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A novel approach to biological control with entomopathogenic nematodes: Prophylactic control of the peachtree borer, *Synanthedon exitiosa*

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ABSTRACT

Generally, microbial control agents such as entomopathogenic nematodes are applied in a curative manner for achieving pest suppression; prophylactic applications are rare. In this study, we determined the ability of two *Steinernema carpocapsae* strains (All and Hybrid) to prophylactically protect peach trees from damage caused by the peachtree borer, *Synanthedon exitiosa*, which is a major pest of stone fruit trees in North America. In prior studies, the entomopathogenic nematodes *S. carpocapsae* and *Heterorhabditis bacteriophora* caused field suppression when applied in a curative manner to established *S. exitiosa* populations. In our current study, nematodes were applied three times (at 150,000–300,000 infective juveniles/tree) during September and October of 2005, 2006, and 2007. A control (water only) and a single application of chlorpyrifos (at the labeled rate) were also made each year. The presence of *S. exitiosa* damage was assessed each year in the spring following the treatment applications. Following applications in 2006, we did not detect any differences among treatments or the control (possibly due to a low and variable *S. exitiosa* infestation of that orchard). Following applications in 2005 and 2007, however, the nematode and chemical treatments caused significant damage suppression. The percentage of trees with *S. exitiosa* damage in treated plots ranged from 0% damage in 2005 to 16% in plots treated with *S. carpocapsae* (Hybrid) in 2007. In control plots damage ranged from 25% (2005) to 41% (2007). Our results indicate that nematodes applied in a preventative manner during *S. exitios*'s oviposition period can reduce insect damage to levels similar to what is achieved with recommended chemical insecticide treatments.

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1. Introduction

When applied inundatively, microbial control agents (bacteria, protozoa, fungi, and entomopathogenic nematodes) are generally used in a curative manner to control an existing pest population (Tanada and Kaya, 1993; Lacey and Kaya, 2007). Due to economic constraints, and in some cases a lack of persistence in the environment, field application of microbial control agents in a preventative manner is relatively rare (Shapiro-Ilan et al., 2002; Lacey and Shapiro-Ilan, 2008). In this study, we investigated the feasibility of using prophylactic microbial control when applying entomopathogenic nematodes for control of the peachtree borer, *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae).

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) kill insects with the aid of a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990). Infective juveniles (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their bacterial symbionts, which are primarily responsible for killing the host within 24–48 h, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002). The nematodes molt and complete up to three generations within the host after which IJs exit the cadaver to find new hosts (Kaya and Gaugler, 1993).

Entomopathogenic nematodes are used to control a variety of economically important insect pests such as the black vine weevil, *Otiorhynchus sulcatus* (F.), diaprepres root weevil, *Diaprepes abbreviatus* (L.), fungus gnats (Diptera: Sciaridae), and various white grubs (Coleoptera: Scarabaeidae) (Klein, 1990; Shapiro-Ilan et al., 2002; Grewal et al., 2005a). Additionally, entomopathogenic nematodes are highly virulent to larvae of many species of Sesiidae including several *Synanthedon* spp. (Miller and Bedding, 1982; Deseö and Miller, 1985; Kaya and Brown, 1986; Begley, 1990; Nachtigall and Dickler, 1992; Williams et al., 2002).

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Synanthedon exitiosa (Lepidoptera: Sesiidae), is a serious pest of various *Prunus* spp. including peach (*Prunus persica* L.) (Johnson et al., 2005). In the southeastern US, the majority of *S. exitiosa* moths emerge and mate during late-summer and early fall (Johnson et al., 2005). Mated adult females usually oviposit eggs (200 to 800 in total) on the bark of host plants or on nearby non-host plants. Hatched larvae bore into the trunk of stone fruit trees near the soil surface and tunnel toward roots. Larvae continue to feed below the soil line at the crown and on major roots. Larvae overwinter in the host plant, but can continue to feed during warm periods, and (in the eastern US) complete development in about 1 year. Current management of *S. exitiosa* across the southeastern US relies solely upon post-harvest chemical control, mainly chlorpyrifos (Horton et al., 2008). Due to environmental and regulatory pressures, research toward developing alternative pest control measures are warranted (Tomerlin, 2000). Entomopathogenic nematodes have potential as biocontrol alternatives for *S. exitiosa* suppression.

