Biological Control 82 (2015) 7–12

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Field suppression of the peachtree borer, *Synanthedon exitiosa*, using *Steinernema carpocapsae*: Effects of irrigation, a sprayable gel and application method

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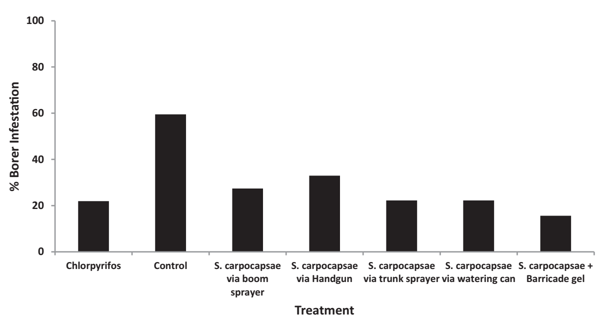
HIGHLIGHTS

- Peachtree borer control using *Steinernema carpocapsae* was similar to chlorpyrifos.
- A sprayable gel, applied after nematodes, may be used in lieu of irrigation.
- This is the first report using the sprayable gel, Barricade®, in soil applications.
- Efficacy was achieved using a boom sprayer, trunk sprayer or watering can.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 August 2014
Accepted 2 December 2014
Available online 8 December 2014

Keywords:
Application method
Entomopathogenic nematode
Formulation
Irrigation
Peachtree borer
Steinernema carpocapsae
Synanthedon exitiosa

ABSTRACT

The peachtree borer, *Synanthedon exitiosa*, is a major pest of stone fruit trees in North America. In prior studies, the entomopathogenic nematode, *Steinernema carpocapsae*, caused substantial reductions in *S. exitiosa* damage when applied by watering can to peach trees that were irrigated regularly. Here we report two additional studies that assessed *S. carpocapsae* efficacy in suppressing *S. exitiosa* damage in peach orchards; one study focused on irrigation requirements and the other on application method. In the first experiment we compared *S. carpocapsae* applied with and without irrigation, and application of a sprayable gel, Barricade®, as a potential replacement for irrigation. In the second experiment, we compared application methods that growers might use including a boom sprayer, handgun, trunk sprayer and watering can (used as a positive control). In both experiments chlorpyrifos was also included as a positive control, and in the application methods experiment an untreated (negative) control was also included. All treatments were applied in the fall of 2012 and 2013 and *S. exitiosa* infestation was assessed following the spring of 2013 and 2014, respectively. In the first experiment, nematodes applied without irrigation did not prevent high levels of infestation levels (75% of trees were infested) whereas nematodes applied with the sprayable gel suppressed damage at the same level as chlorpyrifos (<20% infestation). Thus, our results indicate that the sprayable gel applied to soil around the tree base can enhance entomopathogenic nematode efficacy, and the gel may be used as a substitute for irrigation when applying *S. carpocapsae* for *S. exitiosa* control; this finding may be applicable to similar pests in various cropping systems. This is the first report of direct application of the sprayable gel to soil (previous reports concerned aboveground applications). Also in the first experiment, intermediate levels of damage (31–38% infestation) were observed in plots that received nematodes with irrigation. We suspect that a higher rate of

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http://dx.doi.org/10.1016/j.biocontrol.2014.12.003
1049-9644/Published by Elsevier Inc.

irrigation would have improved efficacy. In the second experiment, the boom sprayer, trunk sprayer and watering can methods of nematode application resulted in *S. exitiosa* infestations that were similar to the chemical insecticide standard treatment (chlorpyrifos) and below levels in the non-treated control, whereas the handgun treatment was not different from the untreated control or chemical standard.

