

Curative Control of the Peachtree Borer Using Entomopathogenic Nematodes

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Abstract: The peachtree borer, *Synanthedon exitiosa* (Say 1823), is a major pest of stone fruit trees in North America. Current management relies upon preventative control using broad-spectrum chemical insecticides, primarily chlorpyrifos, applied in the late summer or early fall. However, due to missed applications, poor application timing, or other factors, high levels of *S. exitiosa* infestation may still occur and persist through the following spring. Curative treatments applied in the spring to established infestations would limit damage to the tree and prevent the next generation of *S. exitiosa* from emerging within the orchard. However, such curative measures for control of *S. exitiosa* do not exist. Our objective was to measure the efficacy of the entomopathogenic nematode, *Steinernema carpocapsae*, as a curative control for existing infestations of *S. exitiosa*. In peach orchards, spring applications of *S. carpocapsae* (obtained from a commercial source) were made to infested trees and compared with chlorpyrifos and a water-only control in 2014 and 2015. Additionally, types of spray equipment were compared: nematodes were applied via boom sprayer, handgun, or trunk sprayer. To control for effects of application method or nematode source, in vivo laboratory-grown *S. carpocapsae*, applied using a watering can, was also included. Treatment effects were assessed 39 d (2014) or 19 d (2015) later by measuring percentage of trees still infested, and also number of surviving *S. exitiosa* larvae per tree. Results indicated that *S. carpocapsae* provided significant curative control (e.g., >80% corrected control for the handgun application). In contrast, chlorpyrifos failed to reduce *S. exitiosa* infestations or number of surviving larvae. In most comparisons, no effect of nematode application method was detected; in one assessment, only the handgun and watering can methods reduced infestation. In conclusion, our study indicates that *S. carpocapsae* may be used as an effective curative measure for *S. exitiosa* infestations.

Key words: application method, curative, entomopathogenic nematode, peachtree borer, *Steinernema carpocapsae*, *Synanthedon exitiosa*.

The peachtree borer, *S. exitiosa* (Lepidoptera: Sesiidae), is a major pest of *Prunus* spp. including peach (*Prunus persica* L.) (Johnson et al., 2005). In the southeastern United States, the majority of *S. exitiosa* moths emerge and mate during late summer and early fall (Johnson et al., 2005). Mated females deposit eggs on the bark of host plants, or sometimes on nearby nonhost plants. Hatched larvae bore into the trunk of stone fruit trees near the soil surface and tunnel into the roots. Larvae feed below the soil surface at the crown and on major roots. The larvae overwinter in the host plant, but can continue to feed during warm periods, and (in the southeastern United States) complete development in about 1 yr. Current management of *S. exitiosa* across the southeastern United States relies solely upon preventative postharvest chemical control, particularly, chlorpyrifos; applications are generally made in the late summer or early fall to prevent or limit damage (Horton et al., 2016).

Although the current practices of chemical insecticide application in the late summer or early fall treatments for *S. exitiosa* control are generally highly effective, missed applications, poor application timing, or other

factors can result in high levels of *S. exitiosa* infestation, which become apparent the following spring. Curative treatments, applied to established infestations, would limit damage to the tree and prevent the next generation of *S. exitiosa* from emerging within the orchard. However, such curative measures for control of *S. exitiosa* do not exist. In this study, we explored the potential to use entomopathogenic nematodes as a curative treatment for *S. exitiosa* control.

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) kill insects with the aid of mutualistic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990; Lewis and Clarke, 2012). Infective juveniles (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or sometimes through the cuticle. After entering the insect's hemocoel, nematodes release their bacterial symbionts, which are primarily responsible for killing the host within 24 to 48 h, providing the nematodes with nutrition, and defending against secondary invaders (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 to attack (Poinar, 1990; Lewis and Clarke 2012).

Entomopathogenic nematodes are commercially available biocontrol agents that are used to control a variety of economically important insect pests, such as the black vine weevil, *Otiorhynchus sulcatus* (F.), diaprepes root weevil, *Diaprepes abbreviatus* (L.), fungus gnats (Diptera: Sciaridae), thrips (Thysanoptera), and various white grubs (Coleoptera: Scarabaeidae) (Klein, 1990; Shapiro-Ilan et al., 2002, 2014; Grewal et al., 2005). Furthermore, entomopathogenic nematodes

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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). The efficacy of entomopathogenic nematode applications, however, can be limited by adverse environmental conditions such as UV radiation or desiccation (Shapiro-Ilan et al., 2006).

