

Microbial Control of Insect Pests in Temperate Orchard Systems: Potential for Incorporation into IPM*

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Key Words

Bacillus thuringiensis, entomopathogenic nematodes, fungi, granulovirus, tree fruit, nut crops

Abstract

Because of their selectivity and safety, microbial control agents (MCAs) appear to be ready-made components of integrated pest management (IPM) systems that do not pose a threat to applicators or the environment and allow other natural enemies to function. Control of several orchard pest insects using MCAs, including viruses, *Bacillus thuringiensis*, fungi, and entomopathogenic nematodes (EPNs), have been demonstrated in apple, pear, stone fruits, citrus, and several nut crops. *B. thuringiensis* is the most used MCA for control of lepidopteran orchard pests. Significant use of EPNs in citrus for control of root weevils is also reported. The granulovirus of codling moth is used increasingly in apple and pear by organic growers, with interest also shown by conventional growers. Although some success has been achieved, in most orchard systems MCAs account for a relatively small proportion of the pest control tactics employed, and in some systems they are not used at all. Research toward improving MCA efficacy and economic competitiveness is required to enhance the role of MCAs in IPM.

IPM: integrated pest management

Entomopathogen: a microorganism or nematode capable of producing disease in an insect

MCA: microbial control agent

INTRODUCTION

The primary means of control of arthropod pests of temperate tree fruits and nuts is through the application of broad-spectrum insecticides. While such interventions are capable of rapidly killing a range of pests, overreliance on chemical pesticides has generated a panoply of problems including safety risks, outbreaks of secondary pests normally held in check by natural enemies, environmental contamination, decrease in biodiversity, and insecticide resistance. In our current climate of increased awareness of the sensitivity of our environment, among both the scientific community and the general public, the development of environmentally safer methods of insect control is desirable. Tightened registration procedures around the world will likely result in a decreased availability of a number of broad-spectrum pesticides, creating new opportunities for alternative methods (including microbials). An integrated pest management (IPM) strategy, in which natural enemies of pest arthropods and other alternative measures play significant roles in crop protection, will minimize negative environmental impacts and other deleterious effects due to insecticide usage while providing a more sustainable approach to pest control. Several entomopathogens (viruses, bacteria, fungi, and nematodes) offer effective means of microbial control that can be combined with other tactics such as mating disruption and the use of reduced-risk pesticides. In addition, microbial control agents (MCAs) are safe for the environment, beneficial insects, applicators, and the food supply, and they can be applied just prior to harvest (88). We provide an overview of microbial control in temperate orchards designed to set the stage for incorporating MCAs into IPM programs for orchard systems.

VIRUSES

Several major groups of viruses infect insects and mites, but baculoviruses have received the most attention and commercial devel-

opment (78, 120). They are reported from Lepidoptera, Coleoptera, Diptera, and Hymenoptera (sawflies), but their use in orchards is exclusively for control of lepidopteran pests. Two distinct groups, nucleopolyhedroviruses and granuloviruses, have been used for experimental and practical control of a limited number of orchard pests. The specificity of viruses used for control of lepidopteran orchard pests is well documented (68). The narrow host ranges of most entomopathogenic viruses can be both a limitation and advantage depending on the suite of arthropod pests in a particular orchard ecosystem and on the controlling effects exerted by predators and parasitoids and other alternatives to broad-spectrum insecticides. Generally, sensitivity to solar degradation necessitates reapplication of virus preparations at fairly short intervals, particularly if the host is multivoltine and if pest population pressure is high (10). Shading and microhabitats on the host plant and in the environment can protect some viruses from solar degradation and enable long-term persistence (30).

BACTERIA

Various spore-forming and nonspore-forming bacteria are pathogens of insects, but only *Bacillus thuringiensis* has been used in orchards. *B. thuringiensis* has been employed to control a multitude of insects in organic and conventional agriculture, forestry, and public health (18). Its safety to pesticide applicators, the food supply, beneficial organisms, and the environment is thoroughly documented (97). A variety of *B. thuringiensis* formulations are commercially produced in several countries with insecticidal activity for species of Lepidoptera, Coleoptera, and Diptera (18, 57), but only those formulations with activity against lepidopteran pests are used in orchards (92). The insecticidal activity of *B. thuringiensis* is due to the crystal protein toxins contained in the parasporal inclusions that are produced at the time of sporulation (35). These toxins basically function as stomach poisons and kill the

insect by disrupting osmotic balance in the midgut epithelium. Damage to the midgut epithelium results in cessation of feeding and gut paralysis and ultimately death. *B. thuringiensis* products are made up primarily of spores and toxins (57), thus differing from most other MCA products (e.g., nematodes, fungi, and viruses), which contain the entire organisms. More detailed descriptions of *B. thuringiensis*, its toxins, and their mode of action are presented by Schnepf et al. (144) and Garczynski & Siegel (57).

