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Insect Pathogens as Biological Control Agents: Do They Have a Future?

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Naturally occurring entomopathogens are important regulatory factors in insect populations. Many species are employed as biological control agents of insect pests in row and glasshouse crops, orchards, ornamentals, range, turf and lawn, stored products, and forestry and for abatement of pest and vector insects of veterinary and medical importance. The comparison of entomopathogens with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost. In addition to efficacy, the advantages of use of microbial control agents are numerous. These include safety for humans and other nontarget organisms, reduction of pesticide residues in food, preservation of other natural enemies, and increased biodiversity in managed ecosystems. As with predators and parasitoids, there are three basic approaches for use of entomopathogens as microbial control agents: classical biological control, augmentation, and conservation. The use of a virus (*Oryctes* nonoccluded virus), a fungus (*Entomophaga maimaiga*), and a nematode (*Deladenus siricidicola*) as inoculatively applied biological control agents for the long-term suppression of palm rhinoceros beetle (*Oryctes rhinoceros*), gypsy moth (*Lymantria dispar*), and woodwasp (*Sirex noctilio*), respectively, has been successful. Most examples of microbial control involve inundative application of entomopathogens. The most widely used microbial control agent is the bacterium *Bacillus thuringiensis.* The discovery of new varieties with activity against Lepidoptera, Coleoptera, and Diptera and their genetic improvement has enhanced the utility of this species. Recent developments in its molecular biology, mode of action, and resistance management are reviewed. Examples of the use, benefits, and limitations of entomopathogenic viruses, bacteria, fungi, nematodes, and protozoa as inundatively applied microbial control agents are presented. Microbial control agents can be effective and serve as alternatives to broad-spectrum chemical insecticides. However, their increased utilization will require (1) increased pathogen virulence and speed of kill; (2) improved pathogen performance under challenging environmental conditions (cool weather, dry conditions, etc.); (3) greater efficiency in their production; (4) improvements in formulation that enable ease of application, increased environmental persistence, and longer shelf life; (5) better understanding of how they will fit into integrated systems and their interaction with the environment and other integrated pest management (IPM) components; (6) greater appreciation of their environmental advantages; and (7) acceptance by growers and the general public. We envision a broader appreciation for the attributes of entomopathogens in the near to distant future and expect to see synergistic combinations of microbial control agents with other technologies. However, if future development is only market driven, there will be considerable delays in the implementation of several microbial control agents that have excellent potential for use in IPM programs.

Key Words: **entomopathogens; microbial control; baculoviruses; entomopathogenic fungi; *Bacillus thuringiensis;* entomopathogenic nematodes.**

INTRODUCTION

Invertebrate pathology, as a vocation, is a recently organized discipline. Its roots, however, can be traced to ancient history with reference to solutions for preventing disease in honey bees and silkworms (Steinhaus, 1956, 1975). The application of microorganisms for control of insect pests was proposed by notable early pioneers in invertebrate pathology such as Agostino Bassi, Louis Pasteur, and Elie Metchnikoff (Steinhaus, 1956, 1975). Several researchers experimented with the use of fungi as microbial control agents in the late 19th century. However, it was not until the development of the bacterium *Bacillus thuringiensis* Berliner that the use of microbes for the control of insects became widespread. Today a variety of entomopathogens are used for the control of invertebrate pests in glasshouse and row crops, orchards, ornamentals, range,

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turf and lawn, stored products, and forestry and for the abatement of pest and vector insects of veterinary and medical importance (Burges, 1981; Tanada and Kaya, 1993; Lacey and Kaya, 2000).

Entomopathogenic organisms used for microbial control include bacteria, viruses, fungi, protozoa, and nematodes. The comparison of entomopathogens with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost. When environmental benefits including safety for humans and other nontarget organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems are taken into account, their advantages are numerous. They also offer some distinct advantages over arthropod biocontrol agents in that most can be applied with conventional equipment and many can be produced with artificial media and stored for extended periods of time. Like arthropod natural enemies, many entomopathogens are specific to certain species or groups of insect pests and some have the potential to provide long-term control. There are also some disadvantages, mostly linked with their persistence, speed of kill, specificity (too broad or too narrow host range), and cost relative to conventional chemical insecticides.

Strategies for the use of entomopathogenic organisms for insect control are basically the same as that for other biological control agents (Harper, 1987). They may be used to augment naturally occurring pathogens (augmentation), conserved or activated in nature (conservation), introduced into pest populations as classical biological control agents to become established and exert long-term regulation of the pest (inoculative release), or used inundatively for rapid short-term control (inundative release). In this presentation we highlight some of the successes of microbial control and speculate on future developments.

NATURALLY OCCURRING PATHOGENS—CAN THEY BE MANAGED?

Like other natural enemies, insect pathogens can exert considerable control of target populations. Epizootics caused by naturally occurring viral and fungal pathogens are often responsible for spectacular crashes of insect pest populations (Evans, 1986; McCoy *et al.*, 1988). The natural epizootics produced by nucleopolyhedroviruses (NPV) of sawflies (*Gilpinia hercyniae* (Hartig) and *Neodiprion* spp.), gypsy moth (*Lymantria dispar* (L.)), and several other insects are often credited with eliminating the need for further interventions (Kaya, 1976; Evans, 1986; Woods and Elkinton, 1987). Because most fungi invade the host insect through the exoskeleton, they are the only significant naturally occurring entomopathogens of phytophagous sucking insects (Latgé and Papierok, 1988; Lacey *et al.*, 1996). Although decimating epizootics caused by protozoa are less frequently observed in pest populations, their function in the regulation of insect populations may be quite significant (Maddox, 1987; Brooks, 1988). The reliance on the natural occurrence of entomopathogens for management of pest insects, however, is risky due to the unpredictability of factors that govern epizootics. Because many pathogens are host-density dependent, epizootics often occur after economic thresholds have been surpassed. Nevertheless, due to their roles in the regulation of pest populations, agricultural practices that foster their conservation and increase their prevalence without encouraging plant pathogens warrant more attention.

An example of the utilization of natural epizootics in integrated pest management (IPM) is presented by Steinkraus and Hollingsworth (1994), in the regulation of the cotton aphid *Aphis gossypii* Glover a significant secondary pest of cotton. The entomophthoralean fungus *Neozygites fresenii* (Nowakowski) Batko often reduces or eliminates the requirement for chemical control of this pest (Steinkraus *et al.*, 1991, 1995). Furthermore, thorough, coordinated surveys for the fungus and communication with growers regarding its prevalence can maximize the impact of the fungus on cotton aphids and minimize the use of pesticides. Epizootics can be predicted at least 1 week in advance. Growers are then advised to refrain from chemical pesticide applications.