Prior research conducted in Georgia peach orchards indicated that preventative applications of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser), can reduce *S. exitiosa* infestations at the same level as chlorpyrifos (Shapiro-Ilan et al., 2009, 2015). Specifically, when nematodes were applied prophylactically in the fall to prevent or limit damage, infestations were reduced by 77% to 100% (Shapiro-Ilan et al., 2009, 2015). We hypothesized that entomopathogenic nematodes would also provide high levels of *S. exitiosa* control when applied curatively. Entomopathogenic nematodes may be particularly capable of providing curative control of established infestations because they are known to enter the wooden galleries and cause mortality in other Sesiidae pests (Lacey and Shapiro-Ilan, 2008; Shapiro-Ilan et al., 2016).

Indeed, some promise in the curative approach has been demonstrated previously (Cossentine et al., 1990; Cottrell and Shapiro-Ilan, 2006). However, Cossentine et al. (1990), using *Heterorhabditis bacteriophora* Poinar, only conducted a single-season experiment (no repeat) and used rubber sponge collars around the base of each trunk to plug the *S. exitiosa* tunnels and create an artificially moist environment for the nematodes. Cottrell and Shapiro-Ilan (2006) also only conducted a one-season trial (no repeat) and applied the nematodes (*S. carpocapsae* and *Steinernema riobrave* Cabanillas Poinar & Raulston) by pouring them onto the base of each tree and covering the site with 2 cm of soil to protect the nematodes from UV radiation and desiccation. Thus, the previous studies (Cossentine et al., 1990; Cottrell and Shapiro-Ilan, 2006), though encouraging, lacked sufficient replication to confirm the approach, and the methodologies used cannot be deemed practical for large-scale commercial application. Therefore, our study expands on the previous.

Our objective was to measure the efficacy of *S. carpocapsae*, as a curative control for existing infestations of *S. exitiosa* in replicated field trials (conducted over 2 yr). Furthermore, expanding beyond prior research on curative *S. exitiosa* control, we compared *S. carpocapsae* treatments to applications of chlorpyrifos (the most popular chemical insecticide used for this pest). Finally, given that application method or equipment can affect nematode efficacy (Hayes et al., 1999; Nilsson and Gripwall, 1999; Brusselman et al., 2010; Shapiro-Ilan et al., 2012), we also compared nematode application using various equipment including a boom sprayer, handgun, and trunk sprayer. The application methods

are akin to what commercial peach growers use (a watering can application was also used as a positive control). In previous research, we found that these application methods did not affect the efficacy of *S. carpocapsae* in preventative applications (Shapiro-Ilan et al., 2015), and in this study we explore the approaches for curative treatment.

MATERIALS AND METHODS

Nematodes and experimental sites: Treatments were applied in the spring of 2014 and 2015 to determine potential for curative control of *S. exitiosa*. *Steinernema carpocapsae* (All strain) was used in the experiments. In both years of the study, treatments included nematodes obtained from a commercial source (e-nema GmbH, Schwentental, Germany) and also produced in vivo at the USDA-ARS laboratory (Byron, GA) according to procedures described by Kaya and Stock (1997). Nematodes were used within 3 wk of receipt and viability on application was >90% for all treatments.

In the first year of the experiment, applications were made in a commercial peach orchard in Fort Valley (32°31'09.99N 83°51'58.01W), Georgia. Peaches (Cresthaven variety) were 2 yr old in 2012 and spaced approximately 5.5 × 4.6 m. The soil was a loamy sand with the percentage sand:silt:clay = 70:20:10, pH = 5.7, and organic matter = 1.5% by weight. In the second year of the experiment, the location was a peach orchard at the USDA-ARS, Southeastern Fruit and Tree Nut Laboratory in Byron Georgia (32°33'42.51N 83°50'35.32W); trees (June Prince variety) were 7 yr old and spaced 6.1 × 6.1 m apart. The soil was a loamy sand with the percentage sand:silt:clay = 76:16:8, pH = 5.6, and organic matter = 1.0% by weight.

