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Pathogens for the Control of Insects: Where Next? [and Discussion]

C. C. Payne, M. Hafez, K. Jones, I. Harpaz, A. E. Akingbohungbe, R. R. M. Paterson and S. M. K. Hag Ahmed

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Pathogens for the control of insects: where next?

By C. C. Payne

Institute of Horticultural Research, Worthing Road, Littlehampton, West Sussex BN17 6LP, U.K.

Insect populations succumb to a variety of infections caused by pathogenic microorganisms (bacteria, fungi, Protozoa) and viruses. The narrow host-range of many of these agents makes them natural candidates for use within integrated pest management systems. Some, such as *Bacillus thuringiensis* and several baculoviruses, may be applied to crops at regular intervals as microbial pesticides, achieving short-term control of a pest population. Longer-term suppression of insect populations requires some degree of persistence of the pathogen in the target host population. Examples of sustained, natural, insect population regulation by microorganisms are rare; regulation demands stable ecosystems and a capacity for the pathogen to spread.

We cannot ignore the fact that many of the microbial pathogens available today fail to meet the expectations of an agricultural industry used to the rapid and broad-spectrum pest knockdown achieved by many chemical pesticides. Despite the many advantages to be gained in selective pest management from the use of naturally occurring strains of insect pathogens, much recent attention has focused on the improvement of strains by genetic manipulation. Significant advances have already been made in the manipulation of bacterial and viral pathogens to increase virulence and modify host range. The environmental persistence of the insect-pathogenic toxin of *B. thuringiensis* has also been extended by inserting the toxin gene into other bacterial hosts and plants. Exciting future opportunities for biological control may be created by such strategies. However, to make responsible use of these manipulated organisms we must understand more about their long-term impact on insect populations and the environment. Such information should come not only from detailed ecological studies of the host–pathogen interaction but also from laboratory and field studies of the frequency and consequences of genetic exchange between modified strains and naturally occurring microorganisms.

1. Introduction

The basic concept of using microorganisms to control insect pests is long-standing with many, largely unsuccessful, attempts in the nineteenth century to exploit fungal diseases for the control of economically important pests (reviewed in Miller *et al.* (1984)). It is now known that a wide range of naturally occurring pathogens of insects (e.g. bacteria, viruses, fungi and Protozoa) can be used as highly selective pest control agents. The development of resistance by certain key pests to chemical pesticides and the increasing production costs for new compounds are encouraging greater interest and investment in such biological control agents. In addition, increased awareness of the adverse environmental consequences of using certain toxic chemical pesticides is further enhancing the desire to control pests with agents that have no damaging effects on non-target species.

Despite these pressures, microbial pest control agents at present form a very small part (less than 1%) of total pesticide sales (Jutsum, this symposium). It is my aim in this paper to

examine the reasons for this and to consider the future use of insect pathogens by methods that could increase their contribution to new integrated pest management systems. To achieve this aim, it is necessary first to consider the properties of pathogens that are currently available.

2. The current status of insect pathogens as biocontrol agents

(*a*) *Bacteria*

Although about 100 insect-pathogenic species of bacteria have been identified, only certain *Bacillus* spp. have shown clear potential as insect-specific pest control agents (Lüthy 1986*a*). Of these, *B. thuringiensis* (*B.t.*), *B. sphaericus* and *B. popilliae* have received most attention. *B.t.* is the only microbial pest control agent that has been commercialized world-wide on a large scale. It is an aerobic, spore-forming bacterium, easy to grow on many media, including those composed of cheap waste products of the fish- and food-processing industry (Lüthy 1986*a*). At sporulation, the bacterium produces both a spore and a large proteinaceous crystal. When the protein crystal is ingested by certain insects, it dissolves in the gut juices and is degraded by proteases to release toxic polypeptides. In the simplest situation, the protein crystal (δ-endotoxin) consists of protoxin molecules with a relative molecular mass (M_r) of about 130000, which are degraded in the insect gut to a protein toxin with an M_r of about 60000. Studies with one strain of *B.t.* var. *kurstaki*, active against Lepidoptera, have suggested that the toxin binds to glycoproteins in the plasma membrane of larval gut epithelial cells, generating small pores in the membrane and destroying regulation of ion exchange (Eller *et al.* 1986). Simultaneously, the muscles of gut and monthparts are paralysed, feeding stops, and death occurs 30 min to 3 days after ingestion (Burges 1986*a*).

Many strains of *B.t.* have now been isolated and classified within more than 20 different varieties by serological techniques. On the basis of their potency for insects, Ellar *et al.* (1986) have grouped these varieties into at least five pathotypes: (*a*) lepidopteran-specific (e.g. var. *thuringiensis*); (*b*) dipteran-specific (e.g. var. *israelensis*); (*c*) coleopteran-specific (e.g. var. *tenebrionis*); (*d*) those active against both Lepidoptera and Diptera (e.g. var. *aizawai*); (*e*) those with no toxicity recorded in insects (e.g. var. *dakota*). Within each of these pathotypes there are marked differences in both specificity and potency.

Recent intensive research on the molecular biology and genetics of *B.t.* toxins is providing insights into the reasons behind the specificity differences. It has been confirmed that the crystal protoxins are single gene products and that the genes are located in many *B.t.* strains on plasmids (Kronstad *et al.* 1982). From the DNA sequence of the protoxin gene from the HD1-Dipel strain of *B.t.* var. *kurstaki* the amino acid sequence has been deduced (Schnepf *et al.* 1985). Comparison of this gene with other cloned protoxin genes from vars. *kurstaki*, *thuringiensis* (= *berliner*), *sotto* and *alesti* has revealed considerable similarities between the deduced structures of the protoxin molecules. In the total of approximately 1170 amino acids, *ca.* 280 at the N-terminus and *ca.* 400–600 at the C-terminus are highly conserved (Ellar *et al.* 1966; Lüthy 1986*b*; Adang *et al.* 1987). The region between these conserved areas, particularly between residues 340 and 617, shows considerable variability in amino acid sequence (Adang *et al.* 1987). The insecticidal activity of the toxin itself (*ca.* 60000 M_r) is located towards the N-terminus and includes most of the conserved N-terminal region and much of the variable region (Lüthy 1986*b*; Adang *et al.* 1987).

Although the relation between toxin structure and biological specificity needs further

scrutiny, variation in potency between different strains could be partly accounted for by differences in the variable region of the toxin. In addition, some *B.t.* strains have been shown to contain more than one distinct toxin gene (three in *kurstaki* HD-1) (Adang *et al.* 1987) with up to five recorded in some strains (Carlton 1986). Although insecticidal activity in some insect species may require more than one toxin, some toxins may have dual specificity (Haider *et al.* 1987). These exciting studies go some way towards understanding the wide variations in *B.t.* specificity and potency, and provide the basis for future *B.t.* strain improvement and genetic engineering (see §4).

Despite the existence of many *B.t.* strains, at present very few varieties are commercially available (table 1). *B.t.* var. *kurstaki* HD-1 has been extensively used to control larvae of pest Lepidoptera as it has a relatively broad activity spectrum (Lüthy 1986*a*). It has now been used widely against lepidopterous pests of horticulture, agriculture and forestry (Burges 1986*a*). *B.t.* var. *israelensis* (Goldberg & Margalit 1977) was recognized as having great potency for larvae of biting blackflies and many species of mosquitoes. Urged on by the need to develop alternative methods to control pesticide-resistant blackfly vectors of onchocerciasis in West Africa, this strain was commercialized within six years of its discovery and has made a major contribution to blackfly and mosquito control (Burges & Pillai 1986). Recently, the potential spectrum of activity of *B.t.* has been increased by the discovery of isolates active against beetles (Krieg *et al.* 1983; Herrnstadt *et al.* 1986).