FUNGI

A diverse spectrum of fungi is reported from insects and mites (63, 195). Some species in the Entomophthorales (e.g., *Neozygites fresenii*, *Entomophaga maimaiga*) and Hypocreales (e.g., *Lecanicillium* spp., *Aschersonia* spp., and *Hirsutella* spp.) produce epizootics in pest populations that often lower pest densities to levels that are not harmful to the crop (181). Commercial development of entomopathogenic fungi has been confined to species in the Hypocreales, most notably *Lecanicillium* spp., *Beauveria bassiana*, *Metarbizium anisopliae*, and *Paecilomyces fumosoroseus* (63). In general, these fungi are inundatively applied to bring about a rapid reduction in the pest population. Because fungi gain access to the host through the cuticle, they are the principal MCAs of sucking insects (Hemiptera, e.g., aphids and whiteflies) and control a variety of insects in other orders such as Coleoptera, Lepidoptera, and Orthoptera (63, 195). Microsporida are now classified with the fungi (76). These obligate pathogens are reported from an assortment of insects including orchard pests but they have not been developed successfully as MCAs because of certain characteristics such as complex life cycles, obligate parasitism, and chronic rather than acute effects.

NEMATODES

Most research on biocontrol using nematodes has been on entomopathogenic nema-

todes (EPNs), which consist of the families Steinernematidae and Heterorhabditidae (59, 66, 182). EPNs kill their hosts with the aid of bacteria carried in the nematode intestine (steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids are associated with *Photorhabdus* spp.) (1, 22). Detailed aspects of the biology and life cycle of EPN are reviewed elsewhere (1, 86). The safety of EPNs to humans and other nontargets has been well recognized and led to their exemption from U.S. EPA pesticide registration (4). EPNs can be mass produced in vivo or in vitro and applied using various standard agricultural equipment (161, 163). Because of their sensitivity to UV degradation and desiccation, EPNs are most suitable for application to soil or cryptic (protected) habitats (163). Most insect targets are in the orders Coleoptera and Lepidoptera, but examples may also be found among other orders such as Blattodea, Diptera, Hymenoptera, Orthoptera, Siphonaptera, and Thysanoptera (66).

MICROBIAL CONTROL OF ORCHARD PESTS

Pome Fruits

Several varieties of pome fruits, including apple, crab apple, pear, and quince, are grown in temperate climates. Apple and pear are the major pome fruit crops in the temperate climate zones. A rich diversity of insects and mites attack these fruits worldwide. Most research on MCAs in pome fruit has been conducted on insect pests of apple.

Codling moth. The most serious insect pest of apple from a global perspective is the codling moth (CM), *Cydia pomonella* (Lepidoptera: Tortricidae) (15). It is also a significant pest of pear, walnut, and other fruits. Shortly after hatching, neonate larvae bore into the fruit and remain there throughout their feeding stages. When larvae are full grown, they leave the fruit in search of

Epizootic: an outbreak of disease in which there is an unusually large number of cases and high morbidity

EPN: entomopathogenic nematode

CM: codling moth

protected habitats in which to spin their cocoons and pupate. There are one to four generations per year depending on climatic conditions. In the fall, diapausing fifth-instar larvae overwinter within the cocoon (hibernaculum) in protected habitats. The two stages most amenable to microbial control are neonate larvae and diapausing cocooned fifth instars. Once larvae have entered the fruit, they are well protected from entomopathogens until they cease feeding and exit. Control of CM in conventional orchards is usually through the application of broad-spectrum insecticides such as azinphos-methyl (Guthion®). Despite control of CM and a variety of other pest insects with fewer applications of broad-spectrum insecticides, hundreds of nontarget species, including beneficial insects, are also killed (47).

The CM granulovirus (CpGV) is the most effective MCA yet developed for control of CM. It was discovered in Chihuahua, Mexico, in 1963 and described by Tanada (184). Its specificity for CM and some closely related species and safety to nontarget organisms are well documented, and its use contributes to the conservation of other natural enemies in orchard agroecosystems (48, 68, 91). The LD₅₀ has been estimated as low as 1.2–5 granules per larva (78, 172), with slightly higher estimates for number of granules per mm² of artificial diet (99, 100). CpGV was registered for commercialization and use in Europe in the late 1980s (36). Three products are now commercially available in North America. Effective control has been reported in apple orchards in Europe, North America, Argentina, Australia, New Zealand, and South Africa (10, 20, 36, 62, 79, 81). Additional virus applications may be required in more southerly latitudes, especially where there is a third CM generation, whereas fewer applications may be required in the shorter growing seasons of northern Europe or Canada. For example, in Nova Scotia, where there is only one CM generation per year, Jaques et al. (80) reported that only two applications of CpGV were usually needed. Control of CM

in pear with CpGV has also been reported (11).

Although use of CpGV has been successful, a number of issues must be considered for its application. Despite the relatively rapid speed of kill, exposed larvae live long enough to damage fruit (10, 48, 61, 82). Damage is due to shallow entries, with larvae usually dying as early instars within the skin of the fruit. Although cosmetic damage to fruit lowers quality and price, apples may still be suitable for processing. A concern of researchers and orchardists regarding CpGV is its sensitivity to solar degradation (8, 51, 62, 82, 89), necessitating relatively frequent application of the virus (7- to 14-day intervals), especially when CM population density is high (10). Formulation to protect CpGV from UV degradation has been investigated by several researchers using adjuvants that include skim milk, molasses, iron filings, lignin, and particle films (9, 12, 14, 89). Development of resistance to CpGV has recently been reported in Germany and France for certain CM populations that have received regular virus applications for several years (45, 52, 143). As with chemical insecticides, management of resistance has been recommended to prevent its spread (143). Because CpGV is used increasingly in North America, the potential for development of resistance should be anticipated. An integrated approach that alternates other soft insect growth regulators, with CpGV should be considered, especially when large-scale use of the virus is implemented within a region.