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1. Introduction

The peachtree borer, *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae), is a major 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 (200–800 in total) on the bark of host plants and even 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 southeastern US) complete development in about 1 year. Current management of *S. exitiosa* across the southeastern US relies solely upon post-harvest chemical control, e.g., chlorpyrifos; applications are generally made in the late summer to prevent or limit damage (Horton et al., 2014). Due to environmental and regulatory concerns, research toward developing alternative pest control measures is warranted. Entomopathogenic nematodes have potential as biocontrol alternatives for *S. exitiosa* suppression (Cossentine et al., 1990; Cottrell and Shapiro-Ilan, 2006; Shapiro-Ilan et al., 2007, 2009).

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; Lewis and Clarke, 2012). 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 (Poinar, 1990; Lewis and Clarke, 2012).

Entomopathogenic nematodes 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), and various white grubs (Coleoptera: Scarabaeidae) (Klein, 1990; Shapiro-Ilan et al., 2002, 2014; Grewal et al., 2005). 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).

Prior research conducted in Georgia peach orchards indicated that the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) can substantially reduce *S. exitiosa* infestations. *S. carpocapsae* (All strain) caused 88% control of *S. exitiosa* when applied curatively in the spring to an existing infestation (Cottrell and Shapiro-Ilan, 2006), and when nematodes were applied prophylactically in the fall to prevent or limit damage, infestations were reduced by 77–100% (Shapiro-Ilan et al., 2009). In both studies (Cottrell and Shapiro-Ilan, 2006 and (Shapiro-Ilan et al., 2009), applications were made by pouring IJs onto the ground at the base of the tree and then covering the application site with a thin layer of soil to protect the nematode from UV radiation and desiccation; each tree was subsequently watered individually for two weeks post-application. This manual approach may be compatible for small or backyard growers, but is not practical for larger commercial growers.

In this study, in an effort to develop optimum methods and parameters for larger scale application of entomopathogenic nematodes for *S. exitiosa* control, we focused on two important aspects: irrigation and method of application. Adequate soil moisture is critical for biocontrol efficacy when using entomopathogenic nematodes; therefore, irrigation prior to and after application is generally recommended (Georgis and Gaugler, 1991; Shapiro-Ilan et al., 2006). However, not all commercial peach orchards in the southeastern US are irrigated. We hypothesized that a sprayable gel, Barricade®, applied to soil at the base of the tree could provide adequate moisture protection for entomopathogenic nematodes and negate the need for irrigation. In previous research, we discovered that the sprayable gel can protect nematodes from desiccation and UV radiation during aboveground applications (e.g., when applied to peach limbs) and thereby enhance control of a closely related pest, the lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson) (Shapiro-Ilan et al., 2010); we reasoned that the gel may have similar utility when applied to the ground. Therefore, in our first experiment, we compared application of nematodes to soil with and without irrigation, and with the sprayable gel. To address the issue of application method, we compared common equipment that commercial growers could use for nematode application including a boom sprayer, trunk sprayer and handgun. These application approaches have been used by growers to apply chemical insecticides for *S. exitiosa* control, and therefore we reasoned they could be easily adapted for nematode application.

2. Materials and methods

2.1. Experiment 1: the effects of irrigation and a sprayable gel

The experiment was conducted in peach orchards with treatment applications being made in 2012 and 2013 (two separate trials). In the first year of the experiment, applications were made in a commercial peach orchard in Fort Valley, Georgia. Peaches (Cresthaven variety) were two years old in 2012 and spaced approximately 5.49 m × 4.57 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, we were informed that the orchard had recently been sprayed with a chemical insecticide for control of *S. exitiosa*, and therefore a new location was used for the 2013 applications. The location was a peach orchard at the USDA, ARS, Southeastern Fruit and Tree Nut Laboratory in Byron Georgia; trees (June Prince variety) were seven years old and spaced 6.1 × 6.1 meters 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. Nematodes, *S. carpocapsae* (All strain) for all applications were cultured *in vivo* according to Shapiro-Ilan et al. (2002, 2014) and used within two weeks of emergence. Viability of nematodes upon application was >95%.

