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REVIEW

Entomopathogenic nematodes against the main guava insect pests

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Abstract The common guava, yellow guava, or lemon guava, *Psidium guajava* L. (Myrtaceae), is native to the Americas and widely cultivated in more than 50 tropical and subtropical countries. There are many insect pests that limit guava production. Biological control should be considered to avoid extensive use of insecticides. Entomopathogenic nematodes can be very effective against life stages in the soil. Therefore, this review aims to summarize most studies carried out for control of guava pests by entomopathogenic nematodes.

Keywords Fruit flies · Guava weevil · IPM · *Psidium guajava* · Microbial control

Introduction

The common guava, yellow guava, or lemon guava, *Psidium guajava* L. (Myrtaceae) is native to the American tropics but is currently grown in more than 50 subtropical and tropical countries. Brazil is the main red guava producer followed by Mexico, whereas India and Pakistan are major producers of white guava (Gould and Raga 2002; Pomar Brasil 2015). Different pests attack fruits, leaves and trunk, causing more or less damage depending on the region or country. In the Americas, the main pests on fruits are the guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), and the fruit flies, *Ceratitis capitata* (Wiedemann), *Bactrocera* spp., and *Anastrepha* spp. (Diptera: Tephritidae). Occasionally, the leaf-footed bug, *Leptoglossus zonatus* Dallas (Hemiptera: Coreidae) can also cause damage. On the leaves, the main pest is the psyllid, *Triozoida limbata* (Hemiptera: Triozidae), which causes damage mainly after pruning, when new shoots start emerging (Souza et al. 2003).

In Asia, Africa and Western Pacific countries, the main pest on fruits are the fruit fly, *Bactrocera* spp., the fruit borer, *Deudorix isocrates* (Fab) (Lepidoptera: Lycaenidae) and *Dichocrocis punctiferalis* (Guenee) (Lepidoptera: Pyralidae), the fruit piercing moth, *Eudocima phalonia* (L.) (Lepidoptera: Noctuidae), the tea mosquito bug, *Helopeltis* spp. (Hemiptera: Miridae), and the atlas moth, *Attacus atlas* (L.) (Lepidoptera: Sturniidae). On the leaves, the main pests are the mealy bug, *Pseudococcus* sp. (Homoptera: Pseudococcidae), the scale insect, *Chloropulvinaria psidii* (Maskell) (Homoptera: Coccidae), and the spiraling whitefly, *Aleurodians dispersus* Russell (Hemiptera: Aleyrodidae). Finally, on the trunk the principal pest is the bark-eating-caterpillar, *Indarbela quadrinotata* Walker (Lepidoptera:

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Metarbelidae) (Sarwar 2006; Haseeb 2007; Muniappan et al. 2012).

Control methods for insect pests involve weekly applications of broad-spectrum insecticides such as organophosphates, focusing mainly in the adult forms. Growing concern over the environmental effects of pesticides has encouraged the development of alternatives. Natural pathogens of insects often play an important role in the regulation of insect populations in agroecosystems (Ignoffo 1985; Steinkraus 2007). However, their main impact on insect pests may occur after economic thresholds are surpassed. Inundatively or inoculatively applied microbial control agents (viruses, bacteria, and fungi) have been developed as alternative control methods for a wide variety of insect pests. These agents play an important role in integrated pest management (IPM) (Lacey et al. 2001; Kaya and Lacey 2007).

Entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* and *Steinernema* (Rhabditida) are obligate parasites of insects (Poinar 1990). These nematodes have a symbiotic relationship with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively (Forst and Clark 2002). Infective juveniles (IJs), the only stage of nematodes found in the soil, enter the hosts through natural openings such as the mouth, anus or spiracles, but IJs of heterorhabditids can also enter through the cuticle. After penetrating into the host's hemocoel, the nematodes release their symbiotic bacteria, which usually kill the host within 24–48 h. The bacteria are also responsible for antibiotic production and for providing nutrition for the nematodes (Dowds and Peters 2002). The nematodes feed, develop, mate, and often complete two to three generations within the insect cadaver. When resources within the cadaver are depleted, a new generation of IJs is produced, which leave the cadaver to search for new hosts (Kaya and Gaugler 1993).

Entomopathogenic nematodes effectively control a variety of economically important insect pests and have excellent potential for control of tropical insect pests (Grewal et al. 2001). This review summarizes the studies carried out with EPNs to control pests or potential pests on guava trees, so they can become part of IPM programs. The following insect pests spend part of their life cycle in the soil, and therefore are potential targets of EPNs.

