WFL Publisher
Science and Technology Food, Agriculture & Environment Vol.1(2) : 326-331. 2003 *www.world-food.net*

The potential role for microbial control of orchard insect pests in sustainable agriculture

Lawrence A. Lacey[1] and David I. Shapiro-Ilan[2]
[1]USDA-ARS, Yakima Agriculture Research Laboratory, Wapato, WA, 98908, USA.
e-mail: llacey@yarl.ars.usda.gov, [2]USDA-ARS, S.E. Fruit and Tree Nut Research Laboratory, Byron, GA, 31008,USA

Received the 11 March 2003, accepted 29 April 2003.

Abstract

Sustainable agriculture will rely increasingly on alternatives to conventional chemical insecticides for pest management that are environmentally friendly and reduce the amount of human contact with pesticides. Microbial control agents, *i.e.* insect pathogens, can provide effective control, conserve biodiversity, and serve as alternatives to chemical insecticides under several conditions. Due to their specificity for insects, insect pathogens including viruses, bacteria, fungi and nematodes are ideal candidates for incorporation into integrated pest management strategies in orchards where their effects on other natural enemies will be minimal. There is also excellent potential for combining microbial control with other soft technologies such as mating disruption. Increased use of microbial control will depend on a variety of factors including improvements in the pathogens (virulence, formulation, delivery, etc.) and an increased awareness of their attributes by growers and the general public. In this review we provide an overview of microbial control of the key insect pests of citrus, pome fruits, stone fruits and nuts.

Key words: Microbial control, orchard, insect pests, entomopathogens, baculovirus, fungi, *Bacillus thuringiensis*, codling moth, citrus root weevils, cherry fruit fly, plum curculio, Mediterranean fruit fly, navel orangeworm, leafrollers, citrus rust mite, pecan weevil.

Introduction

Detrimental consequences of pesticide use are numerous and include concerns over worker and food safety, environmental contamination and decline of biodiversity in agroecosystems. Control of arthropod pests of tree fruit and nuts in most conventional orchards traditionally employs substantial amounts of insecticides to prevent economic damage to crops. The need to reduce pesticide usage while meeting human needs for food and fiber has provided incentive for the development of cost effective alternatives to conventional chemical pesticides. The integrated pest management (IPM) strategy, in which natural enemies (*i.e.*, parasites, predators and pathogens) of pest arthropods and other alternative measures play significant roles in crop protection, is one aspect of sustainable agriculture that attempts to minimize negative environmental impact, and other deleterious effects due to insecticide usage. Because of their specificity for insects, entomopathogens are ideal candidates for incorporation into IPM where their effects on natural enemies will be minimal as compared to most presently used chemical pesticides. Several key pests of fruit and nuts have been controlled with entomopathogens under experimental or operational conditions. In this review we provide an overview of microbial control of the key insect and mite pests in temperate and subtropical tree fruit and nuts. Because the literature on microbial control in orchards is extensive, we have had to restrict our review to major pests.

Citrus

Root weevils of economic importance in citrus primarily include the diaprepes root weevil, *Diaprepes abbreviatus*, blue-green citrus root weevils, *Pachnaeus* spp., fiddler beetles, *Exophthalmus*, spp. and the Fuller rose beetle, *Asynonychus godmani*[1]. Although microbial control studies have been conducted on other citrus root weevils, e.g., the nematode *Steinernema carpocapsae*[2] against *A. godmani*, most has been towards suppression of *D. abbreviatus* and *Pachnaeus* spp.[3-5]. Entomopathogenic nematodes have been researched extensively for control of *D. abbreviatus* and *Pachnaeus* spp. in Florida citrus groves. Among nine nematode species evaluated thus far, *Heterorhabditis indica* and *Steinernema riobrave* were the most virulent to *D. abbreviatus* larvae[6-9]. In several field studies, *S. riobrave* caused > 90% suppression of *D. abbreviatus* and *Pachnaeus* spp[6,10,11]. Currently, *H. indica* and *S. riobrave* are both being marketed commercially for control of *D. abbreviatus* and *Pachnaeus* spp. Factors that can affect efficacy include rate of application (generally rates of ≥100 infective juveniles [IJs] per cm^2 are required)[5,12], formulation[13], and soil type[14,15].

Stability and favorable soil conditions (moisture, aeration, texture) make Florida citrus groves amenable to entomopathogenic nematode recycling and persistence. Indeed, endemic nematode populations exist[16] and can provide significant *D. abbreviatus* suppression (e.g., 7 - 42%)[12]. However, inundative applications of nematodes (i.e., *H. bacteriophora, H. indica, S. carpocapsae*, and *S. riobrave*) to citrus have resulted in poor persistence, reaching pre-treatment levels within two weeks post-application[10,12]. Discovery of other species that are both virulent and persistent would be beneficial. A newly discovered nematode species, *Steinernema diaprepesi* (isolated from *D. abbreviatus*) is persistent and provides substantial control of *D. abbreviatus* in Florida citrus groves where it is endemic[17].

In addition to entomopathogenic nematodes, entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have been isolated from, and tested against, *D. abbreviatus* larvae, but were efficacious only at high rates of application[18-20]. The effectiveness of *B. bassiana* and *M. anisopliae* may, however, be increased through a synergistic relationship between the fungi and sublethal doses of the insecticide, imidacloprid (chloronicotinyl)[20]. A number of other important citrus pests have been studied for suitability to microbial control. The fungus, *Hirsutella thompsonii* has received considerable attention as a microbial control agent of the citrus rust mite, *Phyllocoptruta* *oleivora*[4,21-23], but commercialization was short-lived (lasting a few years and terminating in 1985) due to variation in field efficacy[24]. As a potential alternative to direct application of the fungus, isolation and application of toxins associated with *H. thompsonii* were found to have promise for *P. oleivora* control[25]. Potential for the control of certain fruit flies (Diptera: Tephritidae), such as the Mediterranean fruit fly, *Ceratitus capitata,* and the Caribbean fruit fly, *Anastrepha suspense,* and other species has been demonstrated using Hyphomycete fungi[26-30] and entomopathogenic nematodes[31-34]. Entomopathogenic fungi have also shown promise in controlling certain homopteran pests, e.g., *Aschersonia* spp. for control of whitefly populations[35-37] and *B. bassiana* for control of the brown citrus aphid, *Toxoptera citricida*[38].