Under field conditions, entomopathogenic nematodes caused significant *S. exitiosa* mortality and suppressed damage when applied in a curative manner to late-instar infestations (Cossentine et al., 1990; Cottrell and Shapiro-Ilan, 2006). Application of *H. heliothidis* (=*bacteriophora*) to peach trunks in mid-June significantly reduced the number of adult *S. exitiosa* that emerged from feeding sites by approximately 80% (Cossentine et al., 1990). Additionally, 88% control of *S. exitiosa* larvae was obtained with *Steinernema carpocapsae* (Weiser) in a field trial conducted in the spring (Cottrell and Shapiro-Ilan, 2006). Although such curative treatments may contribute to protecting the tree and reducing subsequent populations, substantial damage from larval feeding will have already occurred by the spring and could result in tree death (Johnson et al., 2005). Indeed, to avoid damage, recommendations for control with chemical insecticides are focused on the *S. exitiosa* egg-laying period and directed at newly hatched larvae before they burrow into the cambium (Johnson et al., 2005; Horton et al., 2008). Our objective was to determine if entomopathogenic nematodes could be applied using a similar approach, i.e., with prophylactic applications to reduce or prevent *S. exitiosa* damage.

2. Materials and methods

2.1. Nematode cultures

The two nematode strains used in this study, *S. carpocapsae* (All strain and Hybrid strain) were cultured at 25 °C in last instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (obtained from Webster's Waxie Ranch, Webster, WI) according to procedures described by Kaya and Stock (1997). *Steinernema carpocapsae* (All strain) was chosen based on prior efficacy observed in the laboratory and field (Cottrell and Shapiro-Ilan, 2006), and the Hybrid strain was added for comparison. The Hybrid strain was created by crossing *S. carpocapsae* DD136 and Italian strains; the resulting hybrid exhibited superior efficacy in control of the pecan weevil, *Curculio caryae* (Horn) (Shapiro-Ilan et al., 2005). After harvesting, IJs were stored at 13 °C for <2 weeks before use. Nematode viability was ⩾95% in all experiments.

2.2. Field efficacy trials

Nematodes were applied in the late-summer and early fall during three consecutive years from 2005 to 2007 at the USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory's research farm in Byron, Georgia. The experiments were implemented in a 0.25 ha peach orchard (cultivar: Redskin, soil was a sandy loam) with tree spacing at 2.5 m between trees within rows and 5.0 m between rows. During these experiments, average trunk diameter of test trees ranged from 31 to 108 mm.

In each year the experiment was conducted, nematodes (*S. carpocapsae* All and Hybrid strain) were applied to the same trees three times during *S. exitiosa*'s egg-laying period. In 2005, nematodes were applied September 2, September 23, and October 14 at a rate of 150,000 IJs/tree in the first two applications and 300,000 IJs/tree in the third application. In 2006, nematodes were applied on September 21, September 28, and October 12 at 300,000 IJs/tree in the first two applications and 150,000 IJs/tree in the third application. In 2007, nematodes were applied on September 24, October 1, and October 9 at 300,000 IJs/tree on each application date. The same trees were used each year for each of the given treatments (except that additional trees were added in 2007). Nematodes were applied by pouring approximately 60 ml water suspensions around the base of each tree. The application sites were then covered with about 2 cm of soil from the orchard floor and watered with an additional 2 l of water. Control trees receiving water only were treated the same. Additionally, each year on the date of the first nematode application, a single application of chlorpyrifos (Lorsban 4E, Dow AgroSciences LLC, Indianapolis, IN, USA) was made in a similar manner by applying 237 ml solution/tree, which was based on the recommended label rate, (i.e., 7 L of formulated product/ha [44.9% A.I.]). All trees were then watered three times per week for the following 2 weeks. Precipitation and soil temperatures were monitored each year during the periods that nematodes were expected to be active, i.e., from the first application until 2 weeks after the last application; these data were collected from a weather station located on the USDA-ARS research farm approximately 0.32 km from the application site. The experiments were arranged in a randomized complete block design with four blocks of six trees per treatment in 2005 and 2006, and four blocks of eight trees per treatment in 2007.