Experimental approach: The experiments were organized as randomized complete block designs with four blocks of five treatments and a nontreated control (water only). Blocks were separated by a minimum of 18 m. Each treatment was applied to five trees within each block (total of 20 trees per treatment). Only trees with noticeable signs of active *S. exitiosa* infestation were selected to receive treatments (Cottrell and Shapiro-Ilan, 2006); the five trees that received the same treatment within each block were grouped together continuously in a row. Three treatments included nematodes applied via three different mechanical sprayer types: boom sprayer (part # 45030051 with 140" 7' nozzle, Moose Utility, Janesville, WI), automated trunk sprayer (Anonymous, 2005), and handgun (65 psi, part # 45030048, Moose Utility, Janesville, WI). The nematodes applied in these three treatments (comparing spray equipment) utilized commercially produced nematodes; thus, the approaches were akin to what peach growers would use in commercial applications. As a fourth treatment, laboratory-grown nematodes were applied manually via watering can. This treatment was

considered a positive control because we had previously demonstrated *S. carpocapsae* (All) produced in this manner and applied manually would suppress *S. exitiosa* damage (Cottrell and Shapiro-Ilan, 2006; Shapiro-Ilan et al., 2009). Thus, our goal in including the positive control was to compare commercial-scale options available to growers (mechanical spray equipment and commercially obtained nematodes) to a smaller scale approach that had already been proven to be efficacious. Application of chlorpyrifos (Lorsban, Dow AgroSciences, Indianapolis, IN) was also included for comparison (a fifth treatment), and a nontreated (negative) control of water-only.

Nematodes were applied at a rate of one million IJs per tree (roughly 350 IJs per cm² of soil surface but this varied based on run-off, local topography around the trunk, etc.). The amount of water used per tree for each application varied based on the nature of equipment and was 2,000 ml for the boom sprayer, 600 ml for handgun, 800 ml for trunk sprayer, and 4,085 ml for watering can. All applications (and thus quantities of water usage) were made in a manner that commercial growers employ. Chlorpyrifos was applied in 600 ml via handgun using a recommended rate of 29.57 ml of product per 3,785 ml water (Horton et al., 2016). The nontreated controls received 700 ml water via handgun.

Soil moisture is critical to entomopathogenic nematode survival and infectivity and therefore irrigation is generally recommended in conjunction with nematode applications (Shapiro-Ilan et al., 2006). However, similar to a large proportion of commercial peach acreage in the southeastern United States, the orchards used in our study lacked irrigation. Therefore, to overcome potential desiccation issues, Barricade gel (Barricade International, Inc., Hobe Sound, FL) was applied to all trees receiving nematode treatments. Prior research indicated that this water-retaining gel can be used in lieu of irrigation for nematode applications targeting *S. exitiosa* (Shapiro-Ilan et al., 2015). The gel was sprayed on soil immediately after nematode application to about 1.5-cm thickness in a 60-cm radius around the base of the tree; the application was made using the manufacturer's spray device at the recommended rate (approximately 4% gel).

In 2014, treatments were applied on 16 May and assessed on 24 June. In 2015, treatments were applied 29 May and assessed on 17 June. Treatment effects were assessed by removing soil from around the base of each trunk and looking for signs of active infestation (i.e., living larvae still present) (Johnson et al., 2005). If infestations were present, then the total number of surviving *S. exitiosa* larvae was determined by using a flat screw driver to open each gallery that was then completely explored. Thus, both percentage of infestations remaining active, and total number of live larvae, were recorded and analyzed. Weather parameters were recorded during the experimental period, i.e., from nematode application to treatment assessment, and

included ambient temperature (minimum, maximum, and daily average), soil temperature (10.16-cm depth), and precipitation.

Statistical analyses

Treatment effects were analyzed with analysis of variance (ANOVA). If the ANOVA detected a significant difference ($P \leq 0.05$) then treatment differences were elucidated through Tukey's test (SAS, 2002). Percentage data (percentage of trees with *S. exitiosa* infestation) were arcsine transformed and numerical data (number of *S. exitiosa* per tree) were square root transformed prior to analysis (Southwood, 1978; Steel and Torrie, 1980; SAS, 2002). Nontransformed means are presented in the Results section and associated figures. To estimate the potential level of *S. exitiosa* control that can be obtained, Abbott's (1925) formula was applied to the two treatments with the numerically lowest level of infestation and number of insects per tree.

