their release.

Further research is needed to determine why *Gambusia* and other fish species do not always thrive and effectively control mosquito larvae in apparently suitable habitats.

A search should be conducted to locate new larvivorous fish; more attention should be given to potential mosquito-control species native to developing countries.

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LARVIVOROUS FISH SUPPLY

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CHAPTER III

Invertebrate Predators

Several kinds of invertebrates that in nature prey on mosquito larvae and pupae offer potential for control of vector mosquitoes. One animal of note is a predacious mosquito *Toxorhynchites.** For certain mosquito vectors, especially in the tropics and on islands, they may be effective control agents.

Toxorhynchites is a largely circumtropical genus containing about 60 species of unusually large mosquitoes. The adults do not bite, but the larva is an active, voracious predator, feeding on the larvae of such container-breeding mosquitoes as *Aedes aegypti, A. simpsoni,* and *A. albopictus.* (See Figure 1.)

Little is known about the biology of most species of *Toxorhynchites.* Only a few species have been colonized; the most promising of these is *T. brevipalpis* from East Africa. During development, larvae of this species will consume about 250 *A. aegypti.* About 2 days before pupation the larva goes on a "killing spree," when it kills, but does not consume, all other inhabitants of its container.

Cannibalism may limit control potential; however, the female scatters her 80–100 eggs in numerous containers, thereby reducing the effects of cannibalism.

What is the method for using this control agent?

Toxorhynchites is an effective predator for mosquito larvae in small containers, such as bottles, cans, tires, vases, household water jars, and tree holes. Several of the most important vectors of human disease breed in such places and are especially hard to control because the larval habitats are small, dis-

*Many literature references to *Toxorhynchites* appear under its earlier name *Megarhinus.*

persed, and inaccessible. Female *Toxorhynchites* can find these containers more readily and more frequently than can the mosquito-control worker. The great potential of this method lies in its capability for dispersion.

Techniques for mass production of *T. brevipalpis*, yielding 50,000–100,000 adults per week have been developed. The life cycle in the laboratory, egg to egg, is 20 days. Larvae can be stored at 16°C for more than 8 months; normal growth resumes at 27°C, and the larvae show no adverse effect from storage; thus, larvae can be stockpiled to produce adults for seasonal release.

How is it suitable for developing countries?

Toxorhynchites can be mass-reared with minimal equipment and facilities and distributed by unskilled workers. However, one must be able to mass-produce *A. aegypti* or another mosquito species to grow the *Toxorhynchites;* without sufficient prey, *Toxorhynchites* become cannibalistic.

FIGURE 1 *Toxorhynchites* (large) feeding on *Aedes* larvae (small). (George B. Craig)

What are its advantages compared with other control methods?

Toxorhynchites can be mass-reared, transported, and released as adults. As mentioned earlier, the adults can seek out small-container habitats scattered throughout their flight range. To apply other control techniques, including insecticides, to these sites is extremely difficult.

The cost of this method promises to be competitive with that of chemical insecticides.

What are its limitations compared with other control methods?

Seasonal fluctuations in rainfall may prevent *Toxorhynchites* from controlling much of a vector-mosquito population if the slower-developing predator builds up to effective numbers only toward the end of the wet season (Figure 2). In local situations, mass release at the *beginning* of the wet season might avoid this difficulty.

Field trials in Fiji, Samoa, and Hawaii have shown that *Toxorhynchites*

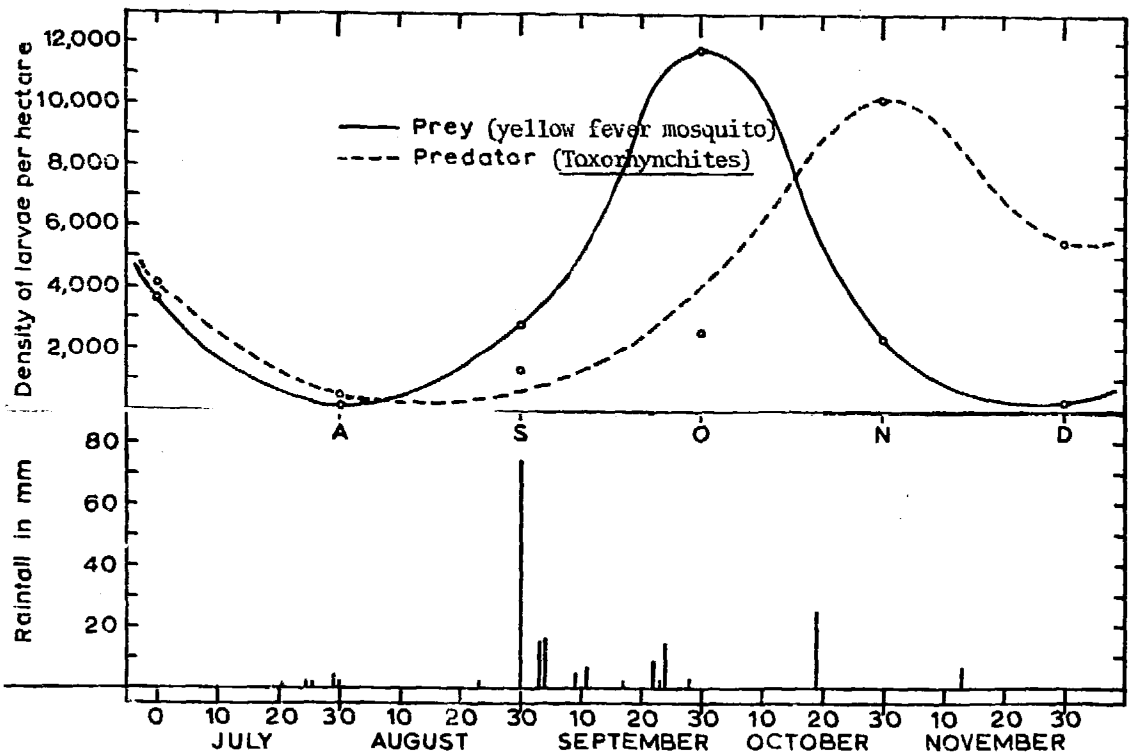

FIGURE 2 Relation between mosquito larvae of *T. brevipalpis* and the yellow fever mosquito *A. aegypti* on which they prey, measured in an old-car dump in Dar-es-Salaam, Tanzania. Prey increase begins with rainfall and is followed by the slower-breeding *Toxorhynchites* which eventually overtakes and then decreases because of its cannibalism. Prey control might be achieved by releasing already-grown predators when the rainy season begins. (Trpis, 1973)

can be transported, propagated in a field insectary,* and established in the target area; but in these trials *Toxorhynchites* did not achieve the desired level of mosquito control. The experiments, however, have been fragmentary and reporting incomplete. Only small numbers were released in "one-shot" experiments; recent evidence suggests that repeated releases are required, possibly on an annual or seasonal basis. Techniques for managing and conserving these predators might enhance their efficiency. One limitation might occur if the released adults are unable to find a large proportion of vector-breeding sites within the target area.

What is the stage of development of this control agent?

This method is ready for extensive field testing. Strains for release trials are now on hand. (See the next question.)

What are the areas in which further research and development could have particular impact?

Toxorhynchites now has enough potential for field investigations, but as a precaution, isolated habitats should be used. Field releases should be undertaken to investigate the most practical way to employ the predator.

Methods of mass production should be extended.

Dispersal should be studied in the field.

Population dynamics of *Toxorhynchites* should be studied in both field and laboratory.

Bionomics of species other than *T. brevipalpis* should be explored.

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CHAPTER IV

Genetic Control

Genetic manipulation of mosquitoes is a potentially powerful method of control. In principle, this approach converts pests or vectors into autocidal biological control agents (lethal to their own kind). Several promising ways to manipulate the hereditary material of mosquitoes are being studied.

What are the methods for using genetic control?

1. Sterile-Male Technique Sterilization of male insects was established as a practical means of insect control in the late 1950's when the screwworm fly *Cochliomyia hominivorax* was controlled in parts of the United States by the release of radiation-sterilized males.

The sterile-male technique has also been applied to mosquitoes: after chemically sterilized males of *Culex pipiens fatigans* were released on a small island off the Gulf Coast of Florida, the natural population was essentially eliminated in 5-6 generations. Excellent results have also been obtained against an isolated population of *Anopheles albimanus* in El Salvador.

2. Cytoplasmic Incompatibility When geographically diverse strains of *C. pipiens* are crossed, it is often found that although mating takes place, the females lay eggs that do not hatch. This incompatibility has recently been traced to microorganisms within the mosquitoes. Incompatible males could be used to eradicate a vector species by the sterile-male method. In this manner infertility was introduced in a population of *C. pipiens* in a village in Burma in 1967, resulting in a high degree of population reduction.

3. Hybrid Sterility Frequently, when closely related species of mosquitoes mate, their chromosomes fail to pair and the hybrid offspring are sterile. The effect is usually greater in male than in female hybrid offspring. It is theoretically possible to produce sterile males in this way for use in an eradication program.

4. *Chromosome Translocations* One possible method of genetic control that has attracted recent attention is the use of chromosome translocations. Normal mosquitoes have three pairs of chromosomes. Radiation can be used to break these pairs into pieces, and the pieces may rejoin in abnormal arrangements. The rearranged chromosomes still contain all the genetic information necessary for survival and growth but cause errors in the formation of sperm and eggs. This can yield healthy and competitive, but sterile, mosquitoes. Moreover, the effect is inherited from partially sterile populations by later generations.

The potential of this method was originally proposed 30 years ago by Serebrovskii in the USSR. Only recently, however, has its use against mosquitoes, tsetse flies, and houseflies been studied and some progress made. Already chromosome translocations have been produced and are being tested in *Aedes, Anopheles,* and *Culex* mosquitoes.

5. *Conditional Lethal Genes* Manipulation of genes that cause behavior that under certain conditions is lethal for the organism is an especially promising way to regulate mosquito populations. Such genes include factors that destroy the mosquito's ability to enter a resting stage to escape harsh environmental conditions, such as freezing and drought. One advantage of using conditional lethal genes is that individuals with the lethal character continue to breed for some time after their release, so the lethal gene is transmitted to future generations.