Treatment effects from all applications were evaluated the following spring, i.e., May 19, 2006, May 24, 2007, and April 16, 2008. On each evaluation date, the presence or absence of *S. exitiosa* infestation on each tree was evaluated according to Cottrell and Shapiro-Ilan (2006). Briefly, soil was removed to approximately 12 cm depth around the base of each tree and examined for signs of infestation, e.g., galleries and frass exudates (Johnson et al., 2005; Cottrell and Shapiro-Ilan, 2006).

2.3. Adult S. exitiosa trap captures

Adult male *S. exitiosa* presence was monitored during 2 years of the experiment, i.e., 2005 and 2007. Trap captures for the local *S. exitiosa* population were used to estimate the potential overlap between the timing of nematode applications and target pest activity (i.e., egg-laying). Due to a shortage in labor we could not conduct the monitoring in 2006. Nine traps (Pherocon 1C trap, Trece Inc., Adair, OK), each baited with a pheromone lure (clearwing borer spp., Scentry Biologicals, Inc., Billings, MT) were set up randomly at the USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory's research farm within 0.48–2.6 km from the research plots. Trap lures were changed every 4 weeks. The traps were checked weekly from June 1 through November 15, and the average capture per trap for each date was calculated.

2.4. Statistical analyses

Treatment effects in field efficacy trials were analyzed using two-way ANOVA; if the *F* statistic was significant ($\alpha = 0.05$) treatment differences were further elucidated through the Student–Newman–Keul's test (SAS, 2001). Percentage mortality was arcsine transformed prior to analysis (SAS, 2001; Steel and Torrie, 1980). Non-transformed means are presented in figures.

3. Results

In field experiments, differences were detected among treatments and the control following nematode applications made in 2005 and 2007, but not 2006 ($F = 84.73$; df = 3,9; $P < 0.0001$ for 2005; $F = 2.37$; df = 3,9; $P = 0.1389$ for 2006, and $F = 7.65$; df = 3,9; $P = 0.0076$ for 2007) (Fig. 1). Following the infestation assessment for the 2005 applications, percentage *S. exitiosa* infestation in the control was higher than in both nematode treatments and the chlorpyifos treatment (0% infestation was observed in these treatments) (Fig. 1). In 2007, percentage infestation in the control was higher than in the nematode and chemical treatments, which were not different from each other (Fig. 1). Average (±SD) daily minimum and maximum soil temperatures (°C) for the application periods, from the first nematode application until 2 weeks after the last application, were 22.1 ± 3.3 and 27.9 ± 3.5 for 2005 (range = 13.3–32.2), 18.4 ± 3.0 and 24.6 ± 3.3 for 2006 (range = 11.6–29.4), and 21.0 ± 2.4 and 27.0 ± 2.5 for 2007 (range = 17.3–30.6), respectively. Average (±SD) precipitation for the application periods (from the first nematode application until 2 weeks after the last one) were 0.63 ± 3.39 mm for 2005, 0.98 ± 3.92 mm for 2006, and 1.45 ± 3.69 mm for 2007.