In the first trial, treatments were applied on September 20, 2012. The treatments included nematodes applied with or without irrigation, or with Barricade (Barricade International, Inc. Hobe Sound FL) in lieu of irrigation. Chlorpyrifos (Lorsban®, Dow Agrosciences, Indianapolis, IN) was applied as a positive control in a manner commonly used by growers, i.e., a handgun application to the trunk base until runoff using a recommended rate of 29.57 ml of product per 3785 ml water (Horton et al., 2014). Given that the experiment was conducted in a commercial orchard the grower cooperator requested that an untreated control not be included; thus, comparisons were made among treatments and the positive control. The experiment was arranged in a randomized complete block design with 4 blocks. Each treatment was applied to four consecutive trees within a row (4 blocks × 4 trees per plot = 16 trees per treatment) and one buffer tree was left between treatments (within a block). Blocks were separated by a minimum of 30 m.

Nematode treatments were applied by pouring 1,500,000 IJs in 100 ml of tap water to the base of a tree, which was immediately followed with 165 ml to water the nematodes in. In the treatment receiving Barricade, the gel was sprayed 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). For the nematode + irrigation treatment, approximately 165–800 ml of water was applied by handgun to runoff targeting a 12.7 cm radius around the trunk; irrigation was applied the day after application and then three times per week for two weeks thereafter (thus 7 additional irrigation events; the other treatments did not receive any additional irrigation). The amount of irrigation varied based on soil moisture (thus impacting the quantity required for run-off), and was within the recommended range of irrigation per unit area (Goldhamer et al., 2001; Taylor and Rieger, 2005).

In the second trial, treatments were applied on September 25, 2013. The treatments and all other application parameters were identical to the first trial. Irrigation, however, was reduced due to a US Government shutdown and during this time federal employees were barred from entering federal facilities. Therefore, rather than seven additional irrigation events, trees in the nematode + irrigation treatment only received three additional waterings (on September 27, 2013 October 3, 2013 and October 5, 2013).

For both trials, treatment effects were assessed in the spring of the year following application, i.e., on April 15, 2013 for the first trial and on April 14, 2014 for the second trial. For each tree, the presence or absence of an *S. exitiosa* infestation was determined as described in Shapiro-Ilan et al. (2009). Briefly, soil was excavated to approximately 12 cm depth around the base of the tree and examined for signs of current infestation, e.g., larvae, active galleries and fresh frass exudates.

2.2. Experiment 2: the effects of application method

This experiment was conducted in a peach orchard at the USDA, ARS, Southeastern Fruit and Tree Nut Laboratory in Byron Georgia; containing trees (June Prince variety) that were 6 year old (at the beginning of the experiment) 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 (the plots were adjacent to the other experiment conducted in Byron, GA and hence soil analyses were the same). *S. carpocapsae* (All strain), which was used in all applications, was obtained from BASF (formerly Becker Underwood, Ames, IA), E-Nema (Schwentinental, Germany) or produced *in vivo* as indicated in the treatment descriptions below. Nematodes were used within 3 week of receipt and viability upon application was >90% for all treatments.

Methods of nematode application included the following equipment: boom sprayer (part # 45030051 with 140″ 7 nozzle, Moose Utility, Janesville, WI), automated trunk sprayer (Anonymous, 2005), handgun (part # 45030048, Moose Utility, Janesville, WI), and watering can (2 gallon, ACE Hardware, Oak Brook, IL). The boom, trunk sprayer and handgun methods were intended for direct comparison to determine relative suitability for commercial grower use; thus all three sprayers utilized nematodes from the same source, i.e., BASF. However, to control for potential differences in commercial nematode products, an additional trunk sprayer treatment was made with nematodes obtained from E-Nema. The watering can treatment used nematodes that were produced *in vivo* in the Shapiro-Ilan laboratory (Shapiro-Ilan et al., 2002, 2014); this treatment was considered a positive control given that we had already 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). Application of chlorpyrifos (at the standard rate as indicated above) was also included as a positive control, and a non-treated (negative) control was also included. The experiment was arranged in a randomized complete block design with three replicate blocks. Each plot was situated in a separated row and contained seven trees (thus there were three blocks × 7 trees = 21 trees per treatment); the blocks were separated by a minimum of 18 m.