6. *Gene Replacement* A vector species need not be eliminated to bring the disease it carries under control. A classic example is the elimination by conventional means of native malaria within the United States in the continued presence of its vector *Anopheles quadrimaculatus*. Deliberately replacing vectors with strains that are of the same species but do not transmit disease might accomplish disease control without reducing the overall number of mosquitoes. Already, genetic differences that render mosquitoes refractory to malaria or filaria parasites–or change their choice of host–have been demonstrated. These findings suggest that practical methods to replace vectors by harmless strains or species may be developed.

Requirements for all Genetic Methods Basic requirements for the practical application of any genetic control technique are

- Technology to rear large numbers of healthy and vigorous insects at reasonable cost;
- Technology to sterilize or introduce genetic defects in insects, without impairing their vigor and mating competitiveness;
- Methods to handle and release the treated insects;
- Extensive knowledge of the ecology and behavior of the vector species (to know when, where, and how many genetically sterile insects to release);
- Suppression, in most cases, of natural populations by other means at the most appropriate time in the seasonal cycle, thereby reducing numbers to levels that make it economically feasible or advantageous to employ genetic techniques (see question on limitations); and
- Assurance that the genetically altered insects do not themselves contribute to the transmission of disease.

How are these methods suitable for developing countries?

These methods could be simple and economical under some circumstances. The considerable labor requirements for mass-rearing the treated insects could be met with local manpower. Initially, however, a highly trained technical staff will be needed to develop the genetic techniques and the facilities and equipment required could be elaborate and costly. Nonetheless, 5 million sterile mosquitoes per week are now being reared in India.

What are their advantages compared with other control methods?

Genetic control techniques are specific for individual species and generally are compatible with other anti-mosquito programs.

Over the years, continuous suppression (or final elimination where feasible) using sterile or genetically altered mosquitoes could well be more effective, more economical, and more acceptable from an ecological standpoint than any other known system of vector control.

What are their limitations compared with other control methods?

Genetic control techniques for particular mosquitoes must be custom-developed for that species in its specific environment. Much information on the ecology of candidate vector species in its natural habitat is required to implement and maintain effective suppression by genetic means. To succeed, programs of genetic control require the assistance of expert supervising personnel with training in the population dynamics and mass rearing of mosquitoes.

Because a given genetic control program will be effective for only one species, it may not provide disease control where several different mosquito vectors are present.

Genetic methods may be most appropriate as a supplement to mosquito-control programs: either to complete, where possible, the elimination of populations or to maintain continuous suppression of populations infiltrating the habitat after a conventional mosquito-control program has been conducted.

Excessive natural populations may make a genetic approach impractical. Success depends on attaining the ratio of released to natural insects required to achieve suppression. The lower the natural population, the fewer insects must be reared and released to attain this ratio. Even during periods of lowest abundance, other ways of reducing natural populations will generally be necessary to make it economical to outnumber them with reared and released insects. However, conventional insecticidal methods can temporarily reduce virtually all vector-mosquito species to low numbers at reasonable cost.

Many technical problems must be overcome to use genetics for control. In particular, insects must be mass-produced and their genetic character altered without adversely affecting their viability and competitiveness.

What is the stage of development of these control methods?

Most of the genetic control techniques are in preliminary stages of development, but field trials are currently under way in India, Kenya, and El Salvador.

What are the areas in which further research and development could have particular impact?

To select and control a vector, extensive knowledge of its genetics and ecology must first be obtained on site. Then, intensive laboratory and field research must be conducted to devise an appropriate genetic control.

Information is needed on absolute numbers of insects present in natural populations during various periods in the seasonal cycles. This becomes the basis for determining if a genetic approach is feasible and practical. Information is needed on the flight range in different ecological areas of vector species that are good candidates for genetic control. Such information is necessary to determine the degree of isolation that is needed to maintain an efficient genetic control program.

The investment in research is large, but the return great. Even if economical mosquito control proves impracticable, the information gathered on mosquito ecology and population dynamics has application in all other mosquito-control techniques.

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CHAPTER V

Parasitic Nematodes

A nematode (nema or roundworm) *Reesimermis nielseni* has been found, in nature, to infect 22 different mosquito species and, in the laboratory, another 33 species—a total including most mosquito genera. *R. nielseni* (and some other nemas) attack only mosquito larvae, although many other species parasitize a wide range of insects.

Mermithid nemas, including *R. nielseni,* spend part of their life cycle as parasites in the body cavity of their hosts, and the rest as free-living worms. A preparasitic nematode larva attaches to a mosquito larva, bores a hole with a probe (stylet), and enters the mosquito's body—all in a few minutes. After its stint inside the host, the nema larva cuts a hole through the insect integument (skin) and emerges through this perforation. The mosquito body fluids leak from the hole, and the mosquito larva always dies. (See Figure 3.)

The postparasitic nemas drop to the bottom of the habitat, molt to adults, and mate. Each female nema lays about 2,500 eggs in the debris on the bottom. The eggs hatch in 4 or more weeks to produce parasitic nemas again.

Young larvae are more susceptible than older ones, probably because their integument is thinner. The larval preparasitic *R. nielseni* is extremely small (about 1.1 mm long). The mature female nema averages about 15 mm, but it is smaller in small hosts and in the presence of a large number of other nemas.

What is the method for using this control agent?

Young nemas may be applied by using the same knapsack sprayers already available for use with chemical insecticides.

Once nemas are successfully established, they remain at the site and do not

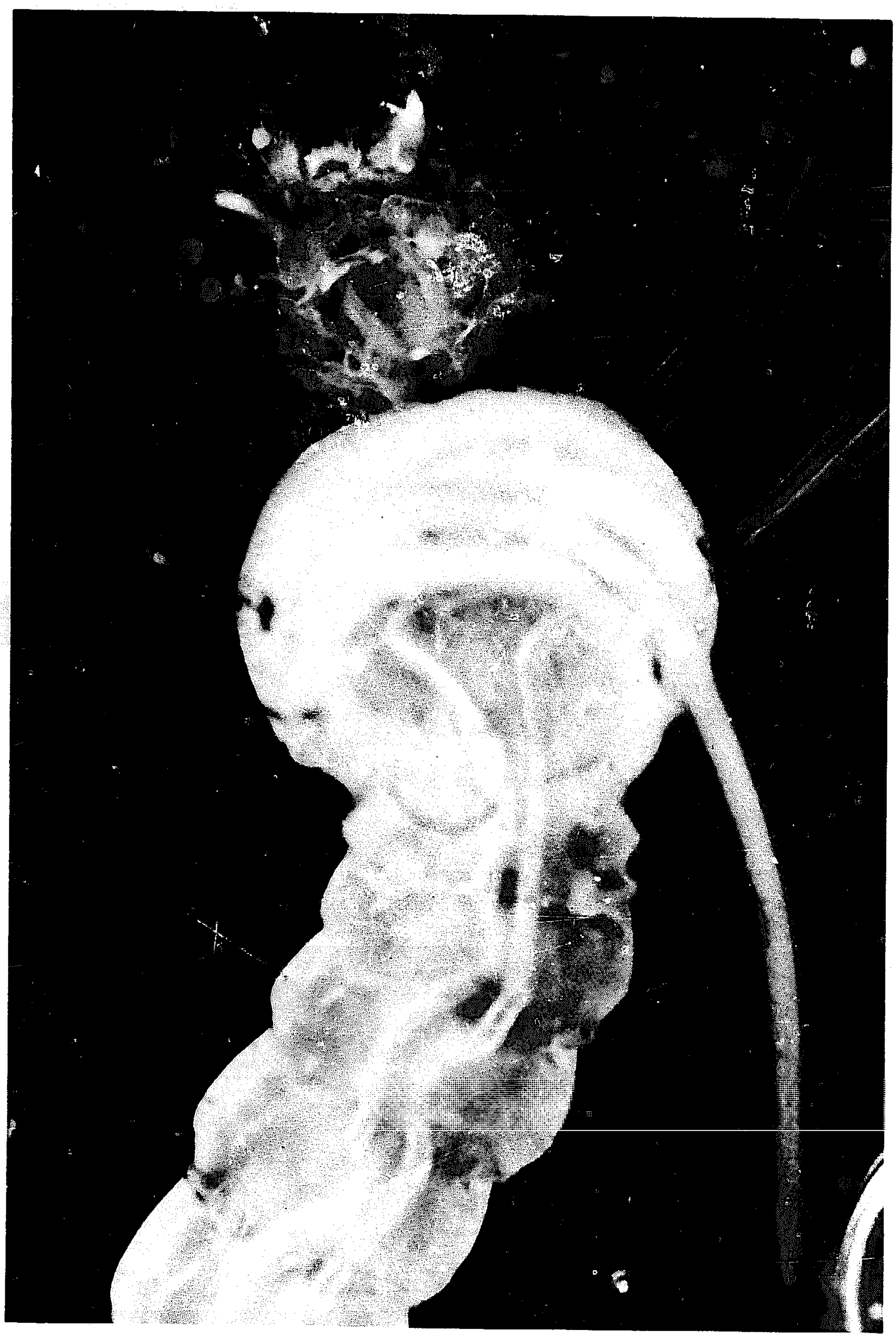

FIGURE 3 Postparasitic nema of *R. nielseni* escaping from a *Culex pipiens fatigans* larva. (Harold C. Chapman)

spread except when the water is accidentally moved from one aquatic habitat to another. This characteristic of remaining in the original habitat reduces the size and frequency of any further treatments.

Currently, nemas must be mass-cultured in living mosquito larvae. With modest laboratory facilities, 250,000 mosquito larvae per week have been used to produce enough *R. nielseni* preparasitic nemas to treat 12 hectares (30 acres) with 1,000 preparasitics per square meter. This dosage will control some anopheline species.

The nematodes can be disseminated in three forms: eggs, preparasitic nemas, or postparasitic nemas. Eggs can be stockpiled (though not for more than 6 months) and disseminated when needed.

How is it suitable for developing countries?

In nature, *R. nielseni* has been found in only three states in the United States; however, other mermithid nematodes are native to regions from the subarctic to the tropics, so, in theory, species can be found for application in any region.

