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Short communication

Susceptibility of the peachtree borer, *Synanthedon exitiosa*, to *Steinernema carpocapsae* and *Steinernema riobrave* in laboratory and field trials

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Abstract

The nematode *Steinernema carpocapsae* (All) strain was significantly more effective against peachtree borer larvae (*Synanthedon exitiosa* [Lepidoptera: Sesiidae]) than *Steinernema riobrave* (7–12) strain in field and laboratory experiments. Eighty-eight percent control of peachtree borer larvae was obtained with *S. carpocapsae* in the field trial when applied at 3×10^5 infective juveniles per tree, and 92% mortality was obtained in the lab assay using 50 infective juveniles per larva.
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1. Introduction

The peachtree borer, *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae), is native to North America where its tree-infesting larvae can inflict serious damage to many species of *Prunus* including peach. Larvae feed on the cambium of trunks and large roots forming galleries that are found from about the soil surface to a depth of nearly 30 cm. Young trees are highly susceptible to severe damage by even a single larva (Johnson et al., 2005).

The majority of *S. exitiosa* moths emerge during late summer and early fall (Becker, 1917). Conventional management of *S. exitiosa* across the southeastern US relies solely upon chemical control, mainly chlorpyrifos, directed at newly hatched larvae before they burrow into the cambium. Although highly efficacious, this practice may have little or no future due to environmental concerns regarding broad spectrum insecticides (Tomerlin, 2000). Therefore, we are exploring alternative methods of control. The focus of this study was on determining the potential of entomopathogenic nematodes to control *S. exitiosa* attacking peach.

Orchard systems generally possess attributes, i.e., favorable soil conditions and shade, amenable to insect suppression using entomopathogenic nematodes (e.g., *Steinernema* spp. and *Heterorhabditis* spp.) (Shapiro-Ilan et al., 2005). We anticipate southeastern peach orchard conditions to be suitable for nematode control of *S. exitiosa* as well. The larval feeding galleries on peach trees are accompanied by one or more external openings used by larvae to extrude frass and other debris (Johnson et al., 2005). Thus, the combination of the soil habitat and portals of entry to feeding galleries should provide entomopathogenic nematodes a favorable environment and means to contact and infect *S. exitiosa* larvae.

Previous research has shown that entomopathogenic nematodes are highly virulent to larvae of many species of Sesiidae (Miller and Bedding, 1982; Capinera et al., 1986; Kaya and Brown, 1986; Nachtigall and Dickler, 1992; Smith-Fiola et al., 1996; Williams et al., 2002). In fact, the application of *Heterorhabditis bacteriophora* (= *heliothidis*)

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Poinar to peach trunks, at a rate of 40,000 nematodes per trunk, in mid-June significantly reduced the number of adult *S. exitiosa* that emerged from feeding sites by 80% (Cossentine et al., 1990). Some reports indicate *Steinernema* spp. to be more virulent to certain *Synanthedon* spp. than *H. bacteriophora* (Deseö and Miller, 1985; Nachtigall and Dickler, 1992), yet steinernematids have not been tested for virulence to *S. exitiosa*.

Therefore, our objective was to determine the susceptibility of *S. exitiosa* larvae to two steinernematids: *Steinernema carpocapsae* (Weiser) (All strain) and *Steinernema riobrave* Cabanillas, Poinar and Raulston (7–12 strain). This was accomplished with a field trial in a peach orchard naturally infested with *S. exitiosa* and in a laboratory assay using field-collected *S. exitiosa* larvae.

2. Materials and methods

2.1. Nematodes

Nematodes, *S. carpocapsae* (All strain) and *S. riobrave* (7–12 strain) were cultured in last instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (obtained from Webster's Waxie Ranch, Webster, WI) at 25 °C within the same environmental chamber per methodology of Kaya and Stock (1997). In our laboratory, each nematode was passed through *G. mellonella* less than seven times before use in either laboratory or field experiments. After harvesting, infective juveniles were stored in 250 ml culture flasks at 13 °C and at a maximum concentration of 1×10^4 infective juveniles per ml for <2 weeks before use. For the field and laboratory experiments, infective juveniles were gravity settled and diluted, respectively, to achieve the treatment rate. Nematode viability was $\geqslant$95% in all experiments.

