THE FUTURE OF MICROBIAL INSECTICIDES AS VECTOR CONTROL AGENTS

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ABSTRACT. Insect vectors of human diseases are subject to diseases of their own caused by viruses, bacteria, fungi, protozoans, and nematodes. Over the past 30 years, many members of these groups have been evaluated as vector control agents, particularly for mosquito control. Most pathogens and nematodes occur primarily in larvae, and are only effective against this stage. The principal candidate control agents studied include iridescent and nuclear polyhedrosis viruses, the bacteria Bacillus thuringiensis and Bacillus sphaericus; the fungi Lagenidium giganteum, Culicinomyces clavosporus, and species of the genus Coelomomyces, the protozoan Nosema algerae, and the mermithid nematode Romanomermis culicivorax. Of these, the only one considered an operational success is the bacterium, Bacillus thuringiensis subsp. israelensis (B.t.i.), which has proven useful for control of both mosquito and blackfly larvae in programs where larviciding has been traditionally employed as a vector control tactic. The reasons for the success of B.t.i. are its cost-effectiveness and relative ease of use, which are due, respectively, to the ability of B.t.i. to be grown on artificial media and the development of formulations that can be applied using conventional insecticide application technology. Because few microbial insecticides are cost-effective, and those that are are only effective against larvae, these agents will likely play only a minor, but in some cases important, role in most future vector control programs.

INTRODUCTION

The use of synthetic chemical insecticides for vector control is in decline due to high costs, the development of resistance in many target populations, and perceived risks to the environment and human health. Although chemical insecticides will remain important in vector control programs, problems they have caused, and the paucity of new types under development, have for some time stimulated interest in alternative control agents. In seeking replacements for chemicals, the pathogens and nematodes that cause diseases fatal to vectors have received considerable study over the past 30 years, particularly with respect to their potential for controlling the mosquito vectors of malaria and filariasis, and the blackfly vectors of onchocerciasis (Fed erici 1981, Chapman 1985, Lacey and Undeen 1986, Guillet et al. 1990, Mulla 1990). These studies have included the search for pathogens and nematodes, the laboratory and field evaluation of those that appeared to have the best potential for operational use, the development and assessment of methods for mass production, and finally implementation in operational control programs followed by evaluation of cost-effectiveness based on control of the target pests or vectors. Overall, these studies have resulted in 3 principal findings: 1) pathogens and nematodes are only effective against the larval stages of vectors, 2) effective control typically requires repeated rather than a single application of the agent during the breeding season, and 3) to be used cost-effectively in vector control programs, a method for mass production of the control agent in vitro must exist. The first finding is simply a biological property of most of the pathogens and nematodes discovered to date; they may occur in adult stages, but seem to take their greatest toll against larvae. The second results from natural diminution, or what might be considered poor recycling potential from the standpoint of vector control. The third results from the relatively large areas that must be treated, and derives from the high costs of mass rearing control agents in organisms, such as mosquito or blackfly larvae, in comparison to the lower costs of mass production for a control agent grown on an inexpensive artificial medium. The latter is only possible at present for some of the bacteria and fungi. As a result of the combination of these findings, only the bacterium, Bacillus thuringiensis subsp. israelensis (B.t.i.), has been used

1 This paper was prepared for oral presentation as part of a symposium, and thus my treatment of the subject has been necessarily brief, particularly with respect to the data that serve as the basis for my opinions. For those readers interested in a more detailed treatment of each of the groups I dealt with, and references to numerous original papers, I recommend the following reviews. For viruses, Federici (1974); for bacteria, Guillet et al. (1990) and Mulla (1990); for fungi, Federici (1981), Hall and Papierok (1982), and McCoy et al. (1988); for protozoa, Henry (1981) and Brooks (1988); and for nematodes, Petersen (1982). The review on microbial control of mosquitoes and blackflies by Lacey and Undeen (1986) is also very worthwhile, as is the book on bacterial control of mosquitoes and blackflies edited by de Barjac and Sutherland (1990).
in operational vector control programs, and only in programs where larviciding has been a traditional method of vector control. Progress in developing microbial for vector control has been slow, and this situation is unlikely to improve soon. Thus, it can be concluded that bacteria are likely to be the only microbial agents used for some time to come in vector control programs, and these will only be used in programs where larviciding has been a successful vector control strategy.

