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Potential for Microbial Biological Control of Coleopteran and Hemipteran Pests of Potato

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ABSTRACT

Numerous insects in the orders Coleoptera and Hemiptera are major pests of potato, *Solanum tuberosum* L., worldwide. Although these pests are currently managed almost exclusively with chemical insecticides, there is continuing demand for alternative controls that pose lower environmental and health risks. Biological control agents represent one such alternative, and in this review we assess the potential for use of various microbial biological control agents for control of Colorado potato beetle, wireworms, aphids, leafhoppers, and psyllids. The Colorado potato beetle (CPB) and wireworms feed by chewing plant tissues and pass substantial portions of their life cycles both above and below ground. Consequently, they can be targeted with a broad range of microbial control agents, including bacteria, fungi, and nematodes. Aphids, leafhoppers, and Psyllids, on the other hand, feed by piercing plants and sucking sap, and most species pass their lives entirely above ground; these pests are susceptible to few pathogens other than fungi. Investigations to date indicate strong potential for microbial control of CPB, using integrated applications of *Bacillus thuringiensis* and *Beauveria bassiana*. Microbial control of wireworms and hemipteran pests is farther from realization. Important constraints include: difficulties in targeting soil-inhabiting pests with microbial control agents, limited efficacy/recycling potential of nematodes applied against wireworms, limited epizootic potential of fungal pathogens in early-season, low-density hemipteran pest populations, and problems with mass production and formulation of key fungal pathogens of Hemiptera. Latest research efforts aimed at overcoming these constraints are reviewed.

Keywords: bacteria, entomopathogens, fungi, nematodes

CONTENTS

INTRODUCTION

An overview of microbial control of insect pests of the potato, *Solanum tuberosum* L., has been presented by Lacey *et al.* (2009). In this chapter, we focus on several of the key coleopteran and hemipteran pests that attack potato. These include the Colorado potato beetle (CPB), wireworms, aphids, leafhoppers, and psyllids.

Biological control is defined as the use of living organisms to suppress pest populations (Eilenberg *et al.* 2001), and we adhere to this concept in defining microbial biological control as the use of living microorganisms in the suppression of pest populations (also termed microbial biocontrol or simply microbial control). This definition notably excludes pesticide products that are based solely on biochemicals produced by, or extracted from, living organisms or microorganisms (e.g., pyrethrums extracted from chrysanthemum flowers, neem oil extracted from the fruits and seeds of the neem tree, and spinosyns produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao). In

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Invited Review

accord with this definition, we contend that only those pesticides based on biological control agents should be referred to as biological pesticides or biopesticides. Our uses of the terms classical-, inoculation (inoculative)-, inundation (inundative)-, and conservation-biological control in describing the basic approaches used in the manipulation of biological control agents adhere also to the definitions proposed by Eilenberg *et al.* 2001). Entomopathogenic nematodes, though technically not microorganisms, are widely treated and viewed as microbial pathogens. These organisms operate essentially as parasitoids; although, the nematodes most commonly applied as microbial control agents (steinernematid and heterorhabditid species) carry symbiotic bacteria that are released into the host, produce a rapidly lethal septicemia and digest host tissues. The nematodes then feed on these bacteria and host tissues.

Efforts have been undertaken to develop and commercialize microbial control agents for all of the aforementioned pests. Results have been exceedingly variable. Greatest progress has been achieved against the CPB, but even in this case, microbial control is not currently a major component of management programs. Challenges to the development and adoption of microbial control have been reviewed by numerous authors (Lacey and Goettel 1995; Gelernter and Trumble 1999; Lacey *et al.* 2001; Wraight and Hajek 2009). Here, we focus on evaluation of the current state of microbial control strategies and technologies relevant to potato pest management.

COLEOPTERAN PESTS

Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Chrysomelidae)

The Colorado potato beetle (CPB) is the most important defoliator of potato worldwide. Large populations of adult CPB emerging from winter hibernation can devastate stands of potato seedlings, and heavy larval infestations can rapidly defoliate and ultimately kill plants of all ages (**Fig. 1**). Readers are referred to Hare (1990) for a detailed description of the biology of this pest. Briefly, adult beetles overwinter in the soil and emerge in the spring in synchrony with the host plant. Eggs are deposited in small clusters on the undersides of foliage over a period of many weeks. Larvae feed in the crop canopy and pass rapidly through four larval instars before entering the soil to pupate. First-generation adults emerge within two weeks. After feeding on potato foliage, these adults, depending on geographic location, either initiate a new reproductive cycle or enter the soil to overwinter. In northern climes, this pest may have one or two generations per year (often only a partial second generation), whereas in warmer regions, three generations may be produced. Because CPB passes significant periods of time both above and below ground, it is vulnerable to attack by a broad range of natural enemies with microbial control potential, including nematodes, bacteria, and fungi.

Nematode pathogens

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae of the order Rhabditida (EPNs) are effective control agents of dozens of insect species in soil and cryptic habitats (Kaya and Gaugler 1993; Georgis *et al.* 2006; Koppenhöfer 2007). The infective juveniles (IJs) or Dauer juveniles of EPNs are free living and actively seek out or wait for potential host insects. Steinernematids and heterorhaditids harbor entomopathogenic bacteria in the genera *Xenorhabdus* and *Photorhabus*, respectively. Soon after the IJs enter the host, usually through natural openings, the bacteria are released within the host insect, killing it within 2 to 3 days (Kaya and Gaugler 1993; Koppenhöfer 2007). The IJs molt to a feeding stage and eventually to the reproductive or adult stages. Depending on the size of the insect host, one or more generations may develop until resources are depleted, at which time IJs exit in search of new hosts. Several EPN species are commercially produced and available for large-scale application. For small-scale experimental testing, EPNs can be produced *in vivo* and on artificial media (Kaya and Stock 1997).

Plate 1 Figs 1-9. (**1**) Complete defoliation of potato by a heavy infestation of Colorado potato beetle. (**2**) Adult Colorado potato beetle killed by *Beauveria bassiana* and supporting outgrowth of the fungus. (**3**) Fourth-instar Colorado potato beetle larva with dark cuticular lesion, a sign of *B. bassiana* infection. (**4**) *Beauveria bassiana*-infected potato beetle larva entering soil to pupate. (**5**) Prepupal larva of Colorado potato beetle in soil pupation cell killed by *B. bassiana* and supporting profuse sporulation. (**6**) Adults (click beetles) and larvae (wireworms) of *Agriotes obscurus* (Coleoptera: Elateridae). (**7**) Relatively light wireworm damage on potato tubers, but even this level of damage would cause the rejection of shipments to North American supermarkets. Wireworm feeding holes (white arrows) give rise to subsurface discoloration and rotting as shown in cross sections. (**8**) Wireworms (*Agriotes obscurus*) infected with *Metarhizium anisopliae*. Lower wireworm exhibits mycelia protruding from intersegmental areas; upper wireworm shows sporulation. (**9**) Mermithid nematode recently emerged from infected wireworm (*Agriotes obscurus*). (**Figs. 1-5**, S. Galaini-Wraight; **Figs. 6-9**, J. T. Kabaluk)

EPNs have been studied for their efficacy against a limited number of potato pest insects. The majority of research with EPNs for control of potato pests has been conducted against CPB. In some of the earliest laboratory tests, Toba *et al.* (1983), exposed larvae of CPB to *Steinernema carpocapsae* (Weiser) and *S. glaseri* (Steiner) in soil. Both nematodes were equally effective, causing 100% mortality of CPB larvae 13 days after applications of 157 IJs/cm^2 of soil. In field-cage tests, *S. carpocapsae* applied to the soil surface at 155 and 310 IJs/cm^2 caused 59 and 71% mortality, respectively, of late fourth-instar, spring generation of CPB.

No infection was obtained following reinfestation of the cage soil with summer-generation larvae. MacVean *et al.* (1982) tested various antidesiccants for protection of *S. carpocapsae* IJs applied to potato foliage. The additives increased retention of IJs on leaf surfaces, yielding 30–60% infection of CPB larvae compared to 10% when the nematodes were applied in water. It was also demonstrated that higher infection levels were achievable when nematodes were applied during the evening versus morning hours. Despite these positive results, applications of EPNs to leaf surfaces are not considered by most researchers to be an effective use of nematodes for CPB control.