Possible use of naturally occurring entomopathogens within the IPM context in other agroecosystems will rely on thorough surveys similar to that reported by Steinkraus and Hollingsworth (1994) and a greater knowledge of the environmental and biological factors that govern epizootics (Fuxa and Tanada, 1987; Harper, 1987).

INOCULATIVE INTRODUCTIONS

The intentional introduction of exotic pathogens as classical biocontrol agents has lagged considerably behind that of predators and parasitoids (Maddox *et al.*, 1992). Regulatory restrictions on their introduction have nearly eliminated classical biological control with exotic pathogens of introduced insect pests in the United States. However, the unintentional or accidental introduction of pathogens, as in the case of the NPV of the European spruce sawfly (*G. hercyniae*), has resulted in significant and ongoing natural control (Balch and Bird, 1944; Bird and Elgee, 1957).

In this section we present three notable cases involving introduced exotic virus, fungus, and nematode species which were intentionally inoculated into habitats where they were previously absent or rare. Other examples of inoculative use of entomopathogens are presented by Tanada and Kaya (1993) and Hajek *et al.* (2000).

The principal characteristics that most successful inoculative agents have in common are persistence in the environment and/or host and ability to cause epizootics and to be transmitted within and between host populations and/or generations. The ecosystems and host plants that have best supported establishment and persistence of introduced entomopathogens are permanent and perennial and can tolerate attack by the targeted insect (i.e., high economic threshold) while inoculum levels increase. Forests are ideal habitats in this sense.

Nonoccluded Virus and the Palm Rhinoceros Beetle

Palm rhinoceros beetles are serious pests of coconut and oil palms throughout the tropics (Bedford, 1980). One species, *Oryctes rhinoceros* (L.), is one of the most important pests of coconut palms in the South Pacific and elsewhere. Adults attack the crown of coconut palms, oil palms, and several other palm species and their feeding can reduce yield and kill seedlings and young and old trees (Bedford, 1980). Larvae of this species develop in rotting palm logs, including the tops of dead standing palms that have been killed by adult beetles or other causes.

Research that followed the discovery and description of the nonoccluded virus of *O. rhinoceros* by Hüger (1966) indicated that the virus had potential for long-term control of the beetle (Bedford, 1980, 1981). Adult beetles become chronically infected and serve as reservoirs and disseminators of the virus (Zelazny, 1973). Healthy adults become infected during mating via oral contact with substrates contaminated with virus by infected mates or by feeding on foliage contaminated by infected adults or in larval breeding sites containing virus-killed larvae (Young, 1974; Zelazny, 1976; Young and Longworth, 1981; Zelazny and Alfiler, 1991). Although there are no external symptoms of the disease in adults and it is not immediately fatal, Zelazny (1973) observed that infected adults died sooner and laid fewer eggs than healthy adults. Transmission to larvae occurs when virus-infected adults defecate in breeding sites. Viral infection in larvae is invariably lethal (Zelazny, 1972, 1976).

Mass production of the virus in *O. rhinoceros* larvae has enabled infection of trapped adults with subsequent autodissemination into larval habitats (Bedford, 1977; Zelazny, 1977, 1978) in several locations where the virus was absent. Direct introduction of the virus into artificial and natural larval habitats has also been successfully used to inoculate beetle populations (Bedford, 1980). Since 1967 the virus has been introduced into coconut plantations in several South Pacific islands and other locations, resulting in significant control of *O. rhinoceros*. The combination of removal or covering of old palm logs that serve as breeding sites and use of the virus has reduced the density of *Oryctes* populations to below economic thresholds in many locations (Bedford, 1980, 1981; Zelazny *et al.*, 1990, 1992; Alfiler, 1992). Bedford (1981), Zelazny *et al.* (1992), and Alfiler (1992) suggest that some old palm logs and larvae should remain to help maintain the virus. Zelazny *et al.* (1992) attribute the success of the virus to its effect on adult longevity and its persistence at low host densities.

Entomophaga maimaiga and Gypsy Moth

Since the introduction of the gypsy moth, *Lymantria dispar* (L.), into North America in the vicinity of Boston, Massachusetts in 1869, it has spread westward and southward and continues to spread 6 to 9 km yearly (Reardon and Hajek, 1993). It is now found in the western United States, apparently spread by humans. Larvae of the moth feed on a wide range of deciduous trees and during cyclic outbreaks have been responsible for defoliation of trees in up to 2 million ha of forest (Reardon and Hajek, 1993).

The entomophthoralean fungus *Entomophaga maimaiga* Humber, Shimazu & Soper was first introduced into the northeastern United States in 1910 and 1911 for gypsy moth control, but was not recovered. Subsequently, Soper *et al.* (1988) isolated and described a virulent strain of *E. maimaiga* from gypsy moth in Japan. Limited field trials of the fungus in Allegheny State Park, New York in 1985 and Shenandoah National Park, Virginia in 1986 produced no infections (1985) or only very low levels of infection (1986) (R. S. Soper *et al.*, unpublished data cited in Hajek *et al.*, 1995, 1996b). The fungus was not recovered from these sites in 1987 or in 1989–1991 (Hajek *et al.*, 1995). However, epizootics caused by *E. maimaiga* in gypsy moth were reported in 1989 in the northeastern United States by Andreadis and Weseloh (1990) and Hajek *et al.* (1990b). The exact origin of the fungus causing these epizootics is somewhat of a mystery (Hajek *et al.*, 1995). Weseloh (1998) presents evidence for fairly recent origin of the fungus in North America.

Since 1989, the fungus has spread, both as a result of natural factors and as the artificial introduction of resting spores (=azygospores) to new sites (Elkinton *et al.*, 1991; Hajek and Roberts, 1991; Weseloh and Andreadis, 1992a; Smitely *et al.*, 1995; Hajek *et al.*, 1996b; Dwyer *et al.*, 1998; Hajek and Webb, 1999). In 1991 and 1992, Hajek *et al.* (1996b) applied 6×10^5 resting spores of *E. maimaiga* at the base of oak trees in 41 test plots in four eastern states along the southern limit of the gypsy moth. By the summer of 1992, 40 of the release sites were positive for the fungus and natural spread to control sites was observed. Where resultant epizootics have occurred, they have been responsible for significant declines or even collapse of gypsy moth populations (Reardon and Hajek, 1993; Smitley *et al.*, 1995; Hajek *et al.*, 1996b; Hajek, 1997), leaving non-

target lepidopterans virtually unaffected (Hajek *et al.,* 1996a).