Predictions about the future use of microbial insecticides and nematodes in vector control programs are best arrived at by an examination of what we have learned over the past 30 years from the study of these potential control agents. Thus, in this paper I will review the key findings for each of the major pathogen groups and nematodes that led to our current view of their probable use in the future.

**VIRUSES**

All viruses are obligate intracellular parasites, and as such must be grown in living hosts. With insect viruses, this means either in insects (i.e., in vivo) or in cultured insect cells (in vitro) because no methods exist for growing viruses on "artificial" media.

Despite these difficulties, searches were conducted for viruses pathogenic to mosquitoes and blackflies because at the time this research was initiated in the 1960s, it was thought that viruses might work as classical biological control agents (i.e., agents that would lead to long-term control once introduced against previously unexposed populations), or that it might be possible to develop suitable methods for the mass production of viruses using colonized insects. The search for viruses, carried out primarily by members of the USDA’s mosquito research laboratories at Lake Charles, LA, and Gainesville, FL, resulted in the discovery of numerous viral pathogens of larvae (Federici 1974). These included several iridoviruses of floodwater mosquitoes belonging to the genera *Aedes* and *Psorophora*, cytoplasmic polyhedrosis viruses (CPVs), and baculoviruses of the nuclear polyhedrosis virus (NPVs) type, the latter 2 types being isolated primarily from *Aedes* and *Culex* species. Iridoviruses and CPVs have also been reported from larvae of several blackfly species.

Studies of the iridoviruses, or iridescent viruses as they are more commonly known, showed that they infected and replicated in most larval tissues with the exception of the midgut epithelium (Anthony and Comps 1991). However, in laboratory trials these viruses were shown to be only moderately infectious for larvae, with mortality rates typically being less than 25% for 1st and 2nd instars, even when larvae were placed in water containing virions at concentrations of several µg/ml. The NPVs and CPVs were shown to only infect the midgut epithelium, where the NPVs replicated in the nuclei throughout the gut, and the CPVs in the cytoplasm of infected cells in the gastric ceca and posterior stomach. As with the iridescent viruses, the mortality rates obtained with NPVs rarely exceeded 25%, and although higher rates of infection were achieved with CPVs, even larvae heavily infected with this virus type could often survive the infection, pupate, and emerge as adults. Owing to their poor efficacy and lack of suitable methods for mass production, serious research on the development and evaluation of viruses as mosquito or blackfly control agents was discontinued in the 1970s.

**BACTERIA**

Bacteria are relatively simple unicellular microorganisms that lack internal organelles such as a nucleus and mitochondria, and reproduce by binary fission. With few exceptions, most of those discovered in vectors grow readily on a wide variety of inexpensive substrates or artificial media, a characteristic that greatly facilitates their mass production. The bacteria currently used or under development as microbial control agents for vectors are spore-forming members of the bacterial family Bacillaceae. Two species have received considerable study, *Bacillus thuringiensis* subsp. *israelensis* (*B.t.i.*), originally discovered in Israel by Goldberg and Margalit (1977) and the only species currently used operationally, and *Bacillus sphaericus*, which has good larvicidal activity against certain mosquito larvae, but whose operational utility has not been clearly demonstrated.

Both *B.t.i.* and *B. sphaericus* kill larvae through the action of insecticidal proteins that destroy the larva’s midgut epithelium shortly after ingestion, usually within a few hours. These insecticidal proteins are contained within a parasporal body produced when the bacterium sporulates (Fig. 1). In *B.t.i.*, the parasporal body is spherical, and contains 4 proteins with molecular masses of 27, 72, 128, and 134 kDa (Federici et al. 1990). These proteins interact synergistically to yield toxicity comparable to that of chemical insecticides such as DDT. *Bacillus thuringiensis israelensis* has been shown to be toxic to most dipterans of the suborder Nematocera, including mosquitoes (Culicidae), blackflies (Simuliidae), crane flies (Tipulidae), and midges (Chironomidae). One of the advantageous properties of *B.t.i.* is that it is highly insecticidal for many vector species, including species of *Aedes*, *Culex*,...
**Fig. 1.** Electron micrographs of the insecticidal parasporal body produced by *Bacillus thuringiensis* subsp. *israelensis*. a. Sporulating cell with developing parasporal body at the right. Sp, spore; E, exosporium membrane; PB, parasporal body. b. Completely formed parasporal body within a cell just prior to cell lysis. The asterisk indicates the inclusion containing the 65-kDa insecticidal protein. c. Parasporal body liberated from cell by lysis. The arrowheads point to a fibrous envelope that holds together inclusions that contain the different insecticidal proteins characteristic of B.t.i. All micrographs are approximately the same magnification, and the bar in (a) is equal to 250 nm.