Table 1. Principal bacterial pest control agents

species	target pests	commercial products
Bacillus thuringiensis var. *kurstaki*	caterpillars (Lepidoptera)	e.g. Dipel (Abbott), Bactospeine (Philips Duphar), Thuricide (Zoecon), world-wide use.
B.t. var *aizawai*	waxmoth (*Galleria mellonella*)	e.g. Certan (Zoecon), U.S.A.
B.t. var. *israelensis*	mosquitoes and blackflies	e.g. Vectobac (Abbott), Bactimos (Philips Duphar), Teknar (Zoecon), world-wide use
B.t. var. *tenebrionis* *B.t.* var. *san diego*	beetles	in development
Bacillus sphaericus	mosquitoes	in development
Bacillus popilliae	Japanese beetle (*Popillia japonica*)	e.g. Doom (Fairfax Biological Laboratory), Milky Spore (Reuter Labs), U.S.A.

Although *B.t.* is known to cause natural epizootics in enclosed environments (e.g. granaries), it is otherwise not a highly infectious natural pathogen as it generally fails to spread. This means that it must be used as a microbial pesticide, with regular applications needed for adequate pest control. Because it has no contact action and must be ingested to be effective, it should be applied to sites (and at ambient temperatures) where larvae are feeding. In most susceptible species, ingestion of the toxin crystal alone seems sufficient to ensure death. However, efficacy in some insects (e.g. the wax moth, *Galleria mellonella*) is dependent on the presence of at least some *B.t.* spores in the ingested material (Li *et al.* 1987); most commercial products of *B.t.* contain a mixture of spores and crystals. Like other microbial agents applied in the field, the bacterial spore is inactivated quite rapidly by the ultraviolet (UV) component

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of sunlight. Although active crystal toxin will persist on foliage for longer than spores, its half-life may be only about 8 days (Kirschbaum 1985).

Although *B.t. israelensis* is very active against larvae of the Diptera *Anopheles*, *Culex*, *Aedes* and *Simulium*, another bacterium, *Bacillus sphaericus*, is more active against *Culex* and some *Anopheles* spp. *B. sphaericus* is an aerobic, spore-forming bacterium common world-wide in soil and aquatic environments. Like *B.t.*, some *B. sphaericus* strains produce a crystalline protein toxin (*ca.* 40000 M_r) from a putative protoxin precursor of 125000 M_r (Baumann *et al.* 1986). After a mosquito larva ingests the toxin, its gut epithelium distends and the gut is paralysed, followed by cell lysis and larval death (World Health Organization (WHO) 1985). Unlike *B.t.*, some strains of *B. sphaericus* are able to grow saprophytically in heavily-polluted water, providing opportunities for the maintenance or increase of inoculum in the absence of the insect host (WHO 1985).

In contrast to *B.t.* and *B. sphaericus*, *Bacillus popilliae* (isolated from the Japanese beetle *Popillia japonica*) does not produce toxins. It also spreads naturally, and persists even in low density host populations. Spores applied to pastures in the U.S.A. during the 1930s promoted disease in the Japanese beetle population that was still present 25–30 years later. After larvae ingest bacterial spores the progress of the disease is slow, leading eventually to large cadavers packed with spores that are very persistent in the soil. The bacterium is an obligate pathogen and it has not proved possible to produce spores adequately *in vitro*. Two small companies in the U.S.A. produce and market bacterial spores grown in larvae (Klein 1981, 1986).

(*b*) *Viruses*

More than 1600 virus isolates have been recorded causing disease in about 1100 species of insects and mites (Martignoni & Iwai 1986). These viruses can be grouped into several categories on the basis of their morphological and biochemical properties (Payne & Kelly 1981). Among these groups, only baculoviruses have been given extensive consideration as microbial control agents. They share no obvious biochemical properties with viruses of vertebrates, plants or microorganisms, and present isolates are restricted in their host range to a small number of insect orders (principally Lepidoptera and Hymenoptera) and a few crustaceans and mites. Many individual isolates are genus- or species-specific and are often highly virulent. Being obligate intracellular parasites they can be produced only in larvae or insect cells in culture.

Baculoviruses are large, rod-shaped, enveloped, DNA-containing viruses. They are currently classified into three subgroups on the basis of morphological properties. In two groups, granulosis (GV) and nuclear polyhedrosis (NPV) viruses, virus particles are packed within large proteinaceous occlusion bodies (OB) produced late in infection, whereas viruses in a third group (non-occluded baculoviruses) do not produce these OBs. The OBs are stable and provide the means by which virus infectivity is preserved outside the host. Diseased larvae can release up to 10^{10} NPV OBs, or well in excess of 10^{11} of the smaller GV OBs, when they die. Under suitable conditions these can promote the rapid spread and maintenance of infection within susceptible insect populations. None the less, the hopes that epizootics leading to long-lasting control can be achieved by a single application of a baculovirus have not proved realistic, except for a few special cases such as the control of the coconut rhinoceros beetle, *Oryctes rhinoceros* (Bedford 1980). In general, adequate pest control has been achieved only when these viruses have been applied repeatedly (Benz 1981).

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Virus infection in susceptible insects takes place only after larvae eat food contaminated with virus. In GV and NPV infections, the matrix protein of the OBS dissolves in the insect gut, releasing virus particles that infect and multiply in gut epithelial cells. In Lepidoptera, the infection quickly spreads to other tissues, whereas in Hymenoptera (e.g. sawflies), the infection is confined to the gut. Young larvae are consistently more susceptible to virus infection than older larvae (Payne 1982). None the less, death of an insect as a direct consequence of virus infection is unlikely to occur less than 3–4 days after infection, even with the most virulent of currently available virus strains.

As with all biological control agents, several factors influence the efficacy of a virus applied as a microbial insecticide. The most important of these are the deleterious effects of UV radiation from sunlight and adverse plant-surface effects (Entwistle & Evans 1985). Although many baculoviruses are highly host specific and may seem ideal candidates for selective control, pest problems on a single crop can rarely be solved by the application of one highly selective agent because most crops are attacked simultaneously by several different species of Lepidoptera. There is, therefore, considerable interest in some baculoviruses with a less-selective host range. An NPV from the alfalfa semilooper, *Autographa californica* (ACMNPV) has attracted most research, with a recorded host range of 43 species of Lepidoptera in 11 families, many of which are pest species (Payne 1986). ACMNPV is the most extensively characterized insect virus, and recent research has identified prospects for strain improvement of this and related baculoviruses through genetic manipulation, even though the biological bases of variation in host-range and virulence are not yet understood.

At the time of writing, only two baculoviruses are available as commercial products. These are *Neodiprion sertifer* NPV (for the control of pine sawfly) and *Mamestra brassicae* NPV, which infects the cabbage moth and some key noctuid pests of food and fibre crops. Other baculoviruses have attracted, or continue to attract, research and industrial interest (table 2). These include the baculovirus of *O. rhinoceros* which has been successfully introduced into many countries in the South Pacific region for the long-term suppression of rhinoceros beetle (Bedford 1980). The potential use of viruses for the control of forest pests has received much attention. Here, the high economic thresholds of pest damage that can be tolerated allow greater scope for viruses to exert their control over a longer time period than can be permitted on high value agricultural crops.