RESULTS

Weather data indicated that ambient and soil temperatures fell within the range of *S. carpocapsae* activity (Grewal et al., 1994) during the experimental period (Table 1). In 2014, differences among treatments and the control were detected in percentage of active *S. exitiosa* infestations ($F_{5, 15} = 6.44, P < 0.0022$). Percentage active *S. exitiosa* infestation was lower in all nematode treatments compared with the nontreated control, whereas infestation in the chlorpyrifos treatment was not different from the control (Fig. 1). Percentage infestation in plots receiving the watering can treatment was lower than the boom sprayer treatment and chlorpyrifos treatment, but otherwise no differences among treatments were detected. Numerically, the handgun and watering can treatments caused the lowest infestation (20% infestation for both treatments). When Abbott's formula was applied to percentage infestation for the handgun and watering can treatments, the level of control was 75%.

Also in 2014, relative to the nontreated control, the mean number of live *S. exitiosa* per tree was lower in all treatments except chlorpyrifos ($F_{5, 107} = 8.73, P < 0.0001$)

TABLE 1. Mean temperatures (°C) and precipitation (cm) during the period from *Steinernema carpocapsae* application to assessment of surviving *Synanthedon exitiosa*.^a

Parameter	2014	2015
Ambient maximum temperature	31.13	32.13
Ambient minimum temperature	18.29	19.54
Ambient average daily temperature	24.71	25.84
Soil temperature (10.16 cm depth)	26.73	28.36
Precipitation	0.20	0.18

^a In 2014, nematodes were applied 16 May and *S. exitiosa* survival assessed on 24 June; In 2015, nematodes were applied on 29 May and *S. exitiosa* assessed on 17 June.

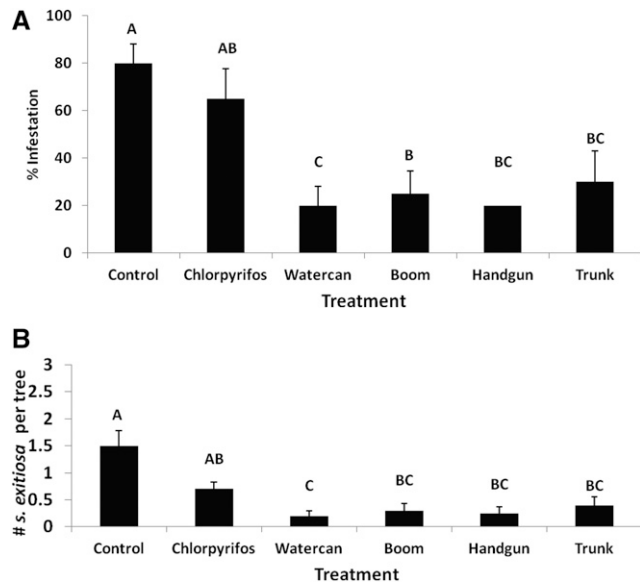


FIG. 1. (A) Percentage infestation and (B) number of surviving *Synanthedon exitiosa* following treatments of *Steinernema carpocapsae* or chlorpyrifos: treatments were applied to existing *S. exitiosa* infestations in the spring of 2014. Nematodes were applied using a boom sprayer, handgun, trunk sprayer, or watering can; chlorpyrifos was applied using a handgun. Control = water only. Treatments effects were assessed 39 d after application. Different letters above bars indicate statistically significant differences (Tukey's test, $\alpha = 0.05$).

(Fig. 1). No differences were detected among nematode treatments. Numerically, the lowest *S. exitiosa* survival was observed in the handgun treatment and watering can; based on Abbott's formula, these treatments provided 83.3% and 86.7% control, respectively.

In 2015, differences among treatments and the control were detected in percentage *S. exitiosa* infestation ($F_{5, 15} = 4.97$, $P < 0.007$) and number of surviving *S. exitiosa* larvae per tree ($F_{5, 107} = 7.58$, $P < 0.0001$) (Fig. 2). The levels of infestation in the nontreated control in 2015 were similar to those found in 2014 (Figs. 1,2). Compared with the nontreated control, percentage *S. exitiosa* infestation was lower in plots receiving *S. carpocapsae* via watering can or handgun application, but other nematode treatments and chlorpyrifos were not different from the control (Fig. 2). Infestation in the handgun treatment was lower than chlorpyrifos but not different from other nematode treatments (Fig. 2). Abbott's formula applied to the two numerically lowest infestation levels, handgun and watering can, indicated 72.2% and 66.7% control, respectively. The mean number of *S. exitiosa* per tree in 2015 was higher in the nontreated control than in all treatments except chlorpyrifos; no differences among nematode treatments were detected (Fig. 2). Abbott's formula indicates 80.1% and 84.6% control in the handgun and watering can treatments, respectively.

