

FORUM

Microbial Control of Arthropod Pests of Tropical Tree Fruits

CLAUDIA DOLINSKI¹ AND LAWRENCE A. LACEY²¹Univ. Estadual do Norte Fluminense/CCTA/LEF, Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brazil²Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Rd., Wapato, WA, 98951, USA

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Controle Microbiano de Artrópodes-Praga em Fruteiras Tropicais

RESUMO - Muitos insetos e ácaros atacam fruteiras nos trópicos. O método tradicional para controlar a maioria dessas pragas é a aplicação de inseticidas químicos. A crescente preocupação sobre os efeitos negativos desses produtos vem encorajando o desenvolvimento de alternativas. Agentes do controle biológico aplicados de forma inundativa ou inoculativa têm sido pesquisados como método de controle alternativo para uma variedade de pragas em grande número de culturas, incluindo fruteiras tropicais. A maioria das pesquisas e aplicações em fruteiras tropicais tem sido feita em citros, banana, côco e manga. Especificamente, há vários casos de sucesso em citros para diferentes ácaros e insetos. Em banana, o controle do moleque-da-bananeira, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) com nematóides e fungus também é considerado de sucesso. *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) é uma das principais pragas em côco e um dos mais importantes exemplos de controle biológico clássico por vírus não-ocluso. As pragas-chave em manga que têm sido controladas com diferentes agentes de controle microbiano são as moscas-das-frutas (Diptera: Tephritidae), com nematóides e fungos, o gorgulho-da-manga, *Sternochetus mangiferae* (Fabricius) (Coleoptera: Curculionidae), com fungos, e vários hemípteros com fungo. O controle microbiano de pragas tem sido bem sucedido também em goiaba, mamão e abacaxi. O desafio que se vislumbra para a maior aplicabilidade dessas tecnologias é o desenvolvimento de combinações compatíveis entre entomopatógenos, predadores e parasitóides juntamente com outras técnicas de controle.

PALAVRAS-CHAVE: Bactéria, vírus, fungo, nematóide

ABSTRACT - A multitude of insects and mites attack fruit crops throughout the tropics. The traditional method for controlling most of these pests is the application of chemical pesticides. Growing concern on the negative environmental effects has encouraged the development of alternatives. Inundatively and inoculatively applied microbial control agents (virus, bacteria, fungi, and entomopathogenic nematodes) have been developed as alternative control methods of a wide variety of arthropods including tropical fruit pests. The majority of the research and applications in tropical fruit agroecosystems has been conducted in citrus, banana, coconut, and mango. Successful microbial control initiatives of citrus pests and mites have been reported. Microbial control of arthropod pests of banana includes banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) (with EPNs and fungi) among others *Oryctes rhinoceros* (L.) is one of the most important pests of coconut and one of the most successful uses of non-occluded virus for classical biological control. Key pests of mango that have been controlled with microbial control agents include fruit flies (Diptera: Tephritidae) (with EPNs and fungi), and other pests. Also successful is the microbial control of arthropod pests of guava, papaya and pineapple. The challenge towards a broader application of entomopathogens is the development of successful combinations of entomopathogens, predators, and parasitoids along with other interventions to produce effective and sustainable pest management.

KEY WORDS: Bacteria, virus, fungus, nematode

A multitude of insect and mite species are pests of tree fruit world wide. The traditional method for controlling most of these pests is the application of chemical pesticides, which has generated complex problems including: insecticide resistance; outbreaks of secondary pests normally held in

check by natural enemies; safety risks for humans and wild and domestic animals; contamination of ground water and riparian habitats; and decrease in biodiversity. Growing concern over the environmental effects of pesticides has encouraged the development of alternatives to broad-

spectrum pesticides. Natural pathogens of arthropods often play an important role in the regulation of insect and mite populations in agroecosystems (Ignoffo 1985, Steinkraus 2007). However, their main impact on pests may occur after economic thresholds are surpassed. Inundatively or inoculatively applied microbial control agents (viruses, bacteria, fungi, and nematodes) have been developed as alternative control methods for a wide variety of arthropod pests (Alves 1998a, Lacey *et al.* 2001, Kaya and Lacey 2007), which include pests of tropical tree fruit. In this review we will explore the use of entomopathogens and nematodes for control of insects and mites of tropical fruit.

Candidate Entomopathogens

Viruses. Many viruses have been identified from hundreds of arthropod species. For most of them, those with particles occluded in protein bodies (OBs) [Baculoviridae, Entomopoxviridae, Reoviridae (Cypoviruses)] have been used successfully in microbial control programs. The Baculoviridae (nucleopolyhedroviruses and granuloviruses) are the most studied and used as microbial control agents (Hunter-Fujita *et al.* 1998, Moscardi 1999, Cory & Evans 2007). They are normally transmitted *per os* and gain access to host tissues via the midgut where the OBs that surround the virus rods are dissolved. The currently unclassified virus of *Oryctes rhinoceros* L. is the most successfully used non-occluded virus. Viruses comprise some of the most host-specific entomopathogens but their main drawbacks are the requirement for *in vivo* production and their sensitivity to ultra-violet degradation.

Bacteria. Although several species of bacteria have been used as microbial control agents of a variety of insects, only *Bacillus thuringiensis* Berliner (Bt) has been used for practical pest control. It is the most widely used inundatively applied microbial control agent (Lacey *et al.* 2001). Several isolates of Bt are commercially produced with activity against Lepidoptera, Coleoptera, and Diptera. The safety of Bt for applicators and vertebrate and invertebrate non-target organisms is well documented (Lacey & Siegel 2000). Its insecticidal activity is associated with delta-endotoxins located in parasporal inclusion bodies (or parasporal crystals) that are produced at sporulation and must be ingested by the target organism in order to be active. A limiting factor of Bt is its fairly narrow host range.

Fungi. The majority of fungi that naturally regulate insect and mite populations is in the order Hypocreales (Ascomycetes, including the vast majority of conidial entomopathogens in more than two dozen genera formerly classified among either the Hyphomycetes or Fungi Imperfecti) and Entomophthorales. The latter are difficult or impossible to mass-produce and, hence, they have not been commercially produced or applied inundatively on a large scale. On the other hand, several asexually reproducing species in the Hypocreales are amenable to mass production and commercialization. The most studied for control of insects and mites belong to the genera *Beauveria*, *Metarhizium*,

Paecilomyces, *Aschersonia*, *Hirsutella*, and *Lecanicillium* (formerly *Verticillium*) (Alves 1998b, Inglis *et al.* 2001, Goettel *et al.* 2005). Because the normal route of invasion is through the cuticle, fungi are especially suitable microbial control agents for sucking insects (Hemiptera).

Phylogenetic studies now confirm that the Microsporidia are highly derived organisms correctly placed among the lower fungi rather than extremely ancient and simple organisms classified with the Protozoa (Hirt *et al.* 1999). Although many microsporidians are common pathogens of arthropods, few have been included in microbial control programs because certain fundamental characteristics (complex life cycles, obligate parasitism, and chronic rather than acute effects) inhibit their use (Solter & Becnel 2007).

Entomopathogenic nematodes (EPNs). Nematodes in the families Steinernematidae and Heterorhabditidae are effective control agents of dozens of insect species in soil and cryptic habitats (Kaya & Gaugler 1993, Georgis *et al.* 2006, Shapiro-Ilan *et al.* 2005). These nematodes are associated with symbiotic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.), which are housed in the intestine of the infective juvenile (IJ) nematode (also referred to as the Dauer stage). The IJ, the only free-living stage, occurs in the soil and searches for an insect host. Upon finding a host, it enters through the mouth, anus or spiracles and penetrates into the body cavity. In the case of heterorhabditids, IJ penetration can occur directly through soft cuticles. In the body cavity, the IJ releases mutualistic bacterial cells, which multiply rapidly and kill its insect host, usually within 48h. In addition, the bacterial cells digest host tissues and produce antibiotics that protect the host cadaver from saprophytes and scavengers, and allow the nematodes to develop and reproduce.

