HISTORY, USE AND FUTURE OF MICROBIAL INSECTICIDES IN URBAN PEST CONTROL

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Abstract—Microbial (bio) insecticides are products based on living micro-organisms which are pathogenic to insects. These micro-organisms have a long history of study and are considered to have great potential as pest control agents. However, in the past research has concentrated on pests of agriculture and, to a lesser extent, on medical and veterinary arthropods. Pests of the urban environment have been less studied but cockroaches, flies, termites and stored product insects have received recent attention.
Selection of a micro-organism for development as a bioinsecticide is based on the following criteria, which are interlinked.

Mammalian safety. Non-infectivity of vertebrates is a primary consideration, but safety also encompasses toxic and allergenic effects. The safety of formulating ingredients and the mode of application must also be considered.

Host specificity. A host range excluding vertebrates is a natural consequence of primary selection criteria, but within the Arthropoda host range can vary widely. A wide host range increases the market potential, but raises problems with effects on non-target organisms: however, these may be less critical in the urban environment than in agriculture.

Ease of production. Some agents (viruses and protozoa, some bacteria) can only be produced *in vivo* and this is very expensive, although cell culture systems are under development and could reduce costs. Others (some bacteria, fungi and nematodes) can be produced using conventional fermentation technology. Production of the desired life stage, eg the conidium in entomopathogenic fungi, may require additional technology.

Ease of formulation and application. These topics are closely linked to production. The production process must generate an active ingredient which is stable and suitable for formulation in acceptable ingredients. The formulation process must generate a stable product which can be applied using approved technology.

Availability of market. Biological pesticides will usually compete with other pest control methods, especially chemical pesticides, and are likely to have the greatest chance of success where limitations on the latter are most severe, for example: where conventional chemicals are withdrawn for safety reasons (eg organochlorines, several organophosphates, methyl bromide); where their use is inimical to other biological methods (eg in the IPM systems in glasshouses); where chemicals are relatively ineffective (eg for some soil pests).

These constraints on the implementation of microbial pesticides will be reviewed and developments in applications for urban pest control will be emphasised.

INTRODUCTION

Microbial insecticides are products derived from microbial pathogens of insects, usually pest species. They rely for their effect primarily on infection and multiplication in the host and therefore by definition they will contain viable infective propagules of the chosen pathogen. *Bacillus thuringiensis* products are the most widely sold; other pathogens which are under development for pest control include viruses, other bacteria, protozoa and fungi. Other arthropods such as mites and ticks and also weeds and plant diseases are targets for microbial pesticides. Entomopathogenic nematodes are usually included in this list and some products are available: nematodes are not microorganisms, but the entomopathogenic species in the Steinernematidae and Heterorhabditidae act as vectors for entomopathogenic bacteria in the genus *Xenorhabdus* and are usually included in reviews of microbial pesticides.

The potential for the use of microbial pesticides has been much reviewed, to the extent that the weight of literature on the subject often appears to exceed the weight of products sold. The enduring interest in microbial pesticides shown by biologists, if not industry, arises from the following observations:

- Many pathogens of invertebrates may cause epizootics in pest populations under natural conditions and therefore appear to offer possibilities for exploitation as pest control agents.
- Many are non-infective to vertebrates and therefore perceived as safe.
- Many are highly specific in their range of infectivity within the invertebrates, raising the hope

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that they could be used more selectively than broad-spectrum chemical pesticides, and thereby avoid some of the well-known problems of induced pest outbreaks and environmental contamination.
- Some can be mass-produced by fermentation methods, raising prospects of economical production technology.

These observations have led to the assumptions that microbial products can be developed, that they will be deployed as substitutes for chemical pesticides and they will be efficacious when so used. The implications of these assumptions, with particular reference to urban pests, are the subject of this paper.

HISTORY

In the history of microbial pesticides, most attention has been focussed on pests of agriculture and on the vectors of human and animal disease. Pests of the urban environment include some from agriculturally important taxa such as beetles, weevils and termites. However, there are many examples of taxa that are primarily or exclusively a problem in the urban environment, for example cockroaches, or those that have a nuisance or public health dimension which is distinct from their agricultural or veterinary importance, for example flies and mosquitoes. This paper will draw on selected examples, especially termites, flies and cockroaches. Some general comments on the main pathogen groups follow. For more information on the major pathogen groups and an exhaustive reference list, see Tanada and Kaya (1993).