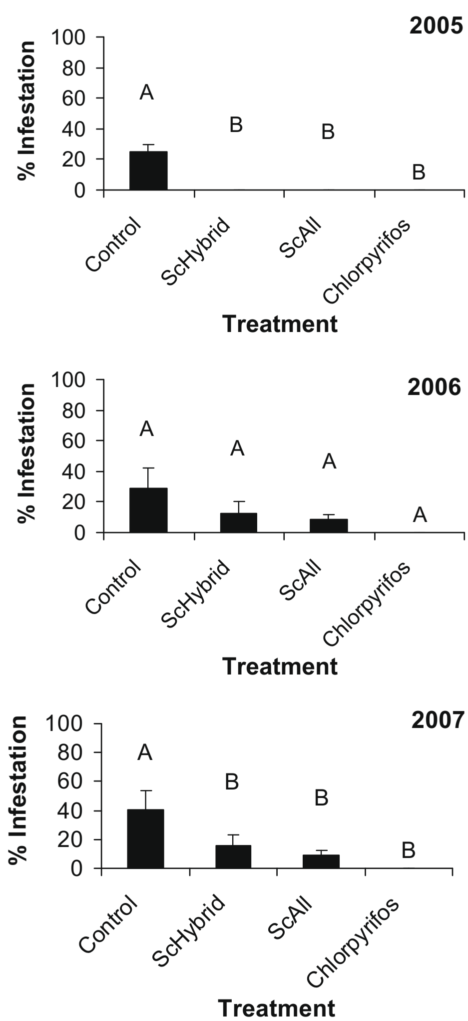

Fig. 1. Average (±SE) percentage of peach trees infested with *Synanthedon exitiosa* larvae in field trials conducted in Byron, Georgia. Nematodes, *Steinernema carpocapsae* (hybrid strain and all strain), were applied three times (at 150,000–300,000 infective juveniles per tree) during September and October of 2005, 2006, and 2007. A control (water only) and a single application of chlorpyrifos (at the labeled rate) were also made each year. The presence of *S. exitiosa* damage was assessed each year in the spring following the treatment applications (approximately 6–7 months after applications were completed). Different letters above bars indicate statistically significant differences (SNK test, $\alpha = 0.05$).

Adult *S. exitiosa* activity based on male captures was detected from June 1 to November 4, 2005 and from June 1 to November 15, 2007 (Fig. 2). In 2005, the bulk of activity was detected between mid-August to mid-October, e.g., between August 16th and October 14th, 74% of the total adult male *S. exitiosa* were captured, and only 40% were trapped during June, July and August (Fig. 2). In 2007, a decline in captures was also obvious by mid-October, but the emergence pattern appeared more spread out, e.g., between August 16th and October 12th, 67% of the total adult male *S. exitiosa* were captured, and more than 55% were trapped during June, July and August (Fig. 2).

A larger proportion of moth captures was detected within the interval of nematode applications in 2005 relative to 2007. In 2005, approximately 48% of the total trap captures occurred prior to the first nematode application (September 2), whereas approximately 77% of captures occurred prior to the first nematode application in 2007 (September 24). Additionally, <1% of the total moths were captured after the last nematode application in 2005 (October 14), whereas approximately 11% were made after the last application in 2007 (October 9).

4. Discussion

Results from our field tests indicate that, *S. carpocapsae*, when applied during egg-laying season, can prevent *S. exitiosa* damage at levels similar to the current recommended chemical insecticide application. Indeed, the levels of *S. exitiosa* damage detected in the nematode treatments (which encompassed three applications per strain each year) did not differ from the chemical insecticide treatment in any of the 3 years of field experiments. However, it must be noted that following the second year of applications (2006), no differences in damage were detected among any of the treatments or the non-treated control. We speculate that this result was due to low yet variable levels of damage across the control and treatments (probably due to a low insect population); thus, to try and overcome the problem and increase power in the experiment, we included additional trees in the experiment in the subsequent year. One might speculate that slightly lower temperatures during the 2006 applications may have contributed to the discrepancy in results among years, but this is unlikely to be the primary explanation because the average maximum and minimum soil temperatures in 2006 were within range of *S. carpocapsae* activity (Grewal et al., 1994), and because a significant treatment effect was also not detected in the chlorpyrifos plots (and temperature would be an improbable cause for this). To avoid the problem of variable *S. exitiosa* population sizes causing a lack of statistical power, we suggest that a maximum number of replicates always be included in future studies; alternatively, the population could be bolstered by obtaining gravid females from the field, and distributing eggs to trees within the experimental plots.