Research and application of entomopathogenic nematodes for control of fruit flies and the guava weevil

Fruit flies

Fruit flies are very important guava pests because the adults lay eggs in the fruit, and the resulting damage done by larvae lowers their quality. Adults can survive for many months, occasionally almost a full year, and males appear to be able to survive much longer than females, even as much as 16 months. The adult female typically oviposits on the fruits when the fruit begins to ripen. Eggs are usually laid in groups of about ten and hatch in 6–12 days. The newly hatched larvae eat and burrow into the fruit pulp, taking on the color of their food so that when small they are overlooked easily. When fully grown, the larvae emerge through prominent exit holes, usually after the fruit has fallen to the ground, and pupate in the soil. Larval development requires approximately three to four weeks, depending largely on temperature conditions during these development periods. The development is faster where comparatively higher temperatures prevail, and as a general rule the shorter the period for fruit maturation the more rapid is the larval development (Aluja 1994).

Many species of *Anastrepha* spp. have been found colonizing guava trees (Zucchi 2008; White and Elson-Harris 1994), such as *Anastrepha bistrigata* Bezzi, *Anastrepha fraterculus* (Wiedemann), *Anastrepha obliqua* (Macquart), *Anastrepha sororcula* Zucchi, *Anastrepha zenildae* Zucchi, *Anastrepha suspensa* Loew, *Anastrepha serpentina* (Wiedemann), *Anastrepha ludens* (Loew), and *Anastrepha striata* Schiner. Another very common fruit fly that attacks guava is *C. capitata* (Canal et al. 1998; Weems 2001; Souza-Filho et al. 2009). Other species as *Ceratitis anonae* (Graham), *Ceratitis cosyra* (Walker), *Ceratitis fasciventris* (Bezzi), and *Ceratitis rosa* Karsch have also been reported on guava fruits (Coperland et al. 2006). The biodiversity of the genus *Bactrocera* was investigated in guava orchards and their surroundings in southern Thailand. Thirty-one species were identified, comprising 14 new records. *Bactrocera papayae* Drew & Hancock and *Bactrocera carambolae* Drew & Hancock were the most abundant species at all sites (Danjuma et al. 2013). Other species have been reported on guava around the world: *Bactrocera*

dorsalis (Hendel), *Bactrocera correcta* (Bezzi), *Bactrocera zonata* (Saunders), *Bactrocera invadens* Drew, Tsuruta and White, and *Bactrocera tryoni* (Froggatt) (José et al. 2013; EPPO 2014; CABI 2015). Control of some of these species by EPNs has been tested, revealing good potential. However, studies also increasingly reveal variance among strains and insect stage tested, associated with unknown and unpredicted abiotic factors.

Anastrepha fraterculus, the South American fruit fly, is of great importance and is considered the key-pest in apple and peach orchards (Kovaleski et al. 2000). It has also been reported on guava (Zucchi 2008). Laboratory, greenhouse, and field experiments in a peach orchard were performed with the objective of selecting efficient indigenous EPN strains from Rio Grande do Sul, Brazil. Laboratory experiments were conducted in 24 well-plates filled with sterile sand and one insect per well, either third instar larvae or pupae. In greenhouse experiments, plastic trays filled with soil collected from the field were used, while in field experiments, holes (3 × 5 cm) were made in soil under the edge of peach tree canopies, where the insects were placed. Among 19 EPN species/strains tested, *Heterorhabditis bacteriophora* Poinar RS88 and *Steinernema riobrave* Cabanillas, Poinar & Raulston RS59 caused the highest *A. fraterculus* larval and pupal mortality. The lethal doses, LD_{50}, of *S. riobrave* on *A. fraterculus* larvae and pupae were 382 (or 347 IJs cm^{-2}) and 112 IJs per larva (or 102 IJs cm^{-2}), respectively. LD_{50} values of *H. bacteriophora* on *A. fraterculus* larvae and pupae were 252 (or 229 IJs cm^{-2}), and 120 IJs per larva (or 109 IJs cm^{-2}), respectively. Consequently, *H. bacteriophora* had a lower LD_{50} on *A. fraterculus* larvae than *S. riobrave*, with similar results for pupae. Experiments carried out in a greenhouse showed no differences in pupal mortality by either nematode at 250 and 500 IJs cm^{-2}. In the field, both strains sprayed on natural and artificially-infested fruit resulted in *A. fraterculus* larval mortality of 51.3, 28.1 and 20, 24.3 %, respectively. Moreover, *H. bacteriophora* RS88 showed better efficacy of host search inside naturally infested fruit, independently of the application method (aqueous suspension or infected cadavers) (Barbosa-Negrisoli et al. 2009).