The other stage of CM that offers potential for control with MCAs is the overwintering or diapausing cocooned larva. After harvest, this stage provides a captive audience representing the entire CM population. If a substantial portion of the population can be eliminated at this stage, little or no CM will be present to produce damage the following spring. Although some research has been conducted on use of the fungus *B. bassiana* for overwintering CM control (49), most research has been on EPNs. Protected habitats, such as those

used by CM and other tortricids for their cocoons in overwintering sites (e.g., under loose bark, in leaf litter, nearby woodpiles, fruit bins left in the orchard), are favorable environmental sites for EPNs. Under ideal conditions of adequate moisture and temperatures above 15°C, *Steinernema carpocapsae* and *S. feltiae* applied at 1–2.5 billion infective juveniles (IJs) per hectare have controlled a high proportion of targeted populations (87, 93, 189). Habitat modification, particularly the use of irrigation before and after treatment, and the use of strip mulches around tree bases are strategies that may enhance or extend the activity of EPNs in orchards and other protected habitats (93, 94). EPNs have also been evaluated for control of cocooned CM in fruit bins when fruit is floated out in drop tanks or when the bins are washed (33, 96). The bins become infested when they are stored in the orchard, but cocooned larvae may remain dormant through cold storage and re-enter orchards the following year (75).

Leafrollers (Lepidoptera: Tortricidae).

After CM, leafrollers and other species of Lepidoptera are the most important pests of apple production that are susceptible to microbial control. Although these are predominantly defoliators, they may also feed on the surface of fruit. *B. thuringiensis* has been used routinely for control of leafrollers, budmoths, and fruitworms (21, 37, 126, 192). A number of factors can affect the performance of *B. thuringiensis* against tortricid pests of apple including temperature, other environmental factors, differences in species and instar susceptibility, spray coverage, and application rate (21, 53, 105–107).

Several viruses (entomopoxviruses, granuloviruses, and nucleopolyhedroviruses) of leafrollers and other orchard pest Lepidoptera have been reported and studied (13, 20, 134, 137, 146, 174, 196). The granulovirus of the summer fruit tortrix moth, *Adoxophyes orana* (AoGV), is commercially produced in Europe and marketed under the name Capex®. AoGV has been extensively tested in Europe and

Japan (36, 146, 174, 196). It has been credited with persistent control (36). Two major problems associated with the virus are its low tolerance for UV radiation and slow rate of kill. Mortality is usually observed in mature larvae.

Control of other lepidopteran pests of apple using EPNs has been reported. A number of other examples include wood-boring insects and other insects with soil stages that are controlled most effectively by *Steinernema* and *Heterorhabditis* spp. (38; see Stone Fruits, below). The apple budmoth (*Platynota idaeusalis*) and oriental fruit moth (*Grapholita molesta*) have similar overwintering strategies to that of CM and could be potential targets for EPNs (see below).

Stone Fruits

Temperate climates foster a variety of important stone fruit crops such as peach, plum, apricot, and cherry. Microbial control has shown success against several key pests of stone fruits including the oriental fruit moth, plum curculio, fruit flies, and various borers.

Oriental fruit moth. The oriental fruit moth (OFM), *Grapholita molesta* (Lepidoptera: Tortricidae), is a prevalent pest of peaches, nectarines, pome (in some regions), and other fruit, causing economic damage by feeding on shoots or by directly infesting fruit. As a defoliator it is susceptible to *B. thuringiensis* (130), but once inside the fruit it is protected from MCAs. Full-grown diapausing larvae spend the winter within cocoons in cryptic habitats. Thus, the overwintering stage may be amenable to control with EPNs, provided there is adequate moisture and temperatures are sufficiently high (>15°C). Under laboratory conditions, *Heterorhabditis marelatus*, *Steinernema carpocapsae*, *S. feltiae*, and *S. riobrave* were pathogenic to OFM diapausing larvae, although no differences in virulence were detected among the nematode species (141). Similar to the approach with CM, application of nematodes to infested fruit bins may substantially reduce

IJ: infective juvenile

OFM: oriental fruit moth

Pathogenic: the potential ability to produce disease

Virulence: the disease-producing power of an organism

populations; application of *S. feltiae* to fruit bins with OFM contained in cardboard strips resulted in 78%–82% OFM mortality (141).