Our *S. exitiosa* trap capture data in 2005 and 2007 indicated that the nematode applications were effective even though the intervals of application and *S. exitiosa* egg-laying periods did not completely overlap with each other, i.e., substantial moth activity was detected prior to the nematode applications (and to some extent after nematode applications). Therefore, *S. carpocapsae* was apparently capable of suppressing damage caused by young *S. exitiosa* larvae that were present prior to nematode application. Such mechanisms of infection parallel nematode activity in other systems such as the ability of *Heterorhabditis* sp. (Alcàzar-1) to protect potato (*Solanum* spp.) tubers from damage caused by neonate and young instar Andean potato weevils (*Premnotrypes* spp.) (Parsa

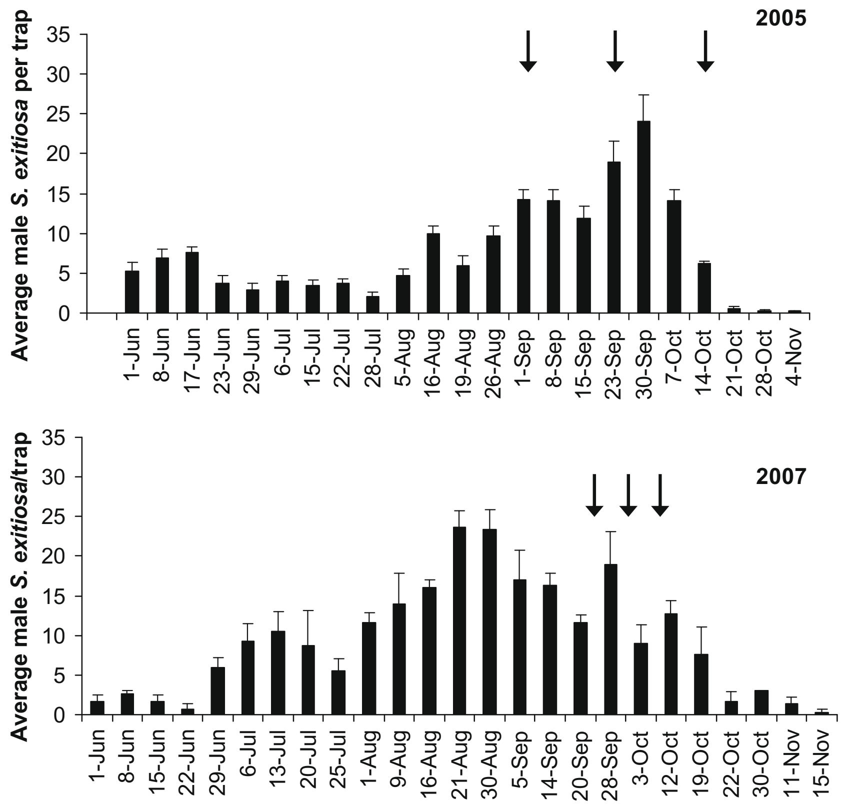

Fig. 2. Average (±SD) capture of male *Synanthedon exitiosa* per trap in 2005 and 2007. Each bar represents the average capture from nine pheromone traps that were placed in the USDA–ARS, Southeastern Fruit and Tree Nut Research Laboratory's research farm in Byron, Georgia. Arrows indicate dates that nematodes, *Steinernema carpocapsae* (Hybrid strain and All strain), were applied for suppression of *Synanthedon exitiosa*.

et al., 2006). Thus, conceivably a single late-season application (possibly with a higher rate) might be as effective as the multiple applications we made in this study. On the other hand, the fact that nematode efficacy in 2005 was arguably superior than in 2007 (because no damage was observed in the 2005 treatments), and that the application period in 2005 encompassed a relatively larger proportion of the *S. exitiosa* emergence period, suggests that a more spread out series of nematode applications (with lower rates) may be the best strategy. Clearly, additional research is required to determine the mechanisms of *S. exitiosa* infection as well as the optimum number and timing of nematode applications.