Bacteria

A notable exception to the neglect of biological pesticides in the main part of the synthetic chemical era, and the only one of commercial importance, was the development of products based on *Bacillus thuringiensis* (BT). This spore-forming bacterium is abundant in soil and on plant surfaces. The varieties of interest for biological insecticide development produce a crystalline proteinaceous toxin with specific activity to specific taxa of pests, eg var. *kurstaki* and others (lepidopterans), var. *israelensis* (dipterans) and var. *tenebrionis* (coleopterans). The toxin is produced during aerobic fermentation and a range of products is available for treating agricultural, forestry and public health pests. The activity of BT depends primarily on the partial solution of the crystal in the appropriate, usually alkaline, midgut of susceptible insects to produce toxic, protoxin fragments which bind to specific sites on the midgut membrane. This causes membrane leakage and ultimately disintegration of the epithelial cells, leading to bacterial invasion of the haemocoel. Although pesticidal activity is sometimes enhanced by the presence of the spores, these are not necessary for the effective action of BT products, which consist of spores and crystalline toxin and are essentially highly specific insecticidal stomach poisons with no deleterious effects on mammals.

BT products dominate the microbial pesticide market and new strains have been identified recently with claimed activity against other taxa. In addition to the well-established niche for BT products in the control of lepidopteran pests, a major market for *B.t.* var. *israelensis* has been the UNDP/World Bank/FAO/WHO programme in West Africa to control river blindness (onchocerciasis), caused by a nematode vectored by the blackfly *Simulium damnosum* which breeds in fast flowing streams. The use of var. *israelensis* products has been integrated with chemical insecticides for water treatment to control the vector (Becker and Margalit, 1993). Another major market has been for the control of lepidopteran defoliators in Canadian forests.

As might be expected with a specific insecticidal toxin, resistance has already been identified to some BT products, especially var. *kurstaki* where this strain has been intensively used against the diamond back moth (*Plutella xylostella*) in Malaysia (Verkerk and Wright, 1994).

Products based on *Bacillus thuringiensis* (BT) are by far the most successful and widely used microbial pesticides, and yet these are on the margins of the definition used in the Introduction, since their efficacy depends largely on the crystalline toxin produced by the bacterium, rather than multiplication of the living pathogen following infection of the host. BT products also contain viable spores of the bacterium and these may contribute to product efficacy, but non-living BT

products derived from asporogenic mutants have also been effective in the control of mosquitoes in the Upper Rhine valley, where environmental constraints restrict the use of spore-containing materials (Krieg *et al.*, 1986). Thus the mode of action of the most familiar example of a biological pesticide is not typical of microbial pesticides.

Other bacteria which have reached the market include *Bacillus popilliae*, *Pseudomonas fluorescens* and *Serratia entomophila*. *B. popilliae* was formerly sold for the control of the Japanese Beetle (*Popillia japonica*) in the USA. However, this bacterium must be produced *in vivo* to generate infective material and the high cost has contributed to the recent loss of these niche products. Many saprophytic soil bacteria such as *Pseudomonas fluorescens* are antagonistic to pathogenic soil-borne fungi and some products have reached the market for treatment of soil disease. However, commercial impact has been small. A unique example of commercialisation is the bacterial product Invade (R), marketed in New Zealand by Monsanto and based on the highly host-specific bacterium *Serratia entomophila* for control of the indigenous soil-dwelling scarabaeid pest *Costelytra zealandica* (Jackson *et al.*, 1992).

Viruses

The arthropod-infecting viruses which have attracted most attention are the occluded nuclear polyhedrosis (NPV), cytoplasmic polyhedrosis (CPV) and granulosis (GV) viruses of insects. The infectious virus particles are embedded in a proteinaceous matrix, the polyhedral inclusion body (PIB), which is visible under the light microscope. Most have very restricted host ranges and are often genus-specific, sometimes species-specific: a notable exception is the *Autographa californica* ("ACAL") virus which infects a wide range of lepidopteran hosts. Lepidoptera and Hymenoptera (sawflies) are particularly affected by these viruses: they are very infectious and at high pest population densities they can cause spectacular epizootics. However, the requirement for *in vivo* production has restricted commercialisation, although some products based on PIBs purified from field-collected material have enjoyed a brief commercial life. There has been no attempt to exploit viruses for control of weeds or plant disease, although the idea has been suggested. Attentuation of the Chestnut blight epidemic in Europe caused by the fungus *Endothia parasitica* has been attributed to infection of the pathogen with a mycovirus.

Protozoa

Most protozoan infections of insects are chronic but some microsporidian infections can be lethal, especially at high doses. Again, *in vivo* production leads to high cost and only one product has been commercialised. "Nolo Bait" containing spores of *Nosema locustae* has been marketed in the USA for control of grasshoppers, but despite being recommended as part of the USDA IPM programme for grasshopper control (Cunningham, 1992), sales have not been large and the product has only been marketed intermittently. Its efficacy is not undisputed.