The nematodes feed on the mutualistic bacterial cells and on degraded host tissues. Depending on host size, there may be one to three nematode generations in the host cadaver. When host nutrients are depleted, the pre-IJs sequester the mutualistic bacterial cells in their intestines. The resulting IJs leave the host and search for new hosts. In the absence of a host, IJs can persist for months in moist soil. However, the IJs have their own natural enemies (*i.e.* nematophagous fungi, predatory mites and other soil predators) and must also contend with abiotic factors such as temperature extremes, low soil moisture, and ultraviolet radiation that affect their survival. Several EPN species are commercially produced and available for large-scale application. For small-scale experimental testing, EPNs can be produced *in vivo* and on artificial media (Kaya & Stock 1997).

Research and Application of Entomopathogens for Control of Arthropod Pests of Tropical Fruits

The literature on microbial control agents of tropical fruit pests has, for the most part, concentrated on key pests of a few major crops (*e.g.* citrus and banana). In this review we bring information on the use of microbial control of arthropod pests of citrus, banana, coconut, mango, guava, papaya and pineapple.

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 climates to temperate habitats around the world. Consequently a huge range of arthropod pests is reported from citrus varieties (Smith & Peña 2002). Successful microbial control of several pests has been reported using fungi, bacteria, viruses and EPNs.

Citrus rust mite (CRM), *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae). This is a major pest of citrus in several countries, including Brazil and the USA. Studies on the use of fungi for its control are limited number. Alves *et al.* (2005) assessed the pathogenicity of five concentrations of *Beauveria bassiana* (Bals.) Vuill. ranging from 10^6 to 10^8 conidia/ml under laboratory conditions (25°C, 12h photophase, 98% RH). Mortality was time and dosage dependent and ranged from 24% to 91% with an LC_{50} of 4.23×10^6 conidia/ml five days after treatment. LT_{50} at the highest concentration was 2.74 days. *Hirsutella thompsonii* Fisher (Entomophthorales: Moliniaceae) is infectious for CRM and several other mite pests of greenhouse crops, coconut and turf (Samson *et al.* 1980, McCoy 1996). In the early 1980's it was mass-produced and formulated by Abbott Laboratories (Chicago, IL, USA) and registered for CRM control in the USA (McCoy & Couch 1982). Under optimal conditions, *H. thompsonii* can control CRM within one to two weeks (McCoy *et al.* 2007). Field applications of mycelia led to production of conidia within 48h and provided suppression of CRM for up to 14 weeks (McCoy *et al.* 1971). McCoy *et al.* (2007) provided protocols for the field evaluation of *H. thompsonii* and other fungi intended for CRM control. Unfortunately, commercial development of the fungus was discontinued by Abbott Laboratories.

Citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae). This mite can be a serious pest of citrus in certain locations. In southern China, Shi & Feng (2006) evaluated the efficacy of four rates of *B. bassiana* (ranging from 1.2×10^{12} to 3.0×10^{13} conidia/ha) and a combination of the fungus with a low rate of pyridaben for control of *P. citri* in orange groves. All of the *B. bassiana* application rates produced significant mortality in *P. citri* and the combinations with pyridaben led to better control. Two applications of $\geq 1.5 \times 10^{13}$ conidia/ha plus low rate pyridaben with a 15 day spray interval resulted in good control of *P. citri* for 35 days with mite density declines of 74-91%. Where *P. citri* is a pest in arid regions, it is not recommendable to use fungi against this pest. McCoy *et al.* (2007) summarized research and results of applications of a non-occluded virus found in *P. citri*. The virus has apparently been responsible for decimating epizootics in *P. citri* populations in Arizona and California (Reed 1981). Shaw *et al.* (1968) reported control of the mite after application of triturated mites infected with the virus. A major limitation of this virus is the need for its mass production in *P. citri*.

Broad mite (BM) or white mite, *Polyphagotarsonemus latus*

(Banks) (Acari: Tarsonemidae). This is a cosmopolitan pest of a variety of plants including citrus, papaya, and mango. It is abundant during warm and humid condition and thus an ideal candidate for control with fungi. However, very little research on microbial control has been conducted on this pest. Cabrera *et al.* (1987) reported natural infection of BM with *H. thompsonii*. Peña *et al.* (1996) assessed the infectivity of *B. bassiana*, *H. thompsonii*, and *Paecilomyces fumosoroseus* (Wize) Brown and Smith conidia in laboratory bioassays on bean leaves. The LC_{50} values for the fungi were 1.16×10^6 , 2.39×10^3 , and 1.29×10^5 conidia/ml, respectively. Mortality due to *B. bassiana* was most rapid in mite densities between 65 and 125 mites/leaf. The efficacy of *B. bassiana* and *P. fumosoroseus* and other agents were also evaluated against BM in a greenhouse test on potted bean plants. Treatments with *B. bassiana* were the most efficacious and persistent, and resulted in 88% mortality.

False spider mite (FSM), *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae). FSM, also known as the red and black flat mite, is a polyphagous widely distributed tropical-subtropical species that has been reported from several hundred plant hosts including citrus, banana, macadamia, orchid, papaya, passion fruit, coffee and tea. FSM is an important citrus pest as it is a vector of the citrus leprosis virus (Childers *et al.* 2003). Rossi-Zalaf & Alves (2006) assessed the activity of 52 isolates of fungi including *B. bassiana*, *Metarhizium anisopliae* (Metsch.) Sorokin, *Paecilomyces* spp., *H. thompsonii*, *Lecanicillium* spp. and others. The most active isolates were all *H. thompsonii*, causing 90-100% mortality six days after treatment. All other species of fungi produced less than 30% mortality six days after treatment. The authors observed conidiogenesis of *H. thompsonii* with development of mycelium and conidiophores emerging from the posterior and anterior parts of mites 120h after spraying with conidia.

Whiteflies and blackflies (Hemiptera: Aleyrodidae). About 30 species of whiteflies and blackflies have been reported attacking citrus worldwide (Smith and Peña 2002), six of which are considered major pests. Franssen (1990), Lacey *et al.* (1996), and Faria and Wraight (2001) summarized the literature on fungi reported from whiteflies.

Most of research on microbial control of whiteflies in citrus has focused on *Aschersonia* spp., which has produced spectacular epizootics in conditions of high humidity and rainfall (Fig. 1a). In Florida, USA, Fawcett (1944) reported epizootics in *Dialeurodes citri* (Ashmead) and *D. citrifolii* (Morgan) in citrus groves during the summer when high humidity promoted conidial sporulation and host infection, and frequent rains enabled effective dispersal of the conidia. Meyerdirk *et al.* (1980) observed *A. aleyrodis* Ashby infecting *D. citri* in Texas. Elizondo & Quezada (1990) published on the distribution of the citrus backfly, *Aleurocanthus woglumi* Ashby, and its natural enemies in four localities in Costa Rica. In addition to parasitoids and predators, mortality due to *A. aleyrodis* was significant. The same agent was unable to control *A. woglumi* following its introduction into and spread through El Salvador (Quezada 1974). Outbreaks of disease