Due to the cost of product and in some cases a lack of persistence in the environment, microbial control agents are usually used to achieve economic pest control in a curative rather than preventative manner. Exceptions in which microbial control agents have been implemented or considered in a prophylactic approach tend to be in protected or controlled environments where prolonged environmental persistence is expected, e.g., in storage facilities or greenhouses (Franz, 1971; McLauglin, 1971; Moorehouse et al., 1993). For example, the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin can be applied as a prophylactic treatment to control *O. sulcatus* in potted plants and in the greenhouse (Moorehouse et al., 1993; Bruck, 2005; Shah et al., 2007). Due to the persistence of *M. anisopliae* in various potting media (Bruck, 2005), it is possible to obtain efficacious prophylactic control, and indeed the approach can be superior to curative treatments, perhaps due to the allowance of more time for conidia to disperse and cause infection (Moorehouse et al., 1993).

Similar to other microbial control agents, in commercial settings, entomopathogenic nematodes are applied almost exclusively in a curative rather than prophylactic manner, i.e., after the problem has been detected rather than before (Grewal et al., 2005a). However, in some cases there is potential for a preventative approach (Grewal et al., 2005b). The approach we used in this study, i.e., using *S. carpocapsae* to prevent *S. exitiosa* damage, may prove to be one of these exceptions. Another example in which preventative control was achieved was reported by Toepfer et al. (2008); damage by the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) was reduced when entomopathogenic nematodes were applied during sowing of corn (*Zea mays* L.). Also, Kim et al. (2004) reported that *S. carpocapsae*, applied for control of a fungus gnat, *Bradysia agrestis* Sasakawa (Diptera: Sciaridae) at the time of sowing, reduced mortality of watermelon plants in a seedling propagation house. For many pest complexes and associated commodities, the cost of entomopathogenic nematode application (even in a curative manner) may be excessive due to low crop value and where the proportion of acreage that must be treated is large (Shapiro-Ilan et al., 2002), and thus, from an economic perspective preventative treatments with entomopathogenic nematodes would not be an option. In the case of *S. exitiosa* control, however, the extremely narrow area that needs to be targeted (i.e., only around the tree base) should increase economic feasibility of a prophylactic approach. For example, if nematodes are applied at the highest rates used in this study the total number of IJs used would be less than 250 million/ha (900,000 IJs/tree × 250 to 275 trees/ha) for all three applications each year. Thus, based on a standard minimum recommended application rate of 25 IJs/cm^2 of treated area (Georgis and Hague, 1991; Shapiro-Ilan et al., 2002, 2006), applications for preventative *S. exitiosa* suppression would require 10-fold less than the amount needed relative to applications requiring that the entire acreage be covered.

Based on regulatory trends, organophosphate insecticide usage on peach will likely continue to decline. Therefore, efficacious and economically sound alternative pest management strategies must be developed. Previous research indicated that curative applications of entomopathogenic nematodes can effectively reduce *S. exitiosa* populations and damage (Cottrell and Shapiro-Ilan, 2006), and in this study we have now demonstrated that preventative treatments are also efficacious. Preventative approaches are especially important for *S. exitiosa* because the insect's activity is often undetected until significant damage has been done (Nielsen, 1981). Overall our research indicates that entomopathogenic nematodes (particularly *S. carpocapsae*) have considerable promise as biocontrol agents for *S. exitiosa*. Additional research is needed to optimize application parameters (timing issues as discussed above as well as rates and methods of application), and to determine if different nematode species or strains might increase the efficiency of the approach.

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