Steinernema carpocapsae (cited as *S. feltiae* before taxonomic revision) and *Heterorhabditis bacteriophora* Poinar (= *H. heliothidis*) were evaluated by Wright *et al.* (1987) against CPB larvae and pupae. In soil under laboratory conditions, *H. bacteriophora* and *S. carpocapsae* ('Mexican' strain) produced the highest mortality 6 days post-treatment. Mortality ranged from 80 to 90% at rates of 79–158 nematodes/cm^2. In a field microplot study, *S. carpocapsae* and *H. bacteriophora* were most effective among four species tested. Adult CPB emergence was reduced by 86–100% after application of 31–93 *H. bacteriophora*/cm^2 and by 88–100% after application of 93–155 *S. carpocapsae* ('Mexican' strain)/cm^2.

Cantelo and Nickle (1992) tested 5 species or strains of EPNs against CPB pre-pupae in potting soil. *Steinernema carpocapsae* ('All' and 'Mexican' strains), *S. feltiae* (strains No. 27 and 980) and *H. bacteriophora* were tested at dosage levels ranging from 10 to 329 IJs/cm^2 in laboratory tests. Each of the five strains produced 100% mortality at 165 IJs/cm^2 and, with the exception of the *S. carpocapsae* 'Mexican' strain, over 98% mortality at 82 IJs/cm^2. Although the authors speculated that field applications would probably require higher rates to obtain the same mortality, they concluded that EPNs have potential to suppress CPB populations, but cost would have to be reduced before being acceptable to growers.

Subsequently, Nickle *et al.* (1994) tested a starch based pelletized formulation (Pesta) of *S. carpocapsae* (All) as a soil treatment in the greenhouse against prepupae of CPB. The IJs survived the pelletization process and the resulting formulation delivered 100,000 viable IJs/g. In the soil, the IJs emerged from the pellets in large numbers and reduced adult emergence of CPB by more than 90%.

Stewart *et al.* (1998) tested the susceptibility of *L. decemlineata* to *S. carpocapsae* (All strain) in the laboratory (18-21°C) and on potatoes in the field in New Brunswick and Prince Edward Island in 1992 and 1993. In the laboratory, applications of 5.0×10^5 *S. carpocapsae*/m^2 against larvae, pupae, and adults resulted in 100% mortality. However, field testing of *S. carpocapsae* in New Brunswick in 1992 reduced *L. decemlineata* populations by only 31% relative to untreated controls, and in 1993, no significant control was observed. Nematode applications on Prince Edward Island did not significantly reduce CPB populations in either year.

Berry *et al.* (1997) compared endemic Oregon species of EPNs and exotic species for control of CPB. In their report, a new species of EPN, *Heterorhabditis marelatus* Liu & Berry, collected from Seaside, OR was effective in reducing emergence of CPB adults from soil treated with the nematode. This species was used as a model by Armer *et al.* (2004a, 2004b) in detailed studies to determine some of the factors that enhance or limit the activity of EPNs against CPB.

Armer *et al.* (2004a) examined the efficacy of *H. marelatus* for control of CPB in laboratory studies and in potato fields in north central Oregon. They tested the hypothesis that increasing nitrogen fertilization would increase foliar alkaloid levels, which would stress the beetle and allow increased nematode infection and reproduction and enable long-term control. Their laboratory studies showed that nematode reproduction was not correlated directly with foliar alkaloid levels; however, the nematode did reproduce more readily in CPB fed on potato plants stressed (stunted) by high levels of fertilizer. Field trials showed that higher nitrogen applications increased field foliar levels of the alkaloids solanine by 35%, and chaconine by 41% over the season, but these treatments did not affect rates of nematode infection or reproduction. No reproducing *H. marelatus* were found in over 300 dead prepupae and pupae from treated plots. It was ultimately concluded that nematode reproduction could not be manipulated through alkaloid stress to the beetle. Results were more encouraging in terms of inundation biological control: across all nitrogen treatments, *H. marelatus* applied twice during the season, resulted in a 50% reduction in adult CPB and a six-fold increase in numbers of dead prepupae in nematode-treated soil versus untreated soil.

Recycling and residual activity has not been observed in most field tests of *Steinernema* and *Heterorhabditus* spp. for CPB control (Toba *et al.* 1983; Armer *et al.* 2004a). Armer *et al.* (2004b) conducted a detailed study to examine the possible factors limiting development and reproduction of *H. marelatus* and its symbiotic bacterium, *Photorhabdus temperata* Fischer-Lesaux *et al.* (identified as *P. luminescens*) in CPB. Although *H. marelatus* killed 98% of treated CPB prepupae, it reproduced in only 1–6% of the beetles. They examined nematode/bacterial inhibition at each step of the developmental cycle and found that encapsulation of the nematodes occurred in only 1.6% of CPB. However, *P. temperata* was negatively affected by a heat-labile factor found in CPB hemolymph. This factor often caused the bacterium to switch from the primary form that produces antibiotics and enzymes that provide nutrients necessary for the normal nematode development to a secondary form that did not produce antibiotics and had reduced enzyme production. The heat-labile factor did not directly affect the nematode. Their data suggest that both the toxic heat-labile factor and a lack of appropriate nutrients altered the CPB-bacterium-nematode interaction. In a subsequent study, Blackburn *et al.* (2007) reported that *P. temperata* did not exhibit phenotype switching in CPB hemolymph but grew abnormally and was inhibited by competing enteric bacteria. Growth of the stressed symbiont was inadequate to support normal nematode development. Though equally detailed investigations have not been conducted with steinernematid species, the above-described findings indicate that, despite being highly susceptible to infection, CPB is not a sustaining host for EPNs. Use of these agents for biological control of this pest may thus be limited to the inundation approach, which continues to be constrained by the unfavorable economics of EPN applications in large-hectarage field crops.

Bacterial pathogens

Development of bacteria for CPB control commenced only relatively recently with the discovery of beetle-pathogenic subspecies of the well-known entomopathogen *Bacillus thuringiensis* Berliner (*Bt*). As with all subspecies of *Bt* that have been developed for pest control, the principal insecticidal activity derives from production of proteinaceous endotoxins. The endotoxins must be ingested to affect the host; all *Bt* endotoxins target the host midgut epithelium and can thus be considered gut poisons. Effects are generally rapid, with lethal doses causing immediate cessation of feeding; mortality of highly susceptible individuals may follow within a few hours (Beegle and Yamamoto 1992; Tanada and Kaya 1993). Susceptibility decreases with age, and late-instar larvae and adults are quite resistant to intoxication (Zehnder and Gelernter 1989; Ferro and Lyon 1991). As has been the case with most commercially developed *Bt*-based biopesticides, those developed for CPB control have included living spores of the pathogen. However, the endotoxins in these products are highly virulent in the absence of spores (Riethmuller and Langenbruch 1989), and thus, the mode of action of these CPB control agents more closely resembles that of materials classified as biochemical or biorational insecticides than as biopesticides. It warrants note, however, that in studies of other subspecies of Bt, toxins associated with the spore coat and/or the capacity of the bacterium to invade the host hemolymph have been shown to contribute to mortality of some hosts (see Glare and O'Callaghan 2000), and the role of spores in insecticidal activity has not been thoroughly investigated for all CPB-active strains of *Bt*.

The first CPB-toxic subspecies of *Bt*, identified as *Bacillus thuringiensis tenebrionis* (*Btt*), was identified in the early 1980s (Krieg *et al.* 1983). It was rapidly commercialized and formulated as a biopesticide for microbial control of CPB, and within 10 years, the gene coding for the insecticidal endotoxin (designated *cry*3A) was engineered into potato plants (Adang *et al.* 1993; Perlak *et al.* 1993). The discovery of *Btt* and the promise of genetically engineered crop plants stimulated much research into the discovery and development of other coleopteran-toxic strains of *Bt*, and additional strains with novel *cry*3 genes (*cry*3B and *cry*3C) coding for toxins active against CPB and other chrysomelid beetle pests were soon identified (see Crickmore *et al.* 1998). The *cry*3B toxin is substantially more toxic against adult CPB than the *cry*3A toxin (Johnson *et al.* 1993); although; it is noteworthy that constant feeding by female beetles on transgenic potato plants producing either *cry*3A or B toxins results in nearly complete inhibition of egg production (Perlak *et al.* 1993; Arpaia *et al.* 2000). As is the case with nearly all *Bt cry* genes, the *cry*3 genes are located on plasmids that can be can be transferred among *Bt* strains via natural conjugation or conjugation-like processes. The discovery of multiple *cry*3 genes has enabled development of transconjugant *Bt* strains with both enhanced toxicity toward CPB and broader toxicity spectra (see Baum *et al.* 1999).