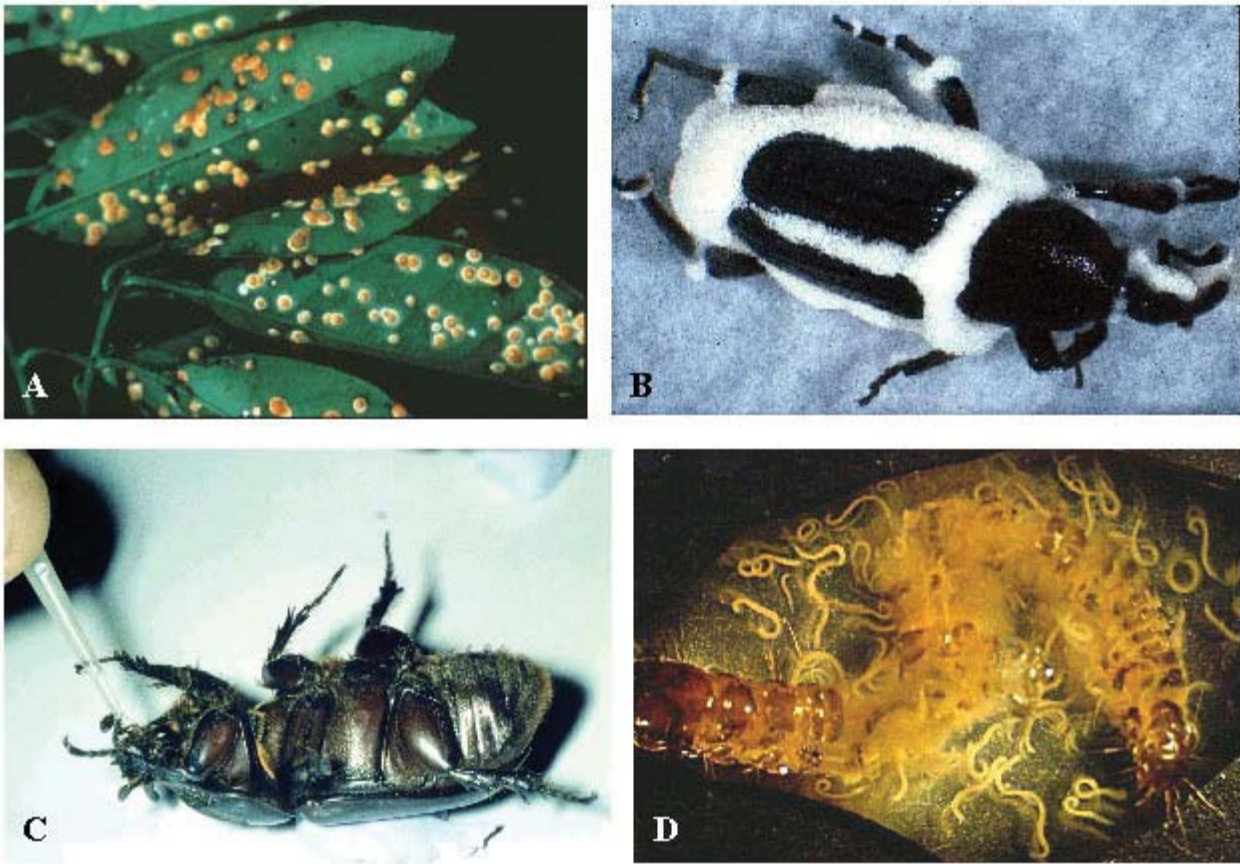


Fig. 1. A) Citrus whiteflies infected with *Aschersonia aleyrodis*. Photo courtesy of G. Xiong; B) Adult banana weevil infected with *Beauveria bassiana*. Photo courtesy of R. Duncan and J. Peña; C) Inoculation of palm rhinoceros beetle with *Oryctes virus*. Photo by C. Prior; D) Dissected banana moth larva showing various life stage of *Steinernema carpocapsae*. Photomicrograph courtesy of R. Duncan and J. Peña.

caused by *Aschersonia* spp. among populations of *Bemisia giffardi* (Kotinsky) on citrus in Taiwan were observed (Yen & Tsai 1969).

In Brazil, the occurrence of *Aschersonia* spp. on whiteflies is very common in all areas where citrus is grown and coincides with the periods of greatest rainfall (Alves 1998b). In addition to natural occurrence, *Aschersonia* spp. has been successfully applied against whiteflies in citrus groves in the Georgia, China and Japan (Ponomarenko *et al.* 1975, Gao *et al.* 1985). In field tests in Japan *Aschersonia* sp. was pathogenic to nymphs, pupae and eggs of *D. citri*, and the mortality increased with the concentration of conidia (Uchida 1970). Ponomarenko *et al.* (1975) introduced several isolates of *Aschersonia* from six countries into orange groves near Adzharia, Georgia, being *A. placenta* Berk & Br. from Vietnam and China the most effective leading to up to 90% parasitism in favorable weather.

Scales (Hemiptera: Coccidae and Diaspididae). These are regarded as the most abundant and injurious citrus pests (Smith & Peña 2002). El-Choubassi *et al.* (2001) observed up to 49% infection of the diaspidid *Parlatoria ziziphi* (Lucas) by *A. aleyrodis* and *A. goldiana* Sacc. & Ellis in Cuba. Gravena

et al. (1988) noted that the main control agent of a diaspidid, *Selenaspidus articulatus* (Morgan), in a grove near São Paulo, Brazil, was *A. aleyrodis*. Yen & Tsai (1969) observed fungal infections caused by *Podonectria coccicola* (Ellis & Everhart) Petch, *Pseudomicrocera henningsii* (Koord.) Petch, and *Sphaerostilbe aurantiicola* (B. & Br.) Petch in the coccids *Chrysomphalus aonidum* (L.), *P. ziziphus* and *Lepidosaphes beckii* (Newm.) in citrus groves in Taiwan. In South Africa, Moore (2002) reported several fungi attacking four scale pests of citrus. No publications on the applied use of fungi for control of scale in citrus were found in the literature.

Aphids (Hemiptera: Aphididae). Aphids, particularly those in the genus *Toxoptera*, are important pests of citrus, especially due to their roles as vectors of diseases such as citrus tristeza. Fungi are important natural enemies of aphids under warm and humid conditions (Latgé & Papierok 1988, Humber 1997) but studies on their use in citrus groves have been limited. Poprawski *et al.* (1999) demonstrated good potential for control of the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), with *B. bassiana*. Application of the fungus in field trials at 2.5×10^{13} and 5.0×10^{13} conidia/ha resulted in 79.8% and 94.4% control, respectively.

Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). This weevil is native to the Caribbean and has become a major pest in Florida citrus since its introduction was first reported in 1964 (Woodruff 1964). Annual losses and cost of control in Florida citrus are thought to exceed \$72 million (Peña *et al.* 2000).

Notching along the margins of young leaves is a typical sign of feeding by the adult. Eggs are laid on older leaves and, after hatching, larvae drop to the ground, enter the soil, and feed on roots for most of the year. Injury caused by the weevil appears to be cumulative; root damage impedes the plant to take up water and nutrients, and can result in tree mortality (Syvertsen & McCoy 1985). In addition, this injury provides an avenue for fungal root rot infections by *Phytophthora* spp. (Graham *et al.* 2003). A single larva can kill young trees, whereas several larvae can cause decline of older, established trees.

Since larvae are below ground, it is difficult to detect them before decline of above ground portions of the host plant is observed (Simpson *et al.* 1996). EPNs (Steinernematidae and Heterorhabditidae) are effective in soil habitats. Because *D. abbreviatus* larvae are vulnerable while entering the soil, numerous studies have been conducted on the use of EPNs for their control. In Florida, EPNs have been marketed for weevil control for over 15 years. Currently, two commercially available species, *Steinernema riobrave* Cabanillas, Poinar & Raulston and *Heterorhabditis indica* Poinar, Karunakar & David are used for control of the weevil. These nematodes appear to be most effective at high temperatures ($27 \pm 2^\circ\text{C}$) in coarse sandy soils. Larval mortality of over 90% has been reported for field trials with *S. riobrave* when applied at 1.2×10^{10} infective juveniles (IJs)/ha (McCoy *et al.* 2002, 2007). Other EPN species, rates and percentage mortality were summarized by Shapiro-Ilan *et al.* (2002). The use of irrigation systems for application of EPNs has been effective in delivering IJs into the zone below trees where larvae enter the soil. Indigenous entomopathogenic fungi infect adults and larvae of *D. abbreviatus* and other weevils in the soil (McCoy *et al.* 2007).

Research conducted by Quintela & McCoy (1998) demonstrated that a commercial oil formulation of *B. bassiana* (Mycotrol) or a combination of the fungus and a sublethal concentration of imidacloprid (a chloronicotinyl insecticide) provided effective control of neonate larvae and teneral adults when applied as a soil barrier. McCoy *et al.* (2007) pointed out that the efficacy of *B. bassiana* as a weevil control agent was limited by its poor persistence in soil. Weathersbee *et al.* (2002) demonstrated larvicidal and sublethal activity of elevated concentrations *B. thuringiensis* subsp. *tenebrionis* for *D. abbreviatus* in artificial diet and potted citrus tests.

Citrus root weevils, *Pachnaeus* spp. (Coleoptera: Curculionidae). *Pachnaeus litus* (Germar) and *P. opalus* (Oliver) are native to Florida and normally are considered minor pests, although they can damage young citrus plants (Tarrant & McCoy 1989). The adults feed on tender foliage of citrus and eggs are laid on mature leaves, often on the same trees on which the adults have been feeding. Neonate larvae drop to the ground and quickly burrow into the

soil where they feed on roots (Bullock *et al.* 1999). Field applications of 5×10^6 *H. bacteriophora* IJs/tree resulted in significant reduction of *P. opalus* adults (76%) as compared to controls (Downing *et al.* 1991). In another experiment, two applications of *S. riobrave* or *S. carpocapsae* at 2×10^6 IJs/tree provided an overall 64% and 53% reduction of *P. litus*, respectively (Bullock *et al.* 1999).