Borers. Several borer pests have shown susceptibility to entomopathogens (16, 66, 85). The clearwing moths (Lepidoptera: Sesiidae) in the genus *Synantbedon*, which are among the most serious of these boring pests, are generally susceptible to EPNs (66). The peachtree borer, *Synantbedon exitiosa* (Lepidoptera: Sesiidae), a major pest in most stone fruits, was controlled using *Heterorhabditis bacteriophora*; an 80% reduction in emergence was observed (31). Cottrell & Shapiro-Ilan (34) observed an 88% reduction in emergence following a late-spring application of *S. carpocapsae* to soil surrounding the tree base at a rate of 3×10^5 per tree to mature infestations. *S. riobrave* was not effective. The high levels of efficacy such as those observed with *S. carpocapsae*, coupled with relatively low rates of application (less than 100 million IJs per ha; 34), suggest that incorporation of *S. exitiosa* control with EPNs into an IPM program could be feasible.

Plum curculio. The plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), is a major pest of stone and pome fruits in North America (139). Laboratory research has indicated that *C. nenuphar* is susceptible to the entomopathogenic fungi *Beauveria* spp. and *Metarhizium anisopliae* (5, 187) and to nematodes (5, 128, 167). Suppression of *C. nenuphar* larvae under field conditions has thus far only been reported using steinernematid nematodes (5, 165, 168). In field trials in peach orchards, when *S. riobrave* was applied to soil infested with *C. nenuphar* larvae (100 IJs/cm²), Shapiro-Ilan et al. (168) observed greater than 90% average suppression of adult emergence. In contrast, Alston et al. (5) observed only low levels of larval control (22%–39%) when applying another steinernematid (*S. feltiae*) at 50–200 IJs/cm² to a northern population of *C. nenuphar*. In laboratory studies the authors determined

that a northern diapausing population of *C. nenuphar* was less susceptible to *S. feltiae* than a nondiapausing southern population. Thus one might expect that *S. feltiae* would produce more positive results in the field when applied to a nondiapausing population, yet in trials conducted in Georgia and Florida, *S. feltiae* failed to suppress adult emergence when the nematodes were applied to soil infested with *C. nenuphar* larvae (168).

The ability of *S. riobrave* to consistently suppress high levels of *C. nenuphar* in the soil could translate into a useful management tactic (168). However, first-generation adults that attack the fruit after overwintering in the orchard or nearby are not likely to be affected by soil application of nematodes, and prevention of damage to the fruit remains a major concern. Furthermore, in some areas such as Georgia, immigration of adult *C. nenuphar* from alternative hosts outside the orchard could be substantial (84). Thus, solely targeting subsequent generations in soil would inherently result in some damage. Nonetheless, it is still conceivable that EPNs could be beneficially incorporated into an IPM program. Continual applications toward the soil-dwelling stages may, over time, reduce the overall population sufficiently (particularly in northern areas where only one generation per year occurs). Additionally, nematode applications could be directed at alternative hosts that serve as reservoirs for the insect. Another approach might be to allow invading adult *C. nenuphar* to attack a suitable trap crop arranged on the perimeter of the orchard, and then apply nematodes to destroy the subsequent generation in the soil. Further testing of EPNs for *C. nenuphar* suppression is warranted.

Fruit flies (Diptera: Tephritidae). Fruit flies are important pests of cherries and a wide variety of other fruit. In cherry-growing areas of the Pacific Northwest of the United States, the western cherry fruit fly, *Rbagoletis indifferens*, is a serious pest of sweet cherries. EPNs and fungi are pathogenic to the western

cherry fruit fly; the level of susceptibility depends on the insect stage (131, 197, 198). In field trials conducted in Washington State, *S. carpocapsae* and *S. feltiae* were equally effective against larvae (59%–85% mortality) when applied to soil under cherry trees at 50–100 IJs/cm². Yee & Lacey (197) proposed the use of EPNs for control of *R. indifferens* in isolated and abandoned lots or in yards of homeowners as a means to deter invasion of commercial orchards. Use of entomopathogenic fungi for control of *R. indifferens* and other fruit flies attacking cherry has not been investigated under field conditions, but such research is warranted. One potential approach is the utilization of attractant traps that enable infection of flies and autodissemination of fungi into fruit fly populations (191).

Nut Crops

A number of nut crops are of importance in temperate orchard systems including almond, filberts, pistachio, pecan, and walnut. Among the significant pests that attack these crops, the navel orangeworm and pecan weevil have been studied the most in terms of amenability to microbial control tactics.

Navel orangeworm (Lepidoptera: Pyralidae). The navel orangeworm, *Amyelois transitella*, is a key pest of pistachios, almonds, and walnuts (140). The larvae infest mature nuts on the tree and mummy nuts on the tree and ground. Entomopathogenic bacteria, virus, and nematodes are pathogenic to *A. transitella*, and potential for their incorporation into IPM programs has been demonstrated (29, 108, 176, 190). Field application of the nematode *S. carpocapsae* to open-hulled almonds during the summer months resulted in >65% mortality in sentinel *A. transitella* (108). Dormant season (winter) application of EPNs to trees, however, resulted in substantially lower *A. transitella* suppression (3). Siegel et al. (175, 176) recently reported on the efficacy of EPNs applied to almond and pistachio

mummy nuts on the ground for control of *A. transitella* larvae. *S. carpocapsae* was more effective than *S. feltiae* and provided >72% mortality at a relatively low rate of 10⁵ IJs/m² (175, 176). EPNs persist well in these orchard environments, offering the potential of recycling within the *A. transitella* population (3, 175). Overall, significant potential is indicated for incorporating ground applications of EPNs into an *A. transitella* control program, yet attention should be given to proper timing and suitable temperatures (176).