(*c*) *Fungi*

There are approximately 100 genera of fungi that contain numerous species pathogenic to insects (Hall & Papierok 1982; Zimmermann 1986). Of these, deuteromycete fungi (table 3) have received most attention as they are among the easiest to produce *in vitro* and several have a broad host range. *Metarhizium anisopliae*, for example, has more than 200 known hosts among Coleoptera, Lepidoptera, Orthoptera and Hemiptera, whereas *Beauveria* spp. have been identified from about 500 host species, principally Lepidoptera and Coleoptera (Hall & Papierok 1982). However, this broad view disguises the fact that individual strains of the same fungal species often have different host ranges or pathogenicities.

The infective propagule in natural deuteromycete infections is the conidiospore. Although a few strains enter the host through the gut or through the respiratory tract, the majority invade insects through the cuticle. Because fungi do not, in general, have to be ingested to be effective, they have potential (unlike *B.t.* or viruses) for the control of sap-feeding arthropods which do not ingest pathogens on the plant surface.

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Table 2. Principle baculovirus candidates for insect pest control

virus	target hosts	crop	status of commercial development
nuclear polyhedrosis viruses			
Anticarsia gemmatalis NPV (Soybean looper)	*A. gemmatalis*	soybean	local production, Brazil
Autographa californica NPV (Alfalfa semilooper)	*Orgyia pseudotsugata* *Trichoplusia ni*	forests cabbage	former commercial trials product, USA.
Gilpinia hercyniae NPV (spruce sawfly)	*G. hercyniae*	forests	—
Heliothis spp. NPV (cotton bollworm)	*Heliothis* spp.	cotton, maize, sorghum	former commercial product (Elcar), U.S.A.
Lymantria dispar NPV (gypsy moth)	*L. dispar*	forests	produced by U.S. Forest Service
Mamestra brassicae NPV (cabbage moth)	*Mamestra*, *Heliothis* and *Diparopsis* spp.	cotton, cabbage and other vegetables	produced commercially in France (Mamestrin)
Neodiprion sertifer NPV (pine sawfly)	*N. sertifer*	forests	produced commercially in U.K. (Virox) and Finland (Monisarmio virus)
Neodiprion lecontei NPV (redheaded pine sawfly)	*N. lecontei*	forests	produced by Canadian Forest Service
Orgyia pseudotsugata NPV (Douglas fir tussock moth)	*O. pseudotsugata*	forests	produced by U.S. (TM–Biocontrol 1) and Canadian Forest Service (Virtuss)
Spodoptera littoralis NPV (cotton leafworm)	*S. littoralis*	cotton	commercial trials product in France (Spodopterin)
Trichoplusia ni NPV (cabbage looper)	*T. ni*	cabbage	former commercial trials product, U.S.A.
granulosis viruses			
Cydia pomonella GV (codling moth)	*C. pomonella*	orchards	commercial trials product, U.S.A. (Decyde)
Plodia interpunctella GV (Indian meal moth)	*P. interpunctella*	stored products (grain)	—
non-occluded baculoviruses			
Oryctes rhinoceros virus (coconut rhinoceros beetle)	*O. rhinoceros*	coconut and oil palm	introduced through Food and Agriculture Organization, United Nation/ South Pacific Commission projects

Fungi are probably more dependent on appropriate microclimate conditions for their success than any other group of microbial insect pathogens. In particular, the high relative humidity (RH) required for spore germination is often very restricting (Drummond *et al.* 1986). Among deuteromycetes infecting terrestrial insects, the lower limit for spore germination is probably about 92% RH and some may require a film of water to germinate.

Specificity of invasion of the insect by a fungal spore may occur at several levels. The pathogen host range may first be influenced by specificity in the ability of spores to adhere to the cuticle. Germination may be influenced not only by humidity but also by the availability of certain nutrients. Successful penetration of the cuticle by the germinating spore probably occurs from a combination of mechanical and enzymic processes (Charnley 1982). Once past

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any insect defence mechanisms operating in the haemocoel, hyphal growth continues, often with the production of a yeast-like phase (blastospores) which allows the fungus to spread through the insect haemocoel. The insect is killed (generally after more than 4 days) after substantial hyphal growth or toxin production or both (Charnley 1982). Sporulation occurs with the development of conidia on the surface of the insect. Some local spread of infection can occur from these individuals.

Table 3. Principal deuteromycete fungal candidates for arthropod pest control

species	main target pests	status of commercial development
Aschersonia aleyrodis	*Trialeurodes vaporariorum* (whitefly)	—
Beauveria bassiana	*Leptinotarsa decemlineata* (Colorado beetle)	product in U.S.S.R. (Boverin)
Beauveria brongniartii	*Melolontha melolontha* (cockchafer)	—
Culicinomyces clavosporus	mosquitoes	—
Hirsutella thompsonii	rust mites	former commercial product (Mycar)
Metarhizium anisopliae	beetles, bugs	product in Brazil (Metaquino)
Nomuraea rileyi	caterpillars	—
Tolypocladium cylindrosporum	mosquitoes	—
Verticillium lecanii	aphids, whitefly	products in U.K. (Vertalec; Mycotal)

Apart from deuteromycete fungi, the Entomophthorales (Zygomycetes) contain by far the most insect-pathogenic isolates. Although the optimal growth temperatures for most Deuteromycetes (20–30 °C) fit them best for use against tropical and subtropical pests, members of the Entomophthorales appear more effective in temperate climates where they often produce extensive, if slow-acting, natural epizootics (Wilding 1981; Wilding *et al.* 1986). Unfortunately, most strains have proved difficult to culture and to store for long periods. Consequently, successful field applications have been relatively few (Wilding *et al.* 1986; Zimmermann 1986).

In the western world, only two insect-pathogenic fungal species are currently produced commercially (table 3). These are *Verticillium lecanii* and *M. anisopliae*. *V. lecanii* was developed to control aphids and whitefly on glasshouse crops (Hall & Papierok 1982), where the protected environment usually maintains the required humidity and temperature levels for effective spore germination and fungal growth. *M. anisopliae* is used as a microbial pesticide for the control of spittle bugs (e.g. *Mahanarva posticata*) on sugar cane in Brazil (Ferron 1981). Whereas *V. lecanii* products are based on blastospores produced by liquid fermentation, other species are applied as conidiospores grown on cereal grain or bran. It is rare for introduced fungal pathogens to recycle or spread extensively or both, over several seasons at the site of introduction. The most frequent method of use for successful pest control is therefore regular application.

(*d*) *Protozoa*

Most insect-parasitic Protozoa of potential interest for pest control are classified within the Microsporida. They have not been successfully used as fast-acting microbial insecticides, and it is unlikely that they will be used in this manner in the future (Maddox 1986*b*). Infection in

susceptible hosts is initiated by ingestion of spores. Microsporida are obligate parasites that require living cells for their development, and the gut or fat body or both provide the main foci of infection. The disease is often not highly pathogenic, but significantly reduces the rate of development of the insect and lowers its fecundity (Wilson 1982; Maddox 1986*a*). Natural spread and survival of the protozoan may be assured in many cases by vertical transmission within eggs of the host.

Although viral and fungal epizootics are considered more common, epizootics of microsporidia regularly occur in many insect species. Thus populations of the European corn borer, *Ostrinia nubilalis*, crash when incidence of the protozoan *Nosema pyrausta* in the insects approaches 100% (Maddox 1986*a*). For microbial control, however, only *Nosema locustae* has received great attention. More than 60 species of grasshopper and cricket are susceptible to this protozoan. Although in nature it is generally uncommon (usually less than 1% of individuals are infected), when spores were applied in a wheat-bran bait, infection levels of up to 40% were obtained (Henry & Oma 1981). In the season after application, infections were also common and the disease spread substantially, suggesting that it has some potential for long-term control. A commercial product (Noloc), based on *N. locustae*, is now manufactured on a small scale in the U.S.A.