Anastrepha obliqua, the West Indian fruit fly, is a significant pest of mango in most of the new world tropics, but it has also been found afflicting guava (Zucchi 2008). Toledo et al. (2005b) tested the effect of temperature, soil texture and depth of the host on the infectivity of *H. bacteriophora* against third instar (early and late stadium) larvae of *A. obliqua*, under laboratory conditions. At the three container depths, late third instars were more susceptible than early third instars. The LC_{50} at 2 cm was 24 IJ cm^{-2} for early stages larvae and 25 IJ cm^{-2} for late. Of the three soil textures evaluated (sandy, sandy loam, and sandy clay), the highest infectivity of both larval ages occurred in sandy-clay soil at 15 % moisture. As for the temperature, the highest infectivity was observed on larvae kept at 24 ± 0.5 °C and the lowest infectivity at 18 ± 0.5 °C in sandy clay soil at 15 % moisture. Infectivity of *H. bacteriophora* was observed during 17 days in sandy clay soil at 16 % moisture, although five days after inoculation, larval mortality decreased as the soil moisture declined. Abiotic studies were also performed with *Steinernema carpocapsae* (Weiser). The LC_{50} values estimated for six-day-old larvae were 9, 20 and 102 IJs cm^{-2} in tubes containing sand depths of 2, 5 and 8 cm, respectively, whereas for eight-day-old larvae, LC_{50} values were 16, 40 and 157 IJs cm^{-2}, respectively. Again, the early third instar larvae at the shallowest dept were the most susceptible. The authors concluded that *S. carpocapsae* can potentially control the West Indian fruit fly in tropical ecosystems with warm temperatures and high soil moisture levels (Toledo et al. 2009).

Anastrepha suspensa, the Caribbean fruit fly or the Caribfly, is a key pest of guava and several other tropical fruits and is distributed within the Greater Antilles, Bahamas and Florida. Beaver and Calkins (1984) reported the evaluation of all stages of *A. suspensa* susceptibility to several steinernematids and heterorhabditids under laboratory conditions. Significantly more larvae and adults of the Caribbean fruit fly were infected and killed by *S. carpocapsae* All, Mexican, and Breton strains, *Heterorhabditis heliothidis* Khan, Brooks, and Hirschmann, and *H. bacteriophora*, ranging from 78.7 to 90.7 % mortality, than by *Steinernema glaseri* (Steiner) with 15.7 % mortality. Fruit fly pupae were less susceptible to nematode infection. They also reported that nematodes multiplied in the host cadavers and began leaving the parasitized hosts within seven days (cycling). Also, the IJs that left the host cadaver were able to infect new *A. suspensa* and *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae.

Anastrepha serpentina, the sapote fruit fly, is intercepted frequently in United States ports of entry in a variety of hosts from several countries. It is an important pest species in Mexico because its larvae infest sapote, sapodilla, willowleaf lucuma, and other fruits including guava (Aluja 1994; Weems 2001). Toledo et al. (2006b) tested the infectivity of *H. bacteriophora* on third instars of this tropical fruit fly, under laboratory conditions. An LC_{50} was estimated at 36.0 ± 5.4 IJ cm^{-2} in cups containing 5 cm of sandy soil, adjusted to 15 % humidity. The LC_{95} was estimated at 686 IJ cm^{-2} and the authors considered this high amount of nematodes to obtain satisfactory control levels. They also stated that other strains should be tested.