Pecan weevil. The pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae), is a key pest of pecan (132, 194). Adults emerge from soil in late July through August to feed on and oviposit in nuts (70). Larvae develop in the nut, and fourth instars drop to the ground, where they burrow to a depth of 8 to 25 cm, form a soil cell, and overwinter. The following year, approximately 90% of larvae pupate and spend the next nine months in the soil cell as adults (70). The remaining 10% of the larval population spend an additional year in the soil as larvae and emerge as adults in the third year (70). Most *C. caryae* adults emerge from soil over a four- to six-week period usually beginning in mid-August (70); larvae emerge from nuts over several months in the autumn and early winter (71, 154). Although some studies have been conducted on virus and bacteria of *C. caryae*, most attention has focused on nematodes and fungi (2, 55, 142, 154, 177).

Early laboratory and field studies with EPNs generally indicated only poor to moderate control against *C. caryae* larvae (127, 153, 180, 186); however, recent greenhouse studies indicated that certain nematode strains can provide high levels of larval suppression. For example, larval survival was reduced to 20% when using the 7-12 strain of *S. riobrave* (156). It is conceivable that strains such as those used in the study by Shapiro-Ilan et al. (156) hold more promise for suppression of *C. caryae* larvae under field conditions.

Adult *C. caryae* may be more amenable to microbial control with EPNs than larvae,

particularly when *S. carpocapsae* is used (152, 154). One approach to control adult *C. caryae* is to apply EPNs in a narrow (1 to 2 m) band around each pecan tree. The adult weevils that crawl to the tree trunk would then be infected as they pass the area of application. If nematodes were applied as such, the cost of application would be reduced relative to treating a broader area (i.e., the entire orchard). *S. carpocapsae* is a good candidate for this application approach because the nematode has an ambushing foraging strategy (sits and waits for a potential host to pass and then attaches to it) and remains near the soil surface when applied there (103). If the banding method is not successful, an alternative approach might be to broadcast EPNs to control *C. caryae* adults under the soil or as they emerge.

In field trials, *S. carpocapsae* caused significant *C. caryae* mortality when applied in a 4-m band from the trunk at 100 IJs/cm² (156). However, a suppression rate of only 25%–50% was observed and generally was only statistically significant during the first week postapplication. Most likely, the nematodes were not exposed to the emerging weevils long enough to ensure infection, and the nematodes did not persist long enough in the soil environment to provide extended control. Thus, methods to enhance field suppression of *C. caryae* (e.g., improved virulence, persistence, or delivery) are required before nematodes can be considered a viable control tactic. Studies suggest that combinations of EPNs with other pathogens could enhance virulence through additive or synergistic interactions (7, 90, 164). Another approach that has been initiated toward enhancing *C. caryae* control with EPNs is strain improvement through hybridization and bacterial transfer (170, 171). Owing to the ability of EPN to recycle in *C. caryae* (159), nematode applications resulting in higher levels of adult *C. caryae* control, along with some level of concurrent larval mortality (due to overlapping generations), may result in cumulative suppression that contributes substantially to overall *C. caryae* population reductions.

A substantial amount of research has focused on the use of Hypocreales fungi for control of *C. caryae* (154, 186). *Beauveria bassiana* is naturally widely distributed in pecan orchards (72, 160) and can cause considerable natural mortality in *C. caryae* (157). The fungus can recycle in the weevil, and transmission of *B. bassiana* from infected *C. caryae* adult to healthy adult or larvae to larvae has been demonstrated (64, 65, 157).

Because of its phenology and susceptibility, adult stage *C. caryae* may be a preferable target for fungal control relative to the larvae (64, 74, 154, 186). Shapiro-Ilan et al. (157) investigated suppression of *C. caryae* adults over a two-week sampling period following application of *B. bassiana* (GHA strain). *B. bassiana* was applied in a 2-m band from the trunk at a rate of 3×10^{10} conidia/m². Up to 95% *B. bassiana*-induced mortality was observed within the first 3 days postapplication, but significant suppression relative to the control did not persist beyond one week post-treatment (157). Thus, a need for improved virulence and persistence is indicated. Improved fungal control of *C. caryae* may be achieved through amelioration of strains or application methods (160).

Based on the levels of weevil mortality observed, it appears the feasibility of incorporating fungus treatments for *C. caryae* control may be high. However, the extent to which fungus-induced weevil mortality translates into economic protection of the crop remains to be tested. Additionally, the issue of compatibility with other management practices such as concurrent application of other pesticides, particularly fungicides, must be raised (73, 83, 185). However, Shapiro-Ilan et al. (169) demonstrated that harmful pesticide effects on *B. bassiana* can be overcome through artificial selection or isolation of naturally resistant strains.