(*e*) *Nematodes*

It may not appear conventional to consider insect-parasitic nematodes alongside microbial pathogens of insects. However, rhabditid nematodes in the genera *Heterorhabditis* and *Steinernema* (= *Neoaplectana*) are mutualistically associated with insect-pathogenic bacteria in the genus *Xenorhabdus* (reviewed in Poiner (1986)). These large Gram-negative, rod-shaped, facultative anaerobic bacteria are held in a pouch in the intestine of the nematode and have been recorded in nature only associated with these nematodes.

Free-living rhabditid nematode larvae in the third of four larval stages are relatively resistant to desiccation and can survive in damp soil without an insect host for several months. When they encounter, or are attracted to, a susceptible host the nematodes enter through the mouth or anus and pass into the haemocoel by penetrating the gut wall. *Heterorhabditis* spp. may enter directly through the cuticle. Once inside the host, the symbiotic bacteria are released and multiply. The insect host is killed by septicaemia within 48 h of invasion. The nematode feeds and develops on the bacteria and decomposing host tissues, and completes its complex life cycle (Wouts 1984). About 10 days after invasion, hundreds or thousands of infective third-stage larval nematodes are released from the cadaver.

In recent years significant advances have been made in the production and formulation of these nematodes (Bedding 1986; Poinar 1986). Both the bacteria and the nematodes can be grown *in vitro* by using diets of homogenized animal tissues or plant products. Unfortunately, phase variation is a common feature of *Xenorhabdus* spp. The primary phase of the bacterium is the phase naturally occurring in the infective nematode. On occasion, this spontaneously converts to a secondary phase bacterium which reduces the capacity for nematode reproduction (Akhurst 1986*b*). Despite this problem, the wide, insect host range of these nematodes, their ability to kill the insect host relatively quickly, and the durability of the infective stage, have made them attractive selective control agents. The ideal targets are insect pests that live in soil and other moist and protected environments (Akhurst 1986*a*). The temperature range of activity for most strains isolated to date is between 10 °C and 32 °C, and low humidity and high solar radiation are limiting factors in their use. Strains of these nematodes are now

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produced commercially on a small scale in the U.S.A. and The Netherlands. At present, these nematodes and their associated bacteria are the only commercially available biological product for use against insect pests in the soil (Poinar 1986).

(*f*) *Strategies for using insect pathogens*

In general, insect pathogens can be utilized in pest control in two ways. They can be applied at frequent intervals as short-term microbial insecticides, or they can be introduced once (or on a limited number of occasions) in the hope or knowledge that they will spread, persist, and hence exert a long-term controlling influence on an insect pest population. Short-term use is an almost inevitable requirement in agricultural situations where crop rotation, cultivation techniques and crop harvesting dilute the pathogen in the ecosystem and interfere with its survival in the environment. In addition, the high quality standards now demanded of much horticultural and agricultural produce impose low pest thresholds which then demand regular spray treatments, whether with chemical or microbial pesticides. In fact, in such situations, few microbial insecticides can compete with the short-term efficacy and speed of kill of most chemical pesticides. In contrast, more stable ecosystems including forests, plantations and pastures provide opportunities for long-term establishment of a pathogen in an insect population.

Because of the different demands of these two strategies the characteristics of pathogens used as microbial insecticides may be quite distinct from those required of agents that must persist for long periods in the pest population or external environment. Pathogens that have potential as microbial insecticides need to be highly virulent and relatively easy to produce. Examples of those that are commercially available include, *B.t.*, NPVs of *N. sertifer* and *M. brassicae*, the fungi *M. anisopliae* and *V. lecanii*, and the nematodes *Heterorhabditis* spp. and *Steinernema* spp. Pathogens effective in long-term, limited release strategies generally have lower virulence but can survive for long periods in the host population or in the environment (e.g. through the production of resistant stages). Examples include *B. popilliae*, *Oryctes* baculovirus, *G. hercyniae* NPV, the fungus *B. brongniartii* and the Protozoa *N. locustae* and *N. pyrausta*. Both groups of agents have important roles in future integrated pest management programmes. Although limited release strategies are not attractive for commercial development it is nevertheless important that such potential uses for insect pathogens are recognized and supported through government or international agency funding if necessary.

3. Overcoming constraints in the wider use of microbial control agents

The demand for alternatives to chemical pesticides has never been greater. The microbial pest control agents described above are specific, harmless to beneficial organisms, non-polluting and, in some cases, will persist to exert long-term control of a pest population. None the less, among the hundreds of potentially useful insect pathogens, very few have been exploited. Some have been developed to, and beyond the point of, commercialization and then discarded in favour of chemical pesticides (e.g. *Heliothis* NPV). It is important to consider the reasons for the restricted use of pathogens, to identify the constraints and to attempt to find methods to optimize their use.

I believe that there are at least two reasons why exploitation of microbial pathogens has been

slow. First, they are not seen as having major potential by commercial companies; market size is often restricted by the inherent target specificity of the microbial agent. Secondly, there is a commonly held view that microbial insecticides are not effective pest control agents. Growers accustomed to the broad spectrum, rapid knockdown and kill of many chemical pesticides find it difficult to accept the slower speed of kill of most microbial pesticides. To improve user acceptance, it is most important that unrealistic claims for the control potential of insect pathogens are not made, and that they are used intelligently, in strategies that exploit their strengths. Unfortunately, this policy has not always been followed, and ignorance of the basic biology of certain pathogens has meant that they have not always been used in the most appropriate manner.

In an attempt to identify future uses for insect pathogens it is necessary to consider in more detail the present constraints on their wider use. Future prospects for the use of genetic manipulation in providing new opportunities for microbial agents are considered in a later section (see §4).

(*a*) *Market potential*

There is no doubt that the limited revenues anticipated from the small potential market size of many insects pathogens provide a major disincentive to their commercial development. Lisansky (1984) considered that greater uptake of microbial pesticides would require a change in market philosophy. Naturally occurring microbial agents are virtually all small-market products, whereas the agrochemical industry generally seeks large-market products to recover its development costs. There are probably very few products based on naturally occurring microorganisms that will be of sufficient value to justify production by major industrial companies (Jutsum, this symposium). Genetic manipulation may, in the longer term, derive microbial strains with altered host range and potency and hence increased potential. Greater financial rewards could be gained by transferring genes that code for toxins from certain insect pathogens into crop plants or other organisms that can deliver the toxin to the pest more effectively than can the original pathogen.

It is likely that the future use of natural pathogen strains will remain in the hands of small companies with low overheads, or international agencies that are largely reliant on government-funded research and development. This balance is unlikely to change in the foreseeable future, as microbial agents are only likely to be adjuncts to, rather than replacements for, chemical pesticides. Developments that could create new market opportunities for the microbial agents include requirements for integrated pest managament programmes brought about by severe pest resistance to chemical pesticides or major 'secondary' pest problems induced by the use of chemicals. Glasshouse and other horticultural crops and, in the longer term, cotton, maize and rice should be worthy of consideration. Environmentally sensitive regions such as forests and public amenity areas where chemical pesticide uses are restricted are also important future markets for insect pathogens.

A further consideration is that microbial pathogens must be able to compete in the market place with other selective pesticides, including growth-regulator type compounds such as diflubenzuron. However, even diflubenzuron has some undesirable effects on non-target species, which are not shared by microbial pathogens (Glen & Phillips 1984). Recent studies in Spain have also indicated that diflubenzuron has extreme persistence on pine needles and reduces populations of non-target Lepidoptera (Soria *et al.* 1986). Apart from selective chemicals, certain pathogens are also likely to compete with each other for the same market.