Anastrepha ludens, the Mexican fruit fly, 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. Its natural distribution includes the Rio Grande Valley of Texas, where populations routinely attain pest status if control measures are not practiced. It is a frequent invader in southern California and Arizona. The Mexican fruit fly is a particular threat to Florida because of its special affinity for grapefruit, of which Florida is one of the world's leading producers (Aluja 1994; Weems 2001). There are different reports of *A. ludens* on guava (CABI 2015). However Birke and Aluja (2011) stated that *A. ludens* and *A. serpentina* do not infest guavas under field conditions. Most probably they do not attack guava in the presence of other more preferable fruits. Lezama-Gutierrez et al. (1996), evaluated the susceptibility of third instar *A. ludens* larvae to various EPN species in pots containing sterile sandy soil, and found higher larval and pupal mortality (90 %) when larvae were exposed to *S. riobrave* and *S. carpocapsae* All. However, Hernández (2003) observed mortality rates of just 22.8, 15.9, 18.4, 17.2 and 17 % of *A. ludens* larvae following treatment with 100 IJs per larva of *Heterorhabditis indica* Poinar, Karunaka and David, *S. carpocapsae* Mexican, *S. carpocapsae* All, *H. bacteriophora* HP88 and *S. riobrave*, under laboratory conditions. On the other hand, pupal mortality was a bit higher with 48.3, 37.1, 32.8, 30.1 and 21.3 %, respectively for the same species. Surprisingly, the LC_{50} and LC_{95} values were much lower than other studies done with fruit flies species, for example *H. indica* with LC_{50} and LC_{95} estimated at 7.2 and 168.1, and *S. carpocapsae* All with 19.3 and 378.77, respectively. In a field test, Toledo et al. (2005a) applied 115 and 345 IJs cm^{-2} in a commercial mango orchard, which resulted in 46.7 % (45.2–48.1) and 76.1 % (74.8–77.3) larval mortality, respectively, considered very satisfactory results for microbial control. In another mango field test, Toledo et al. (2006a) applied *H. bacteriophora* against larvae and pupae. Infection rates reached the highest levels (>70 %) only at the two highest tested concentrations (250 and 500 IJs cm^{-2}) on the soil surface, equivalent to 2.5 and 5×10^{10} IJs ha^{-1}, respectively. The authors concluded that effective control of *A. ludens* would require very high densities of *H. bacteriophora*. Lezama-Gutiérrez et al. (2006) tested the efficacy of *S. carpocapsae* All and *S. riobrave* against last instar larvae in different soil types. Higher rates of larval mortality were observed in sandy loam and loam than in clay. Under field conditions of sandy loam soil, *S. carpocapsae* reduced adult emergence by 64 %, whereas *S. riobrave* reduced adult emergence by only 14 % compared with the control, confirming the importance of soils type when selecting the nematode species and planning fruit fly microbial control strategies. It is certain that biotic and abiotic factors (Shapiro-Ilan et al. 2006) or even low nematode virulence interfered in those studies. In any case, more field studies should be performed.

Anastrepha striata, the American guava fruit fly, is an important pest in the American tropics and subtropics, especially of guavas and other myrtaceous fruits, although it has also been reported to attack mango, orange and peach (CABI 2015). It is considered a pest of quarantine significance by USDA-APHIS-PPQ and many other regulatory agencies (Aluja et al. 1990). So far, no studies have been published of EPNs against this fruit fly, for that reason we need to call attention to neglected pest as this one and declare that more studies are needed.

Ceratitis capitata, the Mediterranean fruit fly or Medfly, has been reported to afflict guava and many other fruits worldwide. Poinar and Hislop (1981) found that adult Mediterranean fruit flies were susceptible to *Steinernema feltiae* (Filipjev) Mexican strain and *H. heliothidis* in laboratory tests, but due to a limited amount of host material, no tests were conducted of larval or pupal susceptibility or of the ability of emerging infective-stage nematodes to infect other hosts. Lindegren and Vail (1986) confirmed that

late third instar larvae (or prepupae) of this fruit fly were also susceptible to *S. feltiae* Mexican strain. They suggested that nematode soil drench applications applied at the base of host plants could be cost-effective for augmentative control of fruit flies. The middle laboratory dose (500 IJs of *S. feltiae* Mexican per larva, which is also about 500 IJs cm^{-2}) appeared to be optimal for this kind of application, since the mean larval mortality at this dosage was 87.1 % (range, 65.8–99.5 %). In further field tests, 97, 94, 79 and 55 % mortality rates of *C. capitata* prepupae were obtained 1, 4, 8 and 14 days after treatment. In another study, prepupae of the Mediterranean fruit fly exhibited a significant mortality response in a papaya field when exposed to concentrations of 5000, 1500, 500, and 150 IJs cm^{-2} of *S. feltiae* Mexican, with the augmentation for eradication of 500 IJ cm^{-2} being applied in soils. The authors concluded that the estimated LC_{50} of 38 IJs cm^{-2} indicates that *S. feltiae* may offer an effective and non-toxic alternative to soil treatments for Mediterranean fruit fly control programs (Lindegren et al. 1990). Gazit et al. (2000) evaluated 12 different species/strains of EPNs against *C. capitata* prepupae. *S. riobrave* Texas and *Heterorhabditis* sp. IS-5 induced >80 % mortality, but *S. riobrave* Texas was the most effective (100 IJs cm^{-2} causing 82 % mortality). The authors concluded that IJ activity was directly related to nematode and insect density. The highest nematode activity was recorded at 1.88 larvae cm^{-2} and decreased at higher larval densities. Moreover, the maximal nematode activity occurred at a density of 150 IJs cm^{-2}. The persistence of this EPN in the soil extended for longer than five days but there was no activity after 14 days. Temperatures ranging between 22 and 41 °C, or moisture levels in the treated soil ranging between 3 and 20 %, had no significant effect on nematode activity, although there was lower activity under cooler conditions (17 °C).

