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Notes and Comments

Pathogenicity of *Steinernema brazilense* (Rhabditida: Steinernematidae) to *Gonipterus platensis* (Coleoptera: Curculionidae) prepupae

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(With 1 figure)

1. Introduction

The genus *Gonipterus* Marelli, 1927 (Gonipterinae: Curculionidae) has about 20 species known as *Eucalyptus* weevils or *Eucalyptus* snout-beetles (Mapondera et al., 2012). The weevil *G. platensis* was the first introduced eucalyptus pest in New Zealand (1890), Africa (1916), South America (1925), Europe (1975) and North America (1994) (Mapondera et al., 2012). The introduction of key pests as *G. platensis* and *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae) is a risk to forest plantations (Reis et al., 2012; Almeida et al., 2018), such as those of eucalypts (Bhattacharya et al., 2003; Clarke et al., 1998). Adults and larvae of the eucalyptus weevil feed preferably on young leaves, buds and developing shoots causing high damage (Clarke et al., 1998). *G. platensis* is the main eucalypt pests in many regions (Reis et al., 2012) and its impact and that of other eucalyptus weevils is poorly studied, mainly due to the difficulties of identifying control factors influencing this crop productivity (Reis et al., 2012).

The egg parasitoid *Anaphes nitens* (Girault, 1928) (Hymenoptera: Mymaridae) has been used for the biological control of *G. platensis* (Hanks et al., 2000), but this pest has been causing high damage levels (Echeverri-Molina and Santolamazza-Carbone, 2010). The life cycle of entomopathogenic nematodes in the soil make them promising biological control agents of insects with part of its life cycle in the soil as eucalyptus weevils (Barbosa-Negrisoli et al., 2010; Grewal, 2000) including *G. platensis*.

Nematodes of the order Rhabditida (Steinernematidae and Heterorhabditidae) can kill insects due to its mutualistic association with *Xenorhabdus* and *Photorhabdus* bacteria (Poinar Junior and Grewal, 2012). These bacteria kill hosts by septicemia in 24 to 48 h and they are been studied to manage insect pests (Poinar Junior and Grewal, 2012). *Heterorhabditis amazonensis* (Rhabditida: Heterorhabditidae) was isolated from soil samples in Amazonas (Andaló et al., 2006) and *Steinernema brazilense* in Mato Grosso, Brazil (Nguyen et al., 2010). In addition, *H. amazonensis*, *S. rarum*, *Oscheios tipulae, Metarhabditis rainai*, were reported in agriculture and native vegetation areas in different Brazilian regions (Brida et al., 2017).

The adaption to new environments and host searching efficiency (Shapiro-Ilan et al., 2005; Achinelly et al., 2016) reinforce the entomopathogenic nematode potential (EPNs) to manage agriculture and forest pests. *Steinernema* and *Heterorhabditis* species (Rhabditida: Steinernematidae/Heterorhabditidae) (Smits, 1996) are efficient against Curculionidae (Manachini et al., 2013). *Steinernema* and *Heterorhabditis* genera were virulent to *Sphenophorus levis* Vaurie (Coleoptera: Curculionidae) adults (Giometti et al., 2011). *Gonipterus platensis* larvae and adults feed on leaves and pupate in the soil when they may have contact with entomopathogenic nematodes.

The objective of this study was to evaluate the pathogenicity of *S. brazilense* to *G. platensis* prepupae.

2. Material and Methods

The experiment was conducted at the Laboratory of Agricultural Nematology of the São Paulo State University (UNESP) in Botucatu, São Paulo state, Brazil. *Steinernema brazilense* was obtained from the Entomopathogenic Nematode Collection of the Entomopathogens "Oldemar Cardim Abreu" Bank of the Biological Institute of São Paulo, São Paulo, Brazil.

Infective juveniles (IJs) of *S. brazilense* IBCBn 06 were multiplied in *D. saccharalis* (third to fifth instar) larvae (Woodring and Kaya, 1988). Five larvae per Petri dish (9-cm diam) lined with filter paper moistened with a suspension of nematodes at the concentration of 500 IJs/cm^2 of Petri dish surface was used. Dead *D. saccharalis* larvae were transferred to white trap (White, 1927) and stored in chamber at 25 °C, 70 ± 80% RH, in the dark. IJs were obtained from this host and collected in a water film

(1 cm deep) in Erlemeyers maintained in BOD chamber at 18 °C, 70 ± 80% RH and used two days after collection. The *G. platensis* prepupae were collected in a eucalyptus plantation in Itatinga (23°11'35.1"S 48°38'32.5"W) São Paulo state, Brazil.