Fungal activity is positively associated with high humidity (Hajek *et al.,* 1990a; Hajek and Soper, 1992) and rainfall (Weseloh and Andreadis, 1992a,b; Weseloh *et al.,* 1993; Smitley *et al.,* 1995; Hajek *et al.,* 1996b). Infection levels were higher where introduced resting spores were watered weekly (Hajek and Roberts, 1991; Hajek *et al.,* 1996b). All larval instars are susceptible to infection by *E. maimaiga* and, although rapid progression of secondary infections characteristic of epizootics depends on adequate population density, the fungus is not strictly density dependent (Hajek *et al.,* 1993). Weseloh and Andreadis (1992b) observed that early stage larvae are infected after germination of overwintering resting spores (primary infections), but late stage larvae become infected by conidia produced in the early instars (secondary infection). A refinement of this model by Weseloh (1999) allowed infection only of 4th and larger instars via germination of resting spores. *E. maimaiga* produces resting spores within cadavers of late instar larvae which are found predominantly on tree trunks (Hajek *et al.,* 1998a). As the cadavers become dislodged, resting spores are leached into the soil in association with rain, with the majority being recovered 0–10 cm from the base of the tree (Hajek *et al.,* 1998b).

In addition to virulence, other biological characteristics of *E. maimaiga* make it an ideal inoculative biocontrol agent that will persist in the environment and recycle in episodic gypsy moth populations. Weseloh and Andreadis (1997) demonstrated staggered germination of resting spores that were assayed yearly over a 6-year period with up to 80% infection produced in gypsy moth larval bioassays each year. The extended survival of the resting spore coupled with variability in germination will ensure persistence of *E. maimaiga* in the environment even in years when gypsy moths are rare or absent.

As gypsy moth spreads further south and west into areas of high-value hardwoods, the potential for greater economic impact could possibly be offset on a sustained basis by the entomopathogenic activity of *E. maimaiga.* The intentional introduction of *E. maimaiga* resting spores from one habitat to another will require permits from the USDA Animal and Plant Health Inspection Service and precautions to avoid the distribution of plant pathogens (Reardon and Hajek, 1993). The development of cost-effective methods for producing resting spores or mycelia in artificial media will be necessary before *E. maimaiga* can be used as an applied microbial insecticide (Reardon and Hajek, 1993). Current methods for production *in vitro* yield considerably fewer spores than *in vivo* methods and loss of virulence has been reported after repeated subculturing (Hajek *et al.,* 1990c).

Deladenus siricidicola and Sirex noctilio

When the woodwasp, *Sirex noctilio* F., a serious pest of Monterey pine, *Pinus radiata* D. Don., was accidentally introduced into New Zealand and Australia without its natural enemies, the devastation to pine plantations was staggering (Bedding, 1993). The most effective natural enemy of the woodwasp is the phaenopsitylenchid nematode, *Deladenus (=Beddingia) siricidicola* Bedding (Bedding and Akhurst, 1974). This species has both a parasitic cycle within the host and multiple free living cycles that feed on *Amylostereum areolatum* (Fr.) Boidin, a symbiotic fungus of *S. noctilio,* within the galleries made by the woodwasp (Bedding, 1967, 1972). Free-living nematodes multiply rapidly when introduced into logs that are infested with *S. noctilio* and *A. areolatum* (Bedding, 1972). When *S. noctilio* larvae are contacted, the nematodes molt into preparasitic adults and mate, and the mated females penetrate the host, beginning the parasitic cycle. The female nematodes remain in the host through its metamorphosis and eventually release nematode juveniles within the pupa of the host. The juvenile nematodes invade the host ovaries and completely sterilize adult female woodwasps. Within the host ovaries, individual eggs are invaded by up to 200 juvenile nematodes. This activity kills woodwasp embryos or interferes with egg development and enables distribution of the nematode to new habitats by ovipositing woodwasps. Levels of *D. siricidicola* parasitism approaching 100% in *S. noctilio* populations have been reported (Bedding, 1993).

A number of factors contributed to the overwhelming success of the *Sirex* control program. Prominent among these are the effectiveness of the nematode in seeking out host larvae, it's multiplication and persistence in the host habitat, dissemination by host females, and the development of mass production of the free-living nematode on artificial medium for inoculation programs (Bedding and Akhurst, 1974). Bedding (1993) estimates potential losses of up to $4 billion in the Australian pine lumber industry if the woodwasp is left unabated.

INUNDATIVE APPLICATIONS

Bacteria

The most widely used inundatively applied microbial control agent is *B. thuringiensis.* The history of its development is presented by Beegle and Yamamoto (1992). Today a number of isolates of the bacterium are commercially produced with activity against Lepidoptera, Coleoptera, and Diptera (Shah and Goettel, 1999). Isolates that are active against chewing lice, plant-parasitic nematodes, and other pests have also been discovered. As of 1998 about 200 *B. thuringiensis-*

based products were registered in the United States alone (Schnepf *et al.*, 1998).

Most of the insecticidal activity of *B. thuringiensis* is associated with the proteinaceous toxins located in parasporal inclusion bodies, also known as parasporal crystals. They are produced at the time of sporulation and account for up to 30% of the total protein content of the bacterium (Höfte and Whiteley, 1989; Aronson, 1993; Agaisse and Lereclus, 1995). Collectively, the toxins found in parasporal crystals are referred to as δ-endotoxins.

B. thuringiensis insecticidal proteins are highly specific insect gut toxins with a superior safety record in regard to their effects on nontarget organisms (Lacey and Mulla, 1990; Melin and Cozzi, 1990; Glare and O'Callaghan, 2000; Lacey and Siegel, 2000) including vertebrates (Saik *et al.*, 1990; Siegel and Shadduck, 1990; Lacey and Siegel, 2000). Their mode of action is thought to involve a cascade of events leading to insect death within several hours following ingestion (Höfte and Whiteley, 1989; Gill *et al.*, 1992; Knowles, 1994; Powell *et al.*, 1995). Cry1 proteins, which are active primarily against larval lepidopteran pests, have been the most extensively studied *B. thuringiensis* insecticidal proteins with respect to their structure and mode of action (Harvey *et al.*, 1986; Ge *et al.*, 1989; Bietlot *et al.*, 1990; Choma and Kaplan, 1990; Knowles and Dow, 1993; Knowles, 1994). The Cry1 proteins (protoxins) which are found in the crystal are biologically inactive. Following ingestion and solubilization in the alkaline midgut, cleavage by gut proteases produces a smaller 60- to 65-kDa activated protein that recognizes specific binding sites at the brush border membrane surface of the epithelial columnar cells lining the gut lumen (Hofmann *et al.*, 1988a,b; Van Rie *et al.*, 1989; Honée *et al.*, 1991). The next steps are pore formation, membrane transport disruption, and cell lysis leading ultimately to insect death (Höfte and Whiteley, 1989; Thomas and Ellar, 1983; Slatin *et al.*, 1990; Knowles and Ellar, 1987; Schwartz *et al.*, 1991, 1993). An extensive review on *B. thuringiensis* insecticidal proteins was recently published by Schnepf *et al.* (1998).