Citrus

Because of the diversity of cultivars and climates in which they are grown, citrus is

perhaps the most widely distributed tree fruit crop, ranging from tropical and subtropical climes to temperate habitats. Consequently, a huge range of arthropod pests are reported from citrus varieties (178). A number of important citrus pests have been studied for suitability to microbial control. The fungus *Hirsutella thompsonii* has received considerable attention as an MCA of the citrus rust mite, *Phyllocoptura oleivora* (115, 116). Commercialization of *H. thompsonii* was short-lived, lasting a few years and terminating in 1985 owing to variation in field efficacy (115). As a potential alternative to direct application of the fungus, isolation and application of toxins associated with *H. thompsonii* have suppressive properties (129), yet commercial development was not pursued probably because of economic constraints. Recently, *B. bassiana* has shown promise for control of *P. oleivora* and other citrus mites (6, 173), but expanded field trials are needed to verify efficacy. Some potential to control the citrus leafminer, *Phyllocnistis citrella*, was indicated with EPNs (17) or *B. thuringiensis* (39). Potential for the control of certain fruit flies (Diptera: Tephritidae), such as the Mediterranean fruit fly, *Ceratitis capitata*, and other species, has been demonstrated using Hypocreales fungi (27, 46, 60, 104) and EPNs (109, 110). Entomopathogenic fungi have also shown promise in controlling certain homopteran pests, e.g., *Aschersonia* spp. for control of whitefly populations (56, 135) and *B. bassiana* for control of the brown citrus aphid, *Toxoptera citricida* (136), but thus far expanded research and implementation of these MCAs have not progressed beyond these initial studies.

Citrus root weevils (Coleoptera: Curculionidae). Citrus root weevils have been controlled with fungal entomopathogens and EPNs. Weevils of economic importance in citrus primarily include the diapaese root weevil, *Diaprepes abbreviatus*, blue-green citrus root weevils, *Pachnaeus* spp., fiddler beetles, *Exophthalmus*, spp., and the Fuller rose beetle, *Asynonychus*

godmanni (114). Although some microbial control studies have been conducted on other citrus root weevils (123), the greatest amount of research has been toward the suppression of *D. abbreviatus* and *Pachnaeus* spp. (44, 119, 162).

S. carpocapsae was the first nematode shown to be pathogenic to *D. abbreviatus* (50, 102, 145) and to be developed commercially for citrus root weevil control (179). Subsequently, *S. riobrave* was particularly virulent to *D. abbreviatus* larvae compared with at least seven other nematode species that were evaluated (26, 43, 150). In several field studies, *S. riobrave* caused >90% suppression of *D. abbreviatus* and *Pachnaeus* spp. (26, 42, 43). Additionally, high levels of virulence were also observed with *Heterorhabditis indica*, especially to early instars (147). EPNs can also cause high levels of *D. abbreviatus* suppression in greenhouses when applied directly in aqueous sprays (149) or when applied in infected host cadavers (166). Both *H. indica* and *S. riobrave* have been commercially developed for application to citrus in Florida. In 1999 more than 19,000 ha were treated with *S. riobrave* (162).

Despite the substantial amount of research and relatively successful biocontrol efforts that have been directed toward *D. abbreviatus* with EPNs, field efficacy can be variable (117, 118, 158). Many factors can affect efficacy, including rate of application (generally high rates of 100 IJs/cm² or more appear to be required for high levels of efficacy) (117, 162), formulation (148), soil type (118, 151), and choice of EPN strain or species (158, 162). For example, recently newly discovered strains of *S. riobrave* that possess higher virulence than the commercialized strain (183) may offer higher efficacy or consistency in *D. abbreviatus* suppression. Also beneficial may be the application or conservation of a newly discovered nematode species, *Steinernema diaprepesi*, which was isolated from *D. abbreviatus*. It appears to persist well and contribute to natural suppression of *D. abbreviatus* in Florida citrus groves where it is endemic (e.g., the nematode can cause >20%

natural mortality during spring months) (41, 125).

Interactions among EPN species can also affect *D. abbreviatus* suppression. Duncan et al. (41) observed suppressed populations of endemic EPN species following application of the exotic species *S. riobrave*. Because endemic nematodes can provide substantial levels of natural control (117), competition between the introduced and endemic species could dampen control of *D. abbreviatus*. Duncan et al. (40) also discovered that free-living bacterivorous nematodes such as *Pellioiditis* sp. can affect *D. abbreviatus* suppression. *D. abbreviatus* mortality increased in the presence of *S. riobrave* and *Pellioiditis* sp. relative to when either nematode was present alone (40). However, relative to when each nematode was present alone, the combination of nematodes resulted in increased populations of free-living bacterivorous nematodes emerging from infected cadavers and decreased populations of *S. riobrave*. The endemic nematode, *S. diaprepesi*, was not similarly affected by free-living bacterivorous nematodes (40). Further elucidation of the biology and ecology of the soil community [such as studies by Duncan et al. (40, 41)] will enhance future microbial control efforts to suppress *D. abbreviatus*.