Fungi

Bacteria, viruses and protozoa infect via the insect gut and must be used in a manner analagous to chemical stomach poisons. By contrast, fungi infect by penetrating the cuticle and can be used as contact pesticides. Most attention has focussed on the mitosporic genera *Metarhizium*, *Beauveria*, *Verticillium*, *Paecilomyces*, *Nomuraea* and *Hirsutella*, although probably more than 700 species in a very wide range of genera are pathogenic to arthropods. The most-favoured species include *M. anisopliae*, *B. bassiana* and *V. lecanii*, all of which have a very wide host range. Individual genotypes, however, are often much more specific. These fungi can be produced *in vitro* on simple substrates. Submerged fermentation usually results in the production of "blastospores", thin-walled hyphal bodies with rapid germination times, but which are susceptible to rapid desiccation and environmental degradation. The natural infectious unit is the conidium, which is normally produced only in air, although some strains of several of the above fungi have been recorded to produce conidia in submerged culture. Products containing fungal material (conidia or fermentation biomass) have been registered recently for several pests, particularly in the USA, including a cockroach trap, Biopath (R) containing *M. anisopliae* and a product for termite control, Bioblast (R) containing the same fungal strain.

Fungi have also been much discussed for the biological control of weeds, but mycoherbicides have not generally been successful. The product Collego (R), a proprietary formulation of the plant pathogen *Colletotrichum gloeosporioides* var. *aeschynomene* was sold for several years in USA for control of the leguminous weed *Aeschynomene virginica*, but has been withdrawn recently. Several other mycoherbicides based on *C. gloeosporioides* have been developed but commercial uptake has been slow. In the author's opinion, these problems are due at least in part to inadequate efficacy and thus improved formulations are the first requirement for greater success. However, the problems of niche marketing for highly specific products should not be underestimated and it appears the problems that led to Collego (R)'s withdrawal were commercial rather than technical.

Nematodes

Nematodes in the Steinernematidae and Heterorhabditidae carry bacteria of the genus *Xenorhabdus* in their oesophagi. The nematode larvae are free-swimming and infect a very wide range of insects. These larvae require free water in order to swim to and penetrate into the orifices of their insect hosts. In the haemocoel they release the bacterium which kills the insect and the nematodes then feed on the decomposing tissues until the substrate is exhausted, whereupon they are liberated to seek new prey. Their host-seeking ability is a great advantage in pest control, but the requirement for free water is a serious limitation. The infectious larvae can be produced by fermentation and several products are currently on the market for control of pests in high value crops such as glasshouse ornamentals. A product has been commercialised very recently in UK for control of slugs. In some countries nematodes do not fall within regulatory requirements for registration, which greatly reduces the costs of commercialisation; however, this situation may not continue.

Most of these pathogens have been known for many decades. The early history of their use as microbial pest control agents is largely one of over-optimistic attempts to control agricultural pests with a variety of agents selected on the basis of field observations of natural pathogen outbreaks, either by introductions or by augmentations of strains already present. These attempts failed because they did not take into account the passive dispersal of most insect pathogens in pest populations: simply placing pathogen inoculum in a host population does not guarantee establishment, spread and control unless the attempt is coincident with fortunate and rare combinations of favourable environment and high host density (Tinsley and Entwistle, 1974).

Pathogen studies declined with the introduction of chemical pesticides, with the exception of continued research on BT. Reliable BT products appeared in the 1960s following research begun before the second world war. These early products had activity only against lepidopterans, but in the 1970s the dipteran-active strains of var. *israelensis* were discovered and marketed for control of mosquitoes and blackflies.

At the same time that more effective BT products were becoming available, a renewed interest occurred in other pathogens, based on the increasing problems arising from chemical pesticide use: environmental damage, pesticide-induced pests, pesticide resistance and public concern. The emphasis was on developing bioinsecticide products for specific agricultural, horticultural and disease vector targets. Several products have been launched since the 1970s and a representative selection chosen to illustrate the range of pathogens under consideration (excluding BT products) are listed in Table 1.

The Biopath (R) trap is included in this list as the most well-known example of a bioinsecticide developed for the control of an urban pest. This product was marketed through supermarkets in USA but is not commercially available in 1996 (D. Miller, pers. comm.). Further discussion of the use of pathogens for control of termites and flies follows.

SOME URBAN PEST CASE STUDIES: TERMITES AND FLIES

Termites

Lund (1971) reviewed microbial control of termites, but found very little to report. Hanel and Watson (1983) achieved some control of termites in Australia by artificially infesting nests with a strain of the fungus *Metarhizium anisopliae*. The strain chosen was from a heterologous host

Table 1.