Laborda et al. (2003) evaluated the *C. capitata* larval and pupal susceptibility to the product Biorend C Foliar®, a mixture of *Steinernema* spp. and chitosan (IDEBIO S.L., Spain) and they found a larval mortality above 90 %, but without effect on pupae. Kepenekci and Susurluk (2006) tested two Turkish strains (*S. feltiae* All and *S. feltiae* S3) against Medfly pupae and observed low mortality caused by both All (26.6 and 33.3 %) and S3 (30 and 40 %) with 50 and 100 IJs, respectively. Almeida et al. (2007) in Brazil tested the pathogenicity of *Heterorhabditis* sp. IBCBn 05 applying 200 IJs per prepupae to the soil and observed adult emergence. The mean number of adults that emerged was 2.4 ± 1.3 when nematodes were applied to ten prepupae in natural soil, compared to 7.6 emerged adults in the control.

In a guava orchard in Brazil, Silva et al. (2010) confirmed that the Mediterranean fruit fly was susceptible to EPNs when exposed to prepupae and one-day old pupae, and *H. indica* IBCB n5 was the most virulent strain, applied at the dosages of 1 and 10 IJs cm^{-2}, with mortality ranging from 66 to 93 %. Another field study was conducted in a guava orchard in Brazil, to test the potential of *H. baujardi* Phan, Subbotin, Nguyen and Moens LPP7 to control *C. capitata* prepupae. The evaluation was conducted in cages constructed over five-years-old guava trees. In each cage, 100 *C. capitata* prepupae and a suspension of 100,000 IJs per 500 ml of water were distributed evenly in the soil. A McPhail trap was placed in each cage and the control efficiency was evaluated by counting the captured adults. The average larval mortality in treated trees was significantly different in relation to control (7.7 and 58.6 %, respectively). When the experiment was repeated the same tendency was reached (30.4 and 87.4 %, respectively) (Minas 2008).

Malan and Manrakhan (2009) tested the potential of *H. bacteriophora*, *Heterorhabditis zealandica* Poinar and *Steinernema khoisanae* Nguyen, Malan and Gozel against prepupae, pupae and adults of *C. capitata* and *C. rosa* under laboratory conditions. Prepupae and adult flies were more susceptible to nematode infection than pupae, and *C. capitata* prepupae were more susceptible to infection than those of *C. rosa*. Larvae of *C. capitata* were severely infected by *H. bacteriophora*, whereas the highest infectivity of *C. rosa* larvae was obtained with *H. zealandica*. In contrast, adults of both species were highly infected by *S. khoisanae*.

The use of EPN strains separately or in combination was tested against the Mediterranean fruit fly under laboratory conditions. Eight strains were used separately or combined: *S. carpocapsae* All, *H. bacteriophora* HP88, *H. baujardi* LPP7, *H. indica* LPP1, *H. indica* LPP14, *Heterorhabditis* sp. LPP9, *Heterorhabditis* sp. LPP17 and *Heterorhabditis* sp. LPP12. The strains *H. baujardi* LPP7, *H. indica* LPP14, *H.* sp. LPP17 and *H.* sp. LPP12 were the most efficient, causing mortality rates ranging between 75 and

98.5 %. The most effective combinations were *H. indica* LPP14 + *H.* sp. LPP9 and *H.* sp. LPP17 + *H.* sp. LPP12 with mortality rates of 60 and 82 %, respectively. The authors concluded that the use of EPNs to control *C. capitata* is feasible either using species individually or in combination, but the highest mortality level were reached with individual strains (Minas et al. 2011).