Apple and Pear

Apple and pear are attacked by a variety of arthropod pests, but successful microbial control has been predominantly against Lepidoptera. In this section we will review the potential of pathogens for control of codling moth (CM), *Cydia pomonella,* and several other species in the family Tortricidae that are collectively referred to as leafrollers. CM is a key cosmopolitan pest of apple, pear, walnut and other fruit[39]. The most vulnerable stages of this pest in regards to microbial control agents are the neonate larvae before they enter the fruit and cocooned larvae. CM overwinter as mature cocooned larvae in cryptic habitats (under bark, in fruit bins, etc.). The entomopathogens with the most promise for incorporation into IPM strategies are the granulovirus of CM (CpGV) and entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.). The CM granulovirus is a highly virulent baculovirus that targets neonate larvae, but must be ingested before or during entry of larvae into fruit. The virus is applied by spraying orchards with approximately 10^{13} virus granules per hectare at 7-14 day intervals when neonate CM larvae are present. The *per os* mode of entry of the virus allows some fruit damage, but larvae often die before penetrating deeply into the fruit[40,41]. Consequently, treated fruit will have as many CM entries as untreated fruit, but some with minute entries will be marketable. The principal objective of treatments, however, is population reduction. The specificity of the virus for CM and some closely related species and safety to nontarget organisms[42,43] can also contribute to the conservation of insect natural enemies and biodiversity in the orchard agroecosystem. Numerous field trials around the world have demonstrated good activity against CM [44-50]. CpGV is now commercially available in Europe and North America. CpGV can be effective in controlling CM, but concerns expressed by growers are: the increased number of shallow entries to fruit; short residual activity of the virus due to UV inactivation; the need for multiple applications; lower efficacy against high density CM populations; and expense. Adjuvants that provide UV protection or increase feeding (thus increase uptake of virus) have reportedly improved the activity of the virus by protecting it from UV degradation or increasing uptake of the virus[51,52]. In addition to efficacy evaluations, investigations are also underway by several researchers to study the role of transovariole transmission of the virus and its latent activity in CM populations. The entomopathogenic nematode, *S. carpocapsae*, has been reported from CM in North America and Eastern Europe[53,54]. Research results indicate good control potential for this and a number of other nematode species when adequate moisture is maintained and temperatures are above 10-15^{0}C[55-59]. Dosages of 1-2 x 10^6 IJs per tree and surrounding area can provide effective control of cocooned larvae under optimal conditions. Protocols for the field evaluation of entomopathogenic nematodes against cocooned stages of CM are presented by Lacey et al.[60]. Fruit bins infested with cocooned CM can be a significant source of invading moths in mid to late summer when they are placed in orchards for harvest. Entomopathogenic nematodes (*S. carpocapsae, S. feltiae*, and others) offer potential for decontaminating fruit bins at the time when they are submerged in drop tanks[61] or sprayed with drenchers used for treating or cooling fruit[62]. Several defoliating tortricid species collectively referred to as leafrollers can be significant pests of apple and pear. The selective control of CM using mating disruption has on occasion resulted in increased numbers of leafrollers which had been coincidentally controlled in conventional orchards by broad spectrum insecticides applied for CM control[63]. Microbial control agents offer a means of control of leafroller larvae to supplement mating disruption for CM and conserve insect natural enemies and other beneficials. The bacterium *B. thuringiensis* (*Bt*) and baculoviruses have been the most extensively studied microbial control agents of leafroller pests in apple. *Bt* has been widely and commonly employed for control of leafrollers in apple and pear and other tree fruit[60,64-71]. It is also one of the most frequently used means of leafroller control in certified-organic orchards[65], and in conventional orchards where resistance to organophosphate insecticides is a concern. Its safety record is well documented having little or no effect on the vast majority of nontarget organisms, including beneficial insects[66]. A variety of factors including temperature, larval age, timing of applications, *Bt* variety, degree of coverage and spray concentration can affect the efficacy of *Bt* formulations against leafroller pests in pome fruit[67-70]. The short residual of *Bt* in combination with rapid foliage growth in the spring and early summer increases the need for several applications to successfully control pests. Environmental factors such as rainfall and sunlight are major determinants of the residual life of *Bt*[71]. There are several viruses reported from leafrollers and other tortricid orchard pests. The most efficacious are baculoviruses including several granuloviruses (GV) and nucleopolyhedroviruses (NPV). Granuloviruses are considerably more specific than many of the NPVs, infecting single species or closely related species. The GV of *Adoxophes orana* (AoGV) has been extensively tested in Europe and Japan[72-75]. This GV is routinely used for control of *A. orana* in certain orchards in Europe. It has been credited with persistent control; however, three major problems associated with it are low tolerance to UV radiation, slow rate of kill and specificity for one pest. Improvement of AoGV activity through formulation is reported by Luisier and Benz[76].