R. nielseni is not yet known to control in the field the vector mosquitoes of any major disease, but laboratory studies have shown that six species of *Anopheles, Aedes,* and *Culex* mosquitoes are susceptible.

At present, nemas can be raised at the cost of about 10 cents per million preparasitics. Since our limited knowledge indicates that a treatment of 1,000 preparasitics per square meter will provide substantial mosquito control, the cost of producing enough nemas to treat an acre would be about 50 cents (U.S.). This cost estimate assumes the availability of minimal laboratory facilities, technical assistance, and appropriate scientific expertise. Technological improvements, including a system of culturing the nema in an artificial environment outside a living mosquito (*in vitro*), will reduce production costs.

What are its advantages compared with other control methods?

R. nielseni can be cultured and sprayed and, as mentioned, will maintain itself once applied. This method is compatible with other control techniques and appears to be an economical control for mosquito larvae.

R. nielseni has never been found to parasitize organisms other than mosquitoes. In initial laboratory experiments in which extremely large numbers (up to 10,000 nemas to one nontarget organism) have been applied, only chaoborids (a mosquito subfamily) were infected. Although no evidence suggests that this nema can harm man, more toxicity data are needed. Toxicity tests using mice are in progress.

What are its limitations compared with other control methods?

R. nielseni will attack only certain species of mosquitoes and is effective only in certain larval habitats. It will not tolerate water that is too alkaline, acid, saline (as in salt marshes), or polluted. In some slightly polluted waters where it can survive, its ability to infect mosquitoes is inhibited. To control mosquitoes the nema needs temperatures above 15°C, since the nema egg diapauses* below this temperature. Little is known about population dynamics of *R. nielseni* in the field.

What is the stage of development of this control agent?

R. nielseni can be economically mass-produced in mosquitoes. Laboratory rearing has produced more than 150 million preparasitic nemas from 250,000 *C. pipiens fatigans* larvae per week.

The chief problem in mass-producing *R. nielseni* is rearing the mosquito larvae on which they grow. It is difficult to determine the diet and quantity of food needed to produce the large mosquito larvae that yield large nematodes capable of maximum egg production. These problems would be avoided if *in vitro* culture could be achieved.

What are the areas in which further research and development could have particular impact?

An important need is to test the nemas for possible hazards to man and other nontarget organisms.

Research is needed to perfect the existing mass-production techniques, in particular to develop *in vitro* systems to culture the nema in an artificial environment.

More information is required concerning the effectiveness of *R. nielseni* under field conditions.

More information is needed on other nematode species, and on their potential for mosquito control.

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CHAPTER VI

Parasitic Protozoans

Microsporida are microscopic protozoans. They are among the most common parasites to cause disease in mosquitoes. Four genera of Microsporida (*Nosema, Pleistophora, Stempellia,* and *Thelohania*) are commonly found in mosquitoes. Despite many attempts in the laboratory, only a few species of Microsporida have ever been deliberately transmitted and established in a mosquito. Among the most promising for mosquito control are certain species of *Nosema*. Transmission of other microsporidans (notably *Thelohania*) by feeding their spores to mosquito larvae has not been successful. Currently, such protozoans are maintained in a mosquito population only by transmission to later generations through infected eggs. Other microsporidans could be useful mosquito-control agents if ways are found to make the spores infectious when ingested. *Nosema* may have potential use in malaria-control programs, since anopheline mosquitoes appear to be its primary hosts (see Figure 4). Furthermore, mosquitoes heavily infected with *Nosema* are reported to develop fewer malaria parasites than noninfected mosquitoes.

What is the method for using this control agent?

Microsporida produce spores that can be stored and then applied with knapsack sprayers. In limited trials, 70–80 percent of a population of mosquitoes (*Anopheles albimanus*) became infected when sprayed with *Nosema stegomyiae* (*algerae*) spores in larval habitats (1,000 spores per ml water). This pathogen can kill larvae, pupae, and adults. Even if the pathogen does not kill the adult females, it may reduce their longevity and disease-transmitting capacity.

Recent studies have shown that one *Nosema* species can be mass-reared in an *in vivo* system (i.e., within living larvae).

How is it suitable for developing countries?

If these pathogens can be mass-cultured and if they prove effective, they could be produced in the developing world and applied in the field by local workers.

What are its advantages compared with other control methods?

Microsporida can be stored as spores for several months under moist, cool conditions; inexpensive procedures for longer storage have yet to be developed. They are easily applied with equipment already present in developing countries. This control technique should prove compatible with other mosquito-control methods.

The species of microsporidans found in nature as pathogens of mosquitoes appear to be host-specific.

What are its limitations compared with other control methods?

Most microsporidans cannot yet be manipulated, even in the laboratory, and toxicological aspects remain to be clarified.

What is the stage of development of this control agent?

Laboratory research is proceeding, and limited field trials are beginning.

What are the areas in which further research and development could have particular impact?

Toxicological studies of any microsporidan being considered for mosquito control are needed to test the effects on mammals and other nontarget organisms. Some species of Microsporida live in other animals, both vertebrate and invertebrate.

Much research is needed, particularly on the microsporidans' nutritional requirements, life cycle, mode of transmission, infectivity and virulence, host-specificity, purification techniques, mass-production, and spore storage.

To determine microsporidans' effectiveness for mosquito control, additional work is needed in the laboratory, in the field, and in different environments.

f *Nosema stegomyiae* in adult *Anopheles quadrimaculatus.* (Claude H. Schmidt)

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CHAPTER VII
Parasitic Fungi

Several fungi that parasitize mosquitoes are being investigated as potential mosquito-control agents. One extensively studied genus is *Coelomomyces,* most species of which infect only mosquito larvae.

In nature, more than 90 percent of a mosquito population may be killed by this infection.

These fungi kill their mosquito-larvae hosts by destroying the fat body* and tissues essential for the development of adults. A few larvae pupate despite the infection and produce infected adults that disseminate the fungus to other water habitats.

The fungus produces infective stages (sporangia) within larvae. (See Figure 5.) Details of the mechanism of infection, which involves the release of motile spores from the sporangia, are still unknown. When larvae die or are destroyed by predators, sporangia are liberated to infect other larvae. The spores and sporangia may be distributed to new sites in dust from dried-up pools. Amphibious animals may also transport them from pool to pool on their skins.

Researchers have learned that other fungi (e.g., species of *Lagenidium*) are exceptionally lethal to mosquitoes, but they do not yet know enough about their side effects to recommend their release as control agents.

What is the method for using this control agent?

Coelomomyces has been successfully used in a pilot field experiment to infect mosquitoes. Sporangia, or mosquito larvae parasitized with *Coelomomyces* in the laboratory, can be employed to infect other mosquitoes in the field. Sporangia are resistant to desiccation, heat, and cold; and both sporangia and the larval cadavers can be stored for later field use.

*Fatty tissue found in insects which serves for food conversion and storage.

Once established, the fungus may be able to maintain itself in the habitat for many years (20 years has been recorded in one instance).

If a fungal species infects either a number of vectors or one important vector, the method should prove practical for disease control.

How is it suitable for developing countries?

Many species and varieties of *Coelomomyces* fungi have been reported from most parts of the world, including tropical Asia, Australia, Africa, and Latin America.

Certain species could be produced in developing countries by existing *in vivo* procedures.

Conventional sprayers already on hand in developing countries—and the personnel already trained to operate them—should prove adequate to disperse the sporangia.

What are its advantages compared with other control methods?

Most species of *Coelomomyces* occur only in mosquitoes. These fungi may persist in larval habitats without need for repeated dosing.

Fungal pathogens can be used in combination with many other mosquito-control techniques.

What are its limitations compared with other control methods?

No effects on nontarget organisms have been demonstrated, but no serious investigation into this question has yet been made. No evidence exists that *Coelomomyces* parasitizes any vertebrate; many workers have handled sporangia and *Coelomomyces*-infected mosquitoes without incurring any known harm.

Antarctic penguins brought to northern zoos often contract fungus-caused lung aspergillosis and certain (entomophthoraceous) fungi can cause disease in insect and vertebrate hosts (including man and horses). Therefore, it must be proved that *Coelomomyces* is without hazard to birds and other wildlife, as well as man and domestic animals. For example, assurance is needed that once problems of mass production are overcome, workers handling large amounts of sporangial concentrates will not be at risk to either allergic reactions or actual infection.

What is the stage of development of this control agent?

Coelomomyces as a mosquito-control agent is still in the experimental stage. A great deal of work remains before it is likely that mass-production