Bactrocera dorsalis, the oriental fruit fly, is one of the most destructive fruit fly pests of East Asia and the Pacific. It is second only to the Mediterranean fruit fly. Its distribution range includes Pakistan and India to southern Japan, Indonesia to Micronesia, and the Mariana Islands and major Hawaiian islands. Recent outbreaks have occurred in southern California and Florida. It is a key pest of mango in India, but it also occurs on guava (CABI 2015). Lindegren and Vail (1986) reported the susceptibility of *B. dorsalis* to *S. feltiae* Mexican strain with mortality ranging from 9 to 85 % when using 50 to 5000 IJs cm^{-2} in soil. Once again the pupae were not susceptible to EPNs. Lin-Jin et al. (2005) tested *S. carpocapsae* All, *S. carpocapsae* A24, *Steinernema feltiae* SN, and *H. bacteriophora* H06 on the oriental fruit fly and observed that the fly population in the following generation of the treated orchard was reduced to 14.6 % compared to the control orchard.

Bactrocera correcta is often referred to as the guava fruit fly. This important pest has not been subjected to tests with EPNs, or results have not been published so far. It is another example of a neglected insect pest that needs investigation.

Bactrocera zonata, the peach fruit fly, has been reported in Asia, India, Pakistan, Nepal, Sri Lanka, Thailand, USA, Egypt, and other African countries and it is considered a main pest on guava (White and Elson-Harris 1994; CABI 2015). Mahmoud and Osman (2007) performed laboratory experiments to determine the efficiency of *S. feltiae* Cross N 33 against second and third instar larvae and one, four and six-days-old pupae of *B. zonata*. Mortality maxima using 800 IJs against five specimens at 72 h were 56 % for second instar larvae and 88 % for third instar larvae, whereas the highest pupal mortality was 56 % for one-day-old pupae, 32 % for four-days-old pupae and 20 % for six-days-old pupae. LC_{50} and LC_{90} values were 299 and 1083 for second instar larvae, 181 and 655 for third instar larvae, 747 and 2705 for one-day old pupae, 1227 and 5017 for four-days-old pupae, and 3114 and 10,048 for six-days-old pupae, respectively. The authors concluded that third instar larvae and one-day-old pupae of *B. zonata* were significantly more susceptible to nematode infection than the other stages. Mahmoud (2007) combined botanical insecticides NSK (Neem seed kernel) extract, NeemAzal T® 5 % and Neemix® 4.5 % with *S. feltiae* Cross N 33 to control the peach fruit fly under laboratory conditions. Of 25 treatment combinations between azadirachtin from NSK extract and *S. feltiae,* 18 gave synergistic responses, four were additive, none were antagonistic and three produced no response. The same number of combinations with NeemAzal T 5 % showed 19 synergistic responses, one additive, none antagonistic and five without any response. Combinations of Neemix 4.5 % and *S. feltiae* showed 11 synergistic responses, five additive, three antagonistic and six without any response. They concluded that Most combinations of the nematode with NSK, NeemAzal T 5 % or Neemix 4.5 % significantly increased host mortality. Other laboratory experiments were performed to evaluate the pathogenic effects of *H. bacteriophora* and *S. carpocapsae* All on the third instar larvae, newly formed pupae and seven-days-old adults of the peach fruit fly by applying 50–250 IJ cm^{-2}. Mortality rates ranged from 9.3 to 42.7 %, and 67.3–100 % for the full-grown larvae treated by *S. carpocapsae* All and *H. bacteriophora*, respectively, whereas ranged from 2.7 to 32.7 %, and 12.7–51.7 for pupae treated by the same nematodes, respectively. Furthermore, the mortality rates varied from 35.0 to 78.7 %, and 41.7–90.3 for adults treated with the same nematodes. The lowest LC_{50} and LC_{90} values were found when larvae were treated by *H. bacteriophora* (28.8 and 167.2, respectively). The authors concluded that although both nematodes were effective on the different stages of *B. zonata*, *H. bacteriophora* was more infective than *S. carpocapsae* and that larvae and adults were more susceptible to nematode infection than the pupae (Fetoh et al. 2011). Soliman et al. (2014) tested *S. riobrave* and *H. bacteriophora* against full-grown larvae and pupae. Larvae were more susceptible to *H. bacteriophora* while one-day-old pupae were highly susceptible to infection with both strains.