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This is true of *B.t.* and baculoviruses. In general, the commercially available *B.t.* var. *kurstaki* HD-1 strain has a broader spectrum of activity than any baculovirus and should prove more effective in situations where a complex of pest Lepidoptera species needs to be controlled. In contrast, because viruses are infectious agents, only small amounts of the most virulent viruses need to be ingested to be effective. This effect in some situations would tip the balance in favour of a viral insecticide.

(*b*) *Patentability*

Industrial property rights provide one way for companies to protect their investment in a new product. The difficulty of protecting natural strains of insect pathogens has been seen as a disadvantage in their commercial exploitation. None the less, patents have been filed on certain novel naturally occurring microorganisms (e.g. *B.t.* var. *tenebrionis*), as well as on production and formulation processes (e.g. with insect-parasitic nematodes) (Bedding 1986). Genetic manipulation offers potential for the development of novel modified strains of insect pathogens with greater scope for patent protection. However, the extent to which large numbers of new patents will be granted, and the will to defend these patents, remain to be tested.

(*c*) *Production*

Microbial pesticides are generally believed to be more expensive to produce than many chemicals. Costs could well fall as demand increases. Thus public pressure in Canada has promoted the use of *B.t.*, in preference to chemical pesticides, for the control of forest pests. Between 1980 and 1983, the average cost of *B.t.* treatments halved to about \$7 ha^{-1}† (Morris *et al.* 1986). In contrast to *B.t.*, viruses must be produced *in vivo*. Depending on the virulence of the virus and the ease with which the insect host can be reared, substantial differences in product cost can arise. Martignoni (1984) lists the 1977 cost of Elcar (*Heliothis* NPV) per hectare dose at \$4.45 (including manufacturing cost plus profit margin) compared with \$42.00 per hectare for TM Biocontrol 1 (NPV of *Orgyia pseudotsugata*; manufacturing cost only). There is little doubt that major savings can be made in *in vivo* production systems through greater automation. Future improvements in virus production could also come from the use of host species that are susceptible to the virus and are easier to rear. As Huber & Miltenburger (1986) point out, it is especially important in such circumstances to authenticate carefully the properties of the pathogen produced. Although large-scale production of insect viruses in cell culture is probably still at least ten years away from achievement, significant advances have been made (Tramper & Vlak 1986), and the exploitation of baculoviruses as gene expression vectors (see §4) provides further motivation to improve insect cell culture systems. At present, major savings in media costs are required. The costs of producing sufficient virus, *in vivo*, to treat 1 ha were cited by Huber & Miltenburger (1986) as \$10–20. The same amount of virus produced by current cell culture techniques would cost \$900. Future savings on media costs and improved yield are also central to the economic mass production of bacterial and fungal pathogens as well as insect-parasitic nematodes. Reduction in manufacturing costs through production improvements is seen as an important component in the future genetic engineering of *B.t.* (Kirschbaum 1985). Research is also underway on the improved production in liquid media of conidia of insect-pathogenic fungi.

Product stability is another important consideration. Most microbial insecticides survive

† 1 hectare = 10^4 m^2.

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well at temperatures of 4 °C or lower but such low-temperature storage is an additional inconvenience and expense for growers accustomed to maintaining chemical pesticides at ambient temperatures. Nevertheless, failure to formulate these biological products effectively and to store them under appropriate conditions has almost certainly contributed to apparent pest control failures in the past. If it proves difficult to produce formulations that retain infectivity, other than by storing them at low temperatures, then growers must be given other incentives to use the products. Alternatively, the pathogens must be employed in control strategies (e.g. as with *Oryctes* virus) where long-term storage outside the laboratory is not required.

Another aspect of product stability is the avoidance of changes in potency during repeated culture of an insect pathogen. The pathogen must be well-characterized, and reliable quality control procedures should be established to ensure that its properties do not change during production. Genetic changes of insect pathogens during passage have been recorded with viruses (Croizier *et al.* 1985; Smith & Crook 1986*a*) and *Bacillus thuringiensis* (Carlton & Gonzalez 1985; Burges 1986*b*).

(*d*) *Host-range and virulence*

The specificity of many microbial agents is seen as advantageous in preserving beneficial species. None the less, the host range of many naturally occurring strains of insect pathogens often limits the potential market size for their use. Thus it has been known for some time that different lepidopteran pests vary in their response to distinct strains of *B.t.* (Dulmage *et al.* 1981), so that few naturally occurring strains are highly pathogenic for all pest species in the complexes of lepidopteran pests that occur on many crops. Similarly, with fungi, important traits for biological control are found separately in different isolates, as with the whitefly- and aphid-pathogenic strains of *V. lecanii* (Hall 1981). Baculoviruses are more specific than *B.t.* or most fungal strains.

The International Strain Screening Programme of new *B.t.* isolates has shown that substantial improvements in biological activity can be obtained by screening naturally occurring strains (Dulmage *et al.* 1981). Further improvements in potency have also been made through the production of new strains by mutation. Although major advances are also likely to come from genetic manipulation, the enormous gene pool of naturally occurring strains of all insect pathogen groups should not be ignored in the search for agents with optimal host-range and virulence characteristics. The first requirement of a screening survey should be the clear identification of target crops and the different regional pest complexes on these crops.

(*e*) *Resistance*

It has often been implied that insects will not develop resistance to microbial control agents. However, as Briese (1986) points out, 'theoretical studies have indicated that intensively applied control measures, whether they be pathogens or chemical insecticides, will invariably select for resistance'. With viruses there is no direct evidence for resistance developing in the field. Huber (1986) detected no difference in susceptibility to *Cydia pomonella* GV (CPGV) between a susceptible laboratory strain of *C. pomonella* and a population collected from an orchard where CPGV had been applied at least four times every year for the previous nine years. However, in a laboratory selection programme, Briese & Mende (1983) recorded a 140-fold increase in the resistance level of *Phthorimaea operculella* larvae to a GV after six generations of selection.

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McGaughey (1985) reported that colonies of *Plodia interpunctella*, obtained from grain storage bins routinely treated with *B.t.* var. *kurstaki*, were less susceptible to *B.t.* than insects from untreated bins. This report would seem to be the only convincing example of the development of resistance of an insect to a microbial pathogen under 'field' conditions. Special circumstances probably prevail in the use of *B.t.* in the stored-grain environment. Under such conditions, the *B.t.* formulation remains stable, and successive generations of *P. interpunctella* can breed in contact with *B.t.* spores and toxin crystals. Consequently, the selection pressure is likely to be much greater than in field-cropping systems. None the less, this example illustrates the need for care in the way in which microbial pathogens are used in future. Control programmes should be managed to slow down the development of resistance and, if resistance develops, remedial action may be required through the selection of more virulent strains of the pathogen and pathogens with different modes of action (Briese 1986).

(*f*) *Persistence*

Some insect pathogens persist in the host population for long periods, or survive as small inocula of relatively stable forms (e.g. spores, occlusion bodies) in protected niches. However, a large proportion of microbial agents, applied as insecticides to the foliage of crop plants, persist for only short periods mainly because they are inactivated by UV light. Many attempts have been made to improve the persistence of different formulations applied to leaves, by the inclusion of compounds that may protect the pathogen from UV (reviewed in Entwistle & Evans (1985)). To a large degree these attempts have been unsuccessful. Although a concerted effort in formulation improvement is still important, other strategies must be considered. Extended persistence of a microbial insecticide becomes of less concern if a satisfactory monitoring system exists for the pest, so that application of the pathogen can be timed more critically. Among those microorganisms that must be ingested to be effective, uptake of the pathogen could be improved by the development of formulations that attract and stimulate the pest to ingest the pathogens. Better targeting of spray deposits (e.g. to underleaf surfaces partly protected from UV and the position where many pests feed most) could extend persistence. Persistence could also be improved through genetic engineering techniques that package the pathogen or its active component into more stable vectors such as other microorganisms or plants (see §4*a*).