DISCUSSION

Our results indicate that spring applications of *S. carpocapsae* can produce high levels of curative control for *S. exitiosa* infestations. In contrast, our results did not support chlorpyrifos as a viable option for curative control. These results were consistent in both locations, i.e., trees that were 2 yr old (2014) and 7 yr old (2015). In

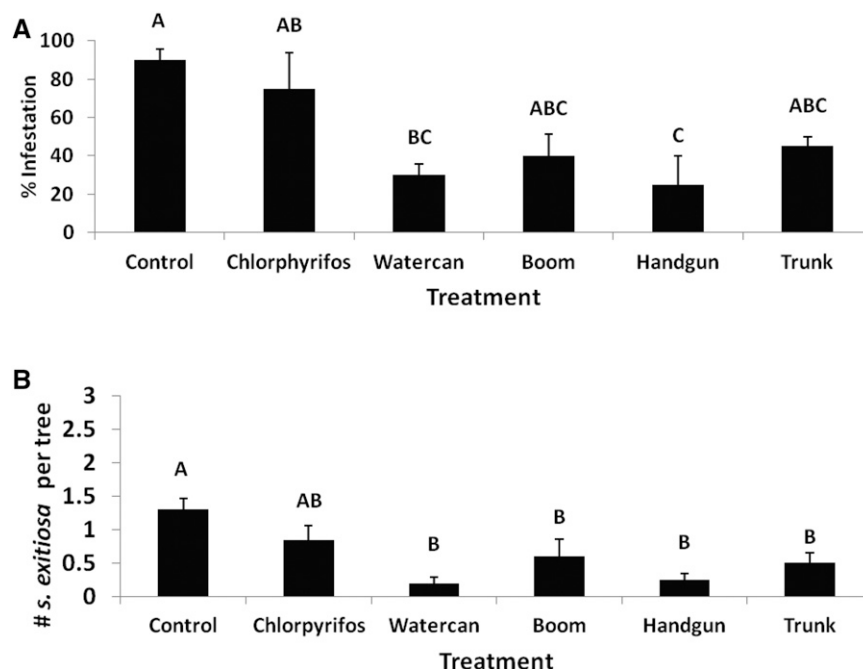


FIG. 2. (A) Percentage infestation and (B) number of surviving *Synanthedon exitiosa* following treatments of *Steinernema carpocapsae*: treatments were applied to existing *S. exitiosa* infestations in the spring of 2015. Nematodes were applied using a boom sprayer, handgun, trunk sprayer, or watering can; chlorpyrifos was applied using a handgun. Control = water only. Treatments effects were assessed 19 d after application. Different letters above bars indicate statistically significant differences within each sample date (Tukey's test, $\alpha = 0.05$).

both years of the study, all the nematode treatments reduced the percentage of trees infested with *S. exitiosa* and/or number of live *S. exitiosa* per tree. We applied the nematodes treatments in May, but conceivably they could be applied earlier in the spring, once temperatures are conducive to infection (e.g., >20°C) (Grewal et al., 1994). Chlorpyrifos did not reduce either percentage *S. exitiosa* infestation level or survival. Chlorpyrifos is the mainstay for *S. exitiosa* in preventative applications applied in the late summer and fall during the oviposition period (Horton et al., 2016). The reason chlorpyrifos was ineffective in spring applications may be that the effects of the chemical are unable to penetrate the roots to reach the larvae.

All of the standard mechanical spray equipment that was tested (boom, handgun, and trunk sprayers) comprising a broad range of output volumes appears to be conducive for nematode application aimed at curative *S. exitiosa* control. Possibly, one might argue that handgun application was superior to the other approaches because it was the only method different from the nontreated control in 2015 regarding percentage infestation. However, in the three other assessments, all treatments were statistically differentiated from the nontreated control. Moreover, no statistical differences in efficacy were detected among the three application methods (when compared to each other) in any of the tests. Thus, we conclude that all three sprayer types are suitable for curative *S. exitiosa* application. Nonetheless, from a practical standpoint, handgun application may be especially suited to a curative approach due to the level of manual control the approach allows. This curative approach allows application to trees with observable infestation, which decreases treatment costs and contrasts with preventative applications applied to the entire orchard (in which case boom or trunk sprayers would be more suitable).

The efficacy of our commercially obtained nematode treatments, applied using mechanical spray equipment, was similar to our positive control (laboratory-grown nematodes applied via watering can). In 2014, the percentage infestation following nematode application via boom sprayer was higher than in the positive control, but no other differences between the spray treatments and positive control were detected throughout the study. Thus, we conclude that the options we tested, which are available to growers for obtaining and applying nematodes on a commercial scale are feasible for use as curative control measures for *S. exitiosa*.