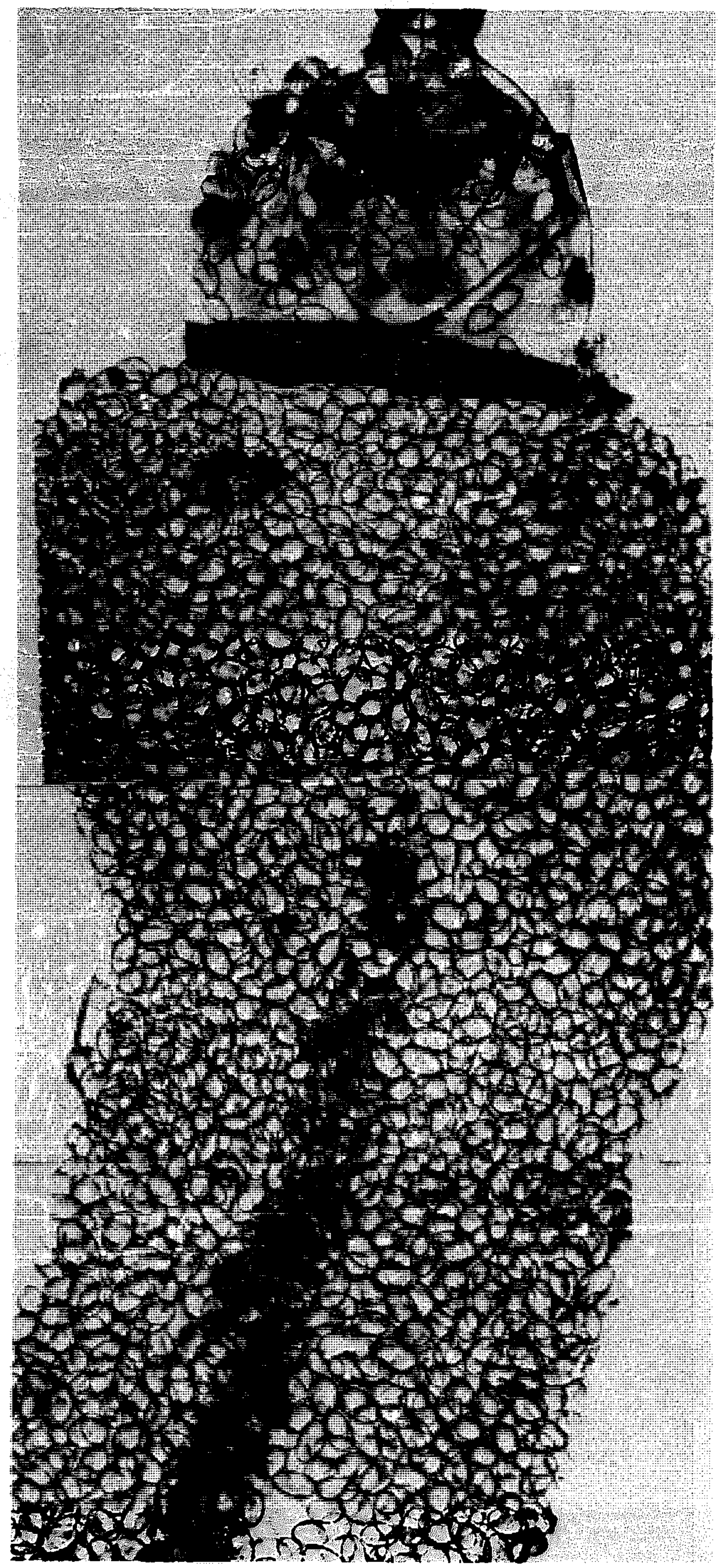

FIGURE 5 A larva of the malarial mosquito *Anopheles quadrimaculatus* almost completely filled with oval sporangia of *Coelomomyces*. Such a larva may contain over 60,000 sporangia, each producing up to 500 active infective stages. (John Couch)

techniques will open the way to practical use of these fungi.

Efforts toward growth in tissue culture are just beginning, and growth on defined media has yet to be obtained. Nonetheless, that heavy larval infections can be built up in the laboratory suggests that eventual scaling up to mass production is feasible. Furthermore, recently achieved *in vitro* survival of the fungi in a mosquito-tissue culture may portend large-scale culture—perhaps by fermentation procedures—and gradual development of a standardizable "microbial pesticide."

What are the areas in which further research and development could have particular impact?

Before *Coelomomyces* can be employed, research must be conducted to

- develop a method to produce *Coelomomyces* sporangia in quantity;
- devise a practical way to distribute them in the field;
- obtain information concerning the specificity of individual species of *Coelomomyces* to individual mosquito species;
- obtain toxicological information on nontarget organisms including man; and
- determine if applications of *Coelomomyces* produce resistance in mosquitoes, thereby killing only susceptible strains and leaving resistant strains to breed and continue transmitting disease.

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CHAPTER VIII
Pathogenic Bacteria

Spore-forming bacteria belong to the family Bacillaceae which has two genera *Bacillus* and *Clostridium.* Both genera include species that produce toxins that act as stomach poisons in insects. A crystalliferous, spore-forming bacterium *Bacillus thuringiensis* (abbreviated *B.t.*) was described in 1915. Today, various strains of this microorganism are cultured on ordinary bacterial media and form the basis of a thriving "microbial insecticide" industry. Spores and proteinaceous crystals of *B.t.* are used in agriculture and forestry as control agents against many pest caterpillars, but the strains used have little or no pathogenicity for mosquitoes. The isolation in 1968 of a virulent strain of *B.t.* (strain BA-068) from dead *Culex tarsalis* mosquito larvae suggests that this bacillus also has potential for mosquito control. This isolate, related to the *B.t.* used in agriculture (identified as *B.t.* var. *thuringiensis* Serotype H-1), is easily produced on standard culture media.

What is the method for using this control agent?

Bacterial insect pathogens, already used successfully in microbial control of several insects, can be handled easily and distributed as spores in the field.

Because spore-forming bacteria produce dormant, desiccation- and heat-resistant, thick-walled, refractile spores, they can survive outside the insect host. In addition to the spore, the crystalliferous bacterium at the time of sporulation produces one or more crystals. (See Figure 6.) When the free-floating spores and crystals of a virulent strain are ingested by a susceptible host insect, the alkalinity of the gut, and enzymes that are present, dissolve the crystal, releasing a toxin which paralyzes the gut. The paralyzed gut changes from alkaline to neutral or slightly acid, causing the spores to germinate, giving rise to free-living bacilli. These bacilli multiply in the still-paralyzed gut until they split the wall and enter the insect's body cavity. This combination of attacks on the host soon kills it. The pathogen then sporulates, giving rise to vast numbers of new spores and crystals which, upon disruption of the cadaver, become incorporated into the debris of the habitat, where they are ingested by another susceptible host.

How is it suitable for developing countries?

To date, *B.t.* BA-068 has been isolated only from a mosquito species in the United States. Its use, however, is not restricted by species or region; it could be used to control various mosquito larvae in many aquatic habitats.

This bacterium is suitable for production in developing countries because locally available resources can be used, possibly including the technology associated with local fermentation industries, such as brewing. In fermenters, it can be produced on a large or small scale. Experience in growing *B.t.* specific to other insects suggests that a scale-up to 10,000-gallon production is practical. This bacillus can be grown on standard artificial media, incorporating a balanced salt mixture, a protein source, and a vitamin source. It can be harvested by either vacuum drying the whole culture—spores, crystals, and spent culture medium—or by centrifugation to a spore slurry, which is then dried or stabilized for storage. The spores are stable, with a shelf life of over 1 year.

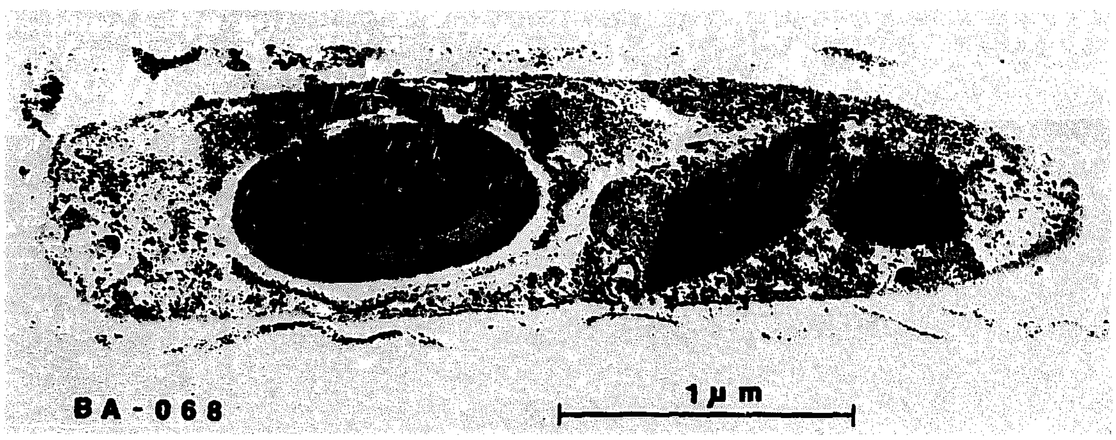

FIGURE 6 Electron micrograph of *Bacillus thuringiensis* (strain BA-068) showing a developing spore and two crystals of toxin. After a mosquito larva ingests spores and crystals, the alkaline stomach contents dissolve crystals, thereby releasing toxins that paralyze and increase the acidity of the larva's gut. Under these conditions, the spore—the reproductive mechanism of the bacterium—can germinate. The resulting bacilli break the gut wall and kill the mosquito larva. (Eldon L. Reeves)

B.t. can be applied in water suspensions, oil-water emulsions, bacterial powders extended with clay, or granular formulations with clay; the choice of materials depends on the method of application. The pathogen is easy to apply with existing insecticide sprayers.

What are its advantages compared with other control methods?

Technology for commercial production of *B.t.* is now available. Under some conditions BA-068 may not have to be applied repeatedly, so costs may be less than those of other biological methods. However, extensive field tests will be required to ascertain the true cost for a given situation.

This bacterium could be employed in combination with most of the other control techniques.

The crystalliferous species of *Bacillus* are generally considered to be environmentally acceptable. There is no indication that they are detrimental to man or plants.

What are its limitations compared with other control methods?

B.t. has been field tested once against mosquitoes. It has not yet been registered by the U.S. Government for mosquito control; however, other strains of *B.t.* have been registered for use against agricultural pests, and this may facilitate its registration.

Most of the bacteria currently recommended for insect control must be applied periodically to maintain control, and if application is frequent, costs may be excessive.

What is the stage of development of this control agent?

Nothing is known of the effectiveness of this bacterium for the control of mosquitoes in developing countries. However, it has been shown to kill larvae of three species of *Aedes* mosquitoes under laboratory conditions at a dosage of 9×10^5 spores per ml of culture water.

Known hosts include at least six species of mosquitoes and five of moths. *Aedes* larvae show greater susceptibility than do *Culex* larvae. Preliminary small-plot field tests (at dosage levels of 9×10^5 spores per ml of standing water) against the pasture mosquito *Aedes nigromaculis* look promising. Extrapolating these dosages from laboratory to field applications, however, indicates they are unrealistically high. By maximizing contact with the larvae, e.g., by granular formulations that concentrate the pathogen in the feeding area at soil level, the dosage can probably be brought down to a practical level.

What are the areas in which further research and development could have particular impact?

Research on this method is needed to

- measure its effectiveness against various mosquito pests and vectors;
- determine the danger to nontarget species;
- develop mass-culture procedures; and
- investigate the costs, benefits, and risks to man and his environment.