Efficacy of *Btt*-based biopesticides was demonstrated in research trials (Ferro and Gelernter 1989; Zehnder and Gelernter 1989; Zehnder *et al.* 1992), and these products were marketed for CPB control in the late 1980s and early 1990s (Gelernter and Trumble 1999). However, because *Bt* toxins degrade rapidly upon exposure to solar radiation, because the CPB-susceptibility profile necessitates targeting of early-instar larvae, and because the beetle has an extended oviposition period, effective use of sprayable *Bt*s generally required carefully timed, multiple applications (Gelernter and Lomer 2000; Navon 2000). These requirements had, from the start, placed the *Bt*-based biopesticides at a significant disadvantage compared to chemical insecticides, and in the mid-1990s, competition from chemical control agents became overwhelming when a new class of insecticides, the neonicotinoids, entered the market. Neonicotinoid insecticides proved not only extraordinarily toxic to both adult and larval CPB, but also exhibited strong systemic activity, which conferred prolonged residual activity. The excellent control of CPB provided by these novel insecticides, combined with public resistance to genetically modified food crops also reduced demand for transgenic *Bt* potatoes, and currently, neither sprayable *Bt*s nor transgenic potato plants play a significant role in CPB management. Nevertheless, the potential for use of *Bt*s as microbial control agents in CPB management remains great, especially in organic or low-insecticide-input potato production systems, in biological IPM programs, and in pesticide-rotation schemes recommended for resistance management (see below). Great potential exists also for continued work toward commercialization of genetically modified potato plants producing one or more of the *cry*3 toxins. Because of the strong capacity of CPB to develop resistance to insecticidal toxins, extensive use of such crops would require development of protocols for resistance management, likely including refuge plantings of non-transgenic potatoes (Roush 1994), strict crop rotations (Roush *et al.* 1990), or pyramiding of *cry*3 genes (Zhao *et al.* 2003); however, the topic of transgenic plants is beyond the scope of this review.

Fungal pathogens

Hypocrealean fungi (Ascomycota) are among the most important natural enemies of chrysomelid beetles (Humber 1996). Species from several of the most prominent genera of insect pathogens, including *Beauveria*, *Metarhizium*, and *Isaria*, have been reported from CPB. These pathogens are commonly associated with the soil and with soil-inhabiting insects, and most reports of natural fungal enzootics and epizootics in CPB populations involve immature beetles undergoing pupation or adult beetles overwintering in the soil (Bajan and Kmitowa 1977; Lipa 1985; Humber 1996). Research into the use of fungi for CPB control was initiated in Eastern Europe in the 1950s. *Beauveria bassiana* (Balsamo) Vuill. and *Isaria farinosa* (Holmskiold) Fr. (= *Paecilomyces farinosus*) were identified as the two most important naturally occurring pathogens (Bajan and Kmitowa 1977). *Beauveria bassiana* was ultimately identified as the more widespread and prevalent of the two species (Lappa 1979), and ultimately became the focus of research and development efforts that have continued to the present.

The mode of action of fungal pathogens differs markedly from that of *Bt*. Insect pathogenic fungi, like bacteria, produce a broad range of toxic metabolites; however, these toxins generally do not exhibit direct insecticidal activity at normal levels of production in the host. Studies suggest important roles in enhancing infection and promoting growth via suppression of the host immune system and in enabling reproduction (sporulation) on the killed host via antibiotic activity against invading saprophytes (Boucias and Pendland 1998). The mode of inoculation and infection by most of the major groups of fungal pathogens also differs fundamentally from that of bacteria. The infectious units of these pathogens, various types of spores or hyphal bodies, initiate infection via germination and production of hyphae that penetrate directly through the body wall of the host. This difference is a great advantage in extending the host range of these pathogens to insects that feed on plant juices via piercing-sucking mouthparts (which precludes these insects as targets of bacterial and viral pathogens with *per os* modes of inoculation), but a major disadvantage with respect to the consequent exposure to potentially unfavorable environmental conditions during the host infection process (fungi require free water or near-moisture-saturated conditions for spore germination and hyphal development). These requirements have important implications with respect to the potential of fungal pathogens for CPB management (see below). The entomopathogenic microsporidia are exceptional in that most species are infectious *per os*, with spores initiating infection via forcible penetration of the epithelial cells of the insect gut.

1. Microsporidian pathogens

Microsporidia, formerly identified as protozoans or protists, are highly reduced obligate intracellular fungal parasites of a broad range of invertebrate and vertebrate animals (James *et al.* 2006). Insects and other arthropods comprise the most common hosts. No insect-pathogenic microsporidia are known to cause disease in healthy, homothermic hosts (Boucias and Pendland 1998). Virulence of entomopathogenic microsporidia is highly variable. In some cases, infection may be rapidly lethal; however, diseases caused by many microsporidia tend to be chronic in nature. This is particularly the case in adult female hosts, where eggs may be infected and the pathogen transovarially transmitted to the offspring. Microsporidia causing both acute and chronic infections have potential as microbial control agents, as even sublethal infections can be highly debilitating to the host, retarding growth and development, reducing activity, altering behavior, and limiting fecundity.

A number of microsporidian species have been identified as pathogens of CPB. Most are of the genus *Nosema*, and the pathologies they produce range from acute to chronic. *Nosema gastroideae* Hostounsky & Weiser and *N. equestris* Hostounsky & Weiser are not naturally occurring pathogens of CPB, but produce severely acute infections in CPB larvae (Hostounsky 1984). This suggests potential for commercialization as sprayable biopesticides; however, mass production of microsporidia is difficult and costly, requiring either living hosts or cell cultures (the infectious spores of the grasshopper pathogen *Paranosema locustae*, one of the few commercialized microsporidian biocontrol agents, are formulated in food baits, as broadcast spray applications targeting plant foliage are not economically feasible). Consequently, few microsporidia have been field tested against CPB. The well-known microsporidian *Vairimoprha necatrix* (Kramer) Pilley has a broad host range, is mass-producible in caterpillar hosts, and is known to infect CPB. Foliar sprays of this pathogen were applied against CPB in one test, but were not effective in reducing the pest population (Jaques and Laing 1988).

Promising alternative strategies for use of microsporidia in insect pest management involve the classical biological control and inoculation biological control approaches based on introductions or releases of control agents into a pest population and reliance on their innate capacities to reproduce and disperse. The above-described species of *Nosema* are so virulent (rapidly lethal) against CPB that pathogen reproduction is limited, minimizing potential for use in such control programs. However, there are other species of microsporidia, most notably *N. leptinotarsae* Lipa and *Endoreticulatus fidelis* (Hostounsky & Weiser) Brooks *et al.*, that are naturally associated with CPB populations (Lipa 1968; O'Neil *et al.* 2005) and possess greater potential for establishment and dispersal. Lipa (1968) described *N. leptinotarsae* as causing depletion of fat reserves in adult CPB, and suggested that infected beetles would exhibit a reduced capacity to overwinter.

There is little active research on use of microsporidia for microbial biological control of CPB. The typically slow action and sublethal nature of these pathogens would necessitate their use in combination with other control agents, and development of integrated pest management systems for CPB is currently impeded by the overwhelming reliance on fast-acting synthetic chemical insecticides.