Treatment applications were made in two consecutive years (two trials): September 25, 2012 and September 26, 2013. The rate of application was the same as in the irrigation experiment, i.e., 1,500,000 IJs per tree. The amount of water used per tree for each application varied based on the nature of equipment (to achieve coverage without runoff) and was 800 ml for boom sprayer, 1600 ml for handgun, 800 ml for trunk sprayer, and 250 ml for watering can. Chlorpyrifos was applied via handgun as described in Experiment 1. After treatments were applied, all nematode plots were watered in the same manner and schedule as described in Experiment 1 (including the reduced level of irrigation in the second year of the experiment). The 2012 and 2013 treatments were assessed as described above on April 16, 2013 and April 16, 2014, respectively.

2.3. Analyses

In Experiment 1 (comparing irrigation and gel), treatment effects within each year were determined separately using ANOVA (Proc GLM, SAS, 2002). Given that the second experiment (application method) was consecutively conducted at the same location, and the interaction between year and treatment was not significant, data from both years were combined and treatment effects were determined by ANOVA (Proc Mixed, SAS, 2002). In both experiments average percentage of infested trees per plot were arcsine transformed prior to analysis (Southwood, 1978; Steel and Torrie, 1980); non-transformed means are presented in the figures. If a significant model and treatment effect was detected in the ANOVA, then the treatment differences were further elucidated through Tukey's test (SAS, 2002). The alpha level for all statistical tests was 0.05. For all experiments (and both years), average daily maximum and minimum ambient temperatures, and precipitation were recorded from the date of application until two weeks following the application.

3. Results

In Experiment 1, the 2012 application resulted in different levels of *S. exitiosa* infestation among treatments ($F = 17.89$; df = 3, 9; $P = 0.0004$) (Fig. 1). When nematodes were applied without

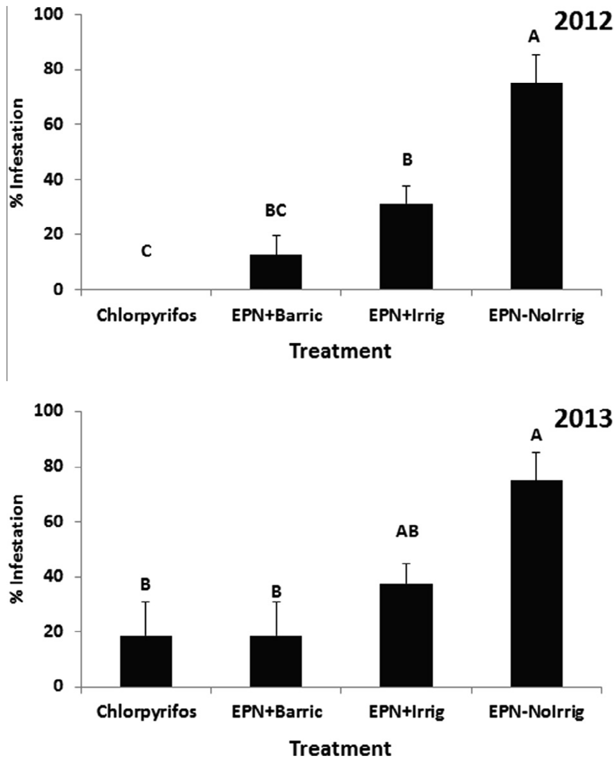

Fig. 1. Percentage of *Synanthedon exitiosa* infestation in peach trees following field application of the entomopathogenic nematode (EPN) *Steinernema carpocapsae*. Applications were made in the fall of 2012 and 2013 and damage was assessed the following spring. Nematodes were applied with or without irrigation (irrig) or with a sprayable gel, Barricade® (Barric). Chlorpyrifos was applied as a positive control. Different letters above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