Bactrocera tryoni, the Queensland fruit fly, is the economically most significant Australian tephritid pest species with a large invasion potential. Beside Oceania, it has also been reported in the USA (CABI 2015). Langford et al. (2014) tested the capacity of

three EPN species with different foraging strategies (*S. feltiae*, *S. carpocapsae* and *H. bacteriophora*) to cause larval and pupal mortality in *B. tryoni* across a range of concentrations (50, 100, 200, 500 and 1000 IJs cm^{-2}), substrate moisture (10, 15, 20 and 25 % w/v) and temperatures (15, 20, 25 and 30 °C). They found that all the EPN species tested caused environment and density-dependent mortality in the third larval instar while pupae were not affected. However, *Steinernema feltiae* caused the highest mortality across different IJ concentrations and over a wider moisture and temperature range when comparing to the other two EPN species.

The guava weevil

Conotrachelus psidii, the guava weevil, occurs throughout the Americas and directly affects the fruit quality causing serious damage. The adults are present in orchards during summer, appearing in September–October and remaining until March. 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. Subsequently, 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 Martinez and Cásares 1982; Bailez et al. 2003). Control methods involve weekly applications of insecticides to suppress adults, and without chemical control the percentage of damaged fruit in heavily infested orchards can reach 100 % (Boscán de Martinez and Cásares 1980). The virulence of nine species/strains of EPNs to fourth instar weevils was assessed in the laboratory. Larval mortality in Petri dish assays with sterile sand at 100 IJs per larva 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 per larva, significant mortality was observed only for *H. baujardi* LPP7 (62.7 %) and *H. indica* Hom1 (68.3 %) at the highest dose. For *H. baujardi* LPP7, the LT_{50} and LT_{90} for 100 IJs were 6.3 and 9.9 days, whereas the LC_{50} and LC_{90} over seven days were 52 and 122.2 IJs. In a greenhouse study with guava trees in 20-l pots (ten weevil larvae per pot), and doses of 500, 1000 or 2000 IJs per pot, or 0.17, 0.35 or 0.7 IJs cm^{-2}, *H. baujardi* LPP7 caused 30 and 58 % mortality at the two highest doses (Dolinski et al. 2006). Del Valle et al. (2008) assessed the susceptibility of the guava weevil to *H. baujardi* LPP7 IJs in the greenhouse and under field conditions, applying the nematodes through insect-cadavers of seventh instar *G. mellonella* larvae. Field persistence of these nematodes in the soil was evaluated through *G. mellonella*-baiting. Insect cadaver concentrations of two, four, and six applied in pots in the greenhouse experiment caused significantly greater mortality than the control. Significant differences were observed in the field between control and treatments only when six cadavers per 0.25 m^2 were applied. IJs from the cadavers persisted six weeks after application in the field, but decreased sharply thereafter.

In 2008, researchers from Universidade Estadual do Norte Fluminense Darcy Ribeiro in Rio de Janeiro state, Brazil started working with a small group of farmers with the objective of establishing an IPM program in guava orchards. Approximately 20 farmers organized in an association named GOIACAM, Associação do Produtores de Goiabas de Cachoeiras de Macacu, paid for the nematode registration and were trained to rear *G. mellonella* themselves. The *G. mellonella* larvae were taken to the university, infected with *H. baujardi* LPP7 and then returned to the farms as infected cadavers for application. Results showed a decrease of 40–70 % in emerged adults, in plots of 9 m^2 where 20 cadavers were used, compared to control trees with no nematode application. When neem cake was also applied below the canopy for larval control, an additive effect occurred, with the adult control reaching almost 80 %. The farmers also initiated cultural control by removing all damaged fruits from the orchards, which reduced the pest inoculum for the following year. Between rows, *Crotalaria juncea* L. and other Fabaceae were planted to increase the soil fertility and serve as refugia for natural enemies. Because insecticides are not being used in those experimental areas, beneficial arthropods such as coccinelids and crisopids are being seen more often within the orchards where nematodes were applied. Using these strategies, the production costs were reduced by 40 % (C. Dolinski, personal communication). Some growers use sprinkler irrigation, so Lara et al. (2008) evaluated the influence of irrigation application of EPNs on the viability, infectivity and host search capability of *H. baujardi* LPP7 IJs. The results demonstrated that the irrigation system did not

adversely affect any of the factors described previously. Today, growers can choose from these different application methods, based on their situation.