2. *Beauveria bassiana*

Beauveria bassiana (*Bb*) is one of the best known of the entomopathogenic fungi and one of the species most widely developed for microbial control of a broad range of insect pests (Faria and Wraight 2007). Commercial-scale *Bb* development efforts were launched in the Soviet Union in the early 1960s. Today, *Bb* is most commonly produced in biphasic fermentation processes, in which hyphal bodies (blastospores) produced in liquid culture are inoculated onto solid substrates (usually barley, rice, or other grains) that support extensive production of aerial conidia. Efficiency of large-scale production systems for this fungus equal or surpass those achieved with any other species of entomopathogenic fungi. This trait, combined with excellent post-production stability (shelf life), has been an important factor stimulating the rapid rate of commercial development seen in recent years (Wraight and Carruthers 1999; Faria and Wraight 2007).

The common occurrence of *Bb* infecting CPB in the soil has stimulated numerous attempts to develop methods and strategies for soil-application of the fungus. Spores of this fungus have been applied to the surface and mixed into the top layers of soil targeting the pupae and overwintering adults (Fedorko *et al.* 1977; Watt and LeBrun 1984; Cantwell *et al.* 1986; Gaugler *et al.* 1989; Jaques and Laing 1988; Schroder and Athanas 1989). Results in most of these studies, however, were not encouraging. Targeting the soil-inhabiting stages of CPB with fungal applications presents a number of technical and other difficulties: 1) vertical movement of fungal spores is limited in some soils, and following overhead spray or drench applications, a large proportion of the inoculum may remain near the surface (Ignoffo *et al.* 1977; Storey and Gardner 1987; Storey *et al.* 1987); thus, insects entering the soil to pupate or overwinter are exposed to high concentrations of inocula for only the brief period required to burrow through this zone of contamination; 2) conidia on the surface of adult CPB are removed by abrasion as the insects dig into the soil (Noronha and Goettel 2009a); this phenomenon was reported also for larvae of *Diaprepes* weevils (Quintela and McCoy 1998) and is likely an important factor determining susceptibility of CPB larvae burrowing into fungus-treated soils; 3) treating a sufficiently deep profile of soil to effect greater exposure is difficult and costly in terms of both the tillage and amounts of inoculum required to establish effective titers of fungus; 4) recent studies indicate that as adult CPB enter diapause, their susceptibility to *Bb* infection decreases markedly (Noronha and Goettel 2009b); 5) overwintering sites in field borders may be difficult to demarcate and not easily accessible or treatable, and 6) field soils may harbor microflora antagonistic to *B. bassiana* (Groden and Lockwood 1991). Thus, while potential exists for manipulation of naturally occurring and augmentatively introduced populations of *Bb* and other fungi for CPB suppression in soils, the focus of CPB control efforts in recent years has turned to foliar sprays targeting the larval stages.

All four instars of the CPB are highly susceptible to *B. bassiana* infection; although, the time required for infected larvae to succumb to infection increases with larval instar (Fargues 1972). Small-scale field tests in which *Bb* was applied against early-instar larvae, typically employing portable sprayers, have, in a some cases, demonstrated high levels of larval control and reasonably good protection from defoliation, supporting yields substantially greater than untreated controls (Fargues *et al.* 1980; Roberts *et al.* 1981; Anderson *et al.* 1988; Poprawski *et al.* 1997; Lacey *et al.* 1999; Wraight and Ramos 2002). However, efforts to reproduce these results on a larger scale and achieve yields comparable to chemical insecticide checks have been unsuccessful (Lipa 1985; Hajek *et al.* 1987). Reasons for this are numerous, including limited persistence of foliar spray deposits (Inglis *et al.* 1995), limited infection potential under dry weather conditions (Lacey *et al.* 1999), technical difficulties associated with spray-targeting of young larvae (Wraight and Ramos 2002), and reduced susceptibility of larvae due to the molting process (Vey and Fargues 1977). However, a perhaps more important constraint relates to the slow action of the pathogen, especially under high-temperature field conditions.

As indicated previously, *Bb* does not produce fast-acting insecticidal toxins like *Bt*. Even under optimal environmental conditions, the pathogen requires several days to kill its CPB host. While the optimal temperature range for *Bb* growth is ca. 23–26ºC, peak numbers of late-instar larvae frequently coincide with periods of very warm weather (e.g., early July in the northeastern USA). Compounding this problem, large larvae tend to congregate and feed in the upper canopy of potato plants, where they are exposed to direct sunlight, especially as defoliation progresses. Solar radiation has been shown to increase body temperatures of exposed larvae several degrees above ambient and to levels > 35°C (May 1982), which are highly inhibitory to *B. bassiana development* and pathogenesis (Inglis *et al.* 1996; Fargues *et al.* 1997; Martin *et al.* 2000). Under such conditions, infections may not become lethal until larvae reach the prepupal stage. This situation is greatly exacerbated by continued feeding of infected larvae (Fargues *et al.* 1994) and continued production of large numbers of eggs by fungus-infected adult beetles (Bajan *et al.* 1977; Fargues *et al.* 1991). It is consequently very difficult, using *Bb* alone, to protect potato crops from economically damaging defoliation, except under weather conditions optimal for disease development.

These numerous constraints on *B. bassiana* efficacy have led most researchers to conclude that effective use of *Bb* for CPB control will require integration with other biological, chemical, or biorational control agents. An integrated microbial biological IPM approach is discussed in the following section.

Integrated microbial biological control of Colorado potato beetle

Two of the most important characteristics of CPB that make it a formidable target for biological control are its high fecundity, expressed during an extended oviposition period that begins very soon after emergence of the potato crop, and its rapid rate of development. The product of these traits is a pest population that 1) emerges early in the season, before insect natural enemy populations are large enough to effect control, 2) is comprised of mixed-age individuals with varying levels of susceptibility to biological control agents, and 3) develops very rapidly to highly destructive levels, challenging the efficacy of slow-acting biological control agents.

Many researchers investigating potential for biological control of CPB have underscored these problems. It is widely recognized that there is no single biological control agent with the capacity to effectively manage this pest and that biological control of CPB will require integration of multiple natural enemies. In terms of potential for integrated microbial control of this pest, studies have focused primarily on *Bt* and *Bb* (Schroder and Athanas 1989; Gallandt 1998; Groden *et al.* 2002; Wraight and Ramos 2007; Wraight *et al.* 2007).

There is a strong rationale for combining use of *Bt* and *Bb* for CPB control. Studies have demonstrated a low-level synergistic effect in terms of total CPB larval mortality when *Bt* and *Bb* are applied in combination. Wraight and Ramos (2005) reported 6–35% greater larval mortality than predicted by independent action of these agents. More significantly, action of these microbes has been recognized as highly complementary (Groden *et al.* 2002; Wraight and Ramos 2005, 2007). *Bt* has very low epizootic potential, whereas *Bb* is an important natural epizootic pathogen of CPB. Susceptibility of CPB to *Bt* decreases with increasing age, whereas *Bb* is virulent against all beetle life stages, except the eggs (Long *et al.* 1998) (**Figs. 2–5**), and the four larval instars are approximately equally susceptible to infection. Finally and perhaps most significantly, *Bt* is a very fast-acting pathogen, and well-timed applications can be effective in protecting potato crops from defoliation by rapidly developing populations of CPB larvae. In contrast, *Bb* acts very slowly; a large proportion of beetles infected as larvae may not succumb to infection until after they enter the soil to pupate (**Figs. 3–5**). The cool, dark conditions below ground greatly hasten fungal development, and the result is a reduction in the number of beetles surviving to adulthood (Wraight, unpublished observations).

The strong complementarity exhibited by these microbes makes them an excellent combination for CPB management, and integrated use of these agents has been under investigation for many years in the USA (Gallandt 1998; Groden *et al.* 2002; Wraight and Ramos 2005, 2007; Lacey, L.A., unpublished observations). Gallandt *et al.* (1998) demonstrated that a bio-intensive pest management strategy, incorporating applications/releases of Bt/Bb mixtures, the botanical insecticide rotenone, and a CPB predator, *Perillus bioculatus*, was effective in controlling insect pests, but was not economically competitive with conventional insecticide-based control programs. Wraight and Ramos (2007) have proposed a *Bt*/*Bb* microbial control program for CPB in the northeastern USA (where the climate limits CPB to just a single complete generation). The program is based on three applications made at approximately 3–4-day intervals: 1) an application of *Bt* alone targeting first-instar larvae, a combined application (tank mix) of *Bt* and *Bb* targeted against second- and third-instar larvae, and an application of *Bb* alone against the late-instar larvae. Demonstration trials of this protocol in 0.2 ha research plots produced excellent control of CPB, with potato yields equivalent to a chemical control check (Wraight and Ramos, unpublished data). As neither of these biological control agents is rapidly effective against adult beetles, success of the proposed bio-based IPM system would be dependent upon an area-wide approach to reduce spring adult populations to manageable levels.