Name (R)	Pathogen	Target	Status
Nolo Bait	*Nosema locustae*	Melanopline grasshoppers	Available in USA
Vertalec	*Verticillium lecanii*	Glasshouse aphids	Available in some European countries
Mycar	*Hirsutella thompsonii*	Citrus rust mite	USA: withdrawn
Biopath	*Metarhizium anisopliae*	Cockroaches	Formerly available in USA
Doom	*Bacillus popilliae*	Japanese beetle	USA only: no longer available
Invade	*Serratia entomophila*	*Costelytra zealandica*	Available in New Zealand only
Virox	*Neodiprion sertifer* Nuclear Polyhedrosis Virus	Sawflies (forestry)	Uncertain
Nemasys	*Steinernema bibionis*	Vine weevils	Available in UK
Nemaslug	*Phasmorhabditis hermaphrodita*	Slugs	Available in UK

(a cercopid) and may not have been of high virulence (Staples and Milner, 1996). More recent work has been carried out by CSIRO, Australia (Staples and Milner, 1996) and EcoScience Corporation (USA) (Miller, 1994). Staples and Milner concluded that there was little prospect for the use of pathogens other than fungi. Certain mound-building and tree-inhabiting species, which are important structural pests in Australia, were amenable to control by direct applications of *M. anisopliae* to the nests, but indirect application via feeding sites has not been successful. Termites are repelled by the live conidia, which may form the basis of a repellent treatment, especially for termites which are pests of agricultural crops.

Both CSIRO and EcoScience Corporation have obtained patents covering their termite control methods. The CSIRO patent claims a method for blowing pure spores of *M. anisopliae* into the termite nest and the use of *M. anisopliae* spores as a termite repellant. The EcoScience Corporation product is Bioblast (R).

In addition to *M. anisopliae*, two species of the very little-studied fungus genus *Cordycepioideus* have been recorded from termites in Mexico (Blackwell and Gilbertson, 1981) and Kenya and preliminary studies on infection biology have been carried out in Kenya. Its apparent high host specificity is of great interest, but it is slow-growing in culture and far from commercial development.

Flies

Until recently, the major pathogens of flies were thought to be entomophthoralean fungi, especially *Entomophthora muscae*. These fungi cannot be grown easily in culture and interest in exploiting them for control was therefore limited. Dipteran-active strains of BT do not seem to have useful activity against muscids. The first record of *Beauveria bassiana* on muscids was made in 1990 (Steinkraus *et al.*, 1990) and more recent work has concentrated on strain selection and formulation (Barson *et al.*, 1994; Geden *et al.*, 1996). Adult flies can be killed, but strategic applications may be better directed against larvae because of the slow speed of kill (6 days). Biological insecticides also have a potentially important role in resistance management (Bateman and Thomas, 1996), which is a particularly important aspect of fly control (Barson *et al.*, 1994).

USE OF PATHOGENS IN URBAN PEST CONTROL

The use of pathogens as microbial insecticides presupposes their development as products. Regulatory restrictions as well as practical considerations dictate this approach. However,

pathogens have been exploited for insect control in other ways, for example "classical" biological control. One very successful example of the "classical" control of an insect pest by a pathogen is the use of the Baculovirus of *Oryctes* to control the coconut rhinoceros beetle *Oryctes rhinoceros* in Pacific and Indian Ocean islands. Another is the recent spread of the fungus *Entomophaga maimaga* in the outbreak populations of the gypsy moth *Lymantria dispar* in the NE states of the USA, following its apparent introduction more than 50 years earlier and an unexplained long period of quiescence or undetected activity. Classical biological control can offer stable and enduring suppression of pest populations at no further cost to the beneficiaries. However, such examples of the use of pathogens are rare and the classical approach is probably of little interest for urban pest control because most urban pests are both universally distributed, and little influenced by the presence of naturally occurring natural enemies. Most use of pathogens therefore implies a commercial process with close analogies to the development of a chemical pesticide.

There are three essential criteria for success in the development of a bioinsecticide: *regulatory compliance, technical efficacy* and *commercial viability.* The requirements to meet these criteria will be considered in the following sections.

Requirement 1: Mammalian safety

Non-infectivity of vertebrates is a primary consideration, but safety also encompasses toxic and allergenic effects. Bioinsecticides must be registered for use and most countries insist on compliance with defined requirements for mammalian safety testing (Laird *et al.,* 1990) based on the concept of the maximum challenge test. Under this system, standard protocols for testing are defined, involving infectivity, toxicity and sensitivity (allergenicity and sensitisation) tests at maximum challenge levels. Levels must be set that are appropriate to biological materials, which are usually of negligible toxicity, but, being proteinaceous, may be indiscriminately allergenic at high doses. Doses may not therefore be the same as those found appropriate for chemical pesticides. Preliminary screening for toxicity/allergenicity may be carried out to select the most appropriate strains (Sherwood *et al.,* 1994), but failure to clear a maximum challenge test may not be absolutely contraindicative: labelling restrictions may be appropriate. However, any indication of mammalian infectivity would normally result in rejection of that agent, and indications of mutagenicity/teratogenicity would be likely to lead to very extensive additional requirements for testing.