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CHAPTER IX

Juvenile Hormone Mimics (Insect Growth Regulators)

Natural and synthetic chemicals that regulate the growth, molting, and general development of mosquitoes and other insects have been discovered. A group of particular interest are the juvenile hormone mimics (JH). The untimely presence of JH chemicals during the larval-pupal and pupal-adult molts causes the production of abnormal or intermediate forms that fail to molt normally and soon die. (See Figure 7.) JH also influence adult mosquito development and maturation. For example, if the mosquito is exposed to JH, then ovarian development, yolk formation, egg maturation in the females and development of the accessory glands in the males may be influenced.

What is the method for using this control agent?

Employing JH is the same as using an insecticide, because they are insecticides, though with different characteristics and modes of action. Unlike existing insecticides, juvenile hormone mimics are not conventional toxicants; instead, they derange normal physiological processes associated with development and metamorphosis.

In use, the main difference between JH and conventional insecticides is that the timing of application is more critical: larvae are susceptible to JH only during a limited part of the molting cycle. Because JH compounds distort development and metamorphosis, their effects may be delayed. For instance, if JH is applied to larvae, the adverse results may not occur until the pupal or adult stage.

How is it suitable for developing countries?

JH compounds are as suitable for developing countries as are conventional chemical larvicides. The equipment and manpower needs are similar.

What are its advantages compared with other control methods?

JH chemicals readily penetrate the insect integument and are biologically active at concentrations well below those of conventional insecticides. Under laboratory conditions, some JH chemicals disrupt development and cause death when topically applied at doses as low as a billionth of a gram (nano-

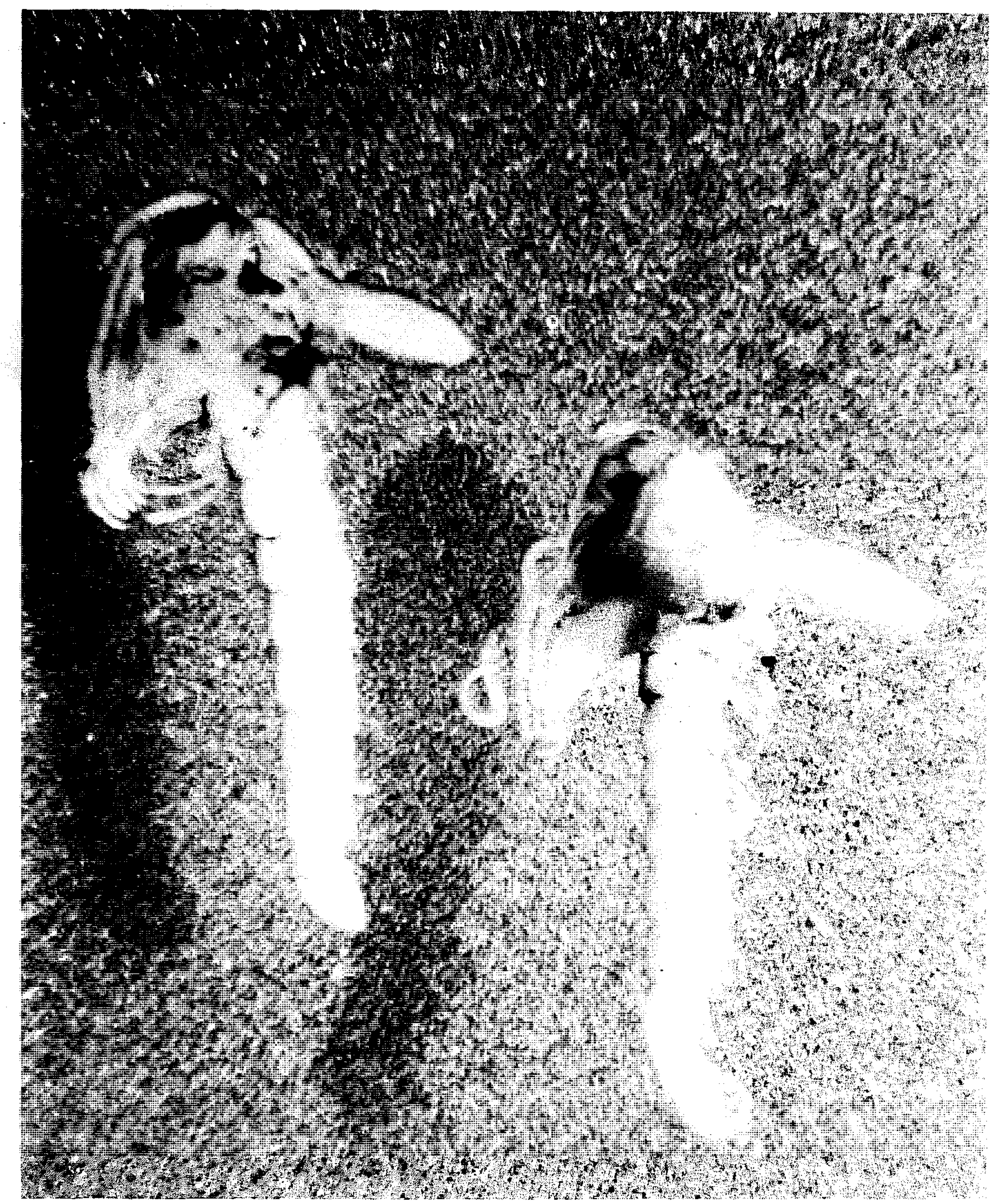

FIGURE 7 Anomalous *Anopheles albimanus* pupae obtained from exposure of larvae to a juvenile hormone compound. (W. L. Jakob and H. F. Schoof)

gram), or when present in the diet or habitat at concentrations of less than 1 part per billion.

The advantage of JH compounds is that they are generally more specific for mosquitoes than are conventional insecticides. They can be applied with standard ground or aircraft equipment. Workers with elementary training can be employed.

These compounds are biodegradable and, so far, have shown little effect on nontarget organisms. Unpublished observations indicate that JH may inhibit development of malarial oocysts in mosquitoes.

Control by JH can probably be used in conjunction with most other control methods.

What are its limitations compared with other control methods?

A primary limitation has been mentioned: application of JH must be timed to coincide with the sensitive stage of the larva; however, slow-release formulations have been developed to greatly reduce this problem. A second limitation is that resistance might develop; another, that the effects of JH compounds on mosquito larvae are not easily assessed.

From most of these compounds, there are no readily observable effects on the larvae. The larvae develop normally into the pupal stage, but the adults fail to emerge. Because most people are now trained to look for either dead larvae or dead adults, assessment methods must be changed, and new techniques for assessing effects will require some retraining of supervisory personnel.

What is the stage of development of this control agent?

JH compounds effective against mosquitoes have been developed. Because many of the more active ones are relatively simple molecules, their commercial production appears to be economically feasible.

In California during 1972, one JH compound applied by aircraft at only 1/20th of a pound per acre gave complete control of field populations of *Aedes nigromaculis.* The same compound has shown a high degree of control when used in the field against other important species of mosquitoes, including *Aedes aegypti* and, under tropical conditions in Panama, *Anopheles albimanus*. Study of nontarget organisms present in the California fields revealed no deleterious effects, except that some aquatic diptera (shore flies and midges) were adversely affected. More recent studies on this JH have shown it to be essentially nontoxic to a wide variety of nontarget organisms (arthropods, fish, birds, etc.).

Numerous other compounds are also being evaluated, and commercially available JH mosquito larvicides can be expected within the next few years. One commercial company has already submitted a petition to the U.S. Environmental Protection Agency for registration of a JH to control a flood-water species of mosquito.

What are the areas in which further research and development could have particular impact?

More extensive field trials are needed with JH compounds to determine their effectiveness and limitations.

More extensive studies are needed to measure the impact of each compound on nontarget species such as fresh-water crustaceans.

Better formulations are needed to extend the life of the active agents in water.

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CHAPTER X

Larvicidal Plants

Earlier in this century several "naturalistic" approaches to mosquito control (e.g., Matheson, 1930) were studied. Worth reconsidering today, they were aimed at learning what makes mosquitoes avoid one waterway and select another in which to breed. This work led to the observation that plants and plant products may play important roles in mosquito development. For example, certain aquatic plants may kill mosquitoes by producing larvidical toxins. A notable group of such plants are the stoneworts (Characeae).

What is the method for using this control agent?

From field and laboratory observations, it appears that appropriate stoneworts in vigorous growths can suppress production of certain mosquitoes by exuding chemicals toxic to larvae into the water.

Highly specialized algae of worldwide distribution, stoneworts occur commonly in quiet waters (both fresh and brackish) with muddy or sandy bottoms in which their anchoring, root-like structures can find a foothold. They range in height from less than 2 cm to more than 2 m. The two main genera are *Chara* and *Nitella*.

How is it suitable for developing countries?

Stoneworts are easily cultivated and transplanted. Some *Chara* beds have been known to exist for 50 years. Production in suitable aquatic nursery beds would require no special equipment or technology. The costs for using this method would be low.