Potential also exists for integrated use of *Bb* with biorational chemical insecticides. One of the most notable of the recently developed biorational materials are the spinosyns. Formulations of spinosyns are now approved for organic crop production systems. These products would be excellent candidates for rotation with *Bt* in a bio-intensive IPM control program. One of the most difficult challenges to development of biologically-based IPM programs in potato has been the emergence of highly virulent new strains of the potato blight pathogen, *Phytophthora infestans*. This problem often requires intensive prophylactic applications of synthetic chemical fungicides, and many of these materials are antagonistic toward beneficial entomopathogenic fungi applied for insect pest control (Loria *et al.* 1983; Majchrowics and Poprawski 1993; Ruano-Rossil *et al.* 2002). Studies have demonstrated, however, that activity of entomopathogenic fungi can be largely preserved if applications are made asynchronously with fungicides (Gardner *et al.* 1984; Jaros-Su *et al.* 1999).

Microbial control agents are generally compatible with a broad range of nontarget arthropods, including beneficial insects deployed for biological control (see Hokkanen and Hajek 2003). In some cases, interactions between microbial and macrobial control agents may be synergistic in terms of pest control (see Wraight 2003). It has been observed, for example, that CPB larvae intoxicated with *Bt* are more susceptible to predation by the pentatomid bug *Perillus bioculatus* (Cloutier and Jean 1998) and parasitism by the tachinid fly *Myiopharus doryphorae* (Riley) (Lopez and Ferro 1995). *Myiopharus* spp. are examples of natural enemies whose populations lag behind the spring populations of CPB (Gollands *et al.* 1991). Use of microbial control agents, as opposed to broad-spectrum chemical insecticides, also has potential to enable greater activity of such control agents against second- and third-generation beetle populations.

Development of resistance to both whole bacterial preparations of *Bt* and individual *cry* toxins has been demonstrated in laboratory studies with a number of insects, including CPB (see Glare and O'Callaghan 2000), and resistance has also become a widespread problem in field populations of diamondback moth, against which *B. thuringiensis kurstaki* and *B. thuringensis aizawai* have been intensively applied (see Roush 2000). Any extensive use of *Bt* for CPB control must, therefore, take into consideration the adoption of rigorous resistance management practices. The above-described integrated use of *Bt* and *Bb* carries the risk of selecting for resistance to both agents simultaneously (Roush 2000). However, the complex mode of action of *Bb* is not based on a virulent insecticidal toxin, and selection for resistance to this natural enemy has not been reported. In addition, the extended oviposition period of CPB, combined with the short half-life of *Bt* foliar applications results in a significant portion of the pest population not being exposed to *Bt* in the proposed integrated spray program. Unexposed individuals essentially comprise a refuge population that maintains a susceptible gene pool. Because CPB adults emerging from over-wintering sites typically invade potato fields by walking, populations tend to concentrate in field edges; thus, another potentially useful strategy for resistance management involves restricting pesticide applications to the edges of potato fields, leaving the untreated areas as refugia (Dively *et al.* 1998).

Wireworms (Elateridae)

With the vulnerability of potato tubers to cosmetic damage, wireworms (larvae of Elateridae (Coleoptera)) (**Fig. 6**) are particularly serious soil pests worldwide (Jansson and Seal 1994; Parker and Howard 2001). New tubers with as few as two holes can be rendered unmarketable as table grade potatoes (**Fig. 7**). In the rare occurrence where wireworms cause quantitative yield loss, it is likely due to feeding damage to the sprouts emerging from the seed tuber, affecting stem growth and new tuber production. Jansson and Seal (1994) list twelve genera of wireworms as pests of potato, the most common being *Agriotes*, *Conoderus*, *Ctenicera*, *Limonius*, and *Melanotus*.

The perception that wireworm problems are on the increase has lead to speculation as to the cause: the increasing demand for unblemished tubers; an increase in the planting of potatoes on land previously cropped to a perennial grass or rotation of potatoes with perennial set-asides (both situations provide oviposition sites for adults and a food supply for new larvae); and a decrease in the availability of persistent organochlorine insecticides that provided long-term control in the past (Parker and Howard 2001). Regardless of the cause, changing values, decreased availability of persistent insecticides, and advances in science are all leading toward the exploration of new reduced-risk insecticides, including microbial insecticides, for wireworm management.

Microbial biological control of wireworms is largely undeveloped, but has always captured the interest of a few researchers and, at times, been in receipt of significant research attention, probably because researchers, pest managers, and agriculturalists frequently observe infected cadavers in their research plots and production fields. Presently, it is receiving significant attention. There are numerous early reports of insect fungal pathogens, mostly *Metarhizium anisopliae* (Metschnikoff) Sorok., infecting wireworms (e.g. Gorham 1923; Rockwood 1950; Fox and Jaques 1958; Tinline and Zacharuk 1960; Fox 1961) (**Fig. 8**). Infections by fungi of other genera, including *Tarichium* (Gorham 1923), *Tolypocladium* (Kabaluk *et al.* 2005), *Beauveria* (Jansson and Seal 1994), *Entomophthora* (Remaudière *et al.* 1976), and *Zoophthora* (Keller 1994) have been mentioned on a few occasions. Reports of epizootic levels have been limited to *Metarhizium* (TK, pers. obs.), *Entomophthora* (Turian 1978), and *Zoophthora* (Keller 1976, 1994). In finding that the frequency and level of *M. anisopliae* were lower in cultivated vs. meadow (alpine meadow/permanent grass) soils, Keller and Schweizer (2001) suggested that wireworm problems in agriculture could be related to a deficiency of *M. anisopliae* in the soil.

Nematode pathogens

Only a few studies have investigated the potential of entomopathogenic nematodes for wireworm biocontrol. Toba *et al.* (1983) reported on the effect of *Steinernema glaseri* and *S. carpocapsae* (cited as *S. feltiae* (Filipjev) under pre-revised taxonomy) against the wireworm *Limonius californicus* (Mannerheim). *Steinernema carpocapsae* caused significantly higher mortality of wireworm larvae than did *S. glaseri*. Complete mortality of the wireworm was not achieved with the highest concentration tested (393 IJs/cm^2). In field tests, *S. carpocapsae* applied to the soil surface at 155 and 310 IJs/cm^2 resulted in only 28 and 29% mortality, respectively. No infection was obtained following reinfestation with summer-generation larvae. Lacey (unpublished data) tested 5 species of EPNs against *Limonius canus* (LeConte) and observed only limited activity of *Steinernema* and *Heterorhabditis* species. *Steinernema carpocapsae*, *H. bacteriophora*, and *S. riobrave* Cabanillas, Poinar, & Raulston produced the highest levels of mortality against small to medium sized larvae at 39, 35, and 24%, respectively. Ansari *et al.* (unpublished data) found that exposing wireworms to *H. bacteriophora* strain UWS1 resulted in 67% mortality in laboratory tests, better than other strains and species tested.

Mermithid nematodes (**Fig. 9**) are common naturally occurring parasites of wireworms (TK, personal observations), and have been reported by Doane *et al.* (1973) and Kabaluk *et al.* (2005). Use of nematodes, either alone or together with other entomopathogens, is an area of research that could offer significant advances in wireworm biocontrol.