In some circumstances, successful pest control will depend on the pathogen establishing itself in the environment. Thus there are considerable future prospects for the use of bacteria, fungi and nematodes for the long-term control of soil-borne pests if satisfactory colonization of the rhizosphere could be achieved. Zimmermann (1986) reported that both *B. bassiana* and *M. anisopliae* are capable of germinating and growing in sterile but not non-sterile soil, presumably because of microbial competition – antagonism with other components of the soil flora. Similar problems may be encountered in the future use of phyllosphere-colonizing insect pathogenic microorganisms (see §4*a*).

(*g*) *Registration and safety*

It is not possible in this chapter to dwell on safety aspects such as the effects of microbial agents on non-target organisms; these are considered in several excellent reviews (Burges 1981; Harrap 1982; Rogoff 1982). Current evidence suggests that the responsible use of the pathogens described above does not adversely affect non-target vertebrates, plants and beneficial invertebrates. None the less, it is entirely appropriate that new insect pathogens should be safety-tested before widespread use. With the advent of genetically manipulated

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microorganisms and *B.t.*-transferred plants risk assessment and management is even more important (see §4*c*).

Lisansky (1984) concluded that recent regulatory improvements in the U.S.A. and the U.K. were likely to make microbial pesticides a more attractive proposition to commercial firms. He estimated then that toxicological testing for a new (naturally occurring) microbial product would cost £40000 compared with £3000000 for a new chemical active ingredient. Even so, such registration costs for small-market products are likely to be a significant part of the total development costs. For some pathogens (e.g. baculoviruses) there are some who advocate the idea of a generic registration for all related products. Other methods of reducing product costs are through the sharing of safety data packages by different companies and Government research and development organizations. Government aid towards the registration of such minor use products is also occurring in the U.S.A. in the form of financial support through the IR-4 scheme.

I have attempted in the above description to identify the major constraints to the more widespread, future use of microbial control agents, and to suggest solutions and opportunities for further research and development. Successful use of microbials is a complex process. The main aim of microbial control programmes is to use the minimum quantity of pathogen sufficient to reduce the pest population below the damage threshold. Although the majority of programmes to date have achieved this on a trial and error basis, approaches to the problems through systems models could provide a strong framework for decision making in the future (Evans 1986). Such an approach, however, will demand access to more quantitative information on host–pathogen interactions than is currently available.

4. Future prospects for the use of genetically manipulated microbial pest control agents

As Dean (1984) wrote in a review of *B.t.* genetics, the prospects for genetic manipulation of pathogens are limited only by the imagination of research workers. However, it is probably most useful to try to examine progress to date in considering what may be achievable within the next ten years. This is not an easy task because much of the research in this area is being done 'behind closed doors' by agrochemical and biotechnology companies. It is clear that some sources see major prospects for genetically manipulated control agents. Estimates of the future U.S. sales of genetically improved biological pesticides show an increase from U.S. $24 M (at 1984 prices) in 1990, to U.S. $455 M in 2025 (Stanford Research Institute International 1984). This contrasts with the present world sales value of existing microbial pesticides which is put at $20 M–40 M (Anon. 1986*b*; Jutsum, this symposium).

In earlier sections, I have identified features of pathogens that may be amenable to improvement through genetic manipulation. These include modifications to virulence, host range, persistence and ease of production. A first requirement is that the pathogen should be amenable to genetic manipulation and, secondly, that it is necessary to understand its mode of action and factors influencing its efficacy. Because basic genetic transformation systems are available at present only for *B.t.* and baculoviruses (among the pathogens described above) I shall restrict detailed discussion to these. However, future scope for genetically modifying the characteristics of insect-pathogenic fungi, *Xenorhabdus* spp. and other bacteria, should not be ignored. Genetic engineering of these agents awaits the development of transformation systems

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and an improved understanding of their biology, particularly of characteristics that govern virulence and host specificity.

(*a*) *Genetic improvement of Bacillus thuringiensis*

There is currently intense research activity in the genetic manipulation of *B.t.* It is an ideal target as the protoxins are known to be single-gene products, the primary structure of the proteins in several strains is known, and the protoxins are made in large amounts and are readily assayed. *B.t.* is also commercially important and shown, from almost 30 years of field use, to be safe. As mentioned above (see §1*a*) the toxin genes are present on plasmids. This fact has permitted the utilization of a non-recombinant methodology to develop novel strains of *B.t.* With a conjugation-like process (Gonzalez *et al.* 1982), plasmids can be exchanged between different parental strains to obtain transconjugants, some of which possess the combined toxic properties of both parents. This technique makes possible the tailoring of *B.t.* strains for improved toxicity and efficacy against a different range of lepidopteran pests. Thus, the transfer of a plasmid from a *B.t.* strain with high potency against *Heliothis armigera* but low activity against *Spodoptera littoralis*, to a recipient *B.t.* strain with the converse potencies, produced a transconjugant with high activity against both these major lepidopterous pests of cotton (Jarrett & Burges 1986). Likewise, Klier *et al.* (1983) constructed a *B.t.* recombinant which had a new combination of Lepidoptera- and mosquito-active toxins. The first field trials of transconjugant *B.t.* strains were done in the U.S.A. and the U.K. during 1986.

The cloning and expression of *B.t.* crystal toxin genes in other organisms was first reported by Schnepf & Whitely (1981), who cloned a *B.t.* var. *kurstaki* toxin gene into *Escherichia coli*. Subsequently, the gene has been cloned into other microorganisms that have potential to act as alternative delivery vectors for the toxin gene. The example that has received most publicity is the cloning by a research team at Monsanto Agricultural Products Co. of a *B.t.* var. *kurstaki* toxin gene into *Pseudomonas fluorescens*, a non-pathogenic rhizosphere-colonizing bacterium (Watrud *et al.* 1985). The aim of this project was to produce an engineered *P. fluorescens* that could be applied as a seed-dressing or soil-inoculant and would protect plants from soil-borne lepidopteran pests. *P. fluorescens* was selected as the delivery vector because it is not pathogenic to humans, was sensitive to clinical antibiotics and sterilants, and had limited potential for genetic exchange and environmental persistence. The toxin gene was inserted into the bacterial chromosome rather than into plasmids (to reduce gene transfer) by direct transposition and homologous recombination by using the transposable element *Tn5*. To further minimize the potential of horizontal gene transfer to other bacteria, the transposon activity of the transformed bacterium was also eliminated. The engineered bacterium produced the 134000 M_r *B.t.* protoxin and the host potency of the engineered bacterium was found to parallel that of the original *B.t.* strain (Watrud *et al.* 1985). Restrictions on the environmental release of genetically engineered microorganisms in the U.S.A. have not yet allowed field trials of this transformed *P. fluorescens* strain.

The concept of using plant microflora as delivery vectors for pesticidal genes is a practical and intellectually satisfying approach to the production of new microbial pesticides. The future introduction of the *B.t.* crystal toxin genes into leaf epiphytes as well as root colonizers would help alleviate the need for insecticide applications to the crop and effectively increase the persistence of the toxin in the environment. In addition to plant colonizing microorganisms, it is likely that attempts are also being made to transfer the *B.t.* var. *israelensis* (dipteran-active)

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toxin gene into prokaryotes that live in water and are present in the feeding zones of mosquito larvae. Lüthy (1986*b*) indicated that research of this kind was being carried out with the blue-green alga (Cyanobacteria) *Anabaena*.

