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Virulence of entomopathogenic nematodes to larvae of the guava weevil, *Conotrachelus psidii* (Coleoptera: Curculionidae), in laboratory and greenhouse experiments

Claudia Dolinski [a,*], Eleodoro Del Valle [a], Robin J. Stuart [b]

[a] *Universidade Estadual do Norte Fluminense Darcy Ribeiro, Laboratório de Entomologia e Fitopatologia, Av. Alberto Lamego, 2000, Pq. Califórnia, Campos dos Goytacazes, RJ 28015-620, Brazil*
[b] *University of Florida, IFAS, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850, USA*

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Abstract

The guava weevil, *Conotrachelus psidii*, is a major pest of guava in Brazil and causes severe reduction in fruit quality. This weevil is difficult to control with insecticides because adults emerge over a long period, and larvae develop to the fourth-instar inside the fruit and move to the soil for pupation. We assessed the virulence of entomopathogenic nematodes to fourth-instar larvae in soil by comparing their susceptibility to nine species or strains: *Heterorhabditis bacteriophora* HP88, *H. baujardi* LPP7, and LPP1, *H. indica* Hom1, *Steinernema carpocapsae* All and Mexican, *S. feltiae* SN, *S. glaseri* NC, and *S. riobrave* 355. In petri dish assays with sterile sand at a concentration of 100 infective juveniles (IJs) of a given nematode species/strain, 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, or *S. riobrave* 355 at concentrations of 100, 200, and 500 IJs, mortality was greater than the control only for *H. baujardi* (62.7%) and *H. indica* (68.3%) at the highest concentration. For *H. baujardi* LPP7 in a petri dish assay, the time required to kill 50 and 90% of the larvae (LT_{50} and LT_{90}) for 100 IJs was 6.3 and 9.9 days, whereas the lethal concentration required to kill 50 and 90% of the larvae (LC_{50} and LC_{90}) over 7 days was 52 and 122.2 IJs. In a greenhouse study with guava trees in 20-L pots, 10 weevil larvae per pot, and concentrations of 500, 1000 or 2000 IJs, *H. baujardi* LPP7 caused 30 and 58% mortality at the two highest concentrations. These results show that *H. baujardi* is virulent to fourth-instar larvae and has potential as a biological control agent in IPM programs.

Keywords: IPM; *Conotrachelus psidii*; *Steinernema*; *Heterorhabditis*; Guava; *Psidium guajava*; Entomopathogenic nematodes; Biological control

1. Introduction

The red guava, *Psidium guajava* L. (Myrtaceae), is native to America and widely cultivated in many tropical and subtropical countries (Menzel, 1985) with Brazil being the world's largest producer. In 2002, 400,000 tons of guavas were produced on 14,000 ha for the fresh market and juice processing (Piedade Neto, 2004). Insect pests such as fruit flies, *Ceratitis capitata* (Wiedeman) and *Anastrepha* spp., and the guava weevil, *Conotrachelus psidii* Marshall, are among the most important limitations for guava production (Barelli and Galli, 1998). In Brazil, the guava weevil is the main pest and can be found in virtually every guava orchard. The adult population peaks during summer (November–March) but adults can be found year round in some areas (Barbosa et al., 2001). Mated females lay eggs in green fruit (3–4 cm diameter) and larvae progress through four instars as the fruit develops. The presence of larvae accelerates fruit maturation and, when the fruit ripens and falls to the ground, the larvae crawl into the soil. After an unknown period of time in the soil, larvae develop into prepupae. The prepupal stage can last up to 6 months before pupation and development into

* Corresponding author. Fax: +55 22 27261549.
E-mail address: claudia.dolinski@censanet.com.br (C. Dolinski).

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the adult, which depends on environmental conditions such as soil moisture and temperature (Bailez et al., 2003; Boscán de Martinez and Cásares, 1982).

Control methods for the guava weevil involve weekly applications of insecticides such as organophosphates to suppress the adults, but no insecticide is currently registered for this pest in Brazil and most of those being used against this pest will be discontinued soon (Agência Nacional de Vigilância Sanitária, 2004; Souza et al., 2003). Without chemical control, the percentage of damaged fruit in heavily infested orchards can reach 100% (Boscán de Martinez and Cásares, 1980). The amount of fruit attacked has been increasing rapidly over the past 3 years perhaps because of the development of insecticide resistance, but 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 and Rolland, 1992). Integrated pest management (IPM) is a reasonable alternative to control various insect pests and could be effective against the guava weevil (Dolinski, 2003). One method of biological control that could be used is the application of entomopathogenic nematodes against life stages in the soil. Preliminary tests indicated that some entomopathogenic nematode species are effective against fourth-instar larvae (Dolinski and Samuels, 2002).