Fuller rose beetle (FRB), *Asynonychus godmani* Crotch (= *Pantomorus cervinus* (Boheman) (Coleoptera: Curculionidae). Adults feed on citrus foliage and cause leaf notching. Larvae develop for 6-10 months in the soil where they feed on the roots. The beetle does not usually cause economic damage but the presence of eggs on exported fruit requires fumigation. Morse & Lindegren (1996) reported the results of field application of rather high rates of *S. carpocapsae* IJs against late-instar larvae under Valencia orange trees. A single application of the Kapow or All strain of *S. carpocapsae*, each applied at 50, 150, and 500 IJs/cm², reduced the number of emerging adult FRB a combined 55% and 38%, respectively, the year following treatment and 79% and 82%, respectively, the 2nd year. Based on EPN recovery six months after application and continued reduction of FRB emergence in the second year, the authors concluded that the EPNs persisted and recycled in the environment.

Citrus fruit borer, *Ecdytolopha auratiana* (Lima) (Lepidoptera: Tortricidae). In the late 1980's, *E. auratiana* became a key pest of citrus in São Paulo, Brazil. Females lay eggs on the fruit surface where neonate larvae enter the fruit and feed for approximately 20 days, rendering them worthless for consumption and processing. When fully grown, larvae leave the fruits and pupate in the soil. Laboratory tests with EPNs against sixth-instar larvae in pots containing sandy soil showed that *H. indica* applied at 1.6 IJ/cm² resulted in 92% mortality (Leite *et al.* 2005).

False codling moth (FCM), *Cryptophlebia leucotreta* Mergé (Lepidoptera: Tortricidae). This is an important pest of citrus in Africa and outlying islands (Moore 2002, Smith & Peña 2002). A granulovirus of FCM has shown promise in field trials. Fritsch (1988) conducted a small-scale field trial of the virus formulated with skimmed milk powder and a wetting agent and applied at 10^8 and 10^9 granules/ml on FCM-infested citrus in Cape Verde, resulting in a 77% reduction of FCM population. Moore (2002) provided a synopsis of research conducted on this virus in South Africa and concluded that it plays a natural regulatory role in FCM populations. Application of the granulovirus at rates of 10^{14} to 10^{15} OBs/ha provided up to 60% reduction of FCM infestations in navel oranges. Subsequently, 17 field trials have been conducted on citrus in three different provinces in South Africa. Where spray coverage was thorough and FCM pressure was moderate, infestation was reduced by 70% following a virus treatment three weeks before harvest (Moore *et al.* 2005). The virus has been registered for use and is now commercially produced in South Africa.

Old world bollworm, *Helicoverpa armigera* (Hübner)

(Lepidoptera: Noctuidae). This insect attacks a wide variety of important crops in portions of Africa, Asia, Australia, and Europe. In South Africa and parts of Asia, it is a serious pest of citrus. *B. thuringiensis* subsp. *kurstaki* (Btk) has been applied for control of *H. armigera* in citrus, but growers have reported dissatisfaction with its efficacy (Moore *et al.* 2004). Field trials of the nucleopolyhedrovirus of *H. armigera* conducted by Moore *et al.* (2004) demonstrated that it was superior to Btk for suppression of the bollworm. Application of 7.26×10^5 and 1.15×10^6 OBs/ml resulted in 100% reduction in bollworms within 14 days of application. Damage to fruit was reduced by up to 75-84% and rejection for export was reduced by 62-96%.

Other lepidopteran pests. The citrus leafminer, *Phyllocnistis citrella* Stainton (Phyllocnistidae) is a significant pest of citrus worldwide. Field application of Bt against the leafminer in the Azores resulted in significant larval mortality 48h after treatment (Dias *et al.* 2005). Shapiro *et al.* (1998) observed that leaf damage and number of *P. citrella* larvae were significantly reduced after 21 days by treatments of Bt plus a wetting agent. Beattie *et al.* (1995) demonstrated only limited potential for control of *P. citrella* with *S. carpocapsae*. Narayanamma & Savithri (2003) and Gopalakrishnan & Gangavisalaksy (2005) reported successful control of the citrus butterfly, *Papilio demoleus* L. (Papilionidae), on sweet orange following applications of Bt at two locations in India. The citrus leafroller, *Cacoecia occidentalis* Walsingham (Tortricidae) is a minor pest of South African citrus. Smith *et al.* (1990) described a granulovirus with potential for its control.

Fruit flies (Diptera: Tephritidae). Some species, such as the Mediterranean fruit fly, *Ceratitidis capitata* Weidemann, are key or major pests of citrus in tropical production areas. Several authors have reported the results of research for control of *C. capitata* and other tephritid species using EPNs and fungi in laboratory studies and field trials in other agroecosystems (see mango section).

Banana

Several varieties of banana and plantain (*Musa* spp.) are grown throughout the tropics and into the sub-tropics. In addition to providing indigenous populations with rich sources of carbohydrate and other nutrients, banana and plantain are valuable export crops. A wide variety of insects and mites attack banana and include species that bore into the trunk, pseudostem, rhizomes, corm and roots, and species that attack flowers, fruits and foliage (Gold *et al.* 2002).

Banana weevil (BW), *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). This weevil is reported as the most important insect pest of banana and plantain (Gold *et al.* 2001). Oviposition takes place at the base of the plant and neonate larvae bore into the corm. Heavy infestations can result in crop failure in newly established stands and reduced yield and shortened life span of plants in established stands (Gold *et al.* 2001, 2002).

Strains of *B. bassiana* have shown good potential to control adult BW (reviewed by Gold *et al.* 2002, 2003) (Fig. 1b). Mortality in adult weevils of up to 60% was reported. Godonou *et al.* (2000) evaluated two formulations of *B. bassiana* (oil palm kernel cake-based formulation of conidia [OPKC] and conidial powder) applied to the planting holes and suckers of banana. Both formulations resulted in 75% mortality in artificially released weevils. Under natural infestation conditions, the OPKC performed better (42% mortality) than the conidial powder (6%). Bautista Filho *et al.* (1995, cited by Alves 1998b) reported up to 61% reduction of BW adults after treatment with *B. bassiana*.

A major constraint to the use of *B. bassiana* is the lack of an economic means of effectively applying the fungus. In Brazil, baits were made of sections of banana pseudostem treated in a suspension of *B. bassiana* conidia, mycelia and medium or with a paste that resulted in application of 5×10^9 conidia/bait. Fifty baits/ha were recommended being baits replaced 15 days, until less than 5 BW are captured/bait. Tinzaara *et al.* (2004) improved targeted delivery of the fungus in and near traps baited with an aggregation pheromone that is attractive to both sexes. *B. bassiana* is transmitted horizontally among BW individuals (Schoeman & Schoeman 1999, Godonou *et al.* 2000, Tinzaara *et al.* 2004), and this greatly improves the dissemination of the fungus within populations. Also, as most mycosed adult cadavers are found in the leaf sheath at the base of plants, the likelihood that ovipositing females and mating pairs to come into contact with recently produced conidia is increased (Tinzaara *et al.* 2004).

Gold *et al.* (2003) reviewed the research of several authors on the potential of endophytic fungi (*Fusarium* spp., *Acremonium* spp., *Geotrichum* spp.) for BW suppression. The most effective species in this role was *Fusarium oxysporum* Schltdl.

Laboratory, greenhouse and field assays demonstrated the activity of EPNs against BW. Rosales & Suarez (1998) evaluated exotic and native EPNs in Venezuela and found some native isolates of *Heterorhabditis* with good potential for BW control. Figueroa (1990) evaluated *S. carpocapsae*, *S. glaseri* (Steiner) and *S. feltiae* (Filipjev) against BW in greenhouse tests in Puerto Rico. The nematodes significantly reduced the number of tunnels made by larvae in plantain corms at 400, 4,000 and 40,000 IJs/four-month-old plant. At the two higher rates, 100% larval mortality was achieved.