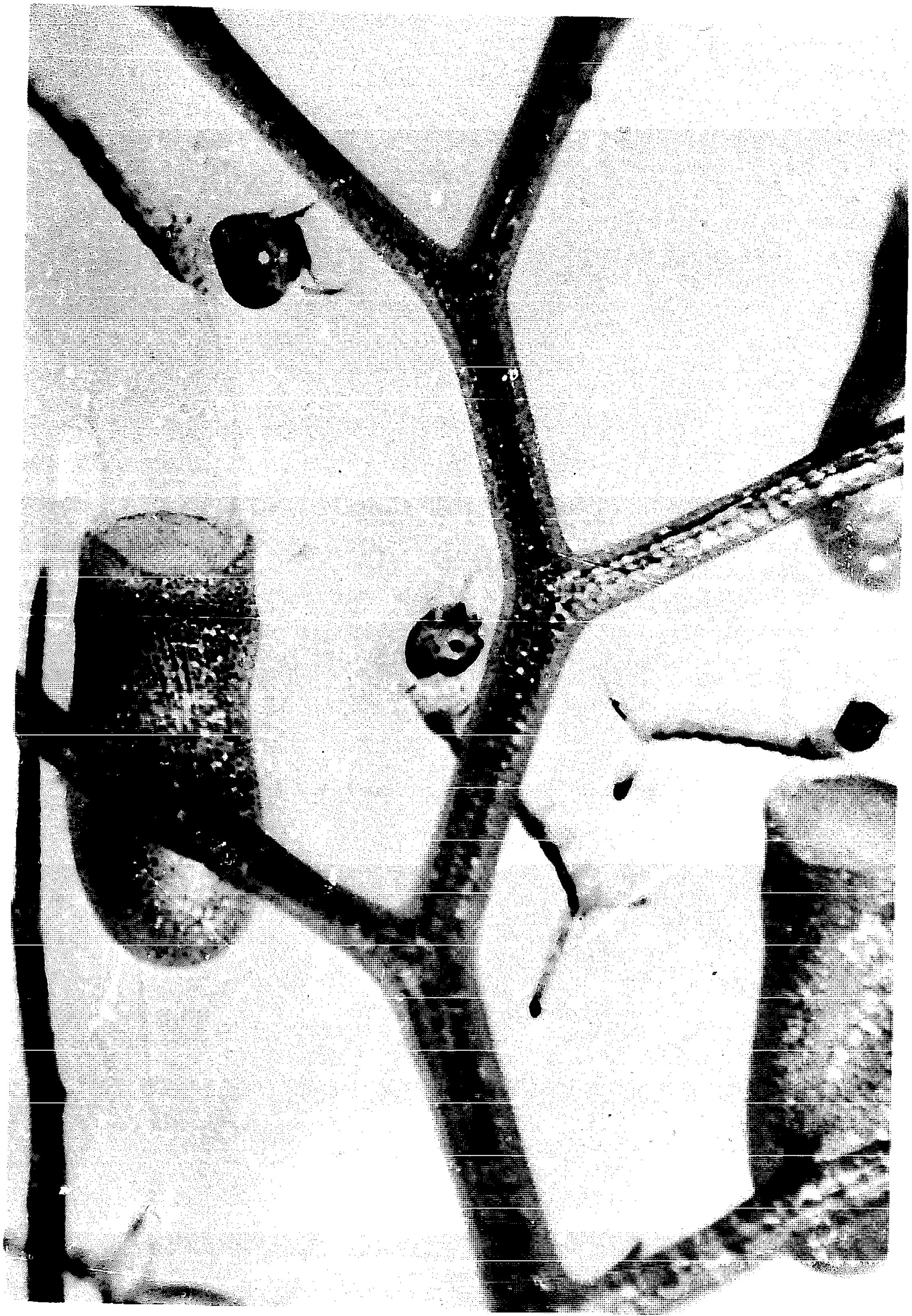

FIGURE 8 *Utricularia* bladders capturing mosquito larvae. (Ernest C. Bay)

What are its advantages compared with other control methods?

Once *Chara* has been introduced and established in an aquatic habitat, a measure of mosquito control might be permanent without further attention or added costs.

What are its limitations compared with other control methods?

Chara will not grow in certain types of larval habitats, particularly highly polluted ones. Various other environmental factors influence the survival and effectiveness of *Chara:* length of day (photoperiod), nutrition, and temperature.

The environmental impact of *Chara* must be thoroughly measured before it is introduced into any new region, because there is always a danger that it will become a pest. Rampant growth of aquatic vegetation is already a major problem in developing countries; although *Chara* species do not threaten as much as water hyacinth, for example, this aspect must be considered. An outline of the problems that could be caused by *Chara* has been published (Yeo, 1972).

What is the stage of development of this control agent?

No recent attempts have been made to establish *Chara* for mosquito control, but many published reports from 50 years ago are summarized by Jenkins (1964).

What are the areas in which further research and development could have particular impact?

Studies are needed to determine the ecological requirements of *Chara* species to grow and be effective larvicidal agents.

An evaluation is needed of any hazards to aquatic ecosystems that might result from introducing stoneworts.

Research to extract and identify the active ingredients from *Chara* and to test this extract as a larvicide could be useful.

Interesting Footnotes

1. A highly speculative but interesting use of plants to reduce mosquito population is the use of bladderwort *Utricularia vulgaris*. Certain carnivorous plants, such as this, capture and ingest small organisms living in aquatic habitats (see Figure 8). Bladders on parts of a single plant 220 cm (nearly 7 feet)

long have been found to contain some 150,000 organisms, including mosquito larvae.

FIGURE 9 Full-grown *Culex peus* larvae attached to mucilage surrounding a *Lepidium flavum* seed 1.5 mm in diameter. When larvae investigate a mucilaginous seed, they may become attached by the oral brushes around their mouthparts. (Eldon L. Reeves)

Some 90 *Utricularia* species are found growing in many bodies of water throughout the world, but studies are needed to learn under what conditions they are effective against mosquitoes and to measure the potential dangers to aquatic ecosystems of introducing this vegetation. Careful analyses will have to be made to determine the costs, benefits, and risks to man and his environment. However, this technique might eventually result in a mosquito-control method requiring minimum maintenance and cost.

2. When the seeds of a few plant species, especially of the Cruciferae (mustard) family, are placed in water, a mucilage exudes from the seedcoat. If mosquito larvae touch these mucilaginous seeds, their mouthparts are entrapped (Figure 9). If held long enough, the larvae drown or starve.

Although the use of seed traps for mosquito control has limited potential, it might have value for mosquito-larvae control in small drinking- or washing-water containers; household pitchers or jars; flower vases; and household ant traps containing water. It is not suitable for widespread mosquito control.

Seeds heated to 121°C for 20 minutes can still capture larvae, but they will no longer germinate and hence will impose no threat of a weed's becoming established.

As many as 27 second instar *Culex* larvae have been observed attached to a single seed. If 5 larvae per seed were captured, 55 million larvae per kilogram would be destroyed if the small seeds of *Capsella bursa-pastoris* were used. The potential capture power of a single kilogram of *Lepidium flavum* seeds may be as high as 118 million larvae.

This method has been tested only under laboratory conditions. Research is needed to find the most effective seeds, to test them against various mosquito species, and to test their inactivation by sediments. Dosage and the effect on water potability must also be determined. If the mucilaginous exudates can be identified and synthesized (or mimicked), then artificial "seeds" that trap mosquitoes more efficiently, with no effect on the water quality, could be produced.

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ESPAÑOL

Introducción y resumen

Las enfermedades transmitidas por insectos afectan por igual a los países desarrollados y a los en vías de desarrollo. En muchas partes del mundo los antiguos azotes de la humanidad—malaria, filariasis, fiebre amarilla y dengue—continúan obstaculizando el progreso.

Si bien en algunos países, los programas de erradicación han tenido efectos dramáticos, en otros no son viables en un futuro cercano debido principalmente a problemas técnicos y a la resistencia a los insecticidas químicos. El ≪control≫ constituye una meta más realista que la erradicación, aunque antes de poder aspirar a lograr un mayor progreso es necesario sumar nuevas técnicas a nuestro arsenal de controles.

En cuanto a número de víctimas, la malaria es posiblemente la enfermedad de más frecuente ocurrencia en el género humano. Cerca de 400 millones de gente vive en áreas donde la malaria es todavía endémica en alto grado; se estima que por lo menos 100 millones de casos ocurren anualmente con un resultado de 1 millón de muertos.*

La filariasis, aunque algo más limitada en cuanto a su distribución que la malaria, es una enfermedad común en regiones tropicales y afecta a más de 250 millones de personas. En años recientes esta enfermedad ha aumentado en forma alarmante a causa del esparcimiento urbano que ha traído consigo un aumento de zonas de cría de vectores de mosquitos—esto es, de mosquitos que transmiten los organismos (patógenos) que provocan la enfermedad.

Pese a que se dispone de una vacuna eficaz, la fiebre amarilla es una de las enfermedades más virulentes del hombre. Esta enfermedad viral está circunscrita a Centro y Sudamérica y a algunas partes de Africa. Su forma urbana es transmitida por un mosquito ≪casero≫ *Aedes aegypti.* Una dificultad que enfrentan los intentos de control que se emprenden es que los huéspedes

*L. J. Bruce-Chwatt, 1971, Bol. OMS, 44:419.

alternativos (tales como los monos) albergan la enfermedad, y de ellos es transmitida al hombre por mosquitos de los bosques.

El dengue, o fiebre quebrantahuesos, se presenta en todas las regiones tropicales y subtropicales del mundo, con frecuencia en extensas epidemias. Al igual que la fiebre amarilla, es producido por un virus cuyo principal agente vector es también el mosquito *Aedes aegypti.* Se carece de vacunas; existen por lo menos cuatro clases de dengues y la inmunidad natural a una no protege contra la infección por otra. Por lo general, el dengue es una enfermedad que postra pero no mata. En los últimos 12 años, empero, se ha descubierto una nueva forma de ella, fiebre hemorrágica, cuya mortalidad asciende hasta un 20% de los niños atacados en el sudeste de Asia.

A más de los agentes patógenos del dengue y de la fiebre amarilla, se conocen más de 80 virus distintos que son transmitidos al hombre por mosquitos. De éstos, 39 provocan enfermedades frecuentes o severas en el hombre. Cabe citar entre las más importantes la fiebre o'nyong-nyong en Africa, la fiebre chikungunya en Asia y Africa, la encefalitis venezolana y la de San Luis en las Américas, y la encefalitis japonesa en el sur y este de Asia.

El control de mosquitos atraviesa en estos momentos una situación de crisis. En los últimos 30 años, el hombre ha dependido casi por completo de insecticidas sintéticos orgánicos. Hoy día, las mismas propiedades que confirieron utilidad a estos productos químicos, han ocasionado graves trastornos ambientales debido a su larga acción residual y a sus efectos tóxicos en una gran diversidad de organismos. Asimismo, muchas campañas de control de vectores han fracasado por la resistencia desarrollada a los pesticidas químicos. Por ejemplo, en algunos lugares de California, los mosquitos transmisores de la encefalitis son virtualmente resistentes a todos los larvicidas.*

Las armas químicas eficaces van desapareciendo y escasean los reemplazos químicos adecuados. La técnica recientemente desarrollada de sustituir pesticidas a medida que van perdiendo su eficacia rara vez ofrece resultados duraderos. No hay casi ya sustitutos porque, en contraste con la pasada época, ha disminuido mucho el desarrollo a escala industrial de nuevos productos pesticidas.