Most countries have requirements of graded complexity, such that small scale field testing (<10 ha in Canada) by trained personnel requires the least regulation, leading to additional requirements for larger-scale tests (up to 1000 ha) and then registration. Canada also imposes additional restrictions according to the concept of the ecozone. Organisms from within Canada, but outside the particular ecozone in which testing is to be carried out, will require more stringent tests.

The USA was the first country to give serious consideration to the special requirements for regulating microbial products and as a result the USA legislation is probably the most enlightened. This is reflected in the large number of products now registered there. Canada does not yet set requirements for registration, only guidelines, and encourages potential producers of bioinsecticides to engage in dialogue with regulators throughout the registration process. In the EU, bioinsecticides are still covered by Directive 91/414/EEC, despite promise of specific legislation. At present, responsibility for the issue of experimental permits still rests with individual member states, but Directive 91/414/EEC requires any individual member state wishing to register a new material to prepare a dossier for submission to all other member states and the EU, which, if it accepts the dossier, will place the material on the positive list of active ingredients.

The safety of formulating ingredients and the mode of application must also be considered as part of the regulatory process. As with the non-target data, failure to "clear" a test may only result in a labelling restriction. In the case of pests in the urban environment, for example, a lack of host specificity might not be seen as a serious impediment, since urban pests such as cockroaches are not controlled by natural enemies and the product is likely to be used in an environment where other beneficial organisms are not threatened. However, potential allergenicity of the active ingredient and formulating ingredients might assume even higher priority in the urban environment where the product is used in close proximity to or within human habitation.

Requirement 2: Host specificity

A host range excluding vertebrates is a natural consequence of primary selection criteria, but within the Arthropoda host range can vary widely. Host specificity data are required by regulatory authorities for microbial pesticides. There is a fundamental dilemma for both manufacturers and regulators concealed in this requirement: specificity is desirable from the environmental perspective, so that non-target and beneficial organisms are not harmed, but such specificity limits the market for the product, to the extent that it may not be economically viable. End-users often want to treat a variety of pests with a single application, which is not possible if the product is very specific. This situation may not apply in the case of urban pests, where treatments are often required for a single, specific pest problem, such as cockroaches, termites or flies.

In the past, the regulatory requirement has usually been to test a few key beneficial organisms such as honey bees and silkworms and a small selection of non-target organisms (eg a fish, a crustacean, an alga, etc). More recently, Canadian regulators have adopted the concept of centrifugal testing, as first proposed for testing biological control agents for weed control (Wapshere, 1975), whereby the closest taxonomic relatives are tested first, followed by those increasingly taxonomically distant from the target, in an attempt to determine the taxonomic limits of host range. This requires an agreed taxonomy, which is not always available, and it is not clear if any bioinsecticides have been tested rigorously using this principle.

Requirement 3: Ease of production

Some agents (viruses and protozoa, some bacteria such as *Bacillus popilliae*) can only be produced *in vivo* and this is very expensive. Registration also requires rigorous quality control on such aspects as product contamination and standard potency and these can be difficult to achieve with *in vivo* systems. Cell culture systems are under development, especially for viruses, and could reduce costs. Others agents (some bacteria, fungi and nematodes) can be produced using conventional fermentation technology. It is important to recognise the distinction between the production of fermentor biomass and the production of infective propagules. Most fermentation technology has been developed to optimise vegetative growth. However, the infective propagules (spores of fungi and bacteria) are often produced only when vegetative growth ceases and the organism switches to the reproductive phase. The production of nematodes using this technology is particularly complex, since conditions must be optimised for two organisms, the nematode and its symbiotic bacterium. The latter is unstable and can easily revert to a non-virulent form.

Production of the desired life stage of some fungi, the conidium, may require additional technology, because conidia are often produced only at the interface with air. Thus the fermentor biomass must be subjected to a second phase during which conidiation will take place. Some strains of fungi including *Metarhizium flavoviride*, *Hirsutella thompsonii* and *Beauveria bassiana* will produce conidia in submerged (single phase) culture, but these propagules may have different physical properties to aerial conidia (eg hydrophilic rather than hydrophobic spore coats) and these can affect formulation properties (Jenkins and Prior, 1993).

Regulatory requirements for registration may include details of the production process. In such cases it will thus not be possible to register a product before it is in production, although this has happened in the USA, where the fungus *Lagenidium giganteum* is registered for use against mosquitoes, but no product yet exists.