Treverrow & Bedding (1993) assayed 32 strains and species of *Steinernema* and *Heterorhabditis* against larvae and adults of BW and reported the greatest activity of the BW strain of *S. carpocapsae* against adults. They also described a method for introducing *S. carpocapsae* IJs into banana corms that involves removing cones (50 mm diam. by 150 mm long) from residual corms with a desuckering gouge and adding 2.5×10^5 IJs/cavity. The cone is then reinserted to produce a protected cavity that is attractive to adult weevils. Treverrow *et al.* (1991) also reported significant mortality (43- 68%) of BW larvae in banana rhizomes after applying *S. carpocapsae* with a water thickener into cuts or holes made in residual rhizomes. Mortality of adult BW attracted to the application sites on treated rhizomes was also observed. In contrast, bi-

monthly treatments with *Heterorhabditis zealandica* Poinar and *S. carpocapsae* applied in a thickened aqueous solution into 200 mm deep incisions in the residual rhizomes of harvested plants from November to May failed to produce adequate control (Smith 1995). The author speculated that the treatments were not effective possibly because of early nematode mortality caused by free water in the spike holes and/or because of the need for more frequent applications. Kermarrec & Mauleon (1989) demonstrated synergy between the insecticide chlordecone and *S. carpocapsae* for control of BW. Other studies on EPNs for BW control were summarized by Gold *et al.* (2002).

West Indian sugarcane borer, *Metamasius hemipterus sericeus* (Olivier) (Coleoptera: Curculionidae). This pest is also known as the silky cane weevil and rotten stalk borer of sugar cane. It can be an important pest of banana in certain areas of the Americas (Giblin-Davis *et al.* 1994, Gold *et al.* 2002). As with BW, fungi and nematodes have potential to control this pest. Peña *et al.* (1995) reported infection of low density populations of the insect by *B. bassiana* in a three-year old banana field in Florida. They observed up to 70% infection when more than ten weevils were captured/trap. Unlike BW, *M. hemipterus sericeus* are strong fliers and could disperse fungi from sources of inoculum (*i.e.* through attractant traps) into neighboring populations. Giblin-Davis *et al.* (1996) evaluated *S. carpocapsae* in palm for control of this pest (see coconut section).

Banana moth, *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae). This insect is only a minor pest of banana, but a serious pest of certain types of palms. Research on EPNs for its control are reported by Peña *et al.* (1990) in the coconut section.

***Opsiphanes tamarindi* Felder (Lepidoptera: Brassolidae).** This is a major defoliator of plantains during the dry season in the region south of Lake Maracaibo, Venezuela. Broad-spectrum insecticides have been ineffective for its control. Briceno (1997) described an IPM system that combined cultural practices, application of Bt against early larval stages, and relying on natural enemies (parasitoids and predators) to control late larval and pupal stages. The seasonal application of Bt helped to eliminate first instars without affecting natural enemies.

Bagworm, *Oiketicus kirbyi* Guilding (Lepidoptera: Psychidae). This is a defoliating pest of banana in Costa Rica and Colombia (Gold *et al.* 2002). Stephens (1962) noted the occurrence of *B. bassiana* and a *Nosema* species in bagworm larvae in Costa Rican bananas.

Coconut

Coconut is significant and sometimes predominant sources of income for several tropical countries. Although a multitude of insects and mites exert varying degrees of economic impact on it, microbial control agents have been used on relatively few of them.

Palm rhinoceros beetles, *Oryctes* spp. (Coleoptera: Scarabaeidae: Dynastinae). These beetles are serious pests of coconut throughout the old world tropics. *Oryctes rhinoceros* (L.) is one of the most important pests of coconut in Southeast Asia and several South Pacific islands (Bedford 1980). Adults attack the heart of plants and feeding can reduce yield and kill trees (Bedford 1980, Zelazny 1983). Larvae develop mainly in rotting palm trunks.

One of the most successful uses of an entomopathogen for classical biological control is reported for *O. rhinoceros*. In 1966, Huger described a non-occluded virus of *O. rhinoceros* from Malaysia that demonstrated potential for long-term control (Huger 1966, 2005). Adults become chronically infected via oral contact with the virus and subsequently serve as reservoirs and disseminators (Huger 1973, Zelazny 1973, Bedford 1981). The midgut epithelial cells of adults become heavily infected (Huger 1973); individual beetles may produce and excrete up to 0.3 mg of virus per day (Monsarrat & Veyrunnes 1976). Viral transmission among adults occurs during mating, when they feed in palms contaminated with feces containing the virus, or in larval breeding sites (Zelazny 1976, Young & Longworth 1981, Zelazny & Alfiler 1991). There are no external symptoms of the disease in adults and it is not immediately fatal (Zelazny 1973). However, it shortens lifespan and reduces fecundity of infected adults. Infected beetles stop feeding, fly less frequently, and the males mate less often (Zelazny 1977). Transmission to larvae occurs when virus-infected adults defecate in breeding sites (Zelazny 1972, 1976). Viral infection in larvae is always lethal. Studies with other *Oryctes* species and *Strategus aloeus* (L.) revealed that the virus is cross-infective to certain other Dynastinae (Lomer 1987). Control of *O. monoceros* (Olivier), a serious coconut pest in Africa, with the *Oryctes* virus was reported by Lomer (1986) and Purrini (1989).

The preferred method for disseminating the virus in coconut plantations has been the infection and release of *O. rhinoceros* adults (Fig. 1C), which resulted in establishment of the virus within larval and adult habitats in several locations in Asia, Africa, and the South Pacific (Bedford, 1981; Zelazny, 1978; Young & Longworth 1981; Jones *et al.* 1998; Huger 2005) where it was previously absent. As few as ten infected beetles can successfully establish the virus on an island (Jones *et al.* 1998). Introduction of virus into artificial and natural larval habitats has also been used successfully to inoculate beetle populations (Bedford 1980). Since 1967, the introduction of virus into coconut plantations in several South Pacific islands and other locations has resulted in significant control of *O. rhinoceros*. Integrated measures that include removal or covering of old palm logs that serve as breeding sites along with inoculative releases of the virus have reduced the density of *Oryctes* populations to below economic thresholds in many locations (Bedford 1980, Zelazny *et al.*, 1992; Alfiler 1992).

Despite the successes of introducing the virus into previously virus-free islands, *O. rhinoceros* remains a serious threat in the coconut and oil palm plantations of Southeast Asia and the Pacific. The prohibition of burning palm logs in the 1990's as a method for maintaining plantation hygiene has dramatically aggravated the problem (Jackson *et al.* 2005,

Ramle *et al.* 2005). In such situations, *O. rhinoceros* outbreaks are not caused by the absence of the virus disease, but by ecological disturbances in the transmission cycle of the virus, like the availability of large number of coconut palms for insect breeding. Under these conditions there is little contact between virus-infected and healthy individuals, whereas a low density of dead-standing palms creates good conditions for spread of the virus (Zelazny & Alfiler 1986, Alfiler 1992, Zelazny *et al.* 1992). Ecological methods for promoting the spread of the virus are to: 1) hide trunks of felled palms from the beetles by promoting the growth of cover crops over them, rather than attempting to burn the trunks, and 2) leave about five dead-standing coconut palms/ha (Zelazny & Moezir 1989).

DNA analysis in Malaysia revealed several distinct viral genotypes with different virulences. The most virulent for larvae and adults (type B) was produced *in vivo* and released into healthy populations. Examination of beetles from the release site and vicinity demonstrated the spread and persistence of type B with concomitant reduction in palm damage (Ramle *et al.* 2005). Decreased control has been reported from other earlier release sites. Jackson *et al.* (2005) reported considerable genetic variation in the virus that suggests its rapid evolution. They recommended a renewed coordinated effort for the selection and distribution of virulent viral strains. Earlier work by Zelazny *et al.* (1990) showed some distinct differences in virulence among strains of the *Oryctes* virus. Marschall and Ioane (1982) demonstrated that re-release of the virus could result in an increase of the infection rate with a reduction of palm damage.

The combined use of the fungus *M. anisopliae* in larval breeding sites and release of virus has also been proposed (Young 1974, Marschall & Ione 1982), but the fungus does not spread well between breeding sites (Zelazny & Alfiler 1986). The *Oryctes* virus has remained the most important biological control agent, however *O. rhinoceros* populations in Java and the southern parts of Sulawesi, Indonesia, are suspected of having developed resistance to the virus (Zelazny *et al.* 1989).