Stone Fruits

Oriental fruit moth, *Grapholitha molesta*, a widespread pest of peaches, nectarines and other fruit, is susceptible to *Bt* when it is feeding on treated foliage[77]. Overwintering larvae (found within cocoons in cryptic habitats) are amenable to control with entomopathogenic nematodes under conditions similar to those required for CM[78]. Various boring insects in the order Lepidoptera are important pests of stone fruits. The clearwing moths (Lepidoptera: Sesiidae) in the genus *Synanthedon*, which are among the most serious of these boring pests, are generally quite susceptible to entomopathogenic nematodes[79-82]. Efficacy with nematodes was also demonstrated for control of the peach fruit borer, *Carposina niponensis* [83,84]. In contrast, the American plum borer, *Euzophera semifuneralis* was not significantly controlled with *S. feltiae* or *H. bacteriophora*[85]. *Bacillus thuringiensis* has been successfully employed to suppress the peach twig borer, *Anarsia lineatella*[86], and the peach fruit borer[82].The plum curculio, *Conotrachelus nenuphar*, a major pest of pome and stone fruits in North America is susceptible to entomopathogenic fungi (*Beauveria* spp. and *M. anisopliae*)[87], and nematodes[87-89]. In preliminary field trials in peach orchards, Shapiro-Ilan et al.[90] observed >90% suppression of *C. nenuphar* larvae with *S. riobrave*. The western cherry fruit fly, *Rhagolites indifferens*, a serious pest of sweet cherries, has been investigated for control using nematodes[91, 92]. In field trials, *S. carpocapsae* and *S. feltiae* provided 59-85% larval mortality when applied to soil under cherry trees at 50-100 IJs/cm^2. Yee and Lacey[92] proposed the use of entomopathogenic nematodes for control of *R. indifferens* in isolated and abandoned lots or in yards of homeowners as a means to reduce invasion of commercial orchards. Several other fruit flies have been investigated for susceptibility to entomopathogenic nematodes and fungi (see Citrus section above).

Nuts

The pecan weevil, *Curculio caryae*, is a key pest of pecans[93,94]. Although some studies have been conducted on virus, bacteria, and protozoan diseases of *C. caryae*, most attention has focused on nematodes and fungi[94-97]. Efficacy of entomopathogenic nematodes for control of *C. caryae* larvae has been shown to be poor to moderate[98-100]. In contrast, adult *C. caryae* appear to be highly susceptible to certain nematode species (e.g., *S. carpocapsae*) [94,101]. Recent field trials indicate *S. carpocapsae* can provide up to 80% control of emerging *C. caryae* adults[90]. Of all the pathogen groups that infect *C. caryae*, fungi have received the most attention. Field studies using *B. bassiana* and *M. anisopliae* have indicated varying levels of efficacy against *C. caryae* larvae and adults[102-104]. Gottwald and Tedders[103] observed < 30 and 6% larval mortality from *B. bassiana* and *M. anisopliae* applications, respectively using relatively high rates (up to 10^7 conidia/g soil). In the same study, higher efficacy was observed toward adults, i.e., 72 and 50% suppression from *B. bassiana* and *M. anisopliae* applications, respectively[103]. Recent studies indicate up to 95% *C. caryae* adult mortality can be achieved through applications of *B. bassiana* during weevil emergence but the duration of the control is generally less than one week[90]. The promising nature of *B. bassiana* as a *C. caryae* control agent, has led to several investigations concerning its ecology including natural distribution in pecan orchards[105,106], transmission[103,107], and interaction with other pesticides[108-110]. Further research is required to explore methods of enhancing the persistence of *C. caryae* control with entomopathogenic fungi. The navel orangeworm, *Amyelois transitella* is a key pest of pistachios, almonds, walnuts and figs. The larvae infest mature nuts on the tree and nut mummies on the tree and ground. All groups of pathogens (virus, bacteria, protozoa, fungi, and nematodes) have shown to have at least some pathogenicity to *A. transitella*[111-113]. Several studies have pursued nematodes as a potential control agent. Summer time field application of the nematode *S. carpocapsae* to open hulled almonds resulted in over 65% mortality in baited *A. transitella*[111]. Dormant season (winter) application of entomopathogenic nematodes to trees, however, resulted in substantially lower *A. transitella* control[114]. Siegel et al. [115] reported high levels of suppression when *S. carpocapsae* and *S. feltiae* were applied to almond and pistachio nut mummies on the ground for control of *A. transitella* larvae. Additionally, *Bt* has been shown to be a viable pesticide alternative in an integrated approach to suppression of *A. transitella*[116,117]. *Bt* has also been successfully employed to suppress some other nut pests such as the peach twig borer, *Anarsia lineatella*[86, 116,118], the fall webworm, *Hyphantria cunea*, and walnut caterpillar, *Datana integerima*, in pecan trees[119].

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 contact with pesticides. Entomopathogens can be effective control agents, conserve biodiversity, serve as alternatives to broad spectrum chemical insecticides under several conditions, and provide options to counteract resistance to conventional insecticides. The integration of entomopathogens into comprehensive IPM programs will not only depend on compatibility with other biological control agents, but also with chemical methods of control and environmental conditions in a given cropping system and their effects on infectivity and persistence of microbial control agents. The increased successful utilization of entomopathogens will require several improvements in the pathogens and their formulation, careful selection of application windows and niches, a better understanding of how they will fit into integrated systems and their interaction with the environment and other components of integrated pest management, as well as an increased awareness of their attributes by growers and the public[120].

Acknowledgements

We thank Don Hostetter, Lisa Neven and Ted Cottrell for review of the manuscript and for providing helpful comments. We also thank Nicole Murphy and Heather Headrick for their help with preparation of the references. This writing was supported in part by the Washington Tree Fruit Research Commission.

References

1McCoy, C. W. 1999. Arthropod pests of citrus roots. In: L. W. Timmer and L. W. Duncan (Eds.). Citrus Health Management. St. Paul: American Phytopathological Society. p. 149-156.