Requirement 4: Ease of formulation and application

Bateman (1994) has emphasised that these topics are closely interlinked with each other and with production. The production process must generate an active ingredient which is stable and suitable for formulation in acceptable materials. The formulation process must then generate a stable product which can be applied using approved technology.

These requirements are well exemplified by recent work on the development of *Metarhizium flavoviride* as a ULV spray for locust and grasshopper control. The active ingredient is conidia of the fungus, suspended in oils of suitable viscosity for application using controlled-droplet

applicators at ULV rates of $5x10^{12}$ conidia/ha in 1–2 l/ha. Conidia are produced in chains on solid substrates at an initial moisture content of approximately 30%, but freshly-harvested conidia are not stable in storage (Moore *et al.*, 1996). The extraction process must separate the conidia as single particles from the substrate, extract the conidia while leaving out any substrate material and, either during extraction or subequently, reduce the moisture content to 5% without affecting viability. The active ingredient is a spore powder to a tightly defined technical specification of moisture content, purity and particle size (Bateman, 1994).

In the urban environment, many formulations and application technologies are possible and it is in this area that ingenuity is likely to be best rewarded. Baits and bait stations in the form of traps are of great interest, particularly in view of the commercial availability of the Biopath (R) cockroach trap marketed until recently in USA by EcoScience Corporation. This trap incorporates a feeding attractant for cockroaches and a living culture of *Metarhizium anisopliae* and is placed in the environment of *Blattella germanica*: cockroaches are attracted to the trap and in the process of walking through it are contaminated with a dose of the fungus. They die several days later, often in humid places where the fungus can sporulate on the cadaver and therefore spread infection to other cockroaches. EcoScience Corporation have obtained a trademark for the term Horizontal Transfer (TM), the process whereby the fungus is transmitted horizontally from one infected insect to another of the same population (Miller, 1994). More recently, the company has withdrawn this product and is experimenting with traps containing oil-based formulations of dried fungal conidia in place of the actively growing culture. The new product is expected to reach the market in 1997 (D. Miller, pers. comm.).

Dusts are of more limited general appeal because of the contamination of the human environment and the possible allergenic effects of inhalation. However, they have promise against termites. Milner and Staples (1996) have shown that a pure, dry powder of *Metarhizium anisopliae* spores is effective when blown into termite nests. The treatment is most effective when the nests are clearly defined and subterranean nest building termites are harder to control. The treatment has been used successfully in Australia and some Pacific Islands (against coconut termites) by CSIRO and in Brazil. In USA, EcoScience Corporation has commercialised "Bioblast", a *Metarhizium* spore powder for termite control. Both CSIRO and EcoScience Corporation have patents published on this method of termite control.

Requirement 5: Availability of market

Biological pesticides will usually compete with other pest control methods, especially chemical pesticides, and are likely to have the greatest chance of success where limitations on the latter are most severe, for example: where conventional chemicals are withdrawn for safety reasons (eg organochlorines, several organophosphates, methyl bromide); where their use is inimical to other biological methods (eg in the IPM systems in glasshouses); where chemicals are relatively ineffective (eg for some soil pests). Since urban pests may be nuisance pests rather than causes of direct economic loss, the sale of microbial products for their control may depend not so much on the actual cost-benefits of control as on less quantifiable criteria such as public pressure for the use of non-toxic pesticides in the human environment. Two major markets for BT products have arisen because of external constraints on the market for chemical control: the onchocerciasis control programme in West Africa adopted BT under the auspices of the United Nations and lepidopteran pests in Canadian forests are treated with BT because of restrictions on the use of cheaper and effective chemicals in the face of environmental concerns.

FUTURE OF BIOINSECTICIDES FOR URBAN PEST CONTROL

There are currently optimistic predictions for an expansion of the whole market for bioinsecticides (Marrone, 1996), but this expansion will necessarily occur from a very small base, because bioinsecticides at present account for only a tiny fraction of the world pesticide market. Three criteria were proposed earlier as essential for success: technical efficacy, regulatory compliance and commercial viability. Of these, regulatory compliance is demonstrably achievable

and will probably not limit success in the long term, though it may become more difficult (Goettel, 1996). However, technical efficacy must be adequate before any enduring commercial success can be achieved and expansion of the market will depend initially on demonstrating better efficacy. Goettel (1996) disputes that regulators should protect consumers from non-efficaceous products. Efficacy data are not required to register a product in the USA, the country where most products have been registered and also the country where the most products have failed. Efficacy data are required for registration in most other countries and in this author's opinion, such a requirement is desirable to establish long-term consumer confidence in biological pesticides.