2.2. Field trial

The field trial was conducted in a 1/3 ha peach orchard with tree spacing at 6.1×6.1 m and 270 trees per ha at the USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA. The scion cultivar was O'Henry budded to Guardian® rootstock. The orchard became infested with *S. exitiosa* during the fall of 2004 and has not received chemical insecticide treatments since becoming infested. During this assay, trunk diameter of test trees ranged from 5 to 10 cm.

We used a randomized complete block design with four blocks of five treatments; each treatment was applied to five trees within each block (total of 20 trees per treatment). Only trees with observable signs of active *S. exitiosa* infestation were selected to receive treatments thus the grouping of five trees receiving the same treatment within each block were always grouped but not necessarily contiguous. The following treatments were applied on May 12, 2005: *S. carpocapsae* (1.5×10^5 or 3×10^5 infective juveniles per tree), *S. riobrave* (1.5×10^5 or 3×10^5 infective juveniles per tree), and a water-treated control. Infective juveniles, in 60 ml of water, were poured around the base of each tree, covered with about 2 cm of soil from the orchard floor and watered with about 2 L of water. Control trees were treated the same. All trees were then watered three times per week for the following two weeks. During the first week of July 2005, (i.e., 7 weeks post-application) all trees were sampled for *S. exitiosa* infestation by removing soil from around the base of the trunk and looking for signs of active infestation (Johnson et al., 2005) and also by opening feeding galleries when obvious signs of infestation were not visible.

2.3. Laboratory assay

A laboratory virulence assay was done using late-instar *S. exitiosa* that had been collected from the field. During late July 2005, peach trees of various age and size were mechanically extracted from orchards for collection of *S. exitiosa* larvae. Each larva collected was meticulously removed from its feeding gallery and held singly in a Petri dish containing a moistened paper towel for <5 days at 10 °C. This was necessary as accumulation of larvae could not be completed in one day. These larvae were all late-instars of uniform size due to the single generation of *S. exitiosa* in central Georgia. The assay arena was a 60 mm inverted Petri dish with filter paper lining the lid (Kaya and Stock, 1997). We used a randomized complete block design (RCBD) with four replications of nine larvae per treatment (each larva in a separate Petri dish) and three treatments. Fifty infective juveniles of either *S. carpocapsae* or *S. riobrave* were applied to each Petri dish in 350 µl of water. The control also received 350 µl of water. Field-collected larvae were randomly assigned to the treatments and then dishes were stored in plastic boxes ($31.37 \times 23.02 \times 10.16$ cm) (Pioneer Plastics, Dixon, KY), containing moistened paper towels, within an environmental incubator at 25 °C. Mortality was assessed at 24, 48, and 72 h after treatment.

2.4. Statistical analyses

Cumulative percentage mortality data from the laboratory assay and percentage infestation data from the field trial both satisfied the assumption of equal variance and were analyzed using ANOVA. Tukey's HSD was used for mean separation when $P < 0.05$ (JMP, 2002).

3. Results and discussion

We found that application of 1.5 and 3 hundred thousand infective juveniles of *S. carpocapsae* to peach trees significantly reduced the percentage of trees with an active *S. exitiosa* infestation compared with both rates of *S. riobrave* and control trees ($F = 23.08$; df = 4, 12; $P < 0.0001$) (Fig. 1). We waited 7 weeks after application to sample trees for *S. exitiosa* because differentiation of active (i.e., with fresh frass and feeding activity) and inactive wounds would be readily apparent and the likelihood of re-infestation during this period was low.