2 Morse, J. G. and J. E. Lindegren. 1996. Suppression of Fuller rose beetle on citrus with *Steinernema carpocapsae*. Fla. Entomol. **79:** 373-384.

3Duncan, L. W., D. I. Shapiro, C. W. McCoy and J. H. Graham. 1999. Entomopathogenic nematodes as a component of citrus root weevil IPM. In: S. Polavarapu (Ed.). Optimal use of Insecticidal Nematodes in Pest Management. New Brunswick: Rutgers University. p. 69-78.

4McCoy, C. W., D. I. Shapiro and L. W. Duncan. 2000. Application and evaluation of entomopathogens for citrus pest control. In: L. Lacey, L. and H. K. Kaya (Eds.). Field Manual of Techniques in Insect Pathology. Dordrecht: Kluwer Academic Publishers. p. 577-596.

5Shapiro-Ilan, D. I., D. H. Gouge and A. M. Koppenhöfer. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus. In: R. Gaugler (Ed.). Entomopathogenic Nematology. New York: CABI. p. 333-356.

6Duncan, L. W. and C. W. McCoy. 1996. Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida:

Steinernematidae: Heterorhabditidae). Environ. Entomol. **25**: 174-178.
[7]Shapiro, D. I., J. R. Cate., J. Pena., A. Hunsberger and C. W. McCoy. 1999. Effects of temperature and host range on suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by entomopathogenic nematodes. J. Econ. Entomol. **92**: 1086-1092.
[8]Shapiro, D. I. and C. W. McCoy. 2000. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. **93**: 1090-1095.
[9]Shapiro, D. I. and C. W. McCoy. 2000. Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae to different rates of entomopathogenic nematodes in the greenhouse. Fla. Entomol. **83**: 1-9.
[10]Duncan, L. W., C. W. McCoy. and C. Terranova. 1996. Estimating sample size and persistence of entomogenous nematodes in sandy soils and their efficacy against the larvae of *Diaprepes abbreviatus* in Florida. J. Nematol. **28**: 56-67.
[11]Bullock, R. C., R. R. Pelosi and E. E. Killer. 1999. Management of citrus root weevils (Coleoptera: Curculionidae) on Florida citrus with soil-applied entomopathogenic nematodes (Nematoda: Rhabditida). Fla. Entomol. **82**: 1-7.
[12]McCoy, C. W., D. I. Shapiro, L. W. Duncan and K. Nguyen. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Contr. **19**: 182-190.
[13]Shapiro, D. I. and C. W. McCoy. 2000. Effect of culture method and formulation on the virulence of *Steinernema riobrave* (Rhabditida: Steinernematidae) to *Diaprepes abbreviatus* (Curculionidae). J. Nematol. **32**: 281-288.
[14]Shapiro, D. I., C. W. McCoy, A. Fares, T. Obreza and H. Dou. 2000. Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus*. Environ. Entomol. **29**: 1083-1087.
[15] McCoy, C. W., R. J. Stuart, L. W. Duncan, and K. Nguyen. 2002. Field efficacy of two commercial preparations of entomopathogenic nematodes against larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Spodosol type soil. Fla. Entomol. **85**: 537-544.
[16]Beavers, J. B., C. W. McCoy and D. T. Kaplan. 1983. Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. Environ. Entomol. **12**: 840-843.
[17]Nguyen, K. B. and L. W. Duncan. 2002. *Steinernema diaprepesi* n. sp. (Rhabditida: Steinernematidae), a parasite of the citrus root weevil *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). J. Nematol. **34**: 159-170.
[18] Beavers, J. B., C. W. McCoy and D. T. Kaplan. 1983. Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. Environ. Entomol. **12**: 840-843.
[19]McCoy, C. W. 1990. Entomogenous fungi as microbial pesticides. In: R. R. Baker and P. E. Dunn (Eds.). UCLA Symposia on Molecular and Cellular Biology, New Series, vol 112. New York: Liss. p. 139-161.
[20]Quintela, E. D. and C. W. McCoy. 1998. Synergistic effect of imidacloprid and two entomopathogenic fungi on the behavior and survival of larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. J. Econ. Entomol. **91**: 110-122.
[21]McCoy, C. W. and T. L. Couch. 1982. Microbial control of the citrus rust mite with the mycoarcaricide, Mycar. Fla. Entomol. **65**: 116-126.
[22]McCoy, C. W., A. J. Hill and R. F. Kanavel. 1972. A liquid medium for the large-scale production of *Hirsutella thompsonii* in submerged culture. J. Invertebr. Pathol. **19**: 370-374.
[23]McCoy, C. W., A. J. Hill and R. F. Kanavel. 1975. Large-scale production of the fungal pathogen *Hirsutella thompsonii* in submerged culture and its formulation for application in the field. Entomophaga **20**: 229-240.
[24]McCoy, C. W. 1996. Pathogens of Eriophyoid mites. In: E. E. Lindquist, M. W. Sabelis and J. Bruim (Eds.). Eriophyoid Mites. Their Biology, Natural Enemies and Control Vol. 6. Amsterdam: Elsevier. pp. 481-490.
[25]Omoto, C. and C. W. McCoy. 1998. Toxicity of purified fungal toxin Hirsutellin A to the citrus rust mite *Phyllocoptruta oleivora* (Ash.). J. Invertebr. Pathol. **72**: 319-322
[26]Castillo, M. A., P. Moya, E. Hernandez and E. Primo-Yufera. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biol. Contr. **19**: 274-282.
[27]Lezama-Gutierrez, R., A. Trujillo-de la Luz, J. Molina-Ochoa, O. Rebolledo-Dominguez, A. R. Pescador, M. Lopez-Edwards and M. Aluja. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): laboratory and field trials. J. Econ. Entomol **93**: 1080-1084.
[28]Ekesi, S., N. K. Maniania, and S. A. Lux. 2002. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontr. Sci. Technol. **12**: 7-17.
[29]Rosa, W. de la, F. L. Lopez and P. Liedo. 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. J. Econ. Entomol. **95**: 36-43.
[30]Dimbi, S., N. K. Maniania, S. A. Lux, S. Ekesi, and J. M. Mueke. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorok and *Beauveria bassiana* (Balsamo) Vuellimin, to three adult fruit fly species: *Ceratitis capitata* Wiedemann, *C. rosa* var *fasciventris* Karsch and *C. cosyra* (Walker) Diptera: Tephritidae. Mycopathologia (in press).
[31]Beavers, J. B. and C. O. Calkins. 1984. Susceptibility of *Anastrepha suspensa* (Diptera: Tephritidae) to steinernematid and heterorhabditid nematodes in laboratory studies. Environ. Entomol. **13**: 137-139.
[32]Lindegren, J. E. and P. V. Vail. 1986. Susceptibility of Mediterranean fruit fly, melon fly, and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in laboratory tests. Environ. Entomol. **15**: 465-468.
[33]Lindegren, J. E., T. T. Wong and D. O. McInnis. 1990. Response of Mediterranean fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in field tests in Hawaii. Environ. Entomol. **19**: 383-386.
[34]Gazit, Y., Y. Rossler and I. Glazer. 2000. Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae). Bicontr. Sci. Technol. **10**: 157-164.
[35]Fawcett, H. S. 1944. Fungus and bacterial diseases of insects as factors in biological control. Botanical Rev. 10: 327-348.
[36]Ponomarenko, N. G., N. A. Prilepskaja, M. Murvanidze, and L. A. Stoljarova. 1975. *Aschersonia* against whitefly. Zashchita Rastenii **6**: 44-45.
[37]Gao, R., Z. Ouyang, Z. Gao and J. Zheng. 1985. A preliminary report on the application of *Aschersonia aleyrodis* for the control of citrus whitefly. Chinese J. Biol. Contr. **1**: 45-46.
[38]Poprawski, T. J., P. E. Parker and J. H. Tsai. 1999. Laboratory and field evaluation of hyphomycte insect pathogenic fungi for control of brown citrus aphid (Homoptera: Aphidae). Environ. Entomol. **28**: 315-321.
[39]Barnes, M. M. 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation, and damage. In: L. P. S. van der Geest and H. H. Evenhuis, (Eds.). Tortricid Pests, Their Biology, Natural Enemies and Control. Amsterdam: Elsevier Science Publishers B. V. p. 313-327.
[40]Glen, D. M. and J. Clark. 1985. Death of *Cydia pomonella* larvae and damage to apple fruit, after field application of codling moth granulosis virus. Entomol. Exp. Appl. **38**: 93-96.
[41]Jaques, R. P. 1990. Effectiveness of the granulosis virus of the codling moth in orchard trials in Canada. Proceedings of the Fifth International Colloquium on Invertebrate Pathology and Microbial Control. Adelaide, Australia. p. 428-430.
[42]Gröner, A, 1990. Safety to nontarget invertebrates of baculoviruses. In: M. Laird, L. A. Lacey and E. W. Davidson, (Eds.). Safety of Microbial Insecticides. Boca Raton: CRC Press. p. 135-147.
[43]Cross, J. V., M. G. Solomon, D. Chandler, P. Jarrett, P. N. Richardson, D. Winstanley, H. Bathon, J. Huber, B. Keller, G. A. Langenbruch and G. Zimmermann. 1999. Biocontrol of pests of apples and pears in North-