A number of insect species are capable of developing resistance to *B. thuringiensis* toxins in the laboratory (McGaughey and Beeman, 1988; Gelernter, 1997), but development in the field has thus far been reported only from diamondback moth, *Plutella xylostella* (L.) (Tabashnik *et al.*, 1990; Ferré *et al.*, 1991; Shelton *et al.*, 1993; Tabashnik, 1994). Several strategies have been proposed for resistance management (Tabashnik *et al.*, 1991; Bosch *et al.*, 1994; Hokkanen and Deacon, 1994; Kennedy and Whalon, 1995; Gelernter, 1997; Schnepf *et al.*, 1998). These include the use of nontreated refugia, high dosage, seed mixtures (transgenics and nontransformed cultivars), and toxin mixtures, and the rotation or alternation of *B. thuringiensis* toxins (summarized by Gelernter, 1997).

B. thuringiensis insecticidal proteins are delivered to insects in formulated products and transgenic plants. Formulated products are prepared from naturally occurring or conjugated strains. Transgenic plants are developed by incorporation of the genes responsible for production of the toxin into the plant genome. In addition to traditional formulations (such as suspensions, wettable powders, tablets, microencapsulation), various alternative means of delivery, including endophytic bacteria such as *Clavibacter xyli* Davis *et al.* (Lampel *et al.*, 1994) or *Bacillus cereus* Frankland and Frankland (Mahaffee *et al.*, 1994), to properly deliver the toxins to the target insects have been investigated. Plant-colonizing bacteria including *Pseudomonas fluorescens* Migula, *P. cepacia* (Burkholder) Palleroni and Holmes, *Rhizobium leguminosarum* Jordan, and *Azosporillium* spp. have also been used to produce and deliver *B. thuringiensis* insecticidal proteins (Obukowicz *et al.*, 1986a,b; Skot *et al.*, 1990; Stock *et al.*, 1990; Udayasuryian *et al.*, 1995; Schnepf *et al.*, 1998). Specific delivery systems based on the hosts developing in aquatic habitats have also been proposed to control mosquito larvae (Porter *et al.*, 1993). These include the cyanobacterium *Agmellenum quadruplicatum* (Stevens *et al.*, 1994), *Synechococcus* sp. (Soltes-Rak *et al.*, 1993), and *Caulobacter crescentus* Poindexter (Thanabalu *et al.*, 1992). While offering some distinct advantages, the use of transgenic plants expressing *B. thuringiensis* toxins raises concerns in regard to the development of and/or exacerbation of resistance. This subject is addressed by several authors in Hokkanen and Deacon (1994) and will not be treated here in the context of microbial control *sensu stricto*.

The largest share of the biopesticide market currently goes to *B. thuringiensis*. Estimates range from US$75 million to US$125 million for recent annual sales worldwide (Soares, 1995; Georgis, 1997; Lisansky, 1997). Varieties of the bacterium are currently used for control of a broad range of crop and forestry pests and larvae of several blood-sucking pests of humans and domestic animals (Lacey and Undeen, 1986; Feitelson *et al.*, 1992; Reardon *et al.*, 1994; Soares, 1995; Evans, 1997; Harris, 1997; Charles *et al.*, 2000; Glare and O'Callaghan, 2000). Application of *B. thuringiensis* to agroecosystems and aquatic environments allows survival of beneficial insects and natural enemies of targeted insects, making it an ideal component of IPM. In agroecosystems it is used against several species of lepidopteran, coleopteran, and some dipteran pests in food and fiber crops. Its use in forestry has increased relative to other interventions, including chemical pesticides (Reardon *et al.*, 1994; Evans, 1997).

B. thuringiensis subsp. *israelensis* de Barjac (*Bti*) is used exclusively or in combination with other interventions for the control of larvae of dozens of species of medically important and pestiferous black flies and mosquitoes around the world (Lacey and Undeen,

1986; Skovmand *et al.,* 2000). A prime example of the successful use of *Bti* occurred in the onchocerciasis control program in West Africa. High levels of resistance to the organophosphate insecticides that were originally employed for control of the *Simulium* vectors of onchocerciasis were threatening the future of the program. The use of *Bti* was instrumental in allowing the program to go forward by providing an alternative intervention and a means of resistance management (Kurtak *et al.,* 1987; Guillet *et al.,* 1990).

Other species of bacteria are used on a much smaller scale for insect control. These include *Paenibacillus* (=*Bacillus*) *popilliae* (Dutky) Pettersson *et al.* and related species and *Serratia entomophila* Grimont *et al.* for control of white grubs (Scarabaeidae) and *Bacillus sphaericus* Neide for control of mosquito larvae. The use of bacteria for scarab control was reviewed by Klein and Jackson (1992) and Klein and Kaya (1995). The requirement for *in vivo* production of *P. popilliae* and lower than expected levels of infection obtained in a number of field trials (Klein and Kaya, 1995) decrease the potential of this bacterium for large-scale control.

B. sphaericus is now commercially produced and has some advantages over *Bti* in that it is more persistent in polluted habitats and may recycle under certain conditions, but has a narrower host range (Lacey and Undeen, 1986; Hougard, 1990; Charles *et al.,* 1996; Nicolas *et al.,* 1994). High levels of resistance have been reported in some populations of *Culex quinquefasciatus* Say to *B. sphaericus* (Rao *et al.,* 1995; Nielsen-Leroux *et al.,* 1995, 1997).

Baculoviruses

A large number of viruses offer potential as microbial control agents of insects (Payne, 1982). Those with the greatest microbial control potential are in the Baculoviridae (nucleopolyhedroviruses [NPV] and granuloviruses [GV]) (Granados and Federici, 1986; Hunter-Fujita *et al.,* 1998). However, information on the potential of other viruses as microbial control agents is somewhat deficient. More than 400 insect species, mostly in the Lepidoptera and Hymenoptera, have been reported as hosts for baculoviruses. Granados and Federici (1986), Adams and McClintock (1991), Tanada and Hess (1991), Tanada and Kaya (1993), Vail (1993), Cunningham (1995), Hunter-Fujita *et al.* (1998), and Vail *et al.* (1999) summarize the literature on the nature of baculoviruses, their mode of action, epizootiology, and use for control of pest insects in forestry and agroecosystems.