Root weevils (Coleoptera: Curculionidae). The rotten sugar cane borer and the Diaprepes root weevil are reported as pests of several palm species (Weissling & Giblin-Davis 1998). Research on the use of EPNs and fungi for control of these pests has been conducted in banana and citrus, respectively. Experiments with *S. carpocapsae* applied at 8×10^6 IJs/palm conducted by Giblin-Davis *et al.* (1996) on *M. hemipterus sericeus*-infested Canary Island date palms (*Phoenix canariensis* Hort. ex Chabaud) resulted in 51% mortality in weevil larvae. Because of high weevil production/palm, Giblin-Davis *et al.* (1996) recommended that EPNs should be applied frequently and over a long period of time for effective management. The precise interval of application and duration of treatments will depend on environmental conditions and population density of the weevil (J. Peña, personal communication).

Mango

Over 260 species of insects and mites have been recorded as pests of mango worldwide (Peña *et al.* 1998, Waite 2002).

Key pests that require regular control measures include fruit flies, seed weevils, tree borers and various Hemiptera.

Fruit flies (Diptera: Tephritidae). Fruit flies are among the most serious pests of tropical fruit and are regarded as the principal pests of mango. *Anastrepha ludens* (Loew) develops in a variety of fruit crops, but is especially damaging in mango and citrus. It is widely distributed in Mexico, most of Central America and southern United States. The Caribbean fruit fly, *Anastrepha suspensa* Loew, is a pest of mango and several other tropical fruits and is distributed within the Greater Antilles, Bahamas and Florida. *Anastrepha obliqua* (Maquart) is a significant pest of mango in Brazil and most of the new world tropics, but it has not yet been tested for susceptibility to entomopathogens. *C. capitata* and other *Ceratitidis* spp. have been reported from mango and many other fruit worldwide. The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a pest of a wide range of fruit in Asia and is a key pest of mango in India. Fruit fly females oviposit in ripening fruit, and larvae burrow into the pulp. Fully grown larvae exit the fruit, usually after it has fallen to the ground, and pupate in the soil.

EPNs and fungi have been evaluated as alternatives to conventional insecticides for control of some important fruit fly pests of mango. Although *A. ludens* is susceptible to a variety of EPN species under laboratory conditions (Lezama-Gutiérrez *et al.* 1996, Toledo *et al.* 2001, 2005), extremely high rates are required for control in the field (2.5×10^2 IJs of *H. bacteriophora*/cm²) (Toledo *et al.* 2006). Similarly, laboratory and field research conducted on the effectiveness of EPNs against *C. capitata*, revealed susceptibility of larvae to several nematode species (Lindgren & Vail 1986, Gazit *et al.* 2000, Laborda *et al.* 2003), but high application rates are required for control in the field (5 to 50×10^2 IJs of *S. carpocapsae*/cm²) (Lindgren *et al.* 1990). Research on the susceptibility of other fruit flies of mango has been limited to the laboratory. Lindgren & Vail (1986) reported on the susceptibility of *B. dorsalis* to *S. carpocapsae* and Beavers & Calkins (1984) reported on the evaluation of *A. suspensa* susceptibility to several steinernematids and heterorhabditids.

Testing of fungi on fruit flies of mango has been predominantly on *M. anisopliae* and *B. bassiana*. Laboratory and field research by Ekesi *et al.* (2002, 2003, 2005) and Dimbi *et al.* (2003a, 2003b) on *M. anisopliae* against *Ceratitidis* spp. elucidated the effect of various factors on the activity of the fungus that included temperature, moisture, gender, life stage and fly species. Mochi *et al.* (2006) investigated the effect of fungicides, acaricides, insecticides and herbicides on *M. anisopliae* activity against *C. capitata* in laboratory exposures in field-collected soil. Significant pupal and adult mortality occurred in soil treated with the fungus with and without pesticides. No larval mortality was observed. Pesticides affected fungal activity slightly with the most significant effect due to the fungicides chlorothalonil and tebuconazole.

Laboratory and field studies on *M. anisopliae* activity against *A. ludens* were reported by Lezama-Gutiérrez *et al.* (2000). When *M. anisopliae* was applied in field cages at

2×10^5 conidia/cm², adult emergence was reduced by up to 43% in loam soil. Castillo *et al.* (2000) studied the activity of strains of *M. anisopliae* and *P. fumosoroseus* against *C. capitata* adults and reported LD₅₀ values of 5.1×10^3 and 6.1×10^3 conidia/fly, respectively, for the two most active strains. They also noted a sublethal effect of the fungi on fecundity. Konstantopoulou and Mazomenos (2005) reported on the laboratory evaluation of *B. bassiana* and *B. brongniartii* against adults of *C. capitata* and Rosa *et al.* (2002) studied the effects of *B. bassiana* on *A. ludens*. In both studies, adult flies were very susceptible to infection by conidia, but Rosa *et al.* (2002) reported negligible effects on larvae and pupae.

Mango seed weevil, *Sternochetus mangiferae* (F.) (Coleoptera: Curculionidae). This is a widespread pest of mango. Eggs are usually laid on green fruit and larvae tunnel to the seed where they develop. Joubert & Labuschagne (1995) reported laboratory and field tests with two strains of *B. bassiana*, but neither strain had an effect on *S. mangiferae*. Shukla *et al.* (1984) described the isolation of a virus infecting larvae of *S. mangiferae* in India. It caused reduction in feeding, sluggishness, browning of the integument and milkiness of the haemolymph. The authors discussed the similarity of the virus to that reported in *O. rhinoceros*.

***Rhytidodera bowringii* White (Coleoptera: Cerambycidae).** Zhou *et al.* (1998) reported on the evaluation of *B. bassiana* against *Rhytidodera bowringii* White. The fungus was isolated from dead *R. bowringii* adults and subsequently used to produce inoculum for field experiments in China. Treatment of two older mango orchards with *B. bassiana* resulted in 84% mortality of the beetle.

Mango mealy bug, *Drosicha mangiferae* Green (Hemiptera: Margarodidae). Srivastava & Fasih (1988) found *B. bassiana* infecting nymphs of *D. mangiferae* in mango orchards in five localities in Lucknow, India. In field trials on infested mango panicles, spray application of a suspension of 4.8×10^6 conidia/ml reduced populations of *D. mangiferae* by 33-100% in ten days. Masarrat & Srivastava (1998) demonstrated the dose-mortality relationship of *B. bassiana* against *D. mangiferae* first-instar nymphs in laboratory assays. Mohan *et al.* (2004) showed insecticidal activity for *Photorhabdus luminescens* (Akhurst) (the symbiotic bacterium isolated from *H. indica*). Application of a formulation of 1.4×10^6 cells/ml of *P. luminescens* on *D. mangiferae*-infested mango twigs resulted in 92.5% mortality of second-instar nymphs after 48h.

Mangohopper, *Amritodus atkinsoni* Lethierry (Hemiptera: Cicadellidae). Vyas *et al.* (1993) reported that a 75 min exposure to 10^9 *M. anisopliae* conidia/g of inert dust caused 100% mortality of *A. atkinsoni* after 96h. Concentrated aqueous suspensions of the fungus (10^9 conidia/ml) were considerably less effective. Srivastava & Tandon (1986) reported natural infection by the fungi *Lecanicillium lecanii* (Zimm.) and *B. bassiana* in populations of the leafhopper *Idioscopus clypealis* (Lethierry) on mangoes in Uttar Pradesh, India.

Guava

Psidium guajava L. is native to the American tropics but is currently grown in more than 50 subtropical and tropical countries. Brazil is the principal red guava producer followed by Mexico whereas India is the major producer of white guava (Gould & Raga 2002). Different pests attack fruits, leaves and trunk, causing more or less damage depending on the region or country. Main pests of fruits are the guava weevil (*Conotrachelus psidii* Marshall) and fruit flies (*C. capitata* and *Anastrepha* spp.). On the leaves, the main pest is a psyllid (*Triozoida* sp.) that causes damage mainly after pruning when new leaves start growing (Souza *et al.* 2003).

Fruit flies (Diptera: Tephritidae). Fruit flies are very important pests in guava because the adults lay eggs in the fruit, and resulting damage by larvae lowers its quality. In Brazil, the main species in guava are *Anastrepha fraterculus* (Wied.), *A. obliqua*, *A. sororcula* (Zucchi), *A. zenildae* (Zucchi) and *C. capitata* (Souza *et al.* 2003). Most of the research on microbial control of these pests has been conducted in other tropical fruit crops, most notably in mango (see mango section).

Guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae). This is a major pest of guava in certain areas in Brazil. Females lay eggs in immature fruit (3-4 cm diameter) and larvae progress through four instars as the fruit develops. Infestation leads to acceleration in fruit maturation and fruit drop when ripe. At this moment, larvae crawl into the soil where they develop into prepupae. Individuals may remain in this stage for up to six months before pupation and development into the adult (Boscán de Martínez & Cásares 1982, Bailez *et al.* 2003). Control methods involve weekly applications of insecticides to suppress adults, but most of those currently in use for guava weevil control will be discontinued soon (Souza *et al.* 2003, Agência Nacional de Vigilância Sanitária 2004). Without chemical control, the percentage of damaged fruit in heavily infested orchards can reach 100% (Bóscan de Martínez & Cásares 1980). The amount of fruit attacked has been increasing over the past three years possibly due to the development of insecticide resistance (reference?). Poorly timed chemical applications and the tendency for adult weevils to hide in the litter around trees and avoid contact with the chemicals could also be involved (Denholm & Rolland 1992).

The virulence of four species/strains of EPNs to fourth-instar larvae was assessed in the laboratory. In petri dish assays with sterile sand at 100 IJs/larva, larval mortality ranged from 33.5% to 84.5%, with the heterorhabditids being the most virulent. In sand column assays with *H. baujardi* LPP7, *H. indica* Hom1, and *S. riobrave* 355 at 100, 200 and 500 IJs/larva, significant mortality was observed only for *H. baujardi* (62.7%) and *H. indica* (68.3%) at the highest dose. For *H. baujardi* LPP7, the LT₅₀ and LT₉₀ for 100 IJs were 6.3 and 9.9 days, whereas the LC₅₀ and LC₉₀ over seven days were 52 and 122.2 IJs (Dolinski *et al.* 2006). In a greenhouse study with guava trees in 20-L pots (ten weevil larvae/pot),

and doses of 500, 1000 or 2000 IJs/pot, *H. baujardi* LPP7 caused 30% and 58% mortality at the two highest doses (Dolinski *et al.* 2006).

In small farms in Cacheiras de Macacu, RJ, Brazil, a combination of different control methods against the guava weevil is being implemented. *H. baujardi* LPP7 is applied in orchards as infected cadavers. Initial results indicate a 40% to 70% decrease in adult weevils by applying 20 cadavers/tree. In addition, removal of all damaged fruit from their orchards helps to reduce pest population in the following year. Another alternative is the weekly application of neem oil against adults and neem cake applied to the soil for control of larvae. By eliminating pesticides, these strategies have effectively reduced production costs by 40%. Recently, fruit quality and yield are being assessed (Dolinski 2006).

Leaf-footed bug, *Leptoglossus zonatus* Dallas (Hemiptera: Coreidae). This is usually a secondary pest on fruits and flowers. Few attempts to control this pest with microbials were published. Three *B. bassiana* isolates and one isolate of *M. anisopliae* were assessed in the laboratory against adults (Grim & Guharay 1998), being *M. anisopliae* NB the most efficient. In a field trial, mineral oil-based ultra low volume controlled droplet applications of *M. anisopliae* NB at 10¹⁰ conidia/tree caused 94% adult mortality. When *B. bassiana* was applied, there was a 28% increase in fruit yield.

Papaya

Carica papaya L. originated in southern Mexico, Central America and northern South America and is cultivated in most tropical countries (Morton 1987a). A total of 134 species of arthropods are reported to attack papaya, some of which are important vectors of major pathogens of papaya (Pantoja *et al.* 2002). Since most fruit production is for exportation and the presence of pesticide residues is not tolerated, the use of alternatives to chemicals, including cultural methods and microbial control, are being increasingly employed.

Fruit flies (Diptera: Tephritidae). Fruit flies comprise the most important pests of papaya in most producing regions. Research on the use of microbial control agents (EPNs and fungi) against several species that attack papaya (*Anastrepha* spp., *Ceratitis* spp., *B. dorsalis*) has been conducted in other tropical fruit crops, most notably in mango (see mango section).

Twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). This is the most important pest in papaya in Brazil, and is responsible for a major portion of the production costs (Alves *et al.* 2002). Because of the high temperatures and humidity in areas where papaya is grown, fungi have potential as microbial control agents of mites. Alves *et al.* (2002) reported use of *B. bassiana* on over 1000 ha of commercial papaya production in Brazil for control of *T. urticae*.

Most of the research on *B. bassiana* and *T. urticae* has been conducted in other crops. Laboratory tests with *B.*

bassiana against eggs, deutonymphs, protonymphs, larvae and adult stages of *T. urticae* on green bean showed positive results (Saenz-de-Cabezón *et al.* 2003). The LC₅₀ for juvenile stages and adults was 3184 and 1949 conidia/ml, respectively. No significant differences in mortality were observed among egg age classes (24-96h-old eggs) at the tested concentrations (1400-22,800 viable conidia/ml).

Natural epizootics of fungi in the genus *Neozygites* have been responsible for spectacular declines in *T. urticae* populations in other cropping systems (reviewed in Steinkraus 2007).

Broad mite, *P. latus*. This is also a significant pest of papaya in some areas (Pantoja *et al.* 2002). Peña *et al.* (1996) investigated the use of *B. bassiana* and other fungi against this pest (see citrus section).

Aphids (Hemiptera: Aphididae). Although aphids do not colonize papaya, they are considered important potential vectors of papaya diseases (Pantoja *et al.* 2002). Many fungi (several Entomophthorales and *L. lecanii*) have resulted in massive natural epizootics in other cropping systems in some of the same aphids that attack papaya (*e.g.*, *Aphis gossypii* Glover, *Myzus persicae* (Sulz.) (Latgé & Papierok 1988, McCoy *et al.* 1988, Steinkraus 2007). Their potential to control aphids and other hemipteran papaya pests warrants further attention.

Other hemipteran pests. Papaya scale, *Philephedra tuberculosa* Nakahara and Gill (Hemiptera: Coccidae) attacks papaya and annona fruits. It is naturally infected by the fungus *L. lecanii*, which can lead to 90% mortality during summer (Peña *et al.* 1987, Peña & Johnson 2006). A variety of mealybugs, whiteflies, and leafhoppers attack papaya (Pantoja *et al.* 2002). Although literature on microbial control of these pests on papaya is scant, several fungi are reported to attack these insects in other agroecosystems, including tropical fruit (McCoy *et al.* 1988, Fransen 1990, Lacey *et al.* 1996, Goettel *et al.* 2005). Research on the use of fungi for control of these species in papaya is warranted.

Pineapple

Ananas comosus (L.) Merrill is native to Brazil, Bolivia, Peru, and Paraguay, and is currently cultivated in most tropical countries (Morton 1987b). It is the third largest fruit crop (after bananas and mango) harvested in the tropics (Petty *et al.* 2002). These authors provided a summary of arthropod pests of pineapple worldwide and considered pink pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) as the key pest worldwide.

Mealy bugs, *Dysmicoccus brevipes* and *D. neobrevipes* (Hemiptera: Pseudococcidae). Mealy bugs cause wilting due to the toxic effect of their feeding. There is potential for using EPNs against this pest based on work done with a closely related species, *D. vaccinii* Miller & Polavarapu (Stuart *et al.* 1997).

Large moth, *Thecla basalides* (Lepidoptera: Lycaenidae).

This insect is an important pest of pineapple in Brazil, mainly in the cultivar 'Pérola'. Females lay eggs from the beginning of flowering until fruit formation. Larvae penetrate the flowers and complete development in 13 to 16 days (Fazolin 2001). In Northern Brazil, the dose of 600 g/ha of Bt is recommended for control (Sanches 2005). In Southern Brazil, Lorenzato *et al.* (1997) reported on the natural enemies of *T. basalides* and effectiveness of insecticides. Application of Bt resulted in effective control.