Prior research indicates that the effects of application equipment vary. In a recent study comparing application equipment for preventative *S. exitiosa* control, handgun, trunk sprayer, boom sprayer, and watering can methods did not differ significantly (Shapiro-Ilan et al., 2015). Similarly, when targeting *Cydia pomonella* (L.), Lacey et al. (2006) did not observe efficacy differences between the uses of a lance applicator versus an airblast

sprayer. In contrast, in other studies, differences in nematode efficacy resulting from application equipment were observed (Hayes et al., 1999; Nilsson and Gripwall, 1999; Brusselman et al., 2010; Shapiro-Ilan et al., 2012). Application equipment can impact various factors that affect entomopathogenic nematode efficacy, such as pressure and recycling time, system environmental conditions, and spray distribution pattern (Shapiro-Ilan et al., 2012). Apparently, the ability of application equipment to affect nematode efficacy can vary with the nematode and host species as well as the cropping system.

In a previous study, Barricade gel was found to be an effective cover formulation when applying *S. carpocapsae* for preventative *S. exitiosa* control (Shapiro-Ilan et al., 2015). The Barricade + nematode treatments caused equal levels of suppression compared to nematode treatments with irrigation and compared with application of chlorpyrifos; nematode application without irrigation failed. Therefore, the use of Barricade applied to the soil surface appears to be an adequate substitute for irrigation, and presumably protects the nematodes from desiccation and/or harmful UV exposure. Barricade was also effective in protecting *S. carpocapsae* from desiccation and/or UV during aboveground applications to peach limbs infested with the lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) (Lepidoptera: Sesiidae) (Shapiro-Ilan et al., 2010). Furthermore, Barricade applied for *S. pictipes* control was effective when applied separately as a cover formulation (as in the current study) or in a single application when tank mixed with nematodes (Shapiro-Ilan et al., 2016). In the current study, Barricade also appeared to be useful for situations where irrigation was not available (though we did not compare the approach directly to irrigation). Similar to the tank mix approach employed by Shapiro-Ilan et al. (2016) for *S. pictipes* control, it may be beneficial to explore single application mixtures of Barricade + nematodes for *S. exitiosa* control. A single application may be more desirable and less expensive than having to apply two materials. If a grower has irrigation within the orchard, then use of irrigation as opposed to Barricade will likely be preferable due to cost. Yet, Barricade or other gels and mulches that may protect the nematodes can provide a viable option for orchards lacking irrigation. The relative efficacy of other gels or mulches should be compared with Barricade for cost and efficacy.

Previous field studies on curative control of *S. exitiosa* (Cossentine et al., 1990; Cottrell and Shapiro-Ilan, 2006) may be considered preliminary in that the research lacked replication in time, and nematodes were applied under conditions that would not be deemed practical to commercial growers. Our findings confirm and expand upon the prior studies by indicating that entomopathogenic nematodes can be commercially applied for curative *S. exitiosa* control in a practical manner. Moreover, the levels of control that we

observed in our study were similar to prior research, which indicated 80% control using *H. bacteriophora* (Cossentine et al., 1990) and 88% control using *S. carpocapsae* (Cottrell and Shapiro-Ilan et al., 2006).

Based on results of the current study as well as previous studies, we can conclude that the use of *S. carpocapsae* for control of *S. exitiosa* is broadly effective in both preventative as well as curative applications. In preventative applications, chlorpyrifos is also highly effective though not different in efficacy from *S. carpocapsae* (Shapiro-Ilan et al., 2009, 2015), yet in curative applications, chlorpyrifos is apparently not effective. Conceivably, other chemical pesticides may be an option for curative control of *S. exitiosa*. However, due to the continued removal or restriction of broad chemical insecticide use in agriculture, including a recent proposal to remove chlorpyrifos (EPA, 2015), alternative approaches such as the use of entomopathogenic nematodes is likely to become more attractive. Furthermore, given that only the base of the tree needs to be treated, the cost of nematodes for *S. exitiosa* control (curative or preventative) promises to be quite inexpensive relative to other entomopathogenic nematode applications that require covering the entire acreage (Shapiro-Ilan et al., 2015). Additional research is needed to determine the efficacy of entomopathogenic nematodes in curative approaches for control of other borer pest species.

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