Fungal pathogens

A few notable pioneering efforts to explore *M. anisopliae* for wireworm control can be attributed to Rockwood (1950) and Fox and Jaques (1958) who, upon their discovery of infections in field populations of wireworms, reapplied inoculum for field-level control, but neither attempt affected wireworm populations. Tinline and Zacharuk (1960) and Zacharuk and Tinline (1968) were the first to report wireworm species x *M. anisopliae/B. bassiana* interactions in wireworm mortality, and since that time, isolate specificity or differential susceptibility of wireworm species to fungal infection has seemed to be the rule when *M. anisopliae* or *B. bassiana* are used to infect wireworms experimentally (Kabaluk *et al.* 2007a; Ansari *et al.* unpublished data; Koelliker, unpublished data). While this limits the host range of specific isolates within and among elaterid genera, it is favorable toward non-target insects. Zacharuk (1973) described in detail the penetration of the wireworm cuticle by *M. anisopliae*, providing significant insight into pathogenesis from a histological perspective.

With significant promise from laboratory results and numerous observations of diseased wireworms in nature, several researchers have recently conducted field trials using *M. anisopliae* for wireworm control in potato (e.g. Kabaluk *et al.* 2002, 2005; Tharp *et al.* 2007; Kuhar and Doughty 2008). The outcomes have been variable and to date, the only successful control of wireworms in any field crop using *M. anisopliae* was reported by Filipchuk *et al.* (1995) who found high efficacy of the product 'Metarizin' on *Agriotes tauricus* Hayden in Russia. Still, Kabaluk *et al.* (2007b) appear to be the only authors to have reported the retrieval of infected cadavers following indundative *M. anisopliae* applications to the field. In cases where crop protection following field applications of *M. anisopliae* have been reported, it is unclear if the effect has been due to infection and mortality of wireworms, repellency, or other factors (Kabaluk and Ericsson 2007a, 2007b).

Despite sparse reports of experimentation with *Beauveria* spp. for wireworm control, a registration was granted in Italy in 2002 for the product 'Naturalis-L' (*B. bassiana* strain ATCC 74040) (http://www.intrachem.com). This product is a liquid formulation of conidia registered for use against several species of *Agriotes* in potato (Ladurner 2007). Pending the positive experience of farmers, it is expected that its registration will expand to other countries.

Bacterial pathogens

Use of bacteria for biological control of wireworms is largely unexplored. There are a few unpublished accounts of testing commercial formulations of *Bacillus* spp., and, undoubtedly, this has been attempted by several researchers as the use of *Bacillus* has expanded to target Coleoptera. However, success at infecting wireworms with bacteria is not widely apparent. Zacharuk (1973) described the invasion of wireworms by, allegedly, *Pseudomonas aeruginosa* (Schröter) Migula and suggested that larvae are susceptible to such an invasion during- and shortly after molting. In reporting the rDNA sequences of 86 bacterial isolates from the gut of the wireworm *Limonius canus*, Lacey *et al.* (2007) suggested that modified *Rahnella aquatilis* Izard, Gavini, Trinel & Leclerc expressing wireworm-active toxins might be useful for wireworm control in potato by treating seed tubers, particularly with the ability of this bacterium to colonize the rhizosphere.

Discoveries in other areas of research offer the opportunity to improve entomopathogens as wireworm pesticides. For example, the chemotropic nature of wireworms (Doane *et al.* 1975) could be adapted to draw wireworms to inoculated media, as has been adapted to insecticide-treated wheat seed (Vernon 2005). Ericsson *et al.* (2007) synergized the efficacy of *M. anisopliae* with the addition of spinosyn, and Kabaluk *et al.* (2005) reported that adult click beetles were as susceptible as larvae to *M. anisopliae* infection.

Given the high degree of species-specificity of *M. anisiopliae* isolates, they could offer possibilities for inoculative or inundative spray applications to field margins and other click beetle refugia, leaving beneficial insects unharmed.

There seems to be a resurgent interest in experimentation with microbials as wireworm pesticides. While new discoveries in technology and knowledge will always offer the opportunity to optimize the isolates at hand, active bioprospecting for superior (in virulence, productivity, and persistence) species and isolates, genetic modification, and concurrent use of two or more entomopathogens could perhaps make the greatest advances in delivering microbial wireworm pesticides to potato farmers at this time.

HEMIPTERAN PESTS

As related in the microbial control introductory chapter, there are a number of major potato pests in the order Hemiptera (in the suborders Sternorrhyncha and Auchenorrhyncha). These include several species of aphids, a number of leafhoppers, and the potato psyllid, *Paratrioza cockerelli* (Sulc) (Zehnder *et al.* 1994). Some hemipterans (most notably the aphids) have extraordinary reproductive potential and can overwhelm host plants; however, the principal damage inflicted by most species results from injection of toxic saliva or vectored plant viruses or phytoplasmas. Hemipteran insects are characterized by a piecing-sucking mode of feeding (Triplehorn and Johnson 2005), and this has precluded them as hosts of viral, bacterial, and microsporidian pathogens, which are infectious *per os*. With respect to biological control, fungal pathogens that infect via direct penetration of the insect cuticle are the only microbes with the potential to regulate hemipteran pest populations.

Aphids (Aphididae)

As vectors of numerous potato viruses, aphids (Hemiptera: Sternorrhyncha) are among the most important pests of potato worldwide (Radcliffe and Ragsdale 2002). Many aphid species feed on potato foliage, including green peach aphid, *Myzus persicae* (Sulzer); potato aphid, *Macrosiphum euphorbiae* (Thomas); buckthorn aphid, *Aphis nasturtii* Kaltenbach; foxglove aphid, *Aulocorthum solani* (Kaltenbach); and cotton or melon aphid, *Aphis gossypii* Glover. The stolon-infesting bulb and potato aphid *Rhopalosiphoninus latysiphon* (Davidson) is also an important virus vector (Robert and Bourdin 2001). These pests, in general, are difficult targets for microbial control using slow-acting pathogens. Most aphids develop rapidly under favorable conditions, with the nymphs undergoing frequent molts, and as mentioned previously, the molting process is an important defense mechanism against cuticle-penetrating fungi. While the non-molting adults are more susceptible to fungal infection, they exhibit virtually no pre-reproductive maturation period and are capable of producing many offspring before infection is established and during the disease incubation period (Wang and Knudsen 1993; Baverstock *et al.* 2006). In addition, most aphid species prefer to feed on new foliage developing at the apex of the host plant, where a large proportion of individuals are exposed to highly insolated/high temperature/low-humidity conditions that are unfavorable for fungal infection and persistence. Finally, applications of fungal pathogens may not reduce aphid populations rapidly enough to protect plants from transmitted viral diseases. Nevertheless, aphid populations worldwide are naturally regulated by a large complex of fungal pathogens belonging to the order Entomophthorales traditionally classified in the class Zygomycetes. *Pandora neoaphidis* (Remaudière & Hennebert) Humber (**Figs. 10, 11**) is by far the most prevalent and widespread species of this complex; however, several other species, including *Neozygites fresenii* (Nowakowski) Remaudière & Keller, *Zoophthora radicans* (Brefeld) Batko (= *E. sphaerosperma*), *Entomophthora planchoniana* Cornu, and *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller, may be important mortality factors depending on the location, time of year, and pest species (Glare and Milner 1991; Pell *et al.* 2001; Steinkraus 2007).

The entomophthoralean fungi are characterized by much more rapid development than the hypocrealean fungi described previously as pathogens of the CPB. Possessing the capacity to sporulate, germinate, and establish infection all within a span of ca. 10 hours, these pathogens are able to exploit brief periods of favorable conditions, such as overnight periods of moderate temperature/high humidity, to establish and spread through aphid populations (Milner and Bourne 1983; Wraight *et al.* 1990). Spectacular epizootics of entomophthoralean fungi frequently overwhelm and rapidly decimate aphid populations in many crops, including potato (see Soper 1981), and observations of these impressive disease cycles have stimulated entomologists to explore the potential to manipulate these pathogens for microbial biological control. These efforts, however, have faced a major constraint in that the primary infectious units of the Entomophthorales are sticky, thin-walled, desiccation-intolerant ballistospores (or highly specialized capilliconidia produced by the ballistospores) that are not readily mass-produced or formulated as sprayable biopesticides (Wilding and Latteur 1987; Pell *et al.* 2001).