En los últimos años se ha mencionado el control biológico como la alternativa más importante al uso de pesticidas. Pese a esto, el control biológico de vectores no pasa de ser un medio de control de enfermedades que ha suscitado gran publicidad pero poco apoyo. Algunos periodistas han descrito el control biológico como una técnica relativamente simple, cuando en realidad es compleja y desafiante.

Antes de que se haga cualquier intento de emplear controles químicos o biológicos, debe elucidarse por completo la ecología del mosquito vector (es decir, sus relaciones con el medio ambiente) y los mecanismos de transmisión de las enfermedades. Se debe poner énfasis en el estudio de los factores am-

*Insecticidas empleados para matar las larvas.

bientales que limitan la reproducción de mosquitos en una dada localidad y en los factores que tornan el habitat* favorable a él. A menudo, un esfuerzo conjunto orientado a reducir el número de lugares de cría del mosquito (programas de ≪reducción de lugares de origen≫) puede constituir una vía inmediata de control de las enfermedades. En la década de los 30 cuando aún no se había empleado el DDT, se logró erradicar de Brasil al mosquito *Aedes aegypti* mediante el empleo de cuantiosos recursos humanos en un programa de eliminación y restricción legal estricta de recipientes caseros de agua. En Panamá, la reducción de los lugares de cría, también mediante el uso masivo de trabajadores que desaguan los charcos que se forman durante la estación de lluvias, ha disminuido considerablemente las poblaciones del mosquito vector de la malaria *Anopheles albimanus.* Por muchos años este programa ha tenido tal éxito que sólo ha sido necesario el uso de pesticidas químicos en pequeña escala, y el DDT todavía es eficaz.

El panel sostiene con firmeza que el primer paso que se emprenda en cualquier proyecto de control de mosquitos y de enfermedades debe consistir en una investigación ecológica destinada a determinar si un cambio menor en el ambiente, o en su defecto un cambio menor asociado a la agricultura o a los modos de vida del hombre puede eliminar la fuente de mosquitos y/o la enfermedad.

Si bien el influir artificialmente en el medio ambiente con miras al control de mosquitos presenta ciertas características atractivas, puede muy bien traer como resultado nuevos problemas. En efecto, el desagüe y relleno de zonas pantanosas con el fin de control de mosquitos puede eliminar el habitat de peces y de otras especies silvestres—este ejemplo ilustra las ventajas de evaluar el costo, beneficios y riesgos implícitos en todo intento de alterar el medio ambiente antes de empezar cualquier programa de control.

En muchos casos el implantar un programa de ≪reducción de lugares de origen≫ no elimina ni los mosquitos ni la enfermedad, y por tanto, es necesario ensayar varias combinaciones de métodos químicos y biológicos como una medida suplementaria de control. En cualquier caso, sin embargo, la información que se recoja respecto a los métodos de reducción de los lugares de origen de mosquitos constituye un prerrequisito a la formulación de un programa de control adecuado.

Puesto que el ambiente y las poblaciones de mosquitos están sujetos a cambios frecuentes, el éxito de todo programa de control—basado ya sea en la reducción de lugares de origen o en controles químicos o biológicos—dependerá de un régimen continuo de vigilancia y de una acción coordinada de seguimiento. Así, por ejemplo, una única liberación de enemigos naturales del mosquito muy pocas veces se traduce en una baja persistente de la población. La vigilancia debe ser siempre una compañera necesaria del control.

Para que tenga éxito, todo programa actual ≪antimosquito≫ debe recurrir a la reducción de los lugares de origen y a la combinación de métodos bio-

*El lugar donde vive y crece en forma natural una planta o especie animal.

lógicos y químicos (control integrado); en tanto que, en la opinión del panel, los programas futuros de control deben emplear un enfoque de sistemas (tecnología del control de plagas). Un enfoque de sistemas consistería en:

1. Reducción de los lugares de origen;
2. Control integrado; y
3. Análisis cuantitativo de los costos y beneficios para el hombre y su ambiente derivados del programa.

Por cierto que estos programas de control de índole tan compleja exigen la participación de biólogos profesionales con experiencia en ecología, entomología, parasitología y genética. Además, el equipo de científicos debe contar con especialistas en economía y técnicas de administración de sistemas y, en algunos casos, en agronomía, horticultura, ingeniería agronómica y en ciencias del suelo.

Para planear cualquier programa de dirección de sistemas de control, es necesario disponer antes de información sobre la ecología del mosquito, la epidemiología de la enfermedad y la gradación de las manipulaciones del medio ambiente y/o técnicas alternativas de control que se apliquen en forma combinada en un medio ambiente particular.

Este informe describe algunas técnicas alternativas de control y evalúa su potencial y limitaciones en el control de mosquitos. En resumen, lo que sigue constituye una breve enumeración de dichas técnicas y del modo cómo operan.

PECES LARVÍVOROS

En charcos, estanques, pantanos, etc., peces de tamaño pequeño, que se alimentan de los huevos, larvas y pupas de mosquito, han mantenido bajo control su cría.

DEPREDADORES INVERTEBRADOS

Destacan entre éstos un grupo de mosquitos de tamaño grande y que no pican (*Toxorhynchites*), cuyas larvas depredadoras devoran las larvas de otras especies de mosquitos vectores de enfermedades. Este tipo de larva depredadora puede resultar muy eficaz para reducir la reproducción de mosquitos dañinos en los agujeros de árboles y en otros recipientes como neumáticos, latas, envases caseros para agua y floreros.

CONTROL GENÉTICO

Como control de sus propias especies, se pueden utilizar mosquitos cuyo material genético ha sido modificado. Por ejemplo, cuando cepas de mosquitos con translocaciones cromosómicas copulan con individuos normales, la reproducción resulta parcialmente inhibida. Igualmente, la puesta en libertad de machos estériles que copulan con hembras normales, se traduce en una reducción de las poblaciones de las generaciones subsiguientes.

NEMATODOS PARÁSITOS

Hay varias especies de nematodos parásitos (gusanos cilíndricos), principalmente mermítidos, que atacan ciertas especies de mosquitos en el estado larvario. Estos diminutos gusanos se prestan a ser reproducidos en masa, y en la actualidad se realizan ensayos de campo para determinar su potencial en el control de mosquitos.

PROTOZOOS PARÁSITOS

En la naturaleza los microsporidios, un grupo de protozoarios que forman esporas, son patógenos comunes de los mosquitos; si se pudiera aumentar su número o mejorar su eficacia, podrían contribuir aún más al control de mosquitos.

HONGOS PARÁSITOS

Muchos hongos, en especial los del genero *Coelomomyces*, destruyen una amplia variedad de larvas de mosquito en una gran diversidad de ambientes acuáticos.

BACTERIAS PATÓGENAS

El *Bacillus thuringiensis* (cepa BA-068), que es una bacteria que forma esporas, es letal en alto grado a ciertas larvas de mosquito. Se presta a ser reproducido en masa e incorporado a habitats acuáticos.

SEUDOHORMONAS JUVENILES (REGULADORES DEL CRECIMIENTO DE INSECTOS)

Las seudohormonas juveniles de insectos obstaculizan y provocan desarreglos en el desarrollo normal de las etapas immaduras del mosquito. La aplicación de dosis bajas de estos compuestos a habitats acuáticos puede impedir que el mosquito complete su ciclo vital.

PLANTAS LARVICIDAS

Bajo ciertas condiciones ambientales, varias plantas larvicidas, incluyendo las algas fijas de la familia Characeae, exudan una toxina en al agua que impide la cría de mosquitos. Asimismo, ciertas semillas que exudan mucilago pueden ahogar a las larvas que se adhieren a ellas.

Al final del capítulo I, se puede encontrar un examen de otras 4 alternativas y algunas referencias generales sobre el control biológico de mosquitos.

FRANÇAIS

Introduction et résumé

Les maladies transmises par les moustiques frappent les pays développés comme les pays en voie de développement. Dans une grande partie du monde, les anciens fléaux de l'humanité—paludisme, filariose, fiévre jaune et dengue—continuent de limiter le progrès.

Dans certains pays, les campagnes d'éradication de ces maladies ont donné des résultats spectaculaires, mais il est des régions où l'éradication ne sera pas possible dans l'avenir prévisible, essentiellement à cause de problèmes d'ordre technique et de la résistance des moustiques aux insecticides chimiques. Il est plus réaliste de chercher à enrayer ces maladies qu'à les supprimer mais, si l'on veut progresser dans ce domaine, il faut ajouter de nouvelles techniques aux moyens dont on dispose pour en contrôler la propagation.

Par le nombre des gens qui en sont atteints, le paludisme est peut-être la maladie des humains la plus répandue. La population des régions où le paludisme est encore endémique est d'environ 400 millions; on estime qu'au moins 100 millions d'entre eux sont atteints chaque année, et qu'un million en meurent.*

Bien qu'elle sévisse dans des régions un peu moins étendues que le paludisme, la filariose est une maladie répandue dans les régions tropicales et qui atteint plus de 250 millions de personnes. Au cours des dernières années elle s'est propagée de façon alarmante, l'urbanisation croissante ayant eu pour conséquence d'accroître les zones de reproduction des moustiques-porteurs—c'est-à-dire ceux qui transmettant les organismes pathogènes.

La fièvre jaune est l'une des maladies les plus virulentes qui frappent les humains, bien qu'il existe un vaccin efficace contre elle. Cette maladie virale sévit seulement an Amérique centrale, en Amérique du Sud et dans quelques régions d'Afrique. Sa forme urbaine est transmise par la moustique ≪domestique≫ *Aedes aegypti.* La lutte contre cette maladie est rendue plus difficile

*L. J. Bruce-Chwatt, 1971, Bulletin de l'OMS. 44:419.

par le fait que des hôtes successifs la portent (par exemple les singes), et la transmettent à l'homme par l'intermédiaire des moustiques de la forêt tropicale.