ern and Central Europe: 1. Microbial agents and nematodes. Biocontr. Sci. Technol. **9**: 125-149.

[44]Falcon, L. A., W. R. Kane and R. S. Bethell. 1968. Preliminary evaluation of a granulosis virus for control of the codling moth. J. Econ. Entomol**.** **61**: 1208-1213.

[45]Huber, J. and E. Dickler. 1977. Codling moth granulosis virus: Its efficiency in the field in comparison with organophosphorus insecticides. J. Econ. Entomol. **70**: 557-561.

[46]Jaques, R. P., J. E. Laing, D. R. Laing and D. S. K. Yu. 1987. Effectiveness and persistence of the granulosis virus of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae) on apple. Can. Entomol. **119**: 1063-1067.

[47]Jaques, R. P., J. M. Hardman, J. E. Laing, R. F. Smith and E. Bent. 1994. Orchard trials in Canada on control of *Cydia pomonella* (Lep.: Tortricidae) by granulosis virus. Entomophaga **39**: 281-292.

[48]Dickler, E. and V. Gehr. 1981. Untersuchungen uber den Einfluss von Apfelwickler Granuloseviren auf die Biozoenose in Apfelanlagen: Analyse des Schalenwicklerkomplexes. Mitt. dtsch. Ges. allg. angew. Ent. **2**: 146-150.

[49]Glen, D. M. and C. C. Payne. 1984. Production and field evaluation of codling moth granulosis virus for control of *Cydia pomonella* in the United Kingdom. Ann. Appl. Biol. **104**: 87-98.

[50]Guillon, M., and G. Biache. 1995. IPM strategies for control of codling moth (*Cydia pomonella* L.) (Lepidoptera: Olethreutidae) interest of CmGV for long term biological control of pests complex in orchards. Med. Fac. Landbouww. Univ. Gent. **60** (3a): 695-705.