The baculovirus virions are enveloped rod-shaped nucleocapsids containing circular, supercoiled, double-stranded DNA. The virions of GVs are individually occluded in a protein matrix (granulin). In the NPVs, singly enveloped (SNPV) or multiply enveloped (MNPV) virions are occluded in a protein matrix (polyhedrin). After ingestion by the host, the occlusion bodies, or polyhedra, are dissolved in the alkaline environment of the host insect's midgut. The liberated virions enter the gut epithelial cells and replicate in the nuclei. Nonoccluded virus particles that are budded from the gut cells into the hemocoel invade other tissues (fat body, tracheal matrix, hypodermis, etc.) within the host. Virus particles that are occluded within polyhedra are generally the infective inoculum for subsequent hosts. Some transmission of baculovirus virions may be facilitated by predators and ovipositing parasitoids via mechanical transmission (Evans, 1986; Gröner, 1990).

As with other biocontrol agents, there are three basic strategies for use of entomopathogenic viruses as microbial control agents (inoculation, augmentation [inundation] and conservation). The use of viral pathogens of insects in most agricultural crops is inundative and does not utilize their full epizootic potential, but takes advantage of their virulence and specificity (Payne, 1982). Baculoviruses registered for use or under development for insect control are presented in Table 1. The NPVs of gypsy moth, the *Helicoverpa/Heliothis* complex, velvet bean caterpillar, *Anticarsia gemmatalis,* and others are or have been applied over fairly large acreages. The NPV of velvet bean caterpillar, for example, has been used to treat ca. 1 million ha of soybeans in Brazil annually (Moscardi and Sosa-Gomez, 2000).

Their efficacy, specificity, and production of secondary inoculum make baculoviruses attractive alternatives to broad-spectrum insecticides and ideal components of IPM systems due to their lack of untoward effects on beneficial insects including other biological control organisms (Huber, 1986; Gröner, 1990; Cunningham, 1995). Unfortunately, this selectivity, often for individual species, coupled with the requirement for and cost of *in vivo* production, has deterred commercial development due to limited market size. Several baculoviruses that have relatively broad host ranges have recently been isolated, partially rectifying this impediment.

Two baculoviruses that have relatively broad host ranges in the Lepidoptera are the NPVs of *Autographa california* (Speyer) (Vail *et al.,* 1971) and *Anagrapha falcifera* (Kirby) (Hostetter and Puttler, 1991). The *Ac*MNPV is active against larvae of 43 species in 11 families of Lepidoptera (Vail *et al.,* 1971, 1999; Cunningham, 1995). Considerable interest has been demonstrated for the abilities of *Ac*MNPV and other baculoviruses as expression vectors for the production of interferon and other biological products of pharmaceutical interest (Luckow and Summers, 1988; Miller, 1988; Shuler *et al.,* 1995).

Some of the drawbacks of the use of entomopathogenic viruses are their relatively slow action compared to that of chemical insecticides, sensitivity to UV light, and the requirement for living systems for production.

TABLE 1

Baculoviruses Registered for Use or under Development for Control of Insect Pests in Agroecosystems, Stored Products, and Forestry

Original host/target species	Selected references
Row crops	
Helicoverpa/Heliothis spp. Bollworms, budworms, corn earworm	Ignoffo (1965), Ignoffo and Couch (1981)
Autographa californica Alfalfa looper and several other lepidopteran species	Vail *et al.* (1971, 1973)
Anticarsia gemmatalis Velvetbean caterpillar	Moscardi and Sosa-Gomez (2000)
Trichoplusia ni Cabbage looper	Ignoffo (1964)
Anagrapha falcifera Celery looper and several other lepidopteran species	Hostetter and Puttler (1991)
Spodoptera spp. Armyworms, cotton leafworm	Cherry *et al.* (1997)
Orchard vineyard	
Cydia pomonella Codling moth	Tanada (1964), Falcon *et al.* (1968)
Harrisina brillians Grape leaf skeletonizer	Stern and Federici (1990)
Oryctes rhinoceros[a] Palm rhinoceros beetle	Zelazny (1978), Bedford (1980)
Stored product pests	
Plodia interpunctella Indian meal moth	Hunter (1970), Vail *et al.* (1991)
Forestry pests	
Lymantria dispar Gypsy moth	Shapiro *et al.* (1981)
Orgyia pseudotsugata Douglas-fir tussock moth	Martignoni and Iwai (1978)
Choristoneura spp. Budworms	Cunningham (1995)
Panolis flammea Pine beauty moth	Entwistle and Evans (1987)
Neodiprion sertifer European pine sawfly	Cunningham (1982)

[a] The *Oryctes* virus has been recently removed from the Baculoviridae and is currently not assigned to a family.

During the time following initial infection, insects continue to feed until the latter stages of infection. Fortunately, the genomes of *Ac*MNPV and other baculoviruses are amenable to genetic manipulation and improvement with recombinant technology. The deletion of certain viral genes that delay host mortality and/or the insertion of others that encode insect-selective toxins, insect hormones, or juvenile hormone esterases or other enzymes has decreased the lag time from ingestion of virus until onset of death in the target insect (Wood, 1991, 1995; Possee, 1993; Miller, 1995; Bonning and Hammock, 1996). The need for and importance of risk assessment of engineered viruses is reviewed by Fuxa (1989, 1990), Miller (1995), and Bonning and Hammock (1996).

Research on formulation and application technology has enabled greater persistence and improved efficacy (Young and Yearian, 1986), but further improvements are needed. Also, additives such as stilbene brighteners and the protein enhancin have the potential to synergize the insecticidal activity of viruses (Corsaro *et al.*, 1993; Shapiro and Dougherty, 1993) and dramatically increase their activity.

Continuing research on methods of production, such as large-scale use of cell lines, that could reduce the cost of production is warranted (Weiss and Vaughn, 1986; Weiss *et al.*, 1994; Granados and McKenna, 1995). In developing countries, where the cost of imported insecticides is high and that of labor is lower, *in vivo* production could provide both a viable means of producing large quantities of virus and a source of employment. The use of baculoviruses for insect control within the IPM context is expected to increase in the coming years, particularly in developing countries and for the control of insects in high-value crops grown on small acreages.