**Anopheles**, and **Simulium**. The proteins in the parasporal body can also destroy the midgut epithelium of adults, although B.t.i. preparations produced commercially are not active against adult flies because the proteins cannot penetrate the cuticle, and methods are not available to induce adult flies to ingest formulations under field conditions. Thus, B.t.i. is only useful at present as a larvicide.

As noted above, B.t.i. is highly insecticidal for many species of mosquito and blackfly larvae, and can be easily grown on artificial media. Moreover, it has proven relatively easy to formulate and has acceptable storage properties. These features are responsible for its operational and commercial success, and are compared with the properties of selected other candidate pathogen and nematode vector control agents in Tables 1 and 2.

For production, B.t.i. is grown by fermentation in batches as large as 50,000 liters, and then formulated as a flowable concentrate, wettable powder, or as granules, though slow-release briquet formulations are also commercially available. In operational vector control programs, such as the World Health Organization’s Onchocerciasis Program in West Africa, flowable concentrates are dropped into rivers during periods when discharge volumes are low (Guillet et al. 1990). When used against *Aedes*, *Culex*, or *Anopheles* species, the preparations used are tailored to the specific habitat being treated, but typically involve the suspension of the particulates near or on the water surface (Mulla 1990). Rates of application are in the range of 200–400 g of technical material per hectare, and vary with the type of formulation, manufacturer, target species, and habitat being treated. The principal products based on
Table 1. Comparison of the efficiency of production relative to application rates for representative bacterial and fungal pathogens evaluated as microbial insecticides for control of mosquito larvae.

<table>
<thead>
<tr>
<th>Agent</th>
<th>LC_50/hectare</th>
<th>Production yield</th>
<th>Medium/hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em> subsp. <em>israelensis</em></td>
<td>200 g technical powder</td>
<td>10 g/liter</td>
<td>20 liters</td>
</tr>
<tr>
<td><em>Bacillus sphaericus</em> 2362</td>
<td>100 g technical powder</td>
<td>10 g/liter</td>
<td>10 liters</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Culicinomyces clavosporus</em></td>
<td>10^{11} conidia</td>
<td>10^{11} conidia/liter</td>
<td>1,000 liters</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em></td>
<td>2 kg conidia</td>
<td>1 kg/25 kg rice</td>
<td>50 kg of rice</td>
</tr>
</tbody>
</table>

1 Refer to the amount of liquid or solid substrate needed to produce the amount of technical material required to achieve population reductions of 90% for 1 ha.
2 Technical powder refers to the unformulated dried solids obtained after fermentation. Of this weight, only approximately 25% consists of insecticidal proteins in *B. thuringiensis*, and 5-10% in *B. sphaericus*; the rest is fermentation solids, bacterial spores, and remnants of bacterial cells.

*B.t.i.* used in vector control programs are Acrobe (American Cyanamid), Skeetal (Novo Nordisk), Teknar (Sandoz), and Vectobac (Abbott Laboratories). Although not used widely for vector control, these products have proven useful and cost-effective in programs that employed larvicides as a vector control tactic. In addition, they are commonly used in industrialized countries for the control of nuisance mosquitoes and blackflies, particularly in environmentally sensitive areas.