[51]Benz, G., C. Fluckiger and D. Zurrer. 1984. Uber die Wirkung von Magermilch auf die Frassintensitat der Eilarven von *Cydia pomonella* (L.) und *Adoxophyes orana* F.v.R. (Lep.,Tortricidae). Mitt. Schweiz. Entomol. Ges. **57**: 233-237.

[52]Ballard, J., D. J. Ellis and C. C. Payne. 2000. The role of formulation additives in increasing the potency of *Cydia pomonella* granulovirus for codling moth larvae, in laboratory and field experiments. Biocontr. Sci. Technol. **10**: 627-640.

[53]Weiser, J., 1955. *Neoaplectana carpocapsae*, n. sp. (Anguillaluata: Steinernematinae), novy cizopasník housenek obalece jablecného, *Carpocapsa pomonnella* L. Bestniik Ceskoslovenske Zoologické Spolecnosti **19**: 44-51.

[54]Poinar, G. O., Jr. 1991. Nematode parasites. In: L. P. S. van der Geest and H. H. Evenhius, (Eds.). Tortricid pests, their biology, natural enemies and control. Amsterdam: Elsevier Science Publishers B. V. p. 273-281.

[55]Kaya, H. K., J. L. Joos, L. A. Falcon, and A. Berlowitz. 1984. Suppression of the codling moth (Lepidoptera: Olethreutidae) with the entomogenous nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae). J. Econ. Entomol. **77**: 1240-1244.

[56]Sledzevskaya, E. R. 1987. Study of the factors determining the activity of the nematode *Neoaplectana carpocapsae* and its efficacy against orchard insect pests. In: M. D. Sonin, (Ed.). Helminths of Insects. New Delhi: Amerind Pub. Co. p. 152-155.

[57]Nachtigall, G. and E. Dickler. 1992. Experiences with field applications of entomoparasitic nematodes for biological control of cryptic living insects in orchards. Act. Phytopathol. Entomol. Hung. **27**: 485-490.

[58]Lacey, L. A. and T. R. Unruh. 1998. Entomopathogenic nematodes for control of codling moth: effect of nematode species, dosage, temperature and humidity under laboratory and simulated field conditions. Biol. Contr. **13**: 190-197.

[59]Unruh, T. R. and L. A. Lacey. 2001. Control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) with *Steinernema carpocapsae*: effects of supplemental wetting and pupation site on infection rate. Biol. Contr. **20**: 48-56.

[60]Lacey, L. A., A. Knight and J. Huber. 2000. Microbial control of lepidopterous pests of apple orchards. In: L. A. Lacey and H. K. Kaya, (Eds.). Field Manual of Techniques for the Evaluation of Entomopathogens. Dordrecht: Kluwer Academic Publishers. p. 557-576.

[61]Lacey, L. A. and R. L. Chauvin. 1999. Entomopathogenic nematodes for control of codling moth in fruit bins. J. Econ. Entomol. **92**: 104-109.

[62]Cossentine, J. E., L. B. Jensen and L. Moyls. 2002. Fruit bins washed with *Steinernema carpocapsae* (Rhabditida: Steinernematidae) to control *Cydia pomonella* (Lepidoptera: Tortricidae). Biocontr. Sci. Technol. **12**: 251-258.

[63]Walker, K. R. and S. C. Welter. 2001. Potential for outbreaks of leafrollers (Lepidoptera: Tortricidae) in California apple orchards using mating disruption for codling moth suppression. J. Econ. Entomol. **94**: 373-380.

[64]de Reede, R. H., P. Gruys and F. Vaal. 1985. Leafrollers in apple integrated pest management under regimes based on *Bacillus thuringiensis*, diflubenzuron or epofenonane. Entomol. Exper. Applic. **37**: 263-274.

[65]Knight, A. L. 1994. Insect pest and natural enemy populations in paired organic and conventional apple orchards in the Yakima Valley, Washington. J. Entomol. Soc. Brit. Columbia **91**: 27-31.

[66]Lacey, L. A. and J. P. Siegel. 2000. Safety and ecotoxicology of entomopathogenic bacteria. In: J.-F. Charles, A. Delécluse, and C. Nielsen-LeRoux, (Eds.). Entomopathogenic Bacteria: From laboratory to field application. Dordrecht: Kluwer Academic Publishers. p. 253-273.

[67]Li, S. Y., S. M. Fitzpatrick, and M. B. Isman. 1995a. Effect of temperature on toxicity of *Bacillus thuringiensis* to the obliquebanded leafroller (Lepidoptera: Tortricidae). Can. Entomol. **127**: 271-273.

[68]Li, S. Y., S. M. Fitzpatrick, and M. B. Isman. 1995b. Susceptibility of different instars of the obliquebanded leafroller (Lepidoptera: Tortricidae) to *Bacillus thuringiensis* var. *kurstaki*. J. Econ. Entomol. **88**: 610-614.

[69]Li, S.Y. and S. M. Fitzpatrick. 1996. The effects of application rate and spray volume on efficacy of two formulations of *Bacillus thuringiensis* Berliner var. *kurstaki* against *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) on raspberries. Can. Entomol. **128**: 605-612.

[70]Knight, A. 1997. Optimizing the use of Bts for leafroller control. Good Fruit Grower **48**: 47-49.

[71]Frye, R. D., C. G. Scholl, E. W. Scholz, and B. R. Funke. 1973. Effect of weather on a microbial insecticide. J Invertebr. Pathol. **22**: 50-54.

[72]Shiga, M., H. Yamada, H. Oho, H. Nakazawa and Y. Ito. 1973. A granulosis virus, possible biological agent for control of *Adoxophyes orana* (Lepidoptera: Tortricidae) in apple orchards. Semipersistent effect of artificial dissemination into an apple orchard. J. Invertebr. Pathol**.** **21**: 149-157.