ROLE OF MICROBIAL CONTROL IN IPM IN TEMPERATE ORCHARD AGROECOSYSTEMS

Control of pest insects using only chemical pesticides has generated a myriad of problems already mentioned in the introduction. Due to their selectivity and safety, MCAs appear to be ready made components of IPM systems that will allow other natural enemies to function and not pose a threat to applicators or the environment. A successful microbial control tactic, i.e., one that reaches implementation, must possess several key elements: sufficiently high levels of efficacy, economical feasibility, and the capacity to fit in with an existing management program and cultural practices. Ef-

ficacy, the level of pest suppression, depends on the ability of the pathogen to persist long enough in the environment to infect the host, the capacity to reach the host (which can rely on the delivery system), and the innate virulence of the pathogen. The specific level of efficacy required for a specific pathogen will vary based on the target pest (and the type of damage it causes) and cropping system. To achieve sufficient efficacy, matching the specific MCA species or strain to a particular target pest is critical. To establish efficacy field tests are required; laboratory virulence does not necessarily translate into ability to achieve suppression in the field. For example, *S. feltiae* and *S. riobrave* exhibited equally high levels of virulence to *C. nenuphar* larvae in the laboratory, but only *S. riobrave* produced high levels of pest control in the field (167, 168); such discrepancies may be due to a variety of factors including suitability to environmental conditions. The importance of economic feasibility cannot be overstated. Regardless of efficacy, a lack of cost competitiveness will prevent MCAs from being incorporated into mainstream pest management programs. For example, the relative high cost of nematodes has prevented implementation in some cropping systems (162), whereas the ease of handling and greater cost competitiveness in *B. thuringiensis* products has facilitated their success.

Among the specific examples of microbial control research cited above, varying degrees of successful and failed implementation can be found. The use of nematodes for control of *D. abbreviatus* is an example of successful implementation. The system possesses all elements for success including high levels of virulence and compatibility with current practices, i.e., nematodes are applied through existing irrigation systems or standard herbicide rigs (162). Use of EPNs for *D. abbreviatus* is cost competitive because the applications are only made under the canopy of the trees (where the insects occur) and not between rows; such reduced area "targeted applications" clearly have merit in enhancing feasibility. An

example of an orchard pest-MCA combination that still has substantial hurdles to overcome before implementation is the use of nematodes for *C. caryae* control. In this case, field efficacy has thus far been low and nematodes are likely to be substantially more expensive than the current chemical alternative.

Even if an MCA is deficient in one or more key elements (efficacy, economics, or compatibility), the MCA might still contribute to pest suppression within an IPM program, albeit not as a stand alone tactic. It is conceivable that incremental contributions to suppression may have their place [e.g., where a pathogen is endemic and thus accrues little or no cost to the grower (181)]. Yet these contributions are, for the most part, overlooked because components of IPM tend only to be evaluated as stand alone tactics without consideration of their interactions with other components of the agroecosystem. The fact is that in a number of systems MCAs are unlikely to serve as stand alone tactics. Therefore, integration with other chemical or biological tactics, or enhancement of efficacy through environmental manipulation (as outlined below) may be the key to incorporating MCAs into temperate orchard IPM.

Combination of Chemical Pesticides, Semiochemical and Microbial Interventions

IPM may employ the judicious use of insecticides when needed. When selective insecticides, such as some of the insect growth regulators, are used for control of orchard pest insects, the negative impact on beneficial insects is reduced (21, 112). Furthermore, the combined use of certain chemicals or other tactics with MCAs can result in an enhanced control effort. For example, sublethal dosages of certain insecticides, such as imidacloprid, may act synergistically in combination with entomopathogens by compromising the targeted insect's defenses (90, 138). Similarly, alternation of CpGV with an organically approved formulation of spinosad (Entrust®)

was reported by Arthurs and Lacey (8) as a strategy used by orchardists for control of CM as well as other pest insects. However, there have been several reports regarding the negative impact of spinosad on parasitoids and some other natural enemies of pest insects (11, 193). The combination of mating disruption using the CM female sex attractant and CpGV to control resistant strains of CM has been successfully utilized in Europe (28, 121, 188) and is a strategy that is being increasingly employed in North America.

Interactions of Entomopathogens with Other Natural Enemies

Competition between microorganisms and multicellular animals for insect hosts is pervasive throughout nature (77). Premature death of the host due to infection is one of the main antagonistic interactions between entomopathogens and parasitoids (23). However, there is evidence for behavioral and biochemical mechanisms that minimize the negative interactions between entomopathogens and insect parasitoids (19, 23). Although there are several reports on the innocuous nature of MCAs toward beneficial insects and other nontarget organisms (48, 68, 97, 155), there are few studies on the specific interactions between entomopathogens and arthropod natural enemies of most orchard pests. Studies by Lacey et al. (98) revealed both antagonism and complementary activity between the EPN *S. carpocapsae* and two ichneumonid idiobiont parasitoids of CM. This research demonstrated the ability of parasitoid females to detect and avoid laying eggs on nematode-infected cocooned CM larvae. The compatibility of the two groups of biological control agents for CM control could be facilitated by careful timing of applications. The ability of parasitoids to avoid EPN-treated larvae and to actively seek out and kill cocooned CM larvae that survived nematode treatments enhances their compatibility. Evidence presented by Arthurs and Lacey (8) indicated that CpGV applications in orchards