With respect to *B. sphaericus*, there are 3 insecticidal proteins, with molecular masses of 43, 52, and 100 kDa, but these do not always occur in all isolates (Baumann et al. 1991, Porter et al. 1993). Interestingly, these proteins are only toxic to mosquitoes, and tend to exhibit the highest toxicity against Culex species. No significant toxicity for *B. sphaericus* has been reported against blackflies or other dipterans. Though the spectrum of activity for *B. sphaericus* is not as broad as for *B.t.i.*, *B. sphaericus* has greater residual activity, and works better in highly polluted waters, although it requires high rates of application, generally greater than 1 g of technical powder/m². It is still considered to hold potential for the species of Culex that vector filarial worms (Hougard 1990, Yap 1990), however, no commercial products based on *B. sphaericus* are currently marketed. This does not mean that for-

Table 2. Summary of property ratings for selected pathogens and nematodes evaluated for vector control.

<table>
<thead>
<tr>
<th>Property</th>
<th>Virus</th>
<th>Bacteria</th>
<th>Fungus</th>
<th>Protozoan</th>
<th>Nematode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iridovirus</td>
<td><em>Bacillus thuringiensis</em> subsp. <em>israelensis</em></td>
<td><em>Culicinomyces clavosporus</em></td>
<td><em>Nosema algerae</em></td>
<td><em>Romaneomermis culicivorax</em></td>
</tr>
<tr>
<td>Broad host/target spectrum</td>
<td>+ +</td>
<td>++++++</td>
<td>+++++</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>Mass production</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>N/P¹</td>
<td>++++++</td>
<td>+</td>
<td>N/P</td>
<td>N/P</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>+</td>
<td>N/A²</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Efficacy/unit weight</td>
<td>+</td>
<td>++++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Safety to nontargets</td>
<td>++++++</td>
<td>++++++</td>
<td>+++++</td>
<td>++++++</td>
<td>++++++</td>
</tr>
<tr>
<td>Formulation</td>
<td>++++++</td>
<td>++++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
</tr>
<tr>
<td>Storage</td>
<td>+</td>
<td>++++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Residual activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

¹ N/P = Not practical or cost-effective with existing technology.
² N/A = Not applicable because technology already exists for cost-effective mass production in vitro.
mulations based on B. sphaericus would not be useful in filariasis control programs, but rather that the market is considered too small to merit commercial development until more cost-effective formulations are devised.

Fungi

Three types of aquatic fungi that attack mosquito larvae have been studied for use as biological control agents, species of Coelomomyces (chytridiomycete fungi), Lagenidium giganteum (an oomycete fungus), and Culcinomycyces clavosporus (a deuteromycete fungus).

The genus Coelomomyces comprises more than 70 species of obligately parasitic fungi that undergo a complex life cycle involving an alteration of sexual (gametophytic) and asexual (sporophytic) generations (Couch and Bland 1985, Whisler 1985). In all species studied, the sexual phase parasitizes a microcrustacean host, usually a copepod, whereas the asexual generation typically develops in a mosquito larva. In the life cycle, a biflagellate zoosporangium infects the hemocoel of a mosquito larva where it produces a sporophyte that colonizes the body and forms resistant sporangia. The larva dies and subsequently the sporangia undergo meiosis, producing uninflagellate meiospores that invade the hemocoel of a copepod host where a gametophyte develops. At maturation, the gametophyte cleaves forming thousands of uniflagellate gametes. Cleavage results in death of the copepod and escape of the gametes, which fuse forming biflagellate zoosporangia that seek out another mosquito host, completing the life cycle. The life cycles of these fungi are highly adapted to those of their hosts. Moreover, as obligate parasites these fungi are very fastidious in their nutritional requirements, and, as a result, no species of Coelomomyces has been cultured in vitro.

Coelomomyces is the largest genus of insect-parasitic fungi, and has been reported worldwide from numerous mosquito species, many of which are vectors of important diseases such as malaria and filariasis. In some of these species, for example, Anopheles gambiae Giles in Africa, epizootics caused by Coelomomyces kill more than 95% of larval populations in some areas (Couch and Umphlett 1963). Such epizootics led to efforts to develop several species as biological control agents over the past 30 years (Federici 1981). However, these efforts have largely been discontinued due to the discovery that the life cycle required a 2nd host for completion, the inability to culture these fungi in vitro, and the development of B.t.i. as a bacterial larvicide for mosquitoes.

Although the difficulties encountered with Coelomomyces make it unlikely that this fungus will ever be developed as a biological control agent, there is still considerable interest in Lagenidium giganteum. This oomycete fungus has 2 important advantages over Coelomomyces; it is easily cultured on artificial media and it does not require an alternate host (Federici 1981, Jaronski et al. 1983). In the life cycle, a motile zoospore invades a mosquito larva through the cuticle. Once within the hemocoel, the fungus colonizes the body over a period of 2–3 days, producing an extensive mycelium consisting largely of nonseptate hyphae. Toward the end of growth, the hyphae become septate, and out of each segment an exit tube forms that grows back out through the cuticle and forms zoosporangia at the tip. Zoospores quickly differentiate in these, exiting out through an apical pore to seek out a new substrate. In addition to this asexual cycle, thick-walled resistant sexual oospores can also be formed within the mosquito cadaver.

Techniques have been developed to produce both zoosporangia and oospores in vitro, and methods are currently being developed to modify existing technology so that the fungus can be mass produced. Several years of field trials in California and North Carolina have shown that the zoosporangia are too fragile for routine use in operational control programs (Merriam and Axtell 1982, Kerwin and Washino 1987). The oospore, however, is quite stable, although germination remains unpredictable. Nevertheless, field results indicate that germination of even a small percentage of oospores can result in the initiation of epizootics that lead to season-long mosquito control. The fungus is, however, quite sensitive to ammonia and chloride ions, and thus is not efficacious in polluted or brackish waters. Nevertheless, although several technical problems related to mass production and formulation remain to be overcome, L. giganteum is a promising candidate for successful commercial development and use in freshwater habitats such as rice fields. Its principal advantage over B.t.i. is that if effective formulations of the oospore can be developed, it appears that in many habitats only a single application would be required per season. Even less frequent application may be possible in some habitats, as evidence suggests that the oospores can overwinter, initiating epizootics the following seasons. The extent to which this occurs and can be relied upon for effective mosquito control remains to be determined. Large-scale field trials have not yet been conducted with L. giganteum, so production costs and rates of application cannot be related to the level and length of vector control. Thus, it is not possible to assess the cost-effectiveness of L. gi-
ganteum as a mosquito or vector control agent at this time.

In addition to Coelomomyces and L. ganteum, the aquatic deuteromycete fungi, Culcicomyces clavosporus and Tolypocladium cylindrosporum, and the terrestrial deuteromycete fungus, Metarhizium anisopliae, have been considered for mosquito control (Federici 1981, Sweeney et al. 1983, Soares and Pinnock 1984). These fungi produce conidia, which adhere to the cuticle of larvae, and then invade and colonize the larva via a germ tube. However, high production costs resulting from inefficient production methods, lack of cost-effective control in the field, and the discovery and development of B.t.i., have eliminated these fungi as serious candidates for development as microbial control agents.

**PROTOZOA**

“Protozoa” is a general term applied to a large and diverse group of eucaryotic unicellular mobile microorganisms that belong to what is now known as the kingdom Protista. Members of this kingdom can be free-living, saprophytic, commensal, symbiotic, or parasitic. The cell contains a variety of organelles, but has no cell wall, and cells vary greatly in size and shape among different species. Feeding is by ingestion or more typically by absorption, and vegetative reproduction is by binary or multiple fission. Both asexual and sexual reproduction occur and the latter can be very complex, and is often useful for taxonomic purposes. Many protozoa produce a resistant spore stage that is also used in taxonomy. The kingdom is divided into a series of phyla based primarily on the mode of locomotion and structure of locomotory organelles, and includes the Sarcomastigophora (flagellates and amoebae), Apicomplexa (sporozoa), Microspora (microsporidia), Acetospora (haplosporidia), and Ciliophora (ciliates). Some types of protozoa, such as the free-living amoebae and ciliates, are easily cultured *in vitro*, whereas most of the obligate intracellular parasites have not yet been grown outside of cells.