Entomopathogenic nematodes (EPNs) in the genera *Heterorhabditis* and *Steinernema* (Rhabditida) are obligate parasites of insects (Poinar, 1990). These nematodes have a symbiotic relationship with bacteria in the genera *Photorhabdus* and *Xenorhabdus*, respectively (Forst and Clarke, 2002). Infective juveniles (IJs), the only stage of the nematodes found in the soil, enter hosts through natural openings such as the mouth, anus or spiracles, but IJs of some species 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 to 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 2–3 generations within the host cadaver. When resources within the cadaver are depleted, a new generation of IJs is produced and leaves the cadaver to search for new hosts (Kaya and Gaugler, 1993).

Entomopathogenic nematodes effectively control a variety of economically important weevil pests including *Diaprepes abbreviatus* (L.), *Conotrachelus nenuphar* (Herbst), *Otiorhynchus sulcatus* (Fabricius) and *Curculio caryae* (Horn) (Olthof and Hagley, 1993; Shapiro and McCoy, 2000; Shapiro-Ilan, 2001a,b; Shapiro-Ilan et al., 2002; Simons, 1981). The objective of our study was to evaluate various species and strains of entomopathogenic nematodes against fourth-instar larvae of *Co. psidii* in laboratory and greenhouse tests as part of an effort to develop entomopathogenic nematodes for an IPM program against the guava weevil.

2. Materials and methods

2.1. Nematodes, larvae, and experimental design

The nematode species and strains used in this study (Table 1) were reared in *Galleria mellonella* (L.) (Pyralidae: Lepidoptera) larvae at 25 °C, according to procedures in Woodring and Kaya (1988). Harvested IJs were kept at 16 °C for less than 1 week before the tests. *G. mellonella* larvae were reared in the laboratory in plastic pots, with a cereal, sugar cane and honey diet (I. Glazer, personal communication). Fourth-instar larvae of *Co. psidii* were derived from guava fruit obtained from orchards in São Francisco de Itabapoana, RJ, Brazil. The fruit was placed in plastic trays (70 × 40 × 10 cm) filled to 4 cm with autoclaved sand and maintained at 25–28 °C. After 3 to 4 days, all larvae left the fruit and were collected and placed in a Gerbox^{TM} (10 × 10 × 5 cm) filled with moist autoclaved sand. For the petri dish, sand column and greenhouse assays, the larvae were used within a few days of leaving the fruit. For the other tests, the larvae were kept at 25 °C for up to 1 month, with only those showing movement being used for testing.

2.2. Petri dish assay

For each replicate, 100 IJs were suspended in 0.5 ml of distilled water and distributed evenly onto a 6 cm-diameter plastic petri dish half filled with autoclaved sand at 10% moisture by weight. One fourth-instar larva of *Co. psidii* was placed in each dish. There were 20 replicates for each nematode strain tested (Table 1) and for the untreated control, which received 0.5 ml of distilled water without nematodes. The petri dishes were placed in plastic bags and incubated in the dark at room temperature (25 ± 2 °C). Larval mortality was recorded after 12 days. All dead insects were transferred to individual modified

Table 1
Species and strains of entomopathogenic nematodes used in this study

Species	Strain	Source/location
Heterorhabditis bacteriophora Poinar	HP88	R. Stuart, University of Florida, Lake Alfred
H. indica Poinar, Karanukar & David	Hom1	R. Stuart, University of Florida, Lake Alfred
H. baujardi Phan, Subbotin, Nguyen & Moens	LPP7[a]	C. Dolinski, Rondônia, Brazil
H. baujardi	LPP1[a]	C. Dolinski, Rondônia, Brazil
Steinernema glaseri (Steiner)	NA	R. Stuart, University of Florida, Lake Alfred
S. feltiae (Filipjev)	SN	R. Stuart, University of Florida, Lake Alfred
S. carpocapsae (Weiser)	All	R. Stuart, University of Florida, Lake Alfred
S. carpocapsae	Mex	R. Stuart, University of Florida, Lake Alfred
S. riobrave Cabanillas, Poinar & Rauston	355	R. Stuart, University of Florida, Lake Alfred

[a] LPP stands for Laboratório de Proteção de Plantas.