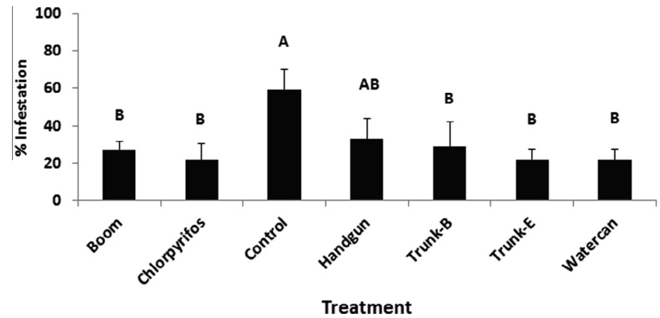

Fig. 2. Percentage of *Synanthedon exitiosa* infestation in peach trees following field application of the entomopathogenic nematode, *Steinernema carpocapsae*. Data shown are combined means from separate field applications made in the fall of 2012 and 2013 (and assessed the following spring of 2013 and 2014, respectively). Nematodes were applied with a boom sprayer, handgun, trunk sprayer or watering can. All nematodes were obtained from BASF except trunk sprayer applications used nematodes from BASF (-B) or E-Nema (-E), and nematodes applied by watering can were produced *in vivo* by USDA-ARS (a positive control). Chlorpyrifos was also applied as a positive control and non-treated plots constituted a negative control. Different letters above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

irrigation, the treatment did not prevent high levels of *S. exitiosa* infestation (75% of trees were infested) (Fig. 1). The levels of infestation observed in the nematode + Barricade treatment and chlorpyrifos treatment were not significantly different from each other and were less than the level observed when nematodes were applied without irrigation. The nematode treatment with irrigation also caused lower infestation levels than nematodes without irrigation, but allowed a higher infestation level compared with the chlorpyrifos treatment (Fig. 1). Results from the 2013 application were similar to the 2012 application, and also indicated a high level of *S. exitiosa* infestation in the no-irrigation treatment, significantly lower infestation in the chlorpyrifos and Barricade treatments (which were not different from each other), and an intermediate level of infestation in the nematodes + irrigation treatment ($F = 5.03$; df = 3, 9; $P = 0.0175$) (Fig. 1). Average daily maximum and minimum temperatures from the time of application through two weeks post-application were 27.9 °C and 16.1 °C for 2012, and 26.3 °C and 14.5 °C for 2013. Total precipitation during the same period was 52.6 mm in 2012 and 7.4 mm in 2013; thus the amount of precipitation in the first year was more than 7× the amount in the second.

Treatment effects were detected in Experiment 2 (comparing nematode application methods) ($F = 4.08$; df = 6, 24; $P = 0.0059$) (Fig. 2). The interaction between year and treatment was not significant ($F = 1.66$; df = 6, 24; $P = 0.1755$). In the combined analysis (across years), lower *S. exitiosa* infestation was observed in all nematode treatments compared with the non-treated control except the handgun treatment (Fig. 2). No differences among application methods were observed, and all nematode treatments (including the handgun treatment) exhibited the same level of infestation as chlorpyrifos (Fig. 2). Average daily maximum and minimum temperatures from the time of application through two weeks post-application were 27.0 °C and 15.4 °C for 2012, and 26.7 °C and 14.1 °C for 2013. Total precipitation during this period was 53.3 mm in 2012 and 5.5 mm in 2013; thus the amount of precipitation in the first year was more than 9× the amount in the second.

4. Discussion

Nematode treatments applied with the sprayable gel Barricade resulted in suppressed *S. exitiosa* infestations that were similar to levels achieved by the chemical standard or nematodes with irrigation and lower than nematodes applied without irrigation. Therefore, it is conceivable for farmers to use a sprayable gel such as Barricade in lieu of irrigation when applying entomopathogenic nematodes for control of *S. exitiosa*. In previous research, we demonstrated that Barricade can enhance the efficacy of aboveground nematode applications (Shapiro-Ilan et al., 2010), and now, in this study we have discovered benefits in applying the sprayable gel to the soil surface around the tree base. Other formulations or adjuvants have also shown promise in protecting entomopathogenic nematodes from adverse environmental conditions during aboveground applications (Glazer et al., 1992; Schroer and Ehlers, 2005; Perry et al., 2012; de Waal et al., 2013). Additional research is needed to compare Barricade with these other formulations as potential replacements for irrigation and to explore the potential for combining formulation components.