As an alternative approach, researchers have attempted point releases of these pathogens (via actively sporulating cultures, mycelial preparations, or laboratory-infected hosts) in aphid-infested fields in an inoculation biological control approach relying on the natural epizootic potential of the pathogen to spread through the pest population. However, in most cases, even when successfully established at strategic locations in aphid-infested fields, the fungi spread slowly and failed to generate field-wide epizootics rapidly enough to protect crops from damage (Wilding 1981; Wilding *et al.* 1986a, 1986b; Poprawski and Wraight 1998). There are numerous interacting factors that govern the initiation and spread of fungal epizootics in aphid populations. Entomophthoralean disease outbreaks usually develop late in crop cycles, after aphid populations have become severely damaging (see Soper 1981). This late occurrence of epizootics has been attributed to both biotic and abiotic factors, especially pest population density and environmental conditions (see reviews by Hajek 1997; Pell *et al.* 2001). Such factors (environmental conditions in particular) are not easily manipulated by pest managers, and initiating epizootics earlier in crop cycles than they would naturally occur is a difficult challenge. Results of inoculative releases are highly unpredictable at best, and unpredictable efficacy remains the single greatest impediment to practical use of these agents for microbial control in field crops.

Recent efforts to develop entomophthoralean fungi for microbial control have focused on production and stabilization of mycelial formulations that could be applied over broader areas to enhance inoculum dissemination and hasten epizootic development. With the exception of *N. fresenii*, the aphid-pathogenic species of Entomopthorales are mass producible as hyphae or hyphal bodies in economical liquid culture media. Individual hyphal elements, like the conidia, are generally too fragile and ephemeral for foliar-spray programs (Wilding and Latteur 1987); however, mycelia have been successfully dried and formulated as various types of granular preparations suitable for various forms of application (Shah *et al.* 1998; Wraight *et al.* 2003; Feng and Hua 2005). Despite these advances, production efficiencies and storage stabilities required to support operational-scale use have not yet been achieved. Most recently, *P. neoaphidis* has been cultured and desiccation stabilized on broomcorn millet seed, a technology that offers considerable potential to improve the efficiency (economics) of entomophthoralean fungus mass production (Hua and Feng 2003; Feng and Hua 2005). Many species of entomophthorales produce thick-walled resting spores that enable the fungus to survive dry or winter seasons. Resting spores have considerable potential as inocula for biological control releases (e.g., Hajek and Roberts 1991), but methods for

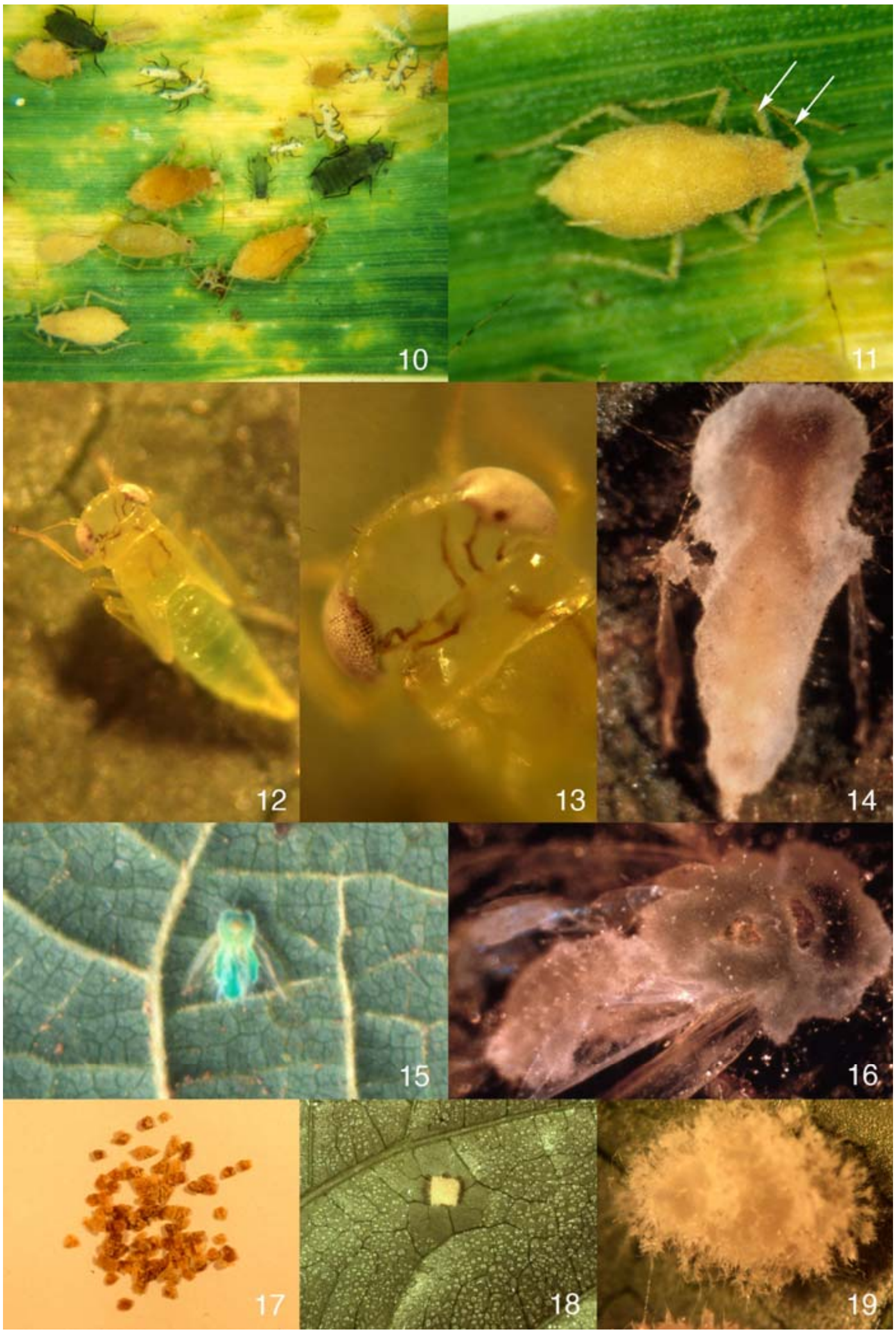

Plate 2 Figs 10-19. (**10**) Epizootic of the entomophthoralean fungus *Pandora neoaphidis* in an aphid population. Patently infected aphids are cream colored; light brown aphid cadavers are overgrown with fungus and supporting production of *P. neoaphidis* ballistospores. *Pandora neoaphidis* is a cosmopolitan species that attacks an extraordinarily broad range of aphid species across many genera (photographed here on *Rhopalosiphum maidis* (M. Goettel)). (**11**) Sporulation of *P. neoaphidis* on aphid host cadaver; note discharged conidia on legs and antennae (M. Goettel). (**12, 13**) Nymph of potato leafhopper, *Empoasca fabae*, infected with the entomophthoralean fungus *Zoopthora radicans*. Fungal hyphae growing in the leafhopper hemocoel were darkened by a host defense reaction (melanotic encapsulation) (S. Galaini-Wraight). (**14**) Nymph of *E. fabae* killed by *Z. radicans* and supporting profuse sporulation. (S. Galaini-Wraight). (**15**) Adult *Empoasca kraemeri* killed by *Z. radicans*; note bright green coloration of fresh fungal outgrowth (S. Wraight). (**16**) Adult *E. kraemeri* killed by *Z. radicans*. Fungal outgrowth from the host cadaver forms a dense mat of specialized hyphae (conidiophores) that actively discharge infectious conidia (S. Galaini-Wraight). (**17**) Laboratory-produced dry granules of *Z. radicans* mycelium (S. Wraight). (**18**) Granule (2 × 2 mm) of *Z. radicans* mycelium inoculated onto a dew-covered leaf (S. Wraight). (**19**) Granule of *Z. radicans* mycelium ca. four hours after inoculation (rehydration) exhibiting a dense growth of conidiophores similar to that produced on an insect host (cf. **Fig. 16**) (S. Wraight).

manipulation of these propagules, including mass production and germinability induction (breaking dormancy), are also lacking.