Fungi

Some 700 species of entomopathogenic fungi have been reported, but only 10 of these have been or are

currently being developed for insect control (Hajek and St. Leger, 1994). A broad range of biologies, from obligate parasitism to opportunistic pathogens that can survive saprophytically in the absence of living hosts, have been documented for the entomopathogenic fungi. In most species of entomopathogenic fungi, access to the host is through the cuticle and may involve complex biochemical interactions between the host and the fungus before germination, penetration, growth, and reproduction of the fungus can occur. The life cycles of obligate parasites, such as those species in the genus *Coelomomyces*, may be quite involved and include intermediate hosts (Couch and Bland, 1985). The Fungi Imperfecti (Deuteromycotina: Hyphomycetes), on the other hand, have simpler life cycles and lack sexual reproduction, and many have considerably broader insect host ranges.

Many entomopathogenic fungi, especially those in the Entomophthorales, are responsible for epizootics that often successfully regulate pest insect populations. Although inoculation of insect populations with entomopathogenic fungi has provided classical biological control of some pests, most notably against the gypsy moth, the most common method of employing fungi for insect control is through inundative means. Most species of entomophthoralean fungi are relatively difficult to produce and their primary conidia are short lived, making timing of inundative applications difficult or impossible. Development of effective methods for production of resting spores and competent mycelia of entomophthoralean species will ultimately increase the utility of these fungi.

Species in the Hyphomycetes demonstrate activity against a broad range of insects pests and are the main contenders for commercial production and use against homopterous pest insects. Several species offer good potential for production on inexpensive artificial media and have good shelf lives. Entomopathogenic Hyphomycetes have been investigated for use against a broad range of insect pests, including whiteflies, aphids, thrips, termites, grasshoppers and locusts, beetles, and others (McCoy *et al.*, 1988; Ferron *et al.*, 1991; Fargues and Maniania, 1992; Khan *et al.*, 1993; Zimmermann, 1993; Devi, 1994; Milner and Prior, 1994; Feng *et al.*, 1994; Goettel *et al.*, 1995, 2000; Lacey *et al.*, 1996; Keller *et al.*, 1997; Milner, 1997). Commercial products based on *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Verticillium lecanii* (Zimmermann) Viegas, and *Paecilomyces fumosoroseus* (Wize) Brown and Smith and experimental isolates of *Metarhizium flavoviride* Gams and Rozsypal, *Nomuraea rileyi* (Farlow) Samson, and *Aschersonia aleyrodis* Webber are currently in use or under development. Several commercial sources of entomopathogenic Hyphomycetes are listed by Shah and Goettel (1999). In conjunction with inundative applications, the endophytic nature of *B. bassiana* in corn offers the potential of season-long control of *Ostrinia nubilalis* (Hübner) and has a suppressing effect on overwintering larvae (Anderson and Lewis, 1991). Despite their somewhat broader host range, the Hyphomycetes still provide a degree of selectivity (McCoy *et al.*, 1988; Goettel *et al.*, 1990).

A complex set of interacting processes, both environmental and biotic, is necessary for or inhibitory to development of epizootics caused by entomopathogenic fungi. These include sensitivity to solar radiation; microbial antagonists; host behavior, physiological condition, and age; pathogen vigor and age; presence of pesticides; and appropriate temperature, humidity, and inoculum thresholds (McCoy *et al.*, 1988; Ferron *et al.*, 1991; Hajek and St. Leger, 1994; Lacey and Goettel, 1995). To take full advantage of the epizootic potential of fungi we need to understand not only the determinants that are critical for fungal virulence and infection but also the techniques to exert control over them through optimization of culture methods, formulation, environmental manipulation, and genetic engineering. Successful use of entomopathogenic fungi as microbial control agents will ultimately depend on the use of the right propagule, formulated in an optimal manner and applied at an appropriate dosage and time. Timing will depend on the presence of susceptible host stages, favorable environmental conditions, and compatible scheduling with other agricultural practices (i.e., linked with irrigation, avoiding fungicides, etc.).

Further improvement in the microbial control activity of entomopathogenic fungi can be expected by their combination with other interventions and technologies, use of other biological control agents, use of environmental manipulation to favor the infection processes, and use of targeted pests to aid in the dissemination of fungus. For example, the use of semiochemicals in traps that attract adult insects and contaminate them with fungal spores will not only control the attracted insects, but also enable autodissemination of the fungi into difficult to treat larval habitats such as soil (Klein and Lacey, 1999), corn ears (Vega *et al.*, 1995), cabbage heads (Furlong *et al.*, 1995), and other habitats (Vega *et al.*, 2000). Quintela and McCoy (1997) reported the synergistic combination of the chloronictinyl insecticide imidacloprid with *M. anisopliae* and *B. bassiana* for control of citrus root weevil, *Diaprepes abbreviatus* (L.), larvae.

The prospects of genetic engineering for improvement of entomopathogenic fungi have steadily increased within the past decade (Ferron *et al.*, 1991; Riba *et al.*, 1994; Charnley *et al.*, 1997; St. Leger and Roberts, 1997), but still lag somewhat behind those of the recombinant technology developed for *B. thuringiensis* and baculoviruses. Developments in the molecular biology of entomopathogenic fungi will provide the tools for elucidating the mechanisms of pathogenesis

and in the future for producing recombinant fungi with enhanced virulence (Charnley *et al.*, 1997). The application of molecular techniques in the study of entomopathogenic fungi is presented in detail by St. Leger and Joshi (1997).

Nematodes

A plethora of nematode species in more than 30 families is associated with insects and other invertebrates (Poinar, 1979, 1990; Kaya and Stock, 1997). The major focus of research and development has been on nematode species in 7 families, Mermithidae, Tetradonematidae, Allantonematidae, Phaenopsitylenchidae, Sphaerulariidae, Steinernematidae, and Heterorhabditidae, because of their potential as biological control agents of insects (Kaya and Stock, 1997). However, the biological control potential of nematodes is not restricted to insects. For example, *Phasmarhabditis hermaphrodita* (Schneider) in the family Rhabditidae suppresses slugs (*Deroceras* sp. and others) which are seedling pests of a number of agricultural crops (Wilson *et al.*, 1993, 1994, 1995; Wilson and Gaugler, 2000).