In Experiment 1, *S. exitiosa* infestations following nematode application with irrigation were intermediate among the other treatments whereas in Experiment 2, nematode applications that received irrigation (which constituted all nematode treatments) resulted in similar levels of infestation compared with the chemical standard (and lower infestation relative to the non-treated control). The latter (Experiment 2) is more consistent with previous findings such as Shapiro-Ilan et al. (2009). In 2013, the reduced level of irrigation and lower precipitation may have led to lower efficacy levels than would have been observed otherwise. It is not clear, however, what led to the lower level of efficacy in the nematode + irrigation treatment observed in 2012.

The nematode treatment without irrigation resulted in failure to control the target pest. This outcome is well supported in prior literature as a large body of evidence indicates that irrigation is generally required to achieve efficacy when using entomopathogenic nematodes in soil (Georgis and Gaugler, 1991; Grewal et al., 2004; Shapiro-Ilan et al., 2002, 2006). Contrarily, in a recent small plot study conducted in apple orchards of West Virginia and Massachusetts, irrigation did not impact nematode efficacy against the plum curculio, *Conotrachelus nenuphar* (Herbst). The lack of irrigation effects in a study by Shapiro-Ilan et al. (2013) may have been attributed to adequate rains and the water holding capacity of the soil. For systems such as the Georgia peach orchards utilized in the present study, where irrigation is clearly required, additional research is needed to determine the optimum level of irrigation for suppression of the target pest.

Although a negative (non-treated) control was not included in Experiment 1 (as was requested by the grower cooperator), our results provide strong evidence of pest suppression in the nematode + Barricade and nematode + irrigation treatments. First, evidence for suppression is indicated in the comparisons to the positive control chlorpyrifos, which has consistently provided control of *S. exitiosa* in numerous field trials and thus may be considered a standard (Yonce, 1980; Shearer et al., 2006; Shapiro-Ilan et al., 2009; Wetly, 2009; Horton et al., 2014). Secondly, evidence is also provided in that nematode applications without irrigation resulted in *S. exitiosa* infestations that were at least 4 fold higher than the nematode + Barricade treatment and at least two fold higher than nematodes with irrigation. It is unclear if a non-treated control would have incurred higher infestations than the nematode treatment without irrigation, yet we believe it is unlikely as the level in the non-irrigated plots was 75%, which was higher than nearby untreated orchards (e.g., in Experiment 2).

All of the nematode application methods we tested resulted in infestations levels that were similar to levels observed in plots receiving chlorpyrifos. However, the handgun application was the only nematode treatment that did not cause lower *S. exitiosa* infestation compared with the non-treated control. Therefore, the handgun method may be deemed less appropriate than the other methods; more research is needed to elucidate why this approach may be less effective and if the approach can be improved. Prior research indicates that the effects of application equipment vary. Certain previous studies did not observe efficacy differences in application methods for entomopathogenic nematodes, e.g., when targeting *Cydia pomonella* (L.), Lacey et al. (2006). Those authors did not observe 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 have been reported (Hayes et al., 1999; Nilsson and Gripwall, 1999; Brusselman et al., 2010; Shapiro-Ilan et al., 2012).

In summary, our experiments indicate that (1) *S. carpocapsae* applications can provide high levels of *S. exitiosa* control at levels similar to the chemical standard (2) a variety of application methods are suitable for nematode application, and (3) Barricade sprayable gel may be used as a substitute for irrigation to ensure nematode efficacy. Given that only the base of the tree needs to be treated, the cost of nematodes for *S. exitiosa* control promises to be inexpensive relative to other entomopathogenic nematode applications that require covering the entire acