BIOLOGICAL CONTROL OF MOSQUITOES AND FLIES IN THE URBAN ENVIRONMENT

NORBERT BECKER[1] AND FRANTISEK RETTICH[2]
[1] German Mosquito Control Association (KABS), 6701 Waldsee., Germany
[2] National Institute of Public Health, Prague.

INTRODUCTION

The uncontrolled growth of many large cities, especially in developing countries, and solutions to the problem generated by this, pose an enormous challenge for governments. In spite of every effort by the state to stem the tide of people moving away from the countryside, by improving the infra-structure within the rural environment, the concentration of people into the cities of tropical and subtropical countries continues at an ever increasing pace.

This rapid expansion raises considerable problems for municipal planning and for the controlled development of these cities. In addition to solving the problem of traffic congestion and, connected with this, of atmospheric pollution, the provision of drinking water and food together with the disposal of sewage and refuse have become critical problems for municipal authorities. In spite of all the efforts of city planners, there is still no solution to these problems in the slums of many cities.

Sewage generally runs untreated into the nearest body of water. Drainage ditches and ponds turn into stinking cesspools. People relieve themselves in simple cesspits and cesspools, as in Africa for example, or along the side of ditches, as in many cities of south-east Asia. Environments develop in which almost the only abundant organisms apart from bacteria and fungi are the immature stages of Diptera such as mosquitoes, scatopsids, syrphids or muscids.

The immature stages of *Culex quinquefasciatus* in particular develop in vast numbers in the polluted water of sewage ditches, pools and cesspits. It is not uncommon for several hundred larvae of this mosquito to be counted in one litre of water. In many settlements in tropical and subtropical countries, the inhabitants are attacked by several hundred mosquito females per night. Unable to get adequate sleep, the working capacity of these people is greatly reduced. But not only is peoples' sense of well-being significantly diminished, but *Cx. quinquefasciatus* is also the most important vector of lymphatic filariasis. More than 90 million people in Asia, Africa and South America are victims of this disease, which is caused by the nematodes *Wuchereria bancrofti, Brugia malayi* and *Brugia timori.* The World Health Organisation estimates that almost a billion people are at present in danger of being infected with *Wuchereria bancrofti* from the bite of *Cx. quinquefasciatus* (WHO 1991). As the pollution of water-bodies increases, *Cx. quinquefasciatus* will find more and more suitable sites for its development within the urban environment, and this will have to lead to increased efforts to combat lymphatic filariasis.

In many cities of the tropics and subtropics, the provision of water for drinking and household purposes is a great problem. In the absence of tap water, water for daily use has to be obtained from wells and then stored indoors in containers. Such containers offer ideal conditions for the development of the immature stages of the yellow-fever mosquito, *Aedes aegypti.* Because of its outstanding ability to adapt to human settlements, *Aedes aegypti* has become the most important vector of dengue and dengue haemorrhagic fever (DHF). Dengue is now the most serious arbovirus disease affecting mankind in terms of its morbidity and mortality. About 1.5 billion people in the tropics, mainly in Asia, the Western Pacific area, the Caribbean, as well as in Central and South America, are at risk of dengue infection (Halstead 1980, 1982; Becker *et al.* 1991). In spite of considerable national and international efforts to suppress these vector-borne diseases, they continue to impede improvements in health and socio-economic development in many tropical countries. Alongside these mosquito-borne diseases which are transmitted mainly in urban areas,

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

malaria should also be mentioned as it continues to threaten half of mankind. The anopheline vectors of malaria colonise a wide variety of standing and running water, such as plant-rich pools and ditches, rice fields, standing water in flooded areas, and even water-filled hoof prints and plant parts. Such types of water-bodies occur only rarely in the more restricted urban environment. In peripheral urban areas, however, there is the danger of infection with a *Plasmodium* species because anophelines occasionally find sites there that are suitable for their offspring and may migrate into such areas.

Mosquitoes are not just a problem of the tropics. They may also cause a considerable nuisance in the temperate latitudes. The species most commonly involved are the so-called floodwater mosquitoes, such as *Aedes vexans* in river valleys that regularly flood, the halophilous species *Ae. caspius* and *Ae. detritus,* which breed particularly in the areas of shallow lagoons that are found along the coasts of South-Europe, Asia Minor and North Africa, or the rock-pool mosquito *Ae. mariae* along the rocky coasts, where mass occurrences can become a great nuisance. These mosquitoes do not breed in urban areas, but during mass occurrences they can migrate over several kilometres and commonly reach the urban environment, where they can become a nuisance in parks and similar facilities. In addition to these, there are mosquitoes in the temperate latitudes that find particularly suitable breeding sites in human settlements. The most important species in this context is *Cx. pipiens,* which is known as the house mosquito because of its presence close to human settlements. This mosquito colonises a wide range of water-bodies, highly polluted as well as relatively pure ones, large or small, standing or flowing, even in the smallest accumulations such as water-filled buckets, flower vases, old tires or gutters. Generally, however, they use the containers in which water is stored for household use. In rural areas, cesspools also provide mass breeding-sites for *Cx. pipiens. Cx. pipiens* can be characterised as the urban mosquito of the temperate latitudes, although it is also not uncommonly found away from human settlements.

Mosquito control programs still rely to a great extent on chemicals. Annual demand now amounts to more than 50,000 tons, with DDT being by far the most commonly-used insecticide. Apart from their beneficial effects, however, these insecticides often have other less desirable ramifications: for example, vectors develop resistance to them, and their non-selectivity frequently causes ecological damage. The ecological and toxicological risks were viewed in a new light after the publication of Rachel Carson's book "Silent Spring". As public awareness of environmental issues arose, the regulations controlling the application of chemicals were tightened. These restrictions gave rise to a worldwide search for new and environmentally safe methods in the 1970's. National and international organisations such as the WHO/TDR supported the development of new integrated control strategies. Macroorganisms such as fish have been used for decades in many mosquito control programs. However, fish and other predators have specific ecological requirements and can only be used where their preferred living conditions are met. This limits their large-scale use in a number of specific habitats. Special attention has therefore been given to the search for microbial control agents. There was a hope, that the inoculation of a pathogen into the water of a breeding-site would achieve a long-term effect as soon as the pathogen would become established in the water and would continue to kill newly-hatching immatures of the vector or pest-species.

Efforts on an international scale have led to the discovery of a great variety of pathogens, including entomopathogenic nematodes, fungi, protozoa, bacteria and viruses (Davidson and Becker, in press).

BIOLOGICAL CONTROL OF MOSQUITOES

Nematodes

Several species of mermithid nematodes have been tested as biological control agents in various parts of the world. Mermithid females lay their eggs in the substrate of the mosquitoes' breeding-site, where they can even survive periods of drought. When flooded and when environmental conditions are suitable, the young nematode larvae hatch from their eggs, penetrate as pre-parasites through the cuticle of the mosquito larva, and in a little over a week grow into post-parasites inside the host larva. The post-parasite leaves its host by boring through the larval

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

cuticle, which leads to the death of the mosquito. In the substrate of the water-body the post-parasites mature eggs, and so within only a few weeks the life-cycle has come full-circle. Appropriate methods for the mass rearing of *Romanomermis culicivorax* were developed. A moist substrate of sand containing eggs for the inoculation of mosquito breeding-sites was available commercially during the 1970' s. Unfortunately, no widespread use of these parasites was possible because of difficulties with transportation and with maintenance of the eggs, as well as the sensitivity of the worms towards particular environmental conditions such as low water temperature or high salinity.

Fungi

Amongst entomopathogenic fungi, the species *Lagenidium giganteum, Coelomomyces* spp. and *Culicinomyces clavisporus* in particular have been intensively studied for the control of mosquito immature stages. *Lagenidium giganteum,* which goes through an asexual and a sexual developmental stage, has shown the most promising results. When introduced into a breeding-site, the motile biflagellated zoospores attach themselves to the larval cuticle. Upon penetration, a fungal mycelium forms inside the larval haemocoel, and this results in the death of the larva. Several further asexual cycles follow on this, or the fungus passes through a sexual cycle at the conclusion of which oospores are formed. These are characterised by their great persistance in the breeding site. The oospores can also be produced in an artificial medium. Unfortunately, it became clear that the oospores only germinate asynchronously, and consequently mosquito populations of high densities are only rarely eliminated by a knock-down effect.

Infections with *Coelomomyces* spp. (Blastocladiales) are known from more than 50 mosquito species. There are more than 20 known species of *Coelomomyces,* and they are characterised by all being obligatory parasites of mosquitoes. At first it proved difficult to infect mosquito larvae in the laboratory. Only when Whistler *et al.* (1974) were able to show that there was an alternation of generations with a simultaneous alternation of hosts, in which copepods as well as mosquito larvae are involved, successful infections regularly did take place. Upon infection, a mycelium forms inside the mosquito larva. Yellowish to brownish coloured sporangia form at the tips of the hyphae, and the haploid zoospores form in these by meioses. After the zoospores are released they infect copepods such as *Acanthocyclops vernalis,* in which a heterothallus is formed which produces isogametes. A biflagellated zygote arises through fusion of the isogametes. This is the first stage to be infectious for mosquito larvae and it penetrates through the cuticle into the larval haemocoel. The initial hopes that a satisfactory parasite for mosquito control had been discovered were dashed by this complex life-cycle and by associated difficulties in the mass production of the fungi.

The imperfect fungus *Culicinomyces clavisporus* can be mass produced in artificial media. When the spores are ingested by mosquito larvae, they penetrate the gut wall and proliferate in the larval body. After the death of the larva, the fungus sporulates on the surface of the cadaver. But due to the lack of persistance and adequate recycling abilities, difficulties in storage and the high dosages required, efficient use of this fungus in mosquito control programs is unlikely.

Protozoa

the Protozoa contain the largest number of mosquito parasites.The best studied are the microsporidian intracellular parasites. Larvae infected with microsporidia can easily be recognised even in the field by their milky-white colour. Despite the great scientific interest of this group of parasites, no one has yet succeeded in using them as microbial control agents. This is mainly because of their complex life-cycle, which makes mass production difficult, as well as their frequently low pathogenicity and persistance.

A common feature of all microsporidia is the development of spores which contain a polar filament internally. This is ejected in the host's gut, and in this way the infectious nucleus is able to penetrate into the host. Two groups of microsporidia as mosquito pathogens are known. The first group, which includes the genera *Nosema* and *Vavraia,* develops asexually and only forms one type of spore. Infection of mosquito larvae can be achieved relatively simply in the laboratory by feeding them with spores. The second group, which includes the genus *Amblyospora,* has a complex developmental cycle. Infection takes place both transovarially and orally. An infected mosquito female transmits the spores in a vertical direction, transovarially to the next generation. In the mosquito larva, the spores divide meiotically and innumerable haploid spores thereby arise in the larval fat-body and are released when the larva dies. If they are now ingested by a female copepod, they reproduce asexually once again. If, on the death of the copepod, the haploid spores are released and ingested by a mosquito larva, the sexual phase begins with fusion of the cells, which again form diploid spores. Female mosquito larvae which are only lightly infected can again transmit the spores transovarially to the larvae of the next generation. This complex developmental cycle prevents any mass-rearing of the parasites and makes it unlikely that these micosporidia will be used in control programs.

Viruses

Viruses are even less suitable for mosquito control than the parasites and pathogens just described, although a number of viruses such as iridescent or parvo-viruses have been isolated from mosquito larvae. No virus has yet been found that can be used as a microbial control agent against mosquitoes.

Bacteria

The discovery of mosquitocidal bacilli such as *Bacillus thuringiensis israelensis* in 1976 and potent strains of *Bacillus sphaericus* in recent years inaugurated a new chapter in the control of mosquitoes and black flies. These bacilli produce protein toxins during sporulation which are highly toxic to mosquito larvae and in the case of *B. thuringiensis israelenis* also against black fly larvae. It could be shown that the selectivity of the bacilli derives from a variety of factors: (1) The protein crystal (inactive protoxin) must be ingested by the target insect, and this depends on their feeding habits. (2) Proteases must then convert the protoxin into biologically active toxins in the alkaline midgut milieu of the target insect. (3) The toxins must then bind to a cell surface receptor (glycoprotein) of the midgut epithelial cells of the target insect. This disturbs the osmoregulatory mechanisms of the cell membrane, thereby swelling and bursting the midgut cells. The nontarget organisms that do not activate the protoxin into the toxin or without the specific receptors on their intestinal cells remain undamaged.

The special properties of these bacilli such as environmental safety, relative ease of mass production, formulation and application, the stability of proper formulations as well as the suitability for integrated control programs based on community participation and the relative low costs for development and registration were the reasons for the fast development and utilization of these bacilli in mosquito and black fly control programs.

The exceptional environmental safety of the bacterial control agents was confirmed in numerous tests. None of the tested taxa such as *Cnidaria, Turbellaria, Rotatoria, Mollusca, Annelida, Acari, Crustacea, Ephemeroptera, Odonata, Heteroptera, Coleoptera, Trichoptera, Pisces, and Amphibia* appeared to be affected when exposed in water containing large amounts of bacterial preparations (Becker and Margalit 1993).

Even within the dipterans, the toxicity of *B. thuringiensis israelensis* is restricted to mosquitoes and to a few nematocerous families. In addition to larval mosquitoes and black flies, only those of the closely related dixids are similarly sensitive to *B. thuringiensis israelensis.* Larval psychodids, chironomids and tipulids generally are far less sensitive than mosquitoes or black flies. In contrary to *B. thuringiensis israelensis,* the toxins of *B. sphaericus* are toxic to a much narrower range of insects. Certain mosquito species, such as *Cx. quinquefasciatus* and *Anopheles gambiae* are highly susceptible whereas *Ae. aegypti* larvae are 100- to 1000-fold less susceptible. Black fly larvae as well as all other insects (exception Psychodidae), mammals, and other nontarget organisms are not susceptible to *B. sphaericus.*

The insecticidal effect of B. *thuringiensis israelensis* emanates from 4 major toxin proteins which are referred to as the CryIVA, B, C and D proteins. A fifth toxin, called the CytA protein, doesn't show the specific binding mechanism which is shown for the Cry proteins. Synergism in the mode of action among these toxins has been postulated from the fact that the individual component toxins are significantly less toxic than the intact crystal containing all the toxins. It is assumed that this synergistic effects also reduce the likelihood of resistance.

In addition to *B. thuringiensis israelensis,* a second spore-forming bacterium, Bacillus sphaericus has become of increasing importance in recent years. The high potential of *B. sphaericus* as a bacterial control agent lies in its spectrum of efficacy and its ability to recycle or to persist in nature under certain conditions, which means that a long-term control can be achieved. In this manner, the time-span between retreatments could be extended and personnel costs reduced. These abilities open up the possibility of the successful and cost-effective control of *Culex* species, particularly of *Cx. quinquefasciatus* the most important vector of lymphatic filariasis and which breeds primarily in highly polluted water-bodies in urban areas.

According to the recent state of knowledge, *B. sphaericus* only kills mosquito larvae, especially those of the genus *Culex,* and its efficacy, like that of *B. thuringiensis israelensis* is based on parasporal protein inclusions, which differ from those of *B. thuringiensis israelensis. The toxin of B. sphaericus* has been shown to be a binary toxin consisting of two proteins, 51.4 kDa and 41.9 kDa. Both are required for a high level of mosquitocidal activity. Receptor binding similar to *B. thuringiensis israelensis* is assumed.

A basic requirement for the successful use of bacterial control agents is the development of effective formulations suited to the biology and habitats of the target organisms. *B. thuringiensis israelensis* preparations can be obtained as wettable powders, fluid concentrates, granules, pellets, briquets, or tablets. The development of suitable formulations based on *B. sphaericus* are also in good progress.

A few hundred grams of powder or even less, a half of a litre to two litres of liquid concentrate or a few kilograms of granules per hectare, are usually enough to kill all mosquito larvae. In some situations a long-term effect can be achieved if higher amounts are used. In recent years, with the production of briquets, pellets, and tablet formulations, progress has been made to achieve a long-term effect. Sustained-release floating granules are being developed. For instance larval *Culex pipiens* can effectively be killed by means of *B. thuringiensis israelensis* briquets. Only 1 briquet per rain barrel produces satisfactory results for more than a month as a result of the slow release effect.

New tablet formulations based on an asporogenous *B. thuringiensis israelensis* strain or *B. thuringiensis israelensis* material sterilized by radiation to prevent contamination of drinking water with spores can successfully used for control of container breeding mosquitoes such as Aedes aegypti as the main vector of dengue. Tablets or briquets based on *B. sphaericus* are very effective to control *Culex* larvae close to human settlements.

Bacterial control agents have a considerable safety advantage over synthetic insecticides because neither the operator nor the occupants of treated sites become exposed to potentially dangerous chemicals. For this reason, such preparations are particularly well suited for use by volunteers.

Applications of bacterial control agents do not harm beneficial animals such as honey bees, silkworms, or aquatic animals such as fish, shrimp or oysters. Such formulations can be used in ecologically sensitive areas. Because they are biodegradable, no toxic residues remain after their use. Their environmental safety permits bacterial control agents to be accepted by both public official and the general public.

Operational aspects in the use of bacilli in mosquito control programs

Before microbial control agents can be used against mosquitoes or flies they must be evaluated for their suitability for the particular situation. The prerequisites needed for this evaluation include

- detailed knowledge of the biology and ecology of the vector or nuisance species;
- most suitable formulations and dosages and
- the best application techniques.

In the preparation phase of the intervention, a baseline collection of entomological and ecological data must be carried out. The following data should be considered:

- the nature of mosquitoes that are present;
- density of larvae;

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

- density of adult insects;
- location of the breeding sites and
- abiotic and biotic conditions of the breeding sites.

The implementation phase of a intervention with bacterial control agents should be designed to assess the following factors: evaluation of the the susceptibility (LC_{50}/LC_{90} values) of the main native mosquito species using different formulations; assessment of the minimum and optimum effective dosage in small scale field tests; testing of the most suitable application technique(s) and formulation(s).

On the basis of the revealed data a mosaic-like intervention strategy for each individual area and situation has to be worked out and the cost-effectiveness of the use of microbial control agents should be subject to evaluation. In addition to an analysis of actual operational costs of such a program, however, subsequent costs due to environmental damage must also be considered.

For successful larval mosquito control, it is most important to map all breeding sites. From the beginning of the control operations the major effort has to be the mapping of all breeding sites with individual numbering of each significant location for quick reference during control and as a basis for communication between the field staff. The operational area should be divided into several districts according to the morphological and topographical structure of the area.

Additional control procedures for the development of an integrated control program have to be tested according to the specific circumstances in the region. Where insecticide usage is reduced or eliminated an integrated approach has to be made using biological control measures combined for instance with environmental management, and the use of predators such as fish. The aim has to be to arrive at a comprehensive control strategy with a mosaic-like structure that takes ecological, epidemiological and sociological aspects into account and in which various methods are integrated. A vital component of each successful vector control program should be not only the inter-disciplinary collaboration between various specialists under the aegis of health services but also the cooperation of the community must be taken into account. Community participation means that the people must become "actors" instead of "spectators". Due to their special properties such as environmental safety and safety for the users bacterial control agents are very suited to be used in integrated control programmes with community participation (Becker, 1992).

The control of breeding sites of *Culex* species or *Aedes aegypti* near the houses has to be based on providing the people concerned with thorough information on the biology of the vector species and tips can be given on how the people can control breeding sites for themselves (e.g. environmental sanitation or using tablets based on bacterial control agents).

BIOLOGICAL CONTROL OF FLIES

The role of the house fly (*Musca domestica*) as a vector of numerous human pathogens has been amply documented in literature. Furthermore, both the house fly and the stable fly (*Stomoxys calcitrans*) cause serious nuisance problems in areas where livestock animals are reared in the vicinity of housing. In temperate latitudes there are serious problems in and near intensive animal units (piggeries, cowsheds, poultry farms, etc.). The flies occur wherever there are accumulations of manure where their maggots can develop successfully.

The frequent use of residual insecticides has resulted in the widespread development of resistance (Jespersen and van Jensen, 1991). Recently resistance even to insect growth regulators (IGR) has developed (Jinliang Shen and Plapp, 1990). Biological control of flies is therefore a pressing need as it may help to reduce the selection pressure of insecticides and may improve overall fly control in general.

There are a few biological agents which have already been found useful in routine fly control. Although they show some promises, further research and development are needed.

Beta exotoxin of Bacillus thuringiensis

B. thuringiensis products containing heat-stable protein-β-exotoxin (Thuringiensin) have been applied against *M. domestica* maggots in dung (Holmberg *et al.*, 1980; Jespersen, 1986; Rupes *et al.*, 1987), but an adequate level of control was only achieved by using rather high dosages. Moreover, β-exotoxin has a less specific range of activity than the safer delta-endotoxin and it is toxic to vertebrates. When administered by injection, the intraperitoneal LD_{50} for mice amounts to 10-20 ug/g body weight, and per os toxicity of some salts of the β-exotoxin in hens is considerable, even at low dosages (3 ug/g body weight) (Sebesta *et al.* 1981). Consequently, as a precautionary measure, β-exotoxin in commercial products using the delta-endotoxin as the active components is banned by law in many countries (Meadows, 1993). The toxicological drawbacks of β-exotoxin may be overcome by the development of safer methods of application (e.g. granules, baits).

Fungi

The search for fungi suitable for the routine biological control of flies should not be limited to rather specific fungi species such as *Entomophthora muscae*. There is evidence that other well-known insect pathogens such as *Metarhizium anisopliae* and *Beauveria bassiana* may be very pathogenic for flies, including the house fly (Rizzo, 1977). *B. bassiana* and *M. anisopliae* have recently been found to be pathogenic for pupae of the tse-tse fly *Glossina morsitans* (Kaya and Mwangi, 1992). Newly discovered fungal toxins such as Tolipin should also be tested against flies.

Protozoa

There appear to be at least some promising protozoan candidates for the microbial control of flies. For example, *Herpetomonas muscarum* (in the house fly or eye gnat: *Hippelates pusio*) and *Crithidia fasciculata* (in *Glossina* spp.) produce an intense haemolytic infection that results in the death of insects (Kramer, 1961; Bailey and Brooks, 1972 a,b; Ibrahim and Molyneux, 1987).

Nematodes

The prospects for use of the nematodes namely *Steinernema* spp. or *Heterorhabditis* spp. in terrestrial (crawling) insects are very promising. However, the application of these entomopathogens in fly control seems still to be rather controversial. Renn et al. (1984) and Geden et al. (1986) found these two nematode genera to be infective for a number of species of Diptera, including *M. domestica*. On the other hand, Mullens et al. (1987) observed no reduction of *Fannia canicularis, F. femoralis,* or *Muscina stabulans* by *Heterorhabditis heliothidis* (NC strain) or *Steinernema feltiae* (Mexican strain).

When *S. feltiae* ("All" strain) was used against *M. domestica*, the nematodes failed to demonstrate parasitism or to bring about any reduction in larval numbers, even when applied to wet manure with host larvae on the surface. Recently, Renn (1990) has seen encouraging signs for the future development of nematode baits (*S. feltiae*).

Parasitic Hymenopterans

Probably the most widely studied biological control agents for flies have been parasitic Hymenoptera of the family *Pteromalidae,* the best known being from the genera *Muscidifurax* and *Spalangia*. In the 1980's, many parasitoides were available commercially. 14 suppliers in California alone were reported by Bezark and Yee (1985), and Poinar and Thomas (1984) listed 10 producers of *M. raptor* and 12 of *Spalangia endius*. However, so far as the authors are aware, no major advances have been achieved towards the goal of a succesful routine use of these parasites for the control of domestic and stable flies. Petersen *et al.* (1983) evaluated weekly releases of *S. endius* on two confined beef feedlots in Nebraska and found that the strains of *S. endius* used were ineffective in controling both house fly and stable fly populations. Meyer *et al.* (1990) terminated field releases of commercial parasites (*S. endius, M. zaraptor, M. raptorellus*) in diaries in southern California. They found increased parasitism in their house fly pupal controls, but the overall effect on parasitism of field-collected stable or house fly pupae or adult fly level was negligible. In Italy Maini et al. (1991) released a total of 300,000 equally distributed male and female adults of *M. zaraptor* in a quantity of 60-824 females per m^2 of substrate and then found that the parasitization of *M.*

Proceedings of the First International Conference on Urban Pests.
K.B. Wildey and Wm H.Robinson (editors). 1993

domestica reached level as high 67.5% in manure pits, while parasitization of *S. calcitrans* was scarce (up to 1.1%).

FUTURE PROSPECTS FOR THE USE OF MICROBIALS

The exceptional qualities of *B. thuringiensis israelensis* and *B. sphaericus* such as high efficacy and the outstanding environmental safety have made the extraordinary development of these bacterial control agents possible. Due to the intensive cooperation between industry, universities and research institutes as well as national and international authorities (especially by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) this methods could be quickly developed and could be put into practice in many mosquito and black fly control programs world-wide.

B. thuringiensis israelensis has long since undergone tests in routine programs. With the development of appropriate formulations an effective and economic control of mosquitoes and black flies is now possible in the majority of cases. As a result, interest in the *B. thuringiensis israelensis* method is increasing worldwide year by year. About 1000 tonnes of *B. thuringiensis israelensis* preparations are now being used annually, with success and with increasing interest. So far there have been no cases of negative effects on the various ecosystems. Microbiological methods offer an ecologically defensible compromise between the desire of man to protect himself from troublesome mosquitoes or black flies and the requirements of contemporary environmental protection policies not to damage highly sensitive ecosystems such as river meadows through the introduction of non-selective methods.

B. thuringiensis israelensis and *B. sphaericus* may also be promising agents in the battle against dangerous diseases such as malaria, filariasis, and arbovirus diseases. Microbial control agents are especially well-suited as larvicides in vector control programs when the target vectors are breeding in well defined breeding sites such as water barrels (container breeder e.g. *Aedes aegypti* or *Culex pipiens*), defined ricefield areas (e.g. *Anopheles sinensis*), lagoons (e.g. *Anopheles sundaicus*), or in defined breeding sites of Cx. quinquefasciatus in urban areas (e.g. cesspits, latrines or waste water ponds and ditches).

Used in suitable formulations, these microbial agents are useful supplements to or replacements for broad-spectrum chemicals.

Further improvements of these microbial preparations, particularly to extent their long-term effect and will further accelerate this process. The ongoing large-scale tests of *B. sphaericus* in the tropics yielded promising results and it is already demonstrated that *B. sphaericus* will be a suitable tool to control *Cx. pipiens* and *Cx. quinquefasciatus,* world-wide the most abundant and wide spread mosquitoes and vectors of dangerous diseases such as lymphatic filariasis. However, further research on parasites and pathogens for the successful use in nuisance and vector control programs is needed.

REFERENCES

Bailey, C.H., and Brooks, W.M. (1972a). Histological observations on larvae of the eye gnat, *Hippelates pusio* (Diptera: Chloropidae), infected with the flagellate *Herpetomonas muscarum*. *J. Invertebr. Pathol*. 19: 342-353.

Bailey, C.H., and Brooks, W.M. 1972b. Effects of *Herpetomonas muscarum* on development and longevity of the eye gnat, *Hippelates pusio* (Diptera: Chloropidae). *J. Invertebr. Pathol.* 20: 31-36.

Becker, N., S. Djakaria, A. Kaiser and Zuhasril. 1991. Efficacy of a new tablet formulation of an asporogenous strain of *Bacillus thuringiensis israelensis* against larvae of *Aedes aegypti*. *Bull. Soc. Vector Ecol.* 16(1): 176-182.

Becker, N. 1992. Community participation in the operational use of microbial control agents in mosquito control programs. *Bull. Soc. Vector Ecol.* 17(2): 114-118.

Becker, N. and J. Margalit. 1993. Use of *Bacillus thuringiensis israelensis* against Mosquitoes and Blackflies. p. 147-170. In: *Bacillus thuringiensis*, An Environmental Biopesticide: Theory and Practice. (Eds. P.F. Entwistle, J.S. Cory, M.J. Bailey, S. Higgs), John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore. pp 311.

Bezark, L. and Yee, H. 1985. Suppliers of beneficial organisms in North America. California Department of Food and Agriculture, Sacramento, Calif.

Davidson, E.W. and N. Becker. 1993. Microbial Control of Vectors in Theory and Practice. In: Biology of Disease Vectors, University Press of Colorado. 307 Boulder, Colorado, pp 660 (in press).

Geden, C.J., Axtell, R.C., and Brooks, W.M. 1986. Susceptibility of the house fly, *Musca domestica* (Diptera: Muscidae), to the entomogenous nematodes *Steinernema feltiae, S. glaseri* (Steinernematidae) and *Heterorhabditis heliothidis* (Heterorhabditidae). *J. Med. Entomol.* 23: 326-332.

Halstead, S.B. 1980. Dengue haemorrhagic fever—a public health problem and a field for research. Bull. WHO 58, 1: 1-21.

Holmberg, A., Sievanen, R. and Carlberg, G. 1980. Fermentation of Bacillus thuringiensis for exotoxin production: process analysis study. *Biotechnol. Bioeng.* 22(8): 1707-1724.

Ibrahim, E.A., and Molineux, D. H. 1987. Pathogenicity of *Crithidia fasciculata* in the haemocoele of *Glossina. Acta Tropica* 44: 13-22.

Jespersen, J. B. 1986. Investigations on biological control agents. *Danish Pest Inf. Lab. Ann. Rep.* 1986 (1987): 51-52.

Jespersen, J. B., and Van Jensen, K.M. 1991. Insecticide resistance in Musca domestica. *Danish Pest Inf. Lab. Ann. Rep.* 1991: 38-44.

Jinliang Shen, and Plapp, F.W. 1990. Cyromazine resistance in the house fly (Diptera: Muscidae): genetics and cross-resistance to diflubenzuron. *J. Econ. Entomol.* 83: 1689-1697.

Kaya, G.P. and Mwangi, E.N. 1982. Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* for larvae of Tsetse flies (*Glossina morsitans*) and adult ticks (*Rhipicephalus appendiculatus*). Abstract for the 25th annual meeting of the Society of Invertebrate Pathology, Heidelberg, Germany, p 307.

Kramer, J.P. 1961. *Herpetomonas muscarum* (Lindey) in the haemocoel of larval Musca domestica (L.). *Entomol. News* 72: 165-166.

Maini,S., Bellini, R. and Galgano, F. 1991. Efficacy of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae) against filth flies in dairy farms in the Po plain area. 6th Ann. Meet. Soc. Vector Ecology. Gvdvllv. p 25.

Meadows, M.P. 1993. *Bacillus thuringiensis* in the environment: ecology and risk assessment. 193-220. In: *Bacillus thuringiensis*, An environmental biopesticide: Theory and Practice. (Eds. P.F. Entwistle, J.S. Cory, M.J. Bailey, S. Higgs), John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore. pp 311.

Meyer, J.A., Mulles, B.A., Cyr, T.A. and Stokes, C. 1990. Commercial and Naturally Occurring Fly Parasitoids (Hymenoptera: Pteromalidae) as Biological Control Agents of Stable Flies and House Flies (Diptera: Muscidae) on California Diaries. *J. Ecol. Entomol.* 83(3): 799-806.

Mullens, B.A., Meyer, J.A. and Georgis, R. 1987. Field tests on insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against larvae of manure-breeding flies (Diptera: Muscidae) on caged-layer poultry facilities. *J. Econ. Entomol.* 80: 438-442.

Patterson, R. S., and Rutz, D.A. 1986. Biological control of muscoid flies. Miscellaneous Publication 61. Entomol. Soc. Am., College Park, Md.

Petersen, J.J., Meyer J.A., Stage, D.A., and Morgan, P.B. 1983. Evaluation of sequential releases of *Spalangia endius* (Hymenoptera: Pteromalidae) for control of house flies and stable flies (Diptera: Muscidae) associated with confined livestock in eastern Nebraska. *J. Econ. Entomol.* 76: 283-286.

Poinar, G.O., and Thomas, G.M. 1984. Laboratory guide to insect pathogens and parasites. Plenum Press. New York. pp 392.

Renn, N. 1990. A comparison of nematode containing baids with commercially available insecticidal baits for the control of adult *Musca domestica* infetations of intensive animal units. Brighton Crop Protection Conference-Pests and Diseases. 3-15.

Renn, N., Barson, G. and Richardson, P.N. 1985. Preliminary laboratory tests with two species of entomophilic nematodes for control of *Musca domestica* in intensive animal units.*Ann. Appl. Biol.* 106: 229-233.

Rizzo, D.C. 1977. Age of three dipteran hosts as a factor governing the pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae. J. Invertebr. Pathol.* 30: 127-130.

Rupes, V. Ryba, J., Hanzlova, H. and Weiser J. 1987. The efficiency of beta-exotoxin of Bacillus thuringiensis on susceptible and resistant house fly Proc. Int. Conf. Medical and Veterinary Diperology. Ceske Budejovice. pp 262.

Sebesta, K. Farkas, J. and Horska, K. 1981. Thuringiensin, the Beta-exotoxin of *Bacillus thuringiensis.* 249-281. In: H.D. Burges (edit.) Microbial control of pests and plant diseases 1970-1980. Academic press.

Tanada, Y. and Kaya, H.K. 1993. Insect pathology. Academic press. pp 666.

WHO (1991). Tropical Diseases. Progress in Research, 1989-1990. Tenth Programme Report. UNDP/WORLD BANK/ WHO. pp 135.