As discussed earlier, the phaenopsitylenchid *D. siricidicola* has been highly successful as a classical biological control agent against the woodwasp *S. noctilio* in New Zealand and Australia (Bedding, 1993). In contrast, the mermithid *Romanomermis culicivorax* Ross and Smith had been used as an inundative agent for mosquito larval suppression (Petersen, 1985). It recycles in certain host habitats and produces high levels of infection in selected target mosquito species, but it is intolerant to polluted, organically enriched, or high-salinity habitats and high temperatures. Furthermore, it could be produced only *in vivo* and could not compete commercially with another biological agent, *B. thuringiensis* subsp. *israelensis*. The tetradonematids, allantonematids, and sphaerulariids have complicated life cycles, are difficult to mass produce, appear to be density independent, and require further studies before they can be used effectively in biological control programs. Currently, the steinernematids and heterorhabditids are receiving the most attention as microbial control agents of soil insects. After *B. thuringiensis*, these nematodes are next in commercial sales at US$2–3 million annually (Georgis, 1997).

The entomopathogenic steinernematid and heterorhabditid nematode species possess many attributes of parasitoids and pathogens. They are analogous to parasitoids because they have chemoreceptors and can actively search for their hosts (Kaya and Gaugler, 1993; Gaugler *et al.*, 1997a). They are similar to pathogens because of their association with mutualistic bacteria in the genera *Xenorhabdus*, for steinernematids, and *Photorhabdus*, for heterorhabditids. The nematode/bacterial complex is highly virulent, killing its host within 48 h through the action of the mutualistic bacteria. These nematodes can be cultured *in vitro*, have a high reproductive potential, and have a numerical, but no functional, response (Kaya and Gaugler, 1993). Moreover, they infect a number of insect pest species, yet pose no threat to plants, vertebrates, and many invertebrates (Akhurst, 1990; Kaya and Gaugler, 1993). They can be mass produced, formulated, and easily applied as biopesticides (Georgis and Manweiler, 1994; Georgis and Kaya, 1998), have been exempt from registration in many countries, are compatible with many pesticides, and are amenable to genetic selection (Kaya and Gaugler, 1993).

The third-stage infective nematode (=dauer stage or infective juvenile) of steinernematids and heterorhabditids has been likened to a guided missile because it carries the "warheads" of the mutualistic bacterial cells in its intestine (Akhurst, 1993). Each species of nematode is associated with a specific bacterium, but some bacterial species are associated with more than one nematode species (Akhurst, 1993; Forst and Nealson, 1996; Burnell and Stock, 2000). The link between the matching of the appropriate phase of these bacteria (the bacterium occurs as phase I or phase II with phase I more suitable for nematode production) and the successful production of efficacious entomopathogenic nematodes is essential (Akhurst, 1993; Forst and Nealson, 1996). The large-scale production of nematodes on solid, monoxenic artificial medium on foam (Bedding, 1984) or particularly on liquid monoxenic media (Friedman, 1990; Ehlers, 1996; Johnigk and Ehlers, 1999) expands the commercial possibilities. However, *in vivo* production using the wax moth, *Galleria mellonella* (L.), or another suitable insect host is still used by some in the cottage industry. Formulation of the infective juveniles in a wettable dispersible granule has permitted storage capability of 6 months at room temperature and has increased the options for their application (Georgis and Manweiler, 1994; Ehlers, 1996).

The entomopathogenic activity of steinernematid and heterorhabditid species has been documented against a broad range of insect pests in a variety of habitats (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). These nematodes are especially efficacious against insects in soil and cryptic habitats (Table 2). They have been used inundatively in a number of high-value cropping systems (Georgis and Manweiler, 1994; Koppenhöfer, 2000). For example, the citrus root weevil, *D. abbreviatus*, in citrus, the black vine weevil, *Otiorhynchus sulcatus* (F.), in nurseries and cranberries, the black cutworm, *Agrotis ipsilon* (Hufnagel), and mole crickets, *Scapteriscus* spp., in turfgrass, and the peach borer moth, *Carposina niponensis* Walsingham, in apples have been successfully controlled.

When an entomopathogenic nematode species is used against a pest insect, it is critical to match the

TABLE 2

Steinernematid and Heterorhabditid Nematodes in Use or Being Developed as Microbial Control Agents of Insects

Nematode families and species[a]	Targeted groups	Selected references
Heterorhabditidae		
Heterorhabditis bacteriophora	Lepidoptera, Coleoptera	Begley (1990), Klein (1990)
Heterorhabditis megidis	Coleoptera	Klein (1990)
Heterorhabditis marelatus	Coleoptera, Lepidoptera	Liu and Berry (1996), Berry *et al.* (1997)
Steinernematidae		
Steinernema carpocapsae	Lepidoptera, Coleoptera, Siphonaptera	Begley (1990), Klein (1990), Georgis and Manweiler (1994)
Steinernema feltiae	Diptera (Sciaridae)	Begley (1990), Klein (1990)
Steinernema glaseri	Coleoptera (Scarabaeidae)	Klein (1990)
Steinernema kushidai	Coleoptera (Scarabaeidae)	Ogura (1993)
Steinernema riobrave	Lepidoptera, Orthoptera	Cabanillas *et al.* (1994)
	Coleoptera (Curculionidae)	Cabanillas and Raulston (1994)
Steinernema scapterisci	Orthoptera (mole crickets)	Parkman *et al.* (1993)

[a] Sources: Gaugler and Kaya (1990), Kaya and Gaugler (1993), and Kaya and Stock (1997).

right nematode species against the insect pest (Bedding, 1990; Kaya and Gaugler, 1993). Some nematode species are more efficacious against a particular insect group than against another insect group. For example, *Steinernema kushidai* Mamiya is effective against scarab grubs and less so against lepidopteran larvae (Mamiya, 1989), and *Steinernema scapterisci* Nguyen and Smart is effective against mole crickets and house crickets, but not effective against other insect groups (Nguyen and Smart, 1991). In addition, foraging behavior of entomopathogenic nematodes can affect their efficacy. Some species are ambushers (e.g., *Steinernema carpocapsae* (Weiser) and *S. scapterisci*) that tend to remain near the soil surface and attach to and infect mobile hosts at the soil–litter interface (Campbell and Gaugler, 1993; Lewis *et al.*, 1993). Other species (e.g., *S. glaseri* (Steiner) and *Heterorhabditis bacteriophora* Poinar) are cruisers that have an active searching strategy and are more effective against less mobile insects in the soil (Lewis *et al.*, 1993; Campbell and Gaugler, 1997).

Although they are used primarily as biopesticides, some species of nematodes persist and recycle in the host habitat, bringing about sustained suppression of some insect pests (Kaya, 1990; Hominick and Collins, 1997). One species, *S. scapterisci*, has been established as a classical biological control agent of mole crickets in Florida, but its impact on the reduction of population levels is still being evaluated (Parkman and Smart, 1996).