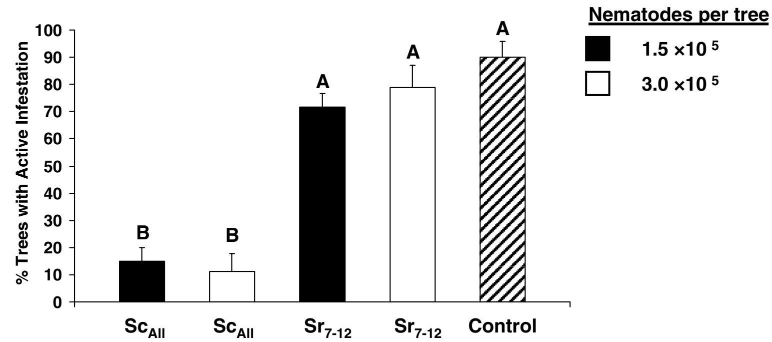

Fig. 1. Results from a field trial showing percentage of peach trees that retained an active infestation of larval peachtree borers, *Synanthedon exitiosa*, seven weeks after treatment with the entomopathogenic nematodes *S. carpocapsae* (All) strain and *S. riobrave* (7–12) strain. Different letters above columns indicate significant difference ($P < 0.05$).

In agreement with our field results, the laboratory virulence assay showed that cumulative percentage mortality of *S. exitiosa* larvae at 72 h after treatment was significantly higher using *S. carpocapsae* compared with *S. riobrave*; mortality induced by both nematode species was significantly greater than control mortality ($F = 38.90$; df = 2,6; $P = 0.0004$) (Fig. 2). No mortality was recorded at 24 h for any treatment, whereas 97% of total mortality due to *S. carpocapsae* occurred between 24 and 48 h. Seventy-five percent of total mortality due to *S. riobrave* occurred between 48 and 72 h.

From the field trial and laboratory assay, it is apparent that *S. carpocapsae* is more virulent to larval *S. exitiosa* than *S. riobrave*. In fact, *S. riobrave* and *Steinernema feltiae* (SN strain) (Filipjev) performed poorly against larval *S. exitiosa* even when three applications were made to the base of peach trees at 4-week intervals (Cottrell and Shapiro-Ilan, unpublished data). It is unlikely that different host-finding strategies exhibited by the 'cruiser' *S. riobrave* and 'ambusher' *S. carpocapsae* (Lewis, 2002) could explain our findings due to the similar results from the field trial and from the laboratory assay where host finding would have been minimized. In addition, *S. exitiosa* larvae are mobile moving up and down in feeding galleries where openings may occur at any point along the length of the gallery.

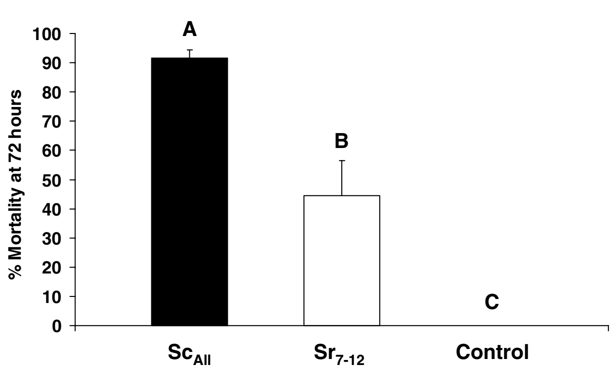

Fig. 2. Cumulative percentage mortality of larval peachtree borers, *Synanthedon exitiosa*, 72 h after treatment with the entomopathogenic nematodes *S. carpocapsae* (All) strain and *S. riobrave* (7–12) strain. Different letters above columns indicate significant difference ($P < 0.05$).

Economical and efficacious alternative pest management strategies must be developed given that organophosphate insecticide usage on peach will continue to decline. Entomopathogenic nematodes may prove to be such an alternative for some peach orchards. Additional field studies are underway to determine nematode strain effects and optimum timing of application to achieve maximum crop protection.

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