were compatible with survival and parasitic activity of the ichneumonid *Mastrus ridibundus*. The models of Begon et al. (19) indicate that coexistence and enhanced biological control are favored by complementary activity between parasitoids and pathogens in terms of their respective qualities. Parasitoids are better suited for exploiting uninfected hosts, particularly in cryptic habitats, because of their abilities of search, whereas most pathogens, such as CpGV, must wait for chance encounters (fungi), proper environmental conditions (EPNs), or well-timed applications (virus, *B. thuringiensis*). According to Begon et al. (19), one of the most important aspects to consider in the integration of pathogens and parasitoids is the stage of the host that is attacked. CpGV normally infects neonate larvae before or during entry into fruit, while *M. ridibundus* searches for and attacks cocooned larvae in cryptic habitats. Although there is minimal effect of microbials on parasitoids, careful timing of applications can further minimize possible negative effects. For example, Cossentine et al. (32) recommended a strategy for using *B. thuringiensis* for control of the obliquebanded leafroller, *Choristoneura rosaceana*, that minimizes impact on its parasitoids.

Ecological Engineering and Agricultural Practices to Increase or Conserve Natural Enemies

Ecological engineering in the context of IPM is the manipulation of agricultural habitats to be less favorable for arthropod pests and more attractive to beneficial insects and other natural enemies (54, 69, 101). The use of environmental modification with mulches and irrigation to enhance the activity of EPNs was presented by Lacey et al. (93, 94). In addition to improvement of nematode persistence and larvicidal activity, mulching can also have a variety of other beneficial effects. In orchard agroecosystems, surface mulches have been used for weed control, improvement in tree vigor, soil nutrient status and biological activity, and have buffered trees against stress re-

sulting from inadequate irrigation. They have also resulted in enhanced biodiversity in orchards, including an increase in the numbers of ground dwelling predators (24, 113, 122). Certain kinds of mulches may be attractive to CM seeking cocooning sites. This in turn may increase predation by ground dwelling predators. However, there may be negative consequences of habitat manipulations. For example, some mulches or other modifications may lead to an increase in intraguild predation (113). An increase in biodiversity may not necessarily translate into an increase in biological control (101). Other agricultural practices that favor MCAs include timing of irrigation to enhance MCA survival (93), postponing application of pesticides that could interfere with natural epizootics (181), and using no till strategies to minimize habitat disturbance (25). The current trend in North America to use high-density trellised plantings as has been the practice in European orchards enables more effective use of EPNs (93). Further investigation of orchard design to maximize pest control and the effectiveness of MCAs and other natural enemies is warranted.

CONCLUSIONS

Sustainable agriculture will rely increasingly on alternatives to conventional chemical insecticides for pest management that are environmentally friendly and reduce the amount of human and environmental contact with pesticides (111). MCAs of orchard pests, in conjunction with other IPM components, can provide effective control of several orchard pests. The challenge we face is to find successful combinations of entomopathogens, predators, and parasitoids with soft and selective insecticides, semiochemicals, and habitat modifications to produce a profitable and sustainable orchard pest management system. Cost-effective suppression of pest populations with MCAs and the establishment and conservation of other natural enemies represent biological solutions that are compatible with other methods such as mating

disruption that could form the foundation of fruit and nut production without disruptive insecticides.

A substantial amount of basic and operational research remains to be done. Basic studies on the fate of entomopathogens in the environment are needed; to a large extent it is not clear why the persistence of certain MCA applications is so short-lived. A variety of applied research endeavors can improve key elements of success (increasing efficacy, reducing production costs, and enhancing compatibility and ease of use) and thereby expand the utility of MCAs. The search for and development of new MCAs appears promising. Also, the discovery of novel matches between existing MCA strains and species and key orchard pests can be fruitful (e.g., the use of

S. riobrave for *D. abbreviatus* and *C. nenuphar*) (162, 165). There is also a need for enhancement of existing MCAs to improve production technology, delivery (including targeted or site-specific applications), virulence, and environmental stability through formulation and strain improvement (67). Strain improvement and other methods of enhancement will be greatly facilitated by established and future genome sequencing projects. Regardless of the improvement approach for MCAs that is employed, an integrated approach for the implementation of IPM components in orchard agroecosystems that is based on pest densities and their relation to economic injury levels will ultimately be required before IPM can be truly implemented (13, 21, 133).

Economic injury level: level of pest density in which the cost of its control equals the value of the crop that is protected by control measures

SUMMARY POINTS

1. Integrating MCAs into orchard IPM will have minimum impact on the actions of other natural enemies and will be safe to farm workers and the environment.
2. Selecting the most effective MCA for a given pest and habitat will rely on a thorough understanding of the biology and ecology of pest and pathogen (strengths and limitations) and the orchard agroecosystem into which it will be applied.
3. Once an effective MCA is selected, enhancement of activity through formulation and proper timing of applications will maximize efficacy.

FUTURE ISSUES

1. An increase in the efficacy and implementation of MCAs will be possible through discovery of new strains, improvement of existing strains through molecular and non-molecular methods, superior application procedures, and improvement of environmental persistence through formulation.
2. Environmental manipulation, including orchard redesign, to improve activity and persistence of MCAs has been demonstrated and should be more thoroughly investigated.
3. Advances in microbial control will also be facilitated through fundamental studies on the microbial ecology, epizootiology, and population dynamics of insect pathogens in orchard systems as well as basic studies on host-pathogen relationships.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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