Genetic improvements in entomopathogenic nematodes may expand their potential as biocontrol agents by increasing search capacity, virulence, and resistance to environmental extremes, among other attributes (Burnell and Dowds, 1996; Gaugler and Hashmi, 1996). Recently, Gaugler *et al.* (1997b), using molecular techniques, have inserted a heat-shock protein into *H. bacteriophora*, resulting in transgenic nematodes that were 18 times better than the wild types at surviving high-temperature stress. Field release of the transgenic and wild-type nematodes showed no differences in their abilities to persist.

Significant advances have been made with these entomopathogenic nematodes, but the high costs associated with production and formulation in comparison to those costs of chemical pesticides and other biologicals (i.e., *B. thuringiensis*) will restrict their use to high-value niche markets and sensitive areas where chemicals cannot be used (Georgis, 1997). However, advances have also been made with new chemistry products (e.g., imidacloprid) that are more environmentally friendly than organophosphates, carbamates, or chlorinated hydrocarbons. Insects will probably become resistant to these new chemical pesticides, and entomopathogenic nematodes and other entomopathogens may play a more important role in IPM. For example, the combination of imidacloprid and entomopathogenic nematodes has shown synergistic activity against 3rd-instar scarabs (Koppenhöfer and Kaya, 1998; Koppenhöfer *et al.*, 2000). Imidacloprid is most efficacious against 1st- and 2nd-instar scarabs, but most damage is done by the 3rd instar. Accordingly, the combination of these two agents may be useful in the management of scarab pests in turf.

Steinernematids and heterorhabditids have been used successfully against a number of soil-inhabiting insect pests. However, this realm of insect nematology is a very young discipline with major contributions being made since the mid-1980s. Kaya and Gaugler (1993) and Gaugler *et al.* (1997a) indicate that there is a need for more in-depth basic information on their biology, including the ecology, behavior, and genetics of these nematodes, to help understand the underlying reasons for their successes and failures as biological control agents. Armed with this information, innovative approaches through genetic engineering and combinations with other control agents offer promise in insect suppression. More traditional approaches of

classical biological control or augmentation with new or previously described species of nematodes may provide population reduction through inoculative releases.

Protozoa

Protozoan diseases of insects are ubiquitous and comprise an important regulatory role in insect populations (Maddox, 1987; Brooks, 1988). They are generally host specific and slow acting, most often producing chronic infections. The biologies of most entomopathogenic protozoa are complex. They develop only in living hosts and many species require an intermediate host. Species in the Microsporida are among the most commonly observed. Their main advantages are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. As inundatively applied microbial control agents, only a few species have been moderately successful (Solter and Becnel, 2000). The grasshopper pathogen *Nosema locustae* Canning is the only species that has been registered and commercially developed (Henry and Oma, 1981). The main disadvantages of the Protozoa as inundatively applied microbial control agents are the requirement for *in vivo* production and low levels of immediate mortality.

THE FUTURE OF INSECT PATHOGENS

Control of pest insects with chemical pesticides has generated several problems including insecticide resistance, outbreaks of secondary pests normally held in check by natural enemies, safety risks for humans and domestic animals, contamination of ground water, decrease in biodiversity, and other environmental concerns. These problems and sustainability of programs based predominantly on conventional insecticides have stimulated increased interest in integrated pest management. Sustainable agriculture in the 21st century will rely increasingly on alternative interventions for pest management that are environmentally friendly and reduce the amount of human contact with chemical pesticides. The mandate of The 1996 Food Quality Protection Act will also influence the development and registration of chemical pesticides in the future. Effective microbial control agents that can fill the void of phased out chemicals exist, but their further development and implementation will require the following advances: improvements in the pathogens, their production, and formulation; better understanding of how they will fit into integrated systems and their interaction with the environment and other IPM components; greater appreciation for their full advantages (efficacy, safety, selectivity, etc.), not simply their comparison with chemical pesticides; and acceptance by growers and the general public. Potential markets and methods for encouraging the use of microbial control agents are presented by Straus and Knight (1997), including the provision of information and education to growers in regard to their use, benefits, and limitations. A number of technical issues that pertain to improvement in biopesticide production, formulation, and application are addressed by Jones and Burges (1997), Jones *et al.* (1997), and Chapple and Bateman (1997).

The role of microbial pesticides in the integrated management of insect pests has been recently reviewed for agriculture (Lacey and Goettel, 1995; Dent, 1997; Georgis, 1997; Tatchell, 1997), forestry (Evans, 1997; van Frankenhuyzen *et al.*, 2000), and public health (Skovmand *et al.*, 2000). In most cases no single microbial control agent will provide sustainable control of an insect pest or complex of pests. As components of an integrated approach, entomopathogens can provide significant and selective insect control. A truly integrated approach in all agricultural practices will be required to obtain the maximum effect from a given intervention or practice without interfering with the effectiveness of other practices (Edwards, 1990). In the not too distant future we envision a broader appreciation for the attributes of entomopathogens and expect to see synergistic combinations of microbial control agents with other technologies (in combination with semiochemicals, soft chemical pesticides, other natural enemies, resistant plants, chemigation, remote sensing, etc.) that will enhance the effectiveness and sustainability of integrated control strategies.

The use of pathogens to suppress populations of pests over large areas containing multiple agricultural and wild host plants has not been adequately explored (Bell and Hardee, 1994). Such an areawide concept could take advantage, for example, of controlling populations of pests before they became economically important in crop plants. Also, in situations in which a crop is a reservoir for polyphagous arthropods but does not sustain economic damage, it acts as a source of populations moving into other crops at levels that would cause economic damage (e.g., cabbage looper movement from cotton to vegetable crops in the fall of the year [Vail *et al.*, 1976]).

Despite our optimistic appraisal of the future of entomopathogens as biological control agents, portions of the biopesticide industry are currently facing financial setbacks (Gaugler, 1997). Although the market for microbial insecticides is growing, it represents only approximately 1–1.5% of the total crop protection market and most of this is due to sales of *B. thuringiensis* (Gaugler, 1997; Georgis, 1997; Lisansky, 1997). Georgis (1997) believes that in the near future microbials will face even stiffer competition from new pesticide chemistries and transgenic plants. Improvements in microbial products, grower awareness of the benefits that microbial control offers, and the need to develop alternatives to conventional chemical insecticides should overcome many of the obstacles that microbial

control is now facing. However, if future development is only market driven, there will be considerable delays in the implementation of several microbial control agents that have good potential for use in IPM programs (Lacey and Goettel, 1995).

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