La dengue sévit dans les régions tropicales et subtropicales du monde entier, souvent en vagues épidémiques. Comme la fièvre jaune, elle est causée par un virus; c'est aussi l'*Aedes aegypti* qui en est le principal porteur. Il n'y a pas de vaccin contre cette maladie; il existe au moins quatre espèces de dengue, et l'immunité à l'une d'entre elles ne protège pas contre les autres. En général, les personnes qui en sont atteintes sont dans un état de prostration mais n'en meurent pas. Toutefois, au cours des douze dernières années, une forme de cette fièvre, découverte récemment, la dengue hémorragique, a causé une mortalité allant jusqu'à 20 pour cent parmi les enfants qui en étaient atteints en Asie du Sud.

Outre ceux qui causent la dengue et la fièvre jaune, plus de 80 virus sont transmis à l'homme par les moustiques. Trente-neuf d'entre eux provoquent des maladies fréquentes ou graves chez l'homme. Les plus importantes sont la fièvre o'nyong-nyong en Afrique, la fièvre chikungunya en Afrique et en Asie, l'encéphalite vénézuélienne et de Saint-Louis sur le continent américain, l'encéphalite japonaise en Asie du Sud et de l'Est.

A l'heure actuelle, la lutte contres les moustiques traverse une crise. Depuis trente ans, l'humanité est presqu'entièrement tributaire des insecticides organiques synthétiques. Aujourd'hui, les mêmes propriétés qui rendaient ces produits chimiques si utiles: leur effet résiduel de longue durée et leur toxicité pour un grand nombre d'organismes divers, ont donné lieu à de graves problèmes d'environnement. En outre, la résistance aux insecticides chimiques a provoqué l'échec de nombreuses campagnes dirigées contre les insectes-porteurs. Par exemple, dans certaines régions de Californie, les moustiques qui transmettent l'encéphalite résistent à pratiquement tous les larvicides.*

Les armes chimiques efficaces sont de moins en moins nombreuses, et les produits chimiques de remplacement adéquats sont rares. La méthode essayée récemment, qui consiste à remplacer par un autre un insecticide devenu inefficace, n'est généralement opérante que pendant une durée limitée. Nous avons à peu près épuisé les remplaçants possibles, le développement industriel de nouveaux produits insecticides étant fortement réduit par rapport à ce qu'il était encore récemment.

L'arme susceptible d'être substituée aux pesticides dont on parle le plus depuis quelques années est le contrôle biologique. Toutefois, s'il est souvent mentionné, le contrôle biologique des porteurs reste un instrument de lutte contre les maladies dont l'emploi n'est pas suffisamment encouragé. Certains rapports ont présenté le contrôle biologique comme étant d'une utilisation assez facile, alors qu'il est en réalité complexe et pose des problèmes.

Il convient, avant d'essayer les méthodes chimiques ou biologiques, de

*Insecticides utilisés pour tuer les larves.

comprendre la manière dont se transmet la maladie. Il faut attirer tout particulièrement l'attention sur les facteurs liés à l'environnement qui limitent la reproduction des moustiques dans un lieu donné et sur ceux qui rendent l'habitat* favorable à leur reproduction. Un effort concerté pour limiter les zones de reproduction des moustiques (programmes dits ≪de reduction à la source≫ peut souvent se révéler de moyen le plus immédiat pour enrayer les maladies. Dans les années 1930, avant la découverte du DDT, l'*Aedes aegypti* avait été exterminé au Brésil par l'utilisation à grande échelle de la main d'oeuvre, dans un programme d'entraves juridiques strictes et l'élimination de l'eau des réservoirs familiaux. A Panama, la réduction des zones de reproduction des moustiques, également par l'utilisation massive de la main d'oeuvre pour assécher les flaques d'eau qui se forment pendant la saison des pluies, a permis d'abaisser considérablement le nombre des *Anopheles albimanus*, porteurs du paludisme. Pendant de nombreuses années, ce programme a été si efficace qu'il n'a pas été nécessaire d'utiliser les insecticides de façon intensive, et que le DDT opère encore dans ces régions.

Le Comité recommande vivement que la première étape de tout projet de lutte anti-moustiques et anti-maladies soit une étude écologique visant à établir si une modification mineure de l'environnement, ou une modification mineure associée à la structure de l'agriculture ou au mode de vie des populations ne permettrait pas d'éliminer l'origine des moustiques ou des maladies, ou des deux à la fois.

Bien qu'à beaucoup d'égards il paraisse souhaitable d'agir sur l'environnement pour éliminer les moustiques, cela peut susciter de nouveaux problèmes. Assécher et combler les marécages pour empêcher la multiplication des moustiques peut avoir pour effet d'éliminer des habitats pour le poisson et la faune sauvage—ce qui montre l'intérêt qu'il y a à évaluer les coûts, les avantages et les risques que comporte toute action sur l'environnement, avant de lancer un quelconque programme d'éradication.

Dans certains cas, les programmes de réduction à la source ne permettent pas d'éliminer l'origine des moustiques ou des maladies, aussi faut-il recourir à diverses combinaisons de méthodes biologiques et chimiques en tant que moyen auxiliaire. Néanmoins, quelles que soient les circonstances, les informations rassemblées pour la réduction des moustiques à la source sont indispensables pour formuler les programmes d'éradication appropriés.

L'efficacité de tout programme d'éradication des moustiques—qui combine la réduction à la source, la lutte chimique et biologique—dépend du maintien d'une surveillance et d'une vérification subséquentes continues, l'environnement et les populations de moustiques étant sujects à des modifications fréquentes. Par exemple, une seule offensive d'ennemis naturels parvient rarement à arrêter la propagation des moustiques pendant longtemps. La surveillance est toujours un complément nécessaire de la lutte contre les moustiques.

*Le milieu géographique propice au développement d'une espèce animale.

De nos jours, les programmes anti-moustiques efficaces doivent faire appel à la réduction à la source et à des combinaisons des méthodes biologiques et chimiques (contrôle intégré); le Comité estime que les programmes anti-moustiques futurs devront utiliser une méthode systématique (technologie du contrôle des insectes). Une méthode systématique comprendrait:

1. Réduction à la source;
2. Contrôle intégré; et
3. Analyse quantitative des coûts et des avantages du programme pour l'homme et son environnement.

Ce type de programme complexe nécessite des biologistes professionnels ayant une connaissance pratique de l'entomologie, de la parasitologie et de la génétique. L'équipe scientifique devra, en outre, comprendre des spécialistes des techniques de l'économie et de l'application des systèmes ainsi que, dans certains cas, de l'agronomie, de l'horticulture, des machines agricoles et de la pédologie.

Pour mettre au point un programme systématique de lutte anti-moustiques, il est indispensable de disposer au préalable d'informations sur l'écologie des moustiques, l'épidémiologie des maladies et sur toute la gamme des interventions sur l'environnement et/ou sur les autres techniques de contrôle qui pourraient être combinées dans un environnement particulier.

Le rapport étudie quelques techniques de contrôle possibles et examine leur potentiel et leurs limitations pour la lutte anti-moustiques. En résumé, la liste ci-dessous énumère brièvement ces techniques et leur application.

POISSONS LARVIVORES

Dans les étendues d'eau, mares, marécages, etc., plusieurs espèces de poissons, d'une taille analogue à celle des vairons, ont limité la reproduction des moustiques en dévorant les oeufs, les larves et les nymphes des moustiques.

PRÉDATEURS INVERTEBRÉS

Parmi les principaux, un groupe de grands moustiques qui ne piquent pas (*Toxorhynchites*), dont les larves rapaces dévorent les larves d'autres espèces de moustiques qui sont porteurs de maladies. Les larves de moustiques rapaces peuvent être particulièrement efficaces pour réduire la reproduction des moustiques-porteurs dans les creux d'arbres et autres abris tels que pneus, boites de conserve, récipients contenant de l'eau et vases de fleurs.

CONTRÔLE GÉNÉTIQUE

Les moustiques dont les caractères génétiques ont été modifiés peuvent être utilisés pour le contrôle de leur propre espèce. Par exemple, lorsque les espéces de moustiques dont les chromosomes se déplacent, s'accouplent avec des moustiques normaux, la reproduction est partiellement suspendue. De même, des males stériles peuvent être lâchés pour s'accoupler avec les femelles normales, ce qui réduit le nombre des générations suivantes.

NÉMATODES PARASITES

Plusieurs espèces de nématodes parasites, et notamment les mermithides, attequent certains moustiques au stade larvaire. Ces minuscules parasites peuvent être élevés en masse, et des essais sont actuellement faits sur place en vue de déterminer leur potentiel pour la lutte anti-moustiques.

PROTOZOAIRES PARASITES

A l'état naturel, les microsporidies, groupe de protozoaires qui produisent des spores, sont des pathogènes communs des moustiques; si l'on peut accroître leur nombre ou les rendre plus virulents, ils peuvent contribuer encore davantage à la lutte anti-moustiques.

FONGUS PARASITES

De nombreux fongus, et particulièrement les membres du genre *Coelomomyces* détruisent une grande variété de larves de moustiques, dans des environnements aquatiques très divers.

BACTÉRIES PATHOGÈNES

Une bactérie qui produit des spores, le *Bacillus thuringiensis* (espèce BA-068), est très meurtrière pour certaines larves de moustiques. On peut en pratiquer l'élevage en masse et l'introduire dans les habitats aquatiques.

IMITATIONS D'HORMONE JUVÉNILE (RÉGULATEUR DE LA CROISSANCE DES INSECTES)

Les imitations d'hormone juvenile des insectes entravent et perturbent le développement normal des moustiques non adultes. L'introduction, en petites quantités, de ces composés dans les habitats aquatiques peut empêcher le moustique d'achever son cycle d'évolution.

PLANTES LARVICIDES

Diverses plantes larvicides, y compris l'algue enracinée, de la famille des Characeae, peut dans certaines conditions d'environnement empêcher la reproduction des moustiques en exsudant une toxine dans l'eau. Par ailleurs, certaines graines qui exsudent du mucilage peuvent noyer les larves qui se collent à elles.

On trouvera, à la fin du Chapitre I, la récapitulation de 4 autres instruments de lutte anti-moustiques, ainsi que quelques indications sur le contrôle biologique des moustiques.

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