[73]Schmidt, A., O. Cazelles and G. Benz. 1983. A granulosis virus of the fruit tortrix, *Adoxophyes orana* F. v. R. (Lep., Tortricidae). Mitt. Scweiz Entomol. Ges. **56**: 225-235.

[74]Blommers, L., F. Vaal, J. Freriks and H. Helsen. 1987. Three years of specific control of summer fruit tortrix and codling moth on apple in the Netherlands. J. Appl. Entomol. **104**: 353-371.

[75]Sekita, N., K. Kawashima, H. Aizu, S. Shirasaki and M. Yamada. 1984. A short term control of *Adoxophyes orana fasciata Walsingham* (Lepidoptera: Tortricidae) by a granulosis virus in apple orchards. Appl. Entomol. Zool. **19**: 498-508.

[76]Luisier, N. and G. Benz. 1994. Improving the efficiency of the granulosis virus of *Adoxophyes orana* F. v. R. for microbiological control. Bull. IOBC SROP 17: **(3**) 274.

[77]Pari, P., G. Carli, F. Molinari and P. Cravedi. 1993. Evaluations de l'efficacité de *Bacillus thuringiensis* Berliner contre *Cydia molesta* (Busck). Bull. OILB/SROP, 16 (**4**) 38-41.

[78]Lacey, L. A. and E. Riga. Unpublished data

[79]Begley, J. W. 1990. Efficacy against insects in habitats other than soil. In: Entomopathogenic Nematodes in Biological Control, R. Gaugler and H. K. Kaya (Eds.). CRC Press, Boca Raton, pp. 215-232.

[80]Cossentine, J. E., F. L. Banham and L. B. Jensen. 1990. Efficacy of the nematode, *Heterorhabditis heliothiodis* (Rhaditida: Heterorhabditidae) against the peachtree borer, *Synanthedon exitiosa* (Lepidoptera: Sesiidae) in peach trees. J.Entomol.Soc.Brit.Columbia., **87**: 82-84.

[81]Gill, S., J. A. Davidson and M. J. Raupp. 1992. Control of peachtree borer

using entomopathogenic nematodes. J. Arborculture **18:**184-187.

[82]Lu, Q. G., N. X. Zhang, Y. Z. Jiang, S. F. Zhang. 1993. Laboratory and field tests on the efficacy of a Chinese produced *Bacillus thuringiensis* wettable powder against peach fruit borer, *Carposina niponensis* (Lep.:Carposinidae). Chinese J. Biol. Contr. **9**:156-159.

[83]Li, Y., Z. Liu, G. L. Zhang, F. Chai and J. Zong. 1993. The application of *Steinernema feltiae agriotes* against the peach fruit borer. Acta Phytophylacica Sinica **20**: 337-342.

[84]Jie,L., L. Nanxin, X. Ruchuang, Z Yiqing and Y. Chunshou. 1994. Applications of a *Steinernema* nematode against peach fruit-borer in apple orchards. Act. Phytophylacica. Sinica**, 21**: 221-224.

[85]Kain, D. F. and A. M. Agnello. 1999. Pest status of American plum borer (Lepidoptera: Pyralidae) and fruit tree borer control with synthetic insecticides and entomopathogenic nematodes in New York State. J. Econ. Entomol. **92**: 193-200.

[86]Barnett, W. W., J. P. Edstrom, R. L. Coviello and F. P. Zalom. 1993. Insect pathogen "Bt" controls peach twig borer on fruits and almonds. California Agric. **47**: 4-6.

[87]Tedders, W. L., D. J. Weaver, E. J. Wehunt and C. R. Gentry. 1982. Bioassay of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Neoaplectana carpocapsae* against larvae of the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae). Environ. Entomol. **11**: 901-904.

[88]Olthof, T. H. and E. C. Hagley. 1993. Laboratory studies of the efficacy of steinernematid nematodes against the plum curculio (Coleoptera: Curculionidae). J. Econ. Entomol. **86**: 1078-1082.

[89]Shapiro-Ilan, D. I., R. F. Mizell, III and J. F. Campbell. 2002. Susceptibility of the plum curculio, *Conotrachelus nenuphar,* to entomopathogenic nematodes. J. Nematol**. 34**: 246-249.

[90]Shapiro-Ilan, D. I. et al. unpublished data.

[91]Patterson Stark, J. E. and L. A. Lacey. 1999. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to steinernematid and heterorhabditid nematodes in laboratory studies. J. Invertebr. Pathol. **74**: 206-208.

[92]Yee, W. L. and L. A. Lacey. 2003. Stage-specific mortality of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to three species of *Steinernema* Nematodes. Biol. Contr**. 27**: 349-356.

[93]Harris, M. K. 1985. Pecan phenology and pecan weevil biology and management. In: Pecan Weevil: Research Perspective, W. W. Neel (ed). Quail Ridge Press, Brandon, MS, pp. 51-58.

[94]Shapiro-Ilan, D. I. 2003. Microbial control of the pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae). Southwestern Entomol. In press.

[95]Sikorowski, P. P. 1985. Pecan weevil pathology. In: Pecan Weevil: Research Perspective, W. W. Neel (Ed). Quail Ridge Press, Brandon, pp. 87-101.

[96]Adams, J. R., T. B. Clark, G. J. Tompkins, W. W. Neel, R. F. Schroder and P. W. Schaefer. 1997. Histopathological investigations on *Rickettsiella*-like sp. and nonoccluded viruses infecting the pecan weevil *Curculio caryae,* the squash beetle *Epilachna borealis,* and the Mexican bean beetle *Epilachna varivestis*. J. Invertebr. Pathol. **69**: 119-124.