The natural control potential of entomophthoralean fungi enables the consideration of classical and conservation biological control strategies in addition to the above-described inoculation approach (Nielsen and Wraight 2008). *Zoophthora radicans* was introduced into Australia for control of spotted alfalfa aphid. The released fungus, originating from Israel, became established, and has suppressed pest populations in some areas (Milner 1985). In the southern USA, a cotton-pest management program has been developed in which prevalence of *N. fresenii* is monitored, with growers being advised to curtail pesticide applications against *A. gossypii* populations predicted to be controlled by natural fungal epizootics (Steinkraus *et al.* 2002; Steinkraus 2007). Though these approaches have not been explored in potato production systems, similar successes are clearly possible. As the entomophthoralean fungi are highly susceptible to many of the fungicides applied for plant disease control in potato, potential also exists for managing fungicide applications to minimize their impacts on populations of beneficial fungi (Lagnaoui and Radcliffe 1998; Ruano-Rossil *et al.* 2002).

Little research has been conducted on the use of broadcast applications of hypocrealean fungi for aphid control in field crops. *Beaveria bassiana* conidia applied via overhead irrigation or conventional spray equipment has been reported to provide up to 65–75% control of Russian wheat aphid populations (Vandenberg *et al.* 2001; Hatting *et al.* 2004). The hypocrealean fungi most widely available as biopesticides (*Beauveria*, *Metarhizium*, *Isaria*, and *Lecanicillium* spp.) have limited epizootic potential in field crop canopies, and thus, study of dried-mycelium formulations of these fungi for inoculation biological control of aphids has also been limited. An alginate pellet formulation of *B. bassiana* was developed (Knudsen *et al.* 1990, 1991) and applied against Russian wheat aphid in field cages. High rates of sporulation (> 95%) were observed on pellets deposited on the soil surface, but the inoculum did not move into the plant canopy, and canopy conditions were considered too dry to support epizootic development (Knudsen and Wang 1998). Similar evaluations have not been conducted in potato, but would be expected to face the same constraints.

Leafhoppers (Cicadellidae)

Many leafhoppers (Hemiptera: Auchenorrhyncha) are important pests of potato. These include numerous *Emposaca* spp.; the aster leafhopper, *Macrosteles quadrilineatus* Forbes; beet leafhopper, *Circulifer tenellus* (Baker); and clover leafhopper, *Aceratagallia sanguinolenta* (Provancher) (Radcliffe and Johnson 1994). The potato leafhopper, *Empoasca fabae* (Harris), is an extremely destructive pest of potato, which through its feeding causes yellowing, curling, and premature senescence of potato foliage. This so called "hopperburn" syndrome results from a plant response to wounding, exacerbated by toxic salivary secretions (Backus *et al.* 2005). Significant damage results from even low-density populations (Radcliffe and Johnson 1994). The other leafhopper species identified above generally cause little direct damage to potato plants, but are important as vectors of potato-pathogenic phytoplasmas and viruses. Potato purple top wilt is a devastating disease caused by a beet leafhopper-transmitted phytoplasma.

Fungal epizootics are encountered much less frequently in leafhopper populations than in aphid populations. With respect to the above-listed potato-pest species, epizootics have been reported only from *Empoasca* spp. The entomophthoralean fungus *Z. radicans* (**Figs. 12–16**) is known as an important natural enemy of *E. fabae* in the USA (McGuire *et al.* 1987a), *E. kraemeri* Ross & Moore in South America (Galaini-Wraight *et al.* 1991), and *E. vitis* Göthe in Europe (Hodge *et al.* 1995).

Z. radicans has proven readily mass producible in liquid culture, and methods for desiccation-stabilization of mycelia (**Figs. 17–19**) were developed more than 20 years ago (McCabe and Soper 1985; Wraight *et al.* 2003). In initial tests, inoculative applications of dry mycelium of this fungus were successful in initiating field-wide epizootics in *Empoasca* populations under favorable environmental conditions (Wraight and Roberts 1987; Leite 1991). Unfortunately, the sensitivity of this pathogen to adverse temperature and moisture conditions (McGuire *et al.* 1987a; Galaini-Wraight *et al.* 1991; Poprawski and Wraight 1998), combined with continuing challenges associated with commercial-scale mass production, formulation, and storage of mycelium-based preparations have impeded its development beyond small-scale research trials.

Z. radicans has also been investigated in the USA as a classical biological control agent. In the mid 1980s, this pathogen was isolated from natural epizootics in *E. fabae* populations in Wisconsin, and introduced into Illinois, where it was not known to occur. No epizootic spread was observed following the introduction, and the effort was presumed unsuccessful; however, epizootics of *Z. radicans* in *E. fabae* populations were reported in the vicinity of the release site during the following two field seasons (McGuire 1987a, 1987b). In contrast, attempts to establish *Z. radicans* in *E. fabae* populations in central New York resulted in low rates of infection and no evidence of recycling (Hodge *et al.* 1995).

Recent studies have demonstrated potential for use of the hypocrealean fungi *M. anisopliae*, and *I. fumosorosea* against *Empoasca* leafhoppers on bean plants (Tounou *et al.* 2003). Spray applications produced high levels of infection by both fungal species under laboratory and greenhouse cage conditions. *Beauveria bassiana* combined with a low ("sublethal") dose of the chemical insectidcide imidacloprid was also reported effective against *E. vitis* in tea plantations in southern China, reducing pest populations by >80% (Pu *et al.* 2005). In other tests, applications of the fungus alone produced 33–60% control (Feng *et al.* 2004). To our knowledge, no virulent leafhopper-pathogenic strains of these common entomopathogenic fungi have been tested against leafhoppers infesting potato crops.

Psyllids (Psyllidae)

The potato or tomato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Sternorrhyncha), is a sporadic but potentially devastating pest of potato in western North America (Cranshaw 1994) and has recently become established in New Zealand (Gill 2006). Feeding activities of this insect include injection of toxic saliva and produce a syndrome of chlorosis and retarded plant growth referred to as psyllid yellows (Cranshaw 1994). This pest is not known to vector any of the major viral or phytoplasma diseases of potato. The potato psyllid has been associated with a new potato tuber disease referred to as "zebra chip" or "papa rayada," but the involvement of a vectored plant pathogen has not been proven (Munyaneza *et al.* 2007). Another species of potato psyllid in South America, *Russelliana solanicolla* Tuthill, was recently confirmed as the first known psyllid vector of a plant virus (Tenorio *et al.* 2003).

Fungal pathogens have not been reported as important natural enemies of potato psyllids; however, there are numerous reports of a great variety of entomopathogenic fungi (entomphthoralean and hypocrealean spp.) infecting various psyllid pests of fruit trees and other woody plants worldwide, and in many cases, pest populations were reported controlled by epizootics of these fungi (Gilliatt 1924; Jaques and Patterson 1962; Arzone 1979; Picco 1985; Villacarlos and Robin 1989; Villacarlos and Wilding 1994; Meyer *et al.* 2007). It is notable that one of the earliest efforts to use an entomophthoralean fungus for biological control of an insect pest involved releases of *Z. radicans* into populations of the apple psyllid, *Psylla mali* Schmidberger, in the USA (Dustan 1927). Orchard crops generally present a more stable and favorable environment for activity of epizootic fungi than annual field crops like potato (at least in situations where intensive applications of fungicides are not required). Nevertheless, these observations, especially in light of the high epizootic potential exhibited by entomophthoralean fungi in field populations of aphids, suggest potential for use of these pathogens against potato psyllids.

Beauveria bassiana, I. *fumosorosea* Wize, *I. javanica* (Friederichs & Bally) Samson & Hywell-Jones (=*P. javanicus*), *Lecanicillium sp.* (identified as *Verticillium lecanii*), *Metarhizium flavoviride* Gams & Rozsypal*, M. anisopliae*, and *Hirsutella* sp. have been found virulent against psyllids in laboratory bioassays (Liu *et al.* 1990; Puterka *et al.* 1994; Meyer *et al.* 2007). *Beauveria bassiana* and *I. fumosorosea* applied against the pear psylla, *Cacopsylla pyricola* (Förster), in orchard tests produced 18–37% mortality (Puterka 1999). We are not aware of any studies assessing the microbial control potential of these or other hypocralean species against potato psylla, but such investigations are warranted.

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