A truly efficacious product could compete effectively in some current markets even at a greater cost than conventional chemicals because there is strong pressure to move away from synthetic insecticides to a "greener" product. However, end-users are most unlikely to accept that biological materials can be as efficacious as general chemical poisons if the comparison is made using conventional indicators such as speed of kill. Therefore some perceptual shifts are needed for wider uptake to occur, particularly in the proportion killed by the initial application and in the speed of kill.

Proportion killed

Typical dose-response slopes for pathogens are much shallower than for chemical pesticides, which means that the difference in dose between the LD50 and the LD95 is very large and may not even be achievable under field conditions (Bateman *et al.*, 1993). Thus bioinsecticides may kill a lower proportion of the pest population than chemicals, simply because insufficient dose can be applied. Improvements to formulation and application technology may increase the proportion of the population that can be killed by a realistic dose (Bateman *et al.*, 1993). Nevertheless, there may be pest control situations where it will never be possible to kill an acceptably high level of the population with the initial application.

However, unlike chemical pesticides, microbial insecticides may have additional effects on pest populations by subsequent multiplication in the initially infected individuals and re-infection of further members of the population (Bateman and Thomas, 1996; Thomas *et al.*, 1995). This cycling in the population has been well recognised by epidemiologists and is the basis of EcoScience Corporation's Horizontal Transfer (TM) mode of action for their Biopath (R) cockroach trap.

There are both biological and social issues involved in the debate over efficacy and the acceptance by consumers of a product which at first inspection seems less effective than the chemical pesticides to which they are accustomed. Complete elimination of urban pests may be desired by clients, but is seldom feasible. Elimination of nuisance pests such as cockroaches is thought desirable and may be achievable with chemical methods, but premises must be maintained pest-free once this is achieved and this requires constant vigilance. The alternative, which may be more realistic, is management of the population, accepting a low level of incidence. It will require a higher standard of monitoring and intervention to maintain the population at the accepted, non-pest level, but this cost of abandoning persistent chemical poisons cannot be avoided.

Speed of kill

Most biological pesticides act slowly compared to chemical poisons. BT kills within 24 h, most fungi and viruses take a week. There are no biological equivalents to conventional insecticides which kill within minutes. However, the requirement for rapid kill may not be an impossible perception to change among many clients. Professional control operators have used slow-acting poisons against rodents for many years with success and the public is able to accept such a time-lag in efficacy. Similarly with termites, the effects of chemical treatment are not instantaneous. Bateman and Thomas (1996) have argued that managing the slow speed of kill is one of the challenges facing the biopesticide industry and some urban pests seem very amenable this challenge: for example cockroaches, termites and stored product pests must all be managed in the long-term and a high initial speed of kill is not essential for consumer acceptance.

Urban pests have a number of features which favour control strategies which use biological insecticides:

- they are pests in the human environment where the perceived hazards of chemical pesticides are of particular concern to the public;
- they are often controlled in isolation from other pests, making them favourable for agents with high specificity;
- they often do not require a high speed of kill, but the prospect of safe, residual action would be very attractive and both these are aspects of the mode of action of biological insecticides;
- the market for some urban pests is large and stable enough to attract commercial interest.

These features should encourage future investment in biological insecticide development. Improvements to efficacy through formulation and application, and reductions in the cost of production, remain major research challenges. In order to improve efficacy while retaining the desired host range, there is also a need to investigate additional strains and species of pathogens. For example, there are no published records of *Metarhizium* spp. isolates from cockroaches, but the screening programme carried out by EcoScience Corporation using isolates from a wide variety of sources revealed a strain with high virulence suitable for commercial development. Since only a small fraction of the potentially available germplasm of most pathogens has been screened, even in the case of well-studied taxa such as *M. anisopliae*, there is clearly scope for the discovery of additional pathogen genotypes with desirable virulence and host range characteristics.

REFERENCES

Barson, G., Renn, N. and Bywater, A.F. (1994). Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. *Journal of Invertebrate Pathology* 64, 107–113.

Bateman, R.P. (1994). Physical properties and atomisation of ULV formulations of myco-insecticides. In Smits, P.H. (ed.) Microbial control of pests. *Fourth European Meeting of the IOBC WPRS. IOBC WPRS Bulletin* 17, 189–192.

Bateman, R.P. and Thomas, M. (1996). Pathogen application against locusts and grasshoppers: insecticide or biological control. *Antenna* 20, 10–15.

Bateman, R.P.,Carey, M., Moore, D. and Prior, C. (1993). The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology* 122, 145–152.

Becker, N. and Margalit, J. (1993). Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies. Pp. 147–170 in Entwistle, P.F., Cory, J.S., Bailey, M.J. and Higgs, S. (eds.) *Bacillus thuringiensis*, an environmental pesticide: theory and practice. John Wiley and Sons, UK.