In a different approach, Mycogen (a San Diego based biotechnology company) are reported to have also cloned the *B.t.* toxin gene into a *Pseudomonas* sp. with the aim of expressing large quantities of the crystal protein and extending its environmental persistence through a bioencapsulation process that kills all the bacteria (Geiser 1986; Barnes & Lavrik 1986). It is understood that clearance has been granted for field trials of this genetically engineered product on the basis that it is classified as a chemical pesticide, containing no live infectious agents. As Lüthy (1986*b*) points out, the encapsulated protoxin produced in this manner must be capable of digestion to the toxin molecule by insect gut juices for it to be effective.

A further, well publicized, genetic engineering strategy for the use of *B.t.* toxin has been to clone the gene into crop plants, with the aim of producing plants immune to attack by certain Lepidoptera. This has now been achieved in tobacco through the transfer of the entire *B.t.* protoxin gene, or the sequence that codes for the toxic fragment, by using modified Ti plasmid vectors (Vaeck *et al.* 1987; Adang *et al.* 1988). This approach is not without its problems. By using a highly susceptible test insect (larvae of the tobacco hornworm, *Manduca sexta*) significant mortality was observed on transformed plants but insecticidal activity exhibited by different transformants was variable (Vaeck *et al.* 1986). In other experiments, the crystal-protein gene messenger (m) RNA transcripts expressed in the plant were truncated, possibly through some difference in mRNA processing in the plant compared with that in the bacterium (Adang *et al.* 1988). Even so, the shortened mRNA must have been sufficient to encode the toxin sequence as insecticidal activity was recorded in the transformed plants.

With this elegant approach now being extended to a range of other plant species, compatibility of the toxin with plant components could be critical. Will expression of the toxin in plant cells reduce crop yield or vigour (Kirschbaum 1985)? Will the toxin interact with plant compounds, e.g. tannins that are believed to bind to and/or inactivate the toxin (Lüthy 1986*a*)? Answers to these questions are awaited with interest. In addition, the potentially high selection pressure that would be imposed on larvae feeding on plants, constitutively expressing an insecticidal molecule, could be seen as a powerful mechanism for the rapid development of resistance in insect populations. Perhaps the insertion of several genes, each coding for a different insecticidal molecule, would be a better long-term strategy? In the meantime, results are eagerly awaited on the first field tests in the U.S.A. of tobacco transformed with *B.t.* toxin (Anon. 1986*a*).

Apart from these exciting developments in the use of new delivery vectors for *B.t.* toxin genes, significant future progress is likely to be made in the construction of new toxins with differences in virulence and host potency through directed mutagenesis and gene ligation.

(*b*) *Genetic improvement of baculoviruses*

Undoubtedly, improvements in baculovirus pathogenicity can be obtained by strain selection. Studies have also shown that genetic recombination occurs between closely related baculoviruses in mixed infections, providing a means of generating new variants (Croizier & Quiot 1981). Perhaps the most intriguing developments have come from studies on baculovirus genetic engineering which illustrate the possibility of introducing new genes into precise positions in baculovirus genomes. Smith *et al.* (1983) were the first to report that a baculovirus

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(ACMNPV) could be used as a vector for the propagation and expression of introduced (passenger) genes. This study made use of the fact that some baculovirus proteins, in particular the matrix protein (polyhedrin) of the occlusion bodies, are produced to high levels during infection. When the gene for this protein was replaced by a gene coding for human β-interferon, the new gene was expressed to a very high level and large quantities of interferon were recovered. Further advances have been made with this system such that recombinant baculoviruses are seen as capable of producing many different protein products (Cochran *et al.* 1986). These studies indicate that baculoviruses can accommodate and express additional DNA sequences, and suggest that new and infectious baculovirus strains could be constructed.

At present, there is no published information on the production of genetically engineered baculoviruses with novel insecticidal properties. None the less, it is likely that the Genetics Institute (Boston, U.S.A.) has a novel recombinant NPV with modified host range and potency which may be field tested within the next two years. In addition, I understand that an NPV of *Spodoptera litura* has been adapted in Japan to grow in the silkworm (to improve production) and then engineered so that the virus infests and kills *S. litura* but does not complete its replicative cycle. In future genetic improvement of NPVs and GVs, it may be necessary to leave the polyhedrin gene intact so that the engineered virus will still produce the occlusion bodies that are important for field stability. Therefore new DNA will have to be inserted into other regions of the viral genome that are not essential for virus replication. The genes that influence virus infectivity and host range have not been identified, and directed phenotypic changes are not yet possible. Genetic control of these properties is likely to prove complex. In the meantime, however, it may be possible to modify a baculovirus so that its speed of kill is increased. The insertion of genes that code for insecticidal toxins (e.g. *B.t.* toxin) or oligonucleotides that may code for insect neuropeptides, could yield such an effect. Problems of how to produce such novel viruses in insects or cell cultures will have to be overcome. One solution could be to produce a conditional lethal mutant (e.g. temperature-sensitive mutant) of the virus which only expresses the toxic gene under specific conditions (e.g. over a defined temperature range).

Finally, viruses themselves may in future be used as gene vectors to transform insects. A complex group of DNA containing, insect viruses (polydnaviruses) are intimately associated with many parasitoids. These could be used to transfer genes that improve the pesticide resistance of a parasitoid, or that modify other components of the parasitoid's behaviour to improve its performance as a biological control agent (Vinson 1986).

(*c*) *Risk assessment and management*

A responsible attitude is required to the environmental release of genetically engineered organisms to satisfy both the regulatory authorities and the public. Not only will there be a need for risk assessment; risk management will also be of prime importance. Containment levels, safety precautions, experimental design and monitoring systems, and risk minimization will need definition based on knowledge of the characteristics of the organism, the risk and type of exposure to non-target organisms, the capability of the organism to survive, multiply and spread, and its interactions with other relevant components of the ecosystem (Heusler 1986). Fortunately, *B.t.* has been in operational use for almost three decades and baculoviruses have also been used extensively in field trials. Both pathogen groups have been subjected to extensive safety tests and there has been no substantiated incident of adverse effects on non-target species.

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Thus these agents are ideal model systems with which to obtain data relevant to making decisions about the release of genetically engineered organisms. Information required by the U.S. Environmental Protection Agency of the *B.t.* toxin gene-transformed *P. fluorescens* have included details of taxonomy, toxicology, persistence, host range and genetic stability (Barnes & Lavrik 1986).

What issues are particularly relevant in the release of novel microbial pesticides? Firstly, general safety of genetically engineered microorganisms can be evaluated by the conventional series of safety tests that would normally be applied for a non-engineered strain. There may be public concern that the release of novel strains not subjected to the process of natural selection could themselves become major pest species. This seems extremely unlikely with either *B.t.* or baculoviruses, natural strains of which have been clearly established as having no adverse effect on vertebrates or plants and restricted pathogenicity in invertebrates. Unfortunately, there is no guarantee that microorganisms, once released, can be destroyed. Suggestions have been made that engineered strains should be produced with limited ability to survive in the field. With most naturally occurring insect pathogens that are used as microbial insecticides, this is already the case.