The nematode *S. brazilense* IBCBn 06 pathogenicity to *G. platensis* prepupae (Woodring and Kaya, 1988) was evaluated in two treatments with five replications, each with four prepupae of this insect per Petri dish (9 cm). Two mL of the suspension of this isolate was applied in aqueous suspension with a pipette, equivalent to a dose of 500 IJ/insect (125 IJ/cm^2) per dish with bottom lined with two filter paper sheets. The control treatment had 2 mL of distilled water per dish. The nematode pathogenicity was evaluated with *D. saccharalis* pupae (Woodring and Kaya, 1988). Petri dishes were sealed with transparent PVC plastic film and stored in BOD chamber at 26 °C and 70% RH in the dark. Dead and live insects were counted after 4 d. Prepupae of *G. platensis* and *D. saccharalis* were rinsed in tap water and individually kept in new Petri dishes (5 cm) with a moistened filter paper for dissection. The number of insect adults, dead prepupae and IJs second generation was quantified under a stereomicroscope.

3. Results and Discussion

Steinernema brazilense IBCBn 06 killed all *G. platensis* prepupae at 4 d after inoculation with infection rates of 10.3 IJs, and a total of 1275.7 IJs produced in the second generation. The *D. saccharalis* pupae parasitism was 100% with infection rate of 15.5, and a total of 7201.9 second generation IJs, proving the high entomopathogenic viability of this nematode. No mortality of *G. platensis* prepupae and *D. saccharalis* pupae was recorded in the controls (Table 1, Figure 1).

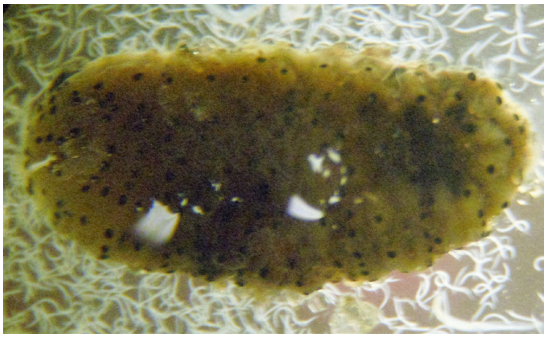

Figure 1. *Gonipterus platensis* (Coleoptera: Curculionidae) prepupae infected by *Steinernema brazilense* IBCBn 06.

The 100% mortality of *G. platensis* prepupae by *S. brazilense* indicates high susceptibility of this weevil stage to parasitism by this nematode. The *S. brazilense* IBCBn 06 pathogenicity was previously reported to important Curculionidae pests as *Anthonomus grandis* (Boheman) (Coleoptera; Curculionidae) (Enrique Cabanillas, 2003), *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae) (Duncan et al., 2003), *Curculio caryae* (Horn) (Coleoptera: Curculionidae) (Shapiro-Ilan et al., 2005). Steinernematidae nematodes live in symbiotic association with bacteria of the genus *Xenorhabdus* (Poinar Junior and Grewal, 2012), responsible for host death and decomposition of their tissues which are used as food for the development this natural enemies (Nermut et al., 2019). Informations on EPNs pathogenicity is important to integrated pest management success (Andaló et al., 2014; Brown and Martin, 2014).

The lower production of *S. brazilense* IBCBn 06 IJs in *G. platensis* pupae than in those of *D. saccharalis* may be due to the larger size of this nematode. This may have caused space and food limitations and a lower offspring number in the first host. The 7201.9 IJs of this nematode produced per *D. saccharalis* pupae confirms results for *H. indica* IBCBn 05 in *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) nymphs (Leite et al., 2003). *S. brazilense* was pathogenic and to *G. platensis* prepupae, however, with a lower production of IJs per host compared to that found with *G. mellonella* larvae with up to 200000 IJs of this microorganism (Dutky et al., 1964).

The nematode *S. brazilense* IBCBn 06 parasitized and killed 100% of *G. platensis* prepupae, showing potential for integrated management of this eucalyptus weevil.

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Table 1. Mortality (%) of *Gonipterus platensis* prepupae (Coleoptera: Curculionidae) and *Diatraea saccharalis* (Crambidae: Lepidoptera) pupae by *Steinernema brazilense* IBCBn 06, number of infecting juveniles (No. IJs) and those produced on the second generation within the host (2nd generation).

Insect	Mortality (%)	No. IJs ± SE	2nd generation ± SE
Gonipterus platensis	100	10.3 ± 2.01	1275.7 ± 474.15
Diatraea saccharalis	100	15.5 ± 4.78	7201.9 ± 2355.88
Gonipterus platensis (control)	0	0	0
Diatraea saccharalis (control)	0	0	0

IJs = Infective juveniles; SE = Standard error.

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