The guava weevil population in the areas where the IPM was implemented in 2008 is very low compared to what it was when we first applied nematodes. We routinely recovered 20–30 adults from each tree prior to initiating GOIACAM, compared to just two or three adults recently. This population reduction reflects directly on the number of damaged fruits, which is now typically 1 %. The weevil is still present, but there is an understanding that it is at a new lower equilibrium in the orchards. A new project is being initiated to convert orchards under both IPM and conventional management to organic management systems. Since the consumption and demand for organically grown fruit is increasing, and because guava planted in these areas is exclusively for direct consumption, there is a desire for less pesticide use. The nematode *H. bacteriophora* LPP30 has been tested against *C. psidii* on conversion to organic and conventional guava cultivation systems, as another potential agent. In guava crops, the population fluctuation of the nematode and the occurrence of the pest were evaluated over one year. Different application methods were tested, with aqueous suspension being the best. A reduction of the weevil population was observed, especially in the conversion to organic area with lower rates of infestation and crop losses. Also, better cycling and persistence of IJs in the area under conversion to organic was observed (Minas 2012).

Silva et al. (2010) evaluated the infectivity of *H. indica* IBCB n5 against the guava weevil under field conditions. It was demonstrated that *H. indica* IBCB n5, applied at the dosages of 1 and 10 IJs cm^{-2}, controlled the guava weevil with mortality ranging from 33 to 50 %.

In Colombia, tests were done under laboratory conditions to assess the effect of seven species of EPNs isolated in the country (*Steinernema websteri* Cutler and Stock JCL006, *Steinernema* sp. 1JCL024, *Steinernema* sp. 2JCL007, *Steinernema* sp. 3JCL027, *Steinernema colombiense* López-Nunes, Plichta, Góngora-Botero, and Stock SNI0198, *H. bacteriophora* HNI0100 and *Heterorhabditis* sp. SL0708) on fourth instar larvae of the guava weevil. The researchers measured the production and the displacement of the most virulent. *Heterorhabditis* sp. SL0708 induced mortality of 85 %, *Steinernema* sp. 1 JCL024 75 % and *S. colombiense* SNI0198 55 %, while mortality was under 25 % for the other species of EPNs. The greatest production of IJs in the weevil was reached with *Heterorhabditis* sp. SL0708, which also showed greater recognition capability to *C. psidii* (Delgado-Ochica and Aponte, 2012).

Conclusion

Sustainable agriculture will rely increasingly on alternatives to synthetic chemical insecticides for pest management that are environmentally responsive and reduce the amount of human contact with hazardous pesticides. Concerns about insecticide resistance as well as human and environmental safety create opportunities for development and use of biocontrol agents such EPNs. EPNs are by no means able to solve alone all major problems of insect pests, and they certainly have an important place in biological insect control. Moreover, EPNs should be viewed as components of IPM, rather than as the only resource available. The problem is to define the ecosystem in which they can effectively play a positive role, and to determine the necessary strategies for the expression of their full potential (Ferron 1985).

As seen previously, there are many successful biocontrol studies using EPNs against the soil stages, such as the guava weevil prepupae or third instar fruit fly larvae. Given that EPNs are biological organisms and are thus sensitive to environmental conditions, caution must be taken in the transfer of technology. Therefore, there are challenges to be met. Aspects that warrant further study and attention are field tests, improved formulation, storage, marketing, and transfer of technology to growers. Further investigations to enhance formulation toward above-ground application against foliar guava pests, and other novel delivery approaches for EPNs, are needed, and goals should be oriented to reducing overall costs while enhancing efficacy. For maximum gains of competitiveness of EPNs, there is a need for an overall program that broadly enhances all critical aspects including strain choice, production, and packaging technology. Identifying markets where expectations are in line with the actual performance of the insect pathogen is also important. For that, there is also a great need of governmental and institutional support (Gelernter and Lomer 2000).

Basically, the key to success is that the approaches using EPNs must be cost competitive and consistently efficacious relative to chemical insecticides. Toward that end, the use of EPNs can be improved by finding well-adapted nematode species or strains that are better able to suppress the target pest in a given area or region. Ideally, EPN use will eventually be optimized specifically for each existing guava pest.

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Claudia Dolinski main research area is biological control of agricultural pests with entomopathogenic nematodes (EPNs). She is focusing on the control of different pests of guava and developing strategies with EPNs as part of an integrated pest management, so that guava growers can use pesticides only as a last resort. She is also interested in enhancing EPNs through selective pressure, mutagenesis and transgenesis (crossings). Her specific goal is to enhance EPNs for tolerance to high soil temperature and desiccation. She also has conducted several lab and field studies aiming to enhance the efficacy of EPNs in the field, mainly on application and formulation technology. She also conducted surveys in crop fields and native Atlantic Forest areas to obtain new EPNs, which are maintained in her nematode collection. In addition to these applied studies, she is interested on basic biological aspects of EPNs, such as embryogenesis and cytogenetics.