[97]Fuxa, J. R., R. Ayyappath and R. A. Goyer. 1998. Pathogens and Microbial Control of North American Forest Insect Pests. Forest Health Technology Enterprise Team, USDA Forest Service, Morgantown, WV.

[98]Nyczepir, A. P., J. A. Payne and J. J. Hamm. 1992. *Heterorhabditis bacteriophora*: a new parasite of pecan weevil *Curculio caryae*. J. Invertebr. Pathol. **60**: 104-106.

[99]Smith, M. T., R. Georgis, A. P. Nyczepir and R. W. Miller. 1993. Biological control of the pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae), with entomopathogenic nematodes. J. Nematol. **25:** 78-82.

[100]Shapiro-Ilan, D. I. 2001. Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. **94**: 7-13.

[101]Shapiro-Ilan, D. I. 2001. Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). J. Entomol. Sci. **36**: 325-328.

[102]Tedders, W. L, D. J. Weaver and E. J. Wehunt. 1973. Pecan weevil: suppression of larvae with the fungi *Metarhizium anisopliae* and *Beauveria bassiana* and the nematode *Neoaplectana dutkyi*. J. Econ. Entomol. **66**: 723-725.

[103]Gottwald, T. R. and W. L. Tedders. 1983. Suppression of pecan weevil (Coleoptera: Curculionidae) populations with entomopathogenic fungi. Environ. Entomol. **12**: 471-474.

[104]Harrison, R. D., W. A. Gardner and D. J. Kinard. 1993. Relative susceptibility of pecan weevil fourth instars and adults to selected isolates of *Beauveria bassiana*. Biol. Contr. **3**: 34-38.

[105]Harrison, R. D. and W. A. Gardner. 1991. Occurrence of the entomogenous fungus *Beauveria bassiana* in pecan orchard soils in Georgia. J. Entomol. Sci. **26**: 360-366.

[106] Shapiro-Ilan, D. I., W. Gardner, J. R. Fuxa, B. W. Wood, K. Nguyen, B. Adams, R. A. Humber and M. J. Hall. 2003. Survey of entomopathogenic nematodes and fungi endemic to pecan orchards of the southeastern US and their virulence to the pecan weevil (Coleoptera: Curculionidae). Environ. Entomol. **32:**187-195.

[107]Gottwald, T. R. and W. L. Tedders. 1984. Colonization, transmission, and longevity of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes) on pecan weevil larvae (Coleoptera: Curculionidae) in soil. Environ. Entomol. **13**: 557-560.

[108]Tedders, W. L. 1981. In vitro inhibition of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* by six fungicides used in pecan culture. Environ. Entomol. **10**: 346-349.

[109]Harrison, R. D. and W. A. Gardner. 1992. Fungistasis of *Beauveria bassiana* by selected herbicides in soil. J. Entomol. Sci. **27**:233-238.

[110]Shapiro-Ilan, D. I., C. C. Reilly, M. W. Hotchkiss and B. W. Wood. 2002. The potential for enhanced fungicide resistance in Beauveria bassiana through strain discovery and artificial selection. J. Invertebr. Pathol. **81**: 86-93.

[111]Lindegren, J. E., F. Agudelo-Silva, K. A. Valero and C. E. Curtis. 1987. Comparative small-scale field application of *Steinernema feltiae* for navel orangeworm control. J. Nematol. **19**: 503-504.

[112]Vail, P. V., D. F. Hoffman, D. A. Streett, J. S. Manning and J. S. Tebbets. 1993. Infectivity of a nuclear polyhedrosis virus isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae) against production and postharvest pests and homologous lines. Environ. Entomol. **22**: 1140-1145.

[113]Cardenas, F. A., P. V. Vail, D. F. Hoffman, J. S. Tebbetts and F. E. Schreiber. 1997. Infectivity of celery looper (Lepidoptera: Noctuidae) multiple nucleopcapsid polyhedrosis virus to navel orangeworm (Lepidoptera: Pyralidae). Environ. Entomol. **26**: 131-134.

[114]Agudelo-Silva, F., F. G. Zalom, A. Hom and L. Hendricks. 1995. Dormant season application of Steinernema carpocapsae (Rhabditida: Steinernematidae) and Heterorhabditis sp. (Rhabditida: Heterorhabditidae) on almond for control of overwintering Amyelois transitella and Anarsia lineatella (Lepidoptera: Gelechidiidae). Fla. Entomol. **78:** 516-523.

[115]Siegel, J., L. A. Lacey, R. Fritts, Jr. and B. Higbee. 2004. Field evaluation of entomopathogenic nematodes against the navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in pistachio and almond nuts. Biol. Contr. *In press*.

[116]Hendricks, L. C. 1994. Almond growing and sustainable agriculture in California. Acta Horticulture **373**: 265-267.

[117]Connell, J. H., F. G. Zalom and W. J. Bentley. 1998. Navel organgeworm control in almonds with *Bacillus thuringiensis*. Acta Horticulture **470**: 547-552.

[118]Epstein, L., S. Bassein, F. G. Zalom and L. R. Wilhoit. 2001. Changes in pest management practice in almond orchards during the rainy season in California, USA. Agric. Ecosyst. Environ. **83**: 111-120.

[119]Tedders, W. L. and H. C. Ellis. 1977. Aerial application of *Bacillus thuringiensis* var. kurstaki (HD-1) to shade and ornamental pecan trees against *Hyphantria cunea* and *Datana integerima*. J. Ga. Entomol. Soc. **12**: 248-250.

[120]Lacey, L. A., R. Frutos, H. K. Kaya and P. Vail. 2001. Insect pathogens as biological control agents: Do they have a future? Biol. Contr. **21**: 230-248.