As might be expected from such a large and diverse group of organisms, many protozoans are parasites of vectors. Those that have been reported most commonly in vector populations are microsporidia (phylum Microspora), and thus it is these, especially members of the genera Nosema and Amblyospora, that have received the most study. These studies have shown that most microsporidians have the general feature of causing chronic diseases. Thus, most cannot be used as fast-acting microbial insecticides. This characteristic is not detrimental in the case of most vectors because it is the adults that are responsible for disease transmission. However, the microsporidia are all obligate intracellular parasites, and there is no easy or cheap way to mass produce them *in vitro*. Moreover, the few field trials that have been conducted indicate that very high rates of application are required to obtain significant reductions in vector populations, at least in mosquito populations, the only vectors they have been tested against. The lack of commercially suitable methods for mass production and poor efficacy have significantly diminished interest in developing protozoans as vector control agents. Fairly extensive studies were carried out with Nosema algerae in the 1970s to evaluate its potential for controlling anophelines, with the aim of reducing the prevalence of malaria, and thus these studies are worth reviewing briefly here.

*Nosema algerae* was originally isolated from Anopheles stephensi Liston, but has a broad host range against anophelines and is capable under experimental conditions of infecting a wide range of other mosquito species. It is also capable of reproduction in the lepidopteran Heliothis zea, and this host has been used to produce spores for field trials (Anthony et al. 1978b). The infectious stage is a spore, within which is a coiled tube, the polar filament, that is responsible for injecting the sporoplasm into larvae after ingestion. Once within the body of a larva, the *Nosema* cells undergo numerous merogonic cycles in larval tissues, and then a sporogonic cycle that results in the development of new spores. The larvae typically die at the end of the disease if they are infected during the early instars. If infected during the 4th instar, larvae will often pupate and adults emerge. These adults have lower fecundity and decreased capacity for malaria transmission (Anthony et al. 1978a). Although the latter results initially indicated some potential for having an impact on disease transmission, field trials carried out in Panama against *Anopheles albimanus* Wied. in the 1970s showed that very high and uneconomic application rates for spores would be required to have any significant impact on disease control. To achieve mosquito infection rates of 86%, 4 applications of spores at a rate of $2.2 \times 10^5$ spores/m² had to be made. To treat a hectare at such a rate would require a minimum of approximately 40,000 lepidopteran larvae to produce the needed amount of spores.

Members of the genus *Amblyospora* also occur commonly in mosquito populations and have received considerable study. Studies of several species of *Amblyospora* have shown that they have complex life cycles, much like those of *Coelomomyces*, requiring an alternate crustacean host (Sweeney et al. 1985). As a result, these parasites...
are also thought to have little potential for development as vector control agents.

**MERMITHID NEMATODES**

Mermithid nematodes are among the largest nematodes attacking insects, and the adult females typically measure from 5 to over 20 cm. In parasitized insects with a translucent cuticle, such as the larvae of mosquitoes and blackflies, the advanced stages of developing nematodes can often be observed within the hemocoel where they appear as long, thin white worms. Mermithids are obligate parasites and have been reported from many different orders of insects as well as from other arthropods such as spiders and crustaceans. However, the only ones seriously considered for use as biological control agents are the species *Romanonermis culicivorax* and *Romanonermis iyengari*, which are capable of parasitizing many species of mosquito larvae (Petersen 1982). The life cycle of *R. culicivorax*, which is quite typical of mermithids, will be used to illustrate the properties that resulted in studies of the biological control potential of this nematode type.

The females of *R. culicivorax* are found in wet soil at the bottom of aquatic habitats in which mosquitoes breed. Here, after mating, the females lay thousands of eggs. The embryo develops into a 1st-instar juvenile over a period of about 1 wk, and afterwards molts to a 2nd-stage juvenile still within the egg. This 2nd stage (preparasite) then hatches out and swims to the water surface where it seeks out and, with the aid of the stylet, invades an early instar mosquito larva via the cuticle. Once within the hemocoel, the immature larva grows over a period of 7–10 days by absorbing nutrients through its cuticle, and molts once during this period. Upon completion of this parasitic phase, the 3rd-stage juvenile punctures its way out through the cuticle of its host, thereby killing the mosquito, and then drops to the bottom of the pond where it matures without feeding over another 7–10 days, finally molting to the adult stage. The adults then mate and the females lay eggs, thus completing the life cycle.