Blackwell, M. and Gilbertson, R.L. (1981). *Cordycepioideus octosporus*, a termite suspected pathogen from Jalisco, Mexico. Mycologia 73, 358–362.

Cunningham, G.L. (1992). APHIS; grasshopper integrated pest management in the United States—a co-operative project with emphasis on biological control. Pp. 21–25 in: Lomer, C.J. and Prior, C. (eds.) Biological control of locusts and grasshoppers. CAB International, UK.

Geden, C.J., Rutz, D.A. and Steinkraus, D.C. (1995). Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. *Biological Control* 5, 615–6221.

Goettel, M. (1996). Editorial. New European Community regulations for microbials: a unique opportunity to learn from past experience and mistakes. *Society of Invertebrate Pathology Newsletter* 28 no. 1, 6–7.

Hanel, H. and Watson, J.A.L. (1983). Preliminary field tests on the use of *Metaharhizium anisopliae* for control of *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). *Bulletin of Entomological Research* 73, 305–313.

Jackson, T.A., Pearson, J.F., O'Callaghan, M.O., Mahanty, H.K. and Willcocks, M.J. (1992). Pathogen to product—development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub *Costelytra zealandica*. Pp. 191–198. In: Glare, T.R. and Jackson, T.A. (eds.) Use of pathogens in scarab pest management. Intercept Ltd, UK.

Jenkins, N.E. and Prior, C. (1993). Growth and formation of true conidia of *Metarhizium flavoviride* in a simple liquid medium. *Mycological Research* 97, 1489–1494.

Krieg, W., Jaeger, K.F., Parg, A., Delzer, J. and Weiser, H. (1986). Biological evaluation of an asporogenic mutant of *Bacillus thuringiensis* serovar. *israelensis*. P.553 in Samson, R.A., Vlak, J. and Peters, D. (eds.) Fundamental and applied aspects of invertebrate pathology. Published by the Foundation of the Fourth International Colloquium of Invertebrate Pathology, P.O. Box 8045, 6700 EM Wageningen, The Netherlands.

Laird, M., Lacey, L.A. and Davidson, E.W. (eds.) (1990). Safety of microbial insecticides. CRC Press Inc., USA.

Lund, A.E. (1971). Microbial control of termites. Pp. 385–386 in: Burges, H.D. and Hussey, N.W. (eds.) Microbial control of insects and mites. Academic Press, London.

Marrone, P. (1996). Guest editorial: it's a great time to start an Ag Biotech company. Society of Invertebrate *Pathology Newsletter* 28 no. 1, 5–6.

Miller, D.W. (1994). Formulation and application of entomopathogenic fungi. Proceedings of the Sixth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, 28 August–2 September, 1995, p. 315. Society of Invertebrate Pathology.

Moore, D., Douro-Kpindou, O.K., Jenkins, N.E. and Lomer, C.J. (1996). Effects of moisture content and temperature on storage of *Metarhizium flavoviride* conidia. *Biocontrol Science and Technology* 6 (in press).

Sherwood, R.L., Johnson, W.D. and Jaronski, S.T. (1994). Acute pulmonary and intraperitoneal toxicity/infectivity and ocular irritation assessment of *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae* and *M.*

flavoviride isolates. P. 116 in Abstracts of the Sixth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, France 28 August–2 September 1994. Society of Invertebrate Pathology, USA.

Staples, J.A. and Milner, R.J. (1996). Biological control of termites: results and experiences within a CSIRO-Project in Australia. *Biocontrol Science and Technology* (in press)

Steinkraus, D.C., Geden, C.J., Rutz, D.A. and Kramer, J.P. (1990). First report of the natural occurrence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica* (Diptera: Muscidae). *Journal of Medical Entomology* 27, 309–312.

Tanada, Y. and Kaya, H.K. (1993). Insect pathology. Academic Press, Inc. San Diego, USA.

Thomas, M.B., Wood, S.N. and Lomer, C.J. (1995). Biological control of locusts and grasshoppers using a fungal pathogen: the importance of secondary cycling. *Proceedings of the Royal Society of London*, B. 259, 265–270.

Tinsley, T.W. and Entwistle, P.F. (1974). The use of pathogens in the control of insect pests. Pp. 115–129 in Price Jones, D. and Solomon, M.E. (eds.) Biology in pest and disease control. Blackwell Scientific Publications, Oxford.

Verkerk, R.H.J. and Wright, D.J. (1994). The potential for induced extrinsic host plant resistance in IRM strategies targeting the diamondback moth. Proceedings of the Brighton Crop Protection Conference—Pests and Diseases 1994, pp. 457–462.

Wapshere, A.J. (1975). A protocol for programmes for biological control of weeds. (PANS) 21, 295–303.