White grubs (Coleoptera: Scarabaeidae). The larval stages of 23 species of scarabs in three sub-families were reported to attack the subterranean organs of pineapple plants in several locations worldwide, with the most serious pests reported from Australia and South Africa (Petty *et al.* 2002). Although no specific studies on the use of microbial control agents have been reported for white grubs in pineapple, pathogens have been successfully applied for their control in other crops (Jackson & Glare 1992, Klein *et al.* 2007). Candidate control agents include fungi [*M. anisopliae*, *Beauveria brongniartii* (Sacc.) Petch], bacteria (Bt, *Paenibacillus* spp., *Serratia entomophila* Bizio), and EPNs (*Heterorhabditis* spp., *Steinernema* spp.).

The Role of Microbial Control in Integrated Pest Management (IPM) in Tropical Fruits

Integrated Pest Management plays a significant role in crop protection being an important aspect of sustainable agriculture that attempts to minimize the negative environmental impacts and other deleterious effects due to the use of chemicals (Huffaker 1985, Dent 2000). Individual components of IPM are often evaluated as stand-alone tactics without consideration of their interactions with other components of the agroecosystem.

An integrated approach that is based on pest densities and their relation to economic injury thresholds will ultimately be required for each cropping system and location before agriculture will be truly sustainable. When selective insecticides are used, the negative impact on beneficial insects is reduced. Biopesticides provide an alternative means of control that further minimizes impacts on beneficials and other non-target organisms (NTOs). This is due to the specific nature of many microbial control agents. Safety testing data for entomopathogens indicate that they are generally safe for most NTOs, especially vertebrates (Laird *et al.* 1990, Akhurst & Smith 2002, Hokkanen & Hajek 2003). However, it will be necessary to determine their effects on the beneficial organisms under the specific conditions in each agroecosystem.

The way in which entomopathogens are utilized, *i.e.* augmentation, inoculative introduction (classical biological control) or conservation, will depend on the characteristics of the pest and the fruit crop in which it causes damage or yield loss. Fruit crops are stable agroecosystems where any of the above strategies for pathogen use could be considered. In addition to the use of commercially available

biopesticides, it may be useful to consider employing native entomopathogens. Surveys should be undertaken in different agro-ecological zones to identify prevailing environmental conditions and the presence of native pathogens and natural enemies that may be better suited for the targeted location than an exotic species or strain (Dolinski & Moino Jr. 2006). On the other hand, an exotic pest may require importation of natural enemies from its native range. In classical biological control, natural enemies, including entomopathogens, are sought in the region of origin of the invasive pest, imported and established in an area where they do not naturally occur. Typically this is a geographic area where the pest has invaded without its natural enemies and there are no effective native natural enemies in the invaded region.

When microbial control agents are formulated as biopesticides, they are predominantly used for inundative applications and often treated much like chemicals, with the expectations that they will perform at the same standards. In general, this has not always been possible. On the other hand, there are biological control agents capable of doing what chemicals are not able to do, *i.e.* EPNs that have a capacity to find their pest host, kill it and reproduce in it. In fact, many entomopathogens have the capacity to reproduce in the host and hence produce secondary inoculum able to attack and kill other individuals in the pest population. This numerical increase response, of which chemicals are incapable, needs to be better exploited in tropical conditions. Several other advantages of entomopathogens over chemicals are presented by Alves (1998c), Lacey *et al.* (2001) and Kaya & Lacey (2007).

The cost of producing natural enemies must be judged in terms of the value of the crop protected by using the agent and in comparison to the cost of competing control options such as chemicals (van Driesche & Bellows 1996). In Europe the costs of biological control agents used in protected crops and horticulture have proven to be economic and comparable to chemicals. In Florida, the use of EPNs is an integral part of IPM in citrus indicating that the benefit/cost relationship is positive. There are crops that have few or no registered pesticides and consumers who prefer to buy pesticide-free produce. In those cases, microbial control is strongly supported.

Interaction of Entomopathogens and Other Biological Control Agents

Parasitoids and predators can interact synergistically/additively (*e.g.*, enhanced transmission and dispersal of insect pathogens) or antagonistically (*e.g.*, parasitism/infection, predation and competition) with entomopathogens. In most studies examining the interaction between entomopathogens and other natural enemies, the pathogen almost always dictates the population dynamics of other guild members (Brooks 1993, Begon *et al.* 1999). Most studies indicate the positive nature of these interactions with respect to the control of insect populations (Brooks 1993, Begon *et al.* 1999, Roy & Pell 2000). Various studies have shown the capacity of parasitoids to identify and avoid oviposition in hosts infected

by the different entomopathogen groups (Brooks 1993). This rejection is usually due to visual changes that occur in hosts and/or chemical cues associated with biochemical changes in hosts late during the disease development. As for predators, this does not appear to be true (Wraight 2003, Koppenhöfer & Grewal 2005). The combination of EPNs with other nematode species, fungi and viruses often results in additive effects on pest mortality, whereas nematode-bacteria interactions range from antagonistic to synergistic (Koppenhöfer & Grewal 2005).

Interaction of Entomopathogens and Chemical Pesticides

Unlike parasitoid, entomopathogens are generally compatible with chemical pesticides (Croft 1990). Exceptions include use of several fungicides and most nematicides with entomopathogenic fungi and EPNs, respectively. Certain combinations of entomopathogens with chemical pesticides can be synergistic, such as reported by Quintela & McCoy (1998) and Koppenhöfer *et al.* (2000) for sublethal concentrations of imidacloprid and fungi or EPNs, respectively. The economic feasibility of such combinations will depend on how much of each component can be reduced compared with their recommended application rates when they are applied individually. The compatibility of chemical pesticides with arthropod natural enemies will be another consideration when integrating pesticides and entomopathogens into a pest management program.

Ecological Engineering and Manipulation of Environment to Enhance Activity and Persistence of Entomopathogens

Ecological engineering in the context of biological control and IPM is the manipulation of agricultural habitats to be less favorable for arthropod pests and more attractive to beneficial organisms (Gurr *et al.* 2004). Under optimal environmental conditions many entomopathogens have the natural ability to cause disease at epizootic levels due to their persistence in the environment and efficient transmission. When an insect pathogen is capable of becoming established in an environment it also has the potential to confer long-term regulation of a pest population.

In order for a disease to become epizootic in an arthropod population three factors are required: presence of the host; presence of the pathogen; and proper environmental conditions (Ignoffo 1985). Habitat manipulation techniques have been employed to optimize environmental conditions to increase entomopathogen (endemic, inoculative release, or inundatively applied) activity and facilitate their persistence. These include augmenting moisture in the habitat and creating habitat refuges such as mulches, hedgerows or grass banks, which maintain soil humidity and temperature favorable to microbial activity, propagation, and survival. Manipulated habitats have also been used to provide host plants for alternate arthropod hosts and nectar sources for parasitoids and other natural enemies.

Microbial agents are susceptible to ultra-violet light, heat, and desiccation, but the effects will vary with the microbial species or strain and habitat. Some are capable of remaining viable for just hours (*e.g.*, non-occluded viruses) or days (*e.g.* fungal conidia, occluded viruses). Others may persist for months (*e.g.*, EPNs, fungal resting spores) or years (*e.g.*, *Paenibacillus* spp., fungal resting spores). In addition to habitat manipulation, formulation of pathogens with humectants, nutrient sources, and UV protectants has been used to enhance persistence under field conditions (Burgess 1998).

Availability of Entomopathogens as a Limiting Factor

Factors that favor accelerated growth and use of biopesticides are their improved performance and cost competitiveness in the face of increasing insect resistance to chemical insecticides, environmental hazards and lack of selective chemical pesticides. Recently, major agrochemical companies have taken a greater interest in microbial pesticides. In all, 281 biopesticides were available on the market in 1993, with active ingredients of bacteria, EPNs, fungi, and viruses (Lisansky & Coombs 1994). Although the market for microbial insecticides is growing, it represents less than 1-1.5% of the total crop protection market (Lacey *et al.* 2001).

Many microbial control agents can be produced on artificial media using fairly simple methods and there are potentially ample markets for them. However, the bottleneck in the use of microbial control in many countries is their local production and availability. Although there are successful examples of important pests being controlled with biopesticides, many microbial control agents are not universally available to growers.

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 hazardous pesticides. Microbial control of arthropod pests of tropical fruits, in conjunction with other IPM components, can provide effective control. The challenge will be to find successful combinations of entomopathogens, predators, and parasitoids along with other interventions. Aspects that warrant further study and attention are improved formulation, storage, marketing, and transfer of technology to growers.

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