Using several different species of colonized mosquitoes and simple rearing techniques, Petersen (1982) and his colleagues were able to develop mass rearing techniques for *R. culicivorax*, and conducted a wide range of trials in which they evaluated this nematode by applying the preparasitic stage in the laboratory and field. In the field studies, rates of parasitism (= mortality) for anopheline larvae as high as 85% were obtained when preparasites were applied at a rate of 2,400/m². Even higher levels of parasitism (96%) were reported by Levy and Miller (1977) when they applied preparasites at a rate of 3,600/m² against floodwater mosquitoes in Florida. When introduced into suitable habitats, *R. culicivorax* can recycle, providing some level of control in subsequent generations of mosquitoes, but this level is typically not sufficient to interrupt the vector potential of mosquitoes or alleviate the annoyance problem. In addition, both laboratory and field studies showed that *R. culicivorax* was quite sensitive to chloride ions, and the methods developed for mass production, storage, shipment, and use were found not to be cost-effective, particularly in comparison to commercial formulations of the bacterial insecticide *B. thuringiensis* subsp. *israelensis*, which was discovered while the development and evaluation of *R. culicivorax* was underway. As a result of its limitations, and the availability of cheaper and more effective alternative biological methods of control, there is little interest today in the further development of *R. culicivorax*, or other mermithids for that matter, as biological control agents.

**SUMMARY AND CONCLUSIONS**

During the past 3 decades, in response to the problems caused by synthetic chemical insecticides, a considerable effort has been made to find and develop pathogens and nematodes as biological control agents, and microbial insecticides for control of vectors. This research has produced a wealth of knowledge about the occurrence, basic biology, and control potential of numerous viruses, bacteria, fungi, protozoa, and nematodes that parasitize vector insects. This knowledge is of value in its own right and serves as a foundation against which to measure the potential of new control agents. However, studies of the various organisms that have been discovered and evaluated as control agents, in combination with an assessment of how they might be used in vector control programs, have demonstrated that most of these agents are not sufficiently cost-effective to be used in such control programs. Moreover, there is no reason to believe this assessment will change over the next 10–20 years, barring some sort of unanticipated breakthrough.

The primary reason for the lack of cost-effectiveness for most pathogens and nematodes that have been evaluated is that methods are not available for their mass production at an acceptable cost. In essence, to achieve levels of vector control that will have a significant impact on the prevalence of human disease, the amounts of vector control agents that would have to be
applied are too high, and thus too costly to produce, even where labor costs are low, using the in vivo methods that are required to produce these agents. Moreover, even when applied at high rates, none of the pathogens or nematodes evaluated persist or recycle at a sufficiently high level to give acceptable rates of vector control for more than 1 month. Despite the numerous potential control agents evaluated, the only cost-effective pathogen identified to date is the bacterium Bacillus thuringiensis subsp. israelensis. Its success is due primarily to its ability to be mass produced in large quantities on relatively inexpensive media using large-scale fermentation technology. Even so, this bacterium is only effective against larvae, and thus its use is limited to vector control programs that have traditionally used larviciding as a tactic. Though limited, the use of B.t.i. has been quite valuable in The World Health Organization’s Onchocerciasis Control Program in West Africa, and may ultimately prove useful in malaria, filariasis, and viral disease control programs that include larviciding. However, B.t.i. is unlikely in the future to be of use against major malaria vectors such as An. gambiae and An. stephensi, where larviciding is not an important control strategy.

The lack of new agents and technologies to control vectors indicates that chemical insecticides will continue to be important, where resistance is not a major problem, in vector control programs. New disease control technologies are being explored, such as disease-refractory transgenic mosquitoes (Collins and Besansky 1994) and releasing transgenic insecticidal organisms used as larval food into breeding habitats. The former tactic has merit, but may ultimately lead to resistance in the population of the disease causing organism, such as the malarial parasites. The latter tactic, based on our experience with the development of resistance to chemical insecticides in larval populations, will almost certainly lead to resistance.

In summary, the insect populations that vector the major diseases of humans, especially given the increasing human populations in areas where these diseases are the most prevalent, offer vector control challenges greater than ever. Pathogens, especially bacteria such as B.t.i., will be of some use in vector control programs, but will not provide a major vector control tactic in the foreseeable future.

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