Of equal relevance is the genetic stability of the novel organism and the consequences of any instability. It is known that *B.t.* plasmids carrying the toxin gene can be transferred in the laboratory to some other bacterial species through conjugation (Gonzalez *et al.* 1982). What is not known is how frequently this occurs in nature, nor how relevant such transfers would be. Likewise, with baculoviruses, recombinants can be obtained between closely related virus strains (Croizier & Quiot 1981; Smith & Crook 1986*a*). It is also known that a number of related but distinct baculovirus genotypes can be isolated from a single infected insect in the wild (Smith & Crook 1986*b*). The potential therefore exists for the transfer of introduced genes at least within a gene pool of related viruses. With what frequency does this occur; to what extent could the genes be transferred to viruses infecting non-target hosts; what would be the consequences of such transfers? These questions can be answered only by ecological investigation of the frequency and consequences of gene transfer. The production of a baculovirus (ACMNPV) 'marked' with a short section of non-coding DNA, and its release in U.K. trials as virus-infected *Spodoptera exigua* larvae is the first attempt to study the molecular epidemiology of a baculovirus strain (Bishop 1986). Such investigations are central to the future successful exploitation of genetically engineered insect pathogens. However, studies with naturally occurring, endemic pathogen strains and insect species would probably be more instructive; neither ACMNPV nor *S. exigua* can be regarded as species endemic to the U.K.

6. Conclusions

The examples cited above illustrate some of the potential uses of microbial pest control agents. Factors constraining their potential were identified and suggestions were made as to how these constraints could be resolved in future. The need to select new pathogen strains, to produce new pathogen strains by genetic engineering and to select more effective use strategies will require additional information on the mode of action of many pathogens and the biology of the host–pathogen relation. Future studies on mode of action will provide data not only of use in selecting the most appropriate strains but also in directing research on future genetic improvement. Because the future registration of genetically engineered strains may be a slow

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process, based on a case-by-case assessment at the outset, there should still be much potential for the exploitation of the naturally occurring variation amongst endemic insect pathogens. In addition, mode-of-action studies (by providing an understanding of the molecular events that allow pathogens to overcome the natural defences of insects) should lead to the discovery of new active molecules that could form the basis for a new generation of synthetic chemical insecticides (Kirschbaum 1985).

Finally, knowledge of the basic biology of the host–pathogen interaction is essential both for the development of new control strategies (Entwistle 1986) and for the future use of genetically engineered agents. Although I was at first concerned that the extensive initial investment of research funds into the genetic manipulation of pathogens would remove resources from basic biological studies, I am now increasingly heartened that investigations into the requirements for the release of genetically engineered microbial pest control agents will now provide the driving force for the essential biological and ecological studies that are still required.

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Discussion

M. Hafez (*Entomology Department, Cairo University, Egypt*). What does Dr Payne think about the efficacy of the polyhedrosis virus of *Spodoptera littoralis* under adverse physical conditions such as excessive heat? Secondly, what about the value of combining this virus with a pheromone against this pest?

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C. C. Payne. I doubt that the temperatures encountered on cotton in Egypt would be sufficient to inactivate the virus but I will ask K. Jones to comment on the first part of the question. I know of no experiments combining the use of virus with an attractant pheromone against *Spodoptera littoralis*. However, some work in the U.S.A. by L. Falcon suggested that virus held in a powdered form in specially constructed light traps could be spread through the insect population by moths attracted to these traps. As viruses have to be ingested to be effective there may also be considerable merit in producing formulations of viruses that attract and stimulate larvae to feed on them.

K. Jones (*Tropical Development and Research Institute, Salisbury, U.K.*). The NPV of *S. littoralis* is extremely virulent against the target for control, that is, first-instar larvae. The LD_{50} against this stage is about one polyhedral inclusion body per larva.

Turning to the persistence of the virus in the field, the effect of field temperatures is insignificant. The temperature on the crop rarely exceeds 40 °C, and such temperatures in the field do not inactivate the virus over the required period for control (*ca.* 2 weeks). A more significant problem is the effect of sunlight: exposure to the ultraviolet region of sunlight rapidly inactivates the virus. However, the site in which the target is located on the undersurface of the leaves provides considerable shade. Thus the virus in this region persists for longer than many studies, in which virus deposits have been exposed to direct sunlight, would suggest. Several UV protectants are in the process of being evaluated and the results of this work should lead to the development of a more persistent formulation.

I. Harpaz (*Department of Entomology, Hebrew University of Jerusalem, Israel*). Will Dr Payne comment on prospects for activating latent baculovirus infections which are quite prevalent among field populations of lepidopterous pest species?

C. C. Payne. Research recently conducted at the Institute of Horticultural Research, Littlehampton, U.K., by I. Smith and N. Crook strongly supports the idea that latent baculovirus infections occur in certain insects, and that these can be activated by certain stresses including 'infection' by another baculovirus. In a series of experiments with a laboratory culture of *Pieris brassicae*, larvae were fed with a very high dose of a granulosis virus (GV) which was believed to have little or no infectivity for this insect species. A few of the larvae died of a GV infection. Virus was purified separately from each infected larva and then characterized by restriction endonuclease analysis of the DNA. Different genotypes (more than 20) were identified from these larvae, which were distinct from the inoculum virus and from each other. This and other experiments have led us to believe that the DNA of the inoculum virus (non-infective, or of low infectivity for *P. brassicae*) is recombining with latent viral DNA sequences contained in the insect population to produce new virus genotypes, some of which have higher virulence for *P. brassicae*. If this explanation is correct (the large number of genotypes obtained tends to rule out contamination or selection from an inoculum of mixed genotypes), then it suggests that recombination between baculovirus DNAs can be a frequent event, at least in laboratory tests. Thus if genetically engineered baculoviruses are to be used in future, foreign genes inserted into the baculovirus DNA could become distributed within a gene pool of related viruses. The potential significance of this should be borne in mind in any risk assessment studies on the release into the environment of genetically engineered baculoviruses.

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A. E. Akingbohungbe (*Department of Plant Science, University of Ife, Nigeria*). Is there any possibility of integrating chemical insecticide use with microbial insecticide? Has there been any work done on compatibility of synergistic interactions?

C. C. Payne. It is often feasible to combine the use of chemical pesticides and microbial insecticides and this would be the aim in an integrated pest and disease management system. Thus the granulosis virus of *C. pomonella* can be tank-mixed with orchard fungicides without adverse effects. However, it would clearly be unwise to integrate fungicides with fungal pathogens of insects, without first testing the compatibility of the two. Synergism between certain microbial and chemical insecticides has been reported, for example, research groups in France have demonstrated that mixtures of some baculoviruses with low doses of synthetic pyrethroids increase larval mortality due to virus.

R. R. M. Paterson (*C.A.B. International Mycological Institute, Kew, U.K.*). In my opinion, a new approach has to be taken with fungal pathogens, which involves more work on the toxins produced by these organisms (and other fungi), to discover selective mycopesticides: would Dr Payne go along with that?

C. C. Payne. There is already some interest in toxins and other secondary metabolites produced by fungal pathogens of insects (e.g. the destruxins of *Metarhizium anisopliae*). Such interest is not only restricted to their potential as pesticides but also their prospective value as biologically active molecules in the chemical and pharmaceutical industry. If one is to make use of such compounds directly as pesticides, careful toxicological testing will be required to ensure that they are selective and harmless to non-target species.

S. M. K. Hag Ahmed (*Wye College, University of London, U.K.*). The first law of control should be to safeguard humans. It seems the use of pathogens may pose a threat to the image of biological control relative to chemical control. It does not seem to be 100% certain that even specific pathogenic microorganisms could not become human pathogens. How can this dilemma be overcome?

C. C. Payne. In using microorganisms as biological control agents one must be sure that they are specific and do not have adverse effects on non-target species. I would like to reassure the questioner that representatives of the agents that I have described in my talk have been shown to be safe to use, through programmes of toxicological and infectivity testing and practical field use. *Bacillus thuringiensis*, for example, has been used commercially for almost 30 years with no substantiated adverse effects on the environment. It is nevertheless important to ensure that appropriate quality control criteria are imposed on microbial agents, both to ensure the identity of the agents themselves and to confirm that no adventitious pathogens of non-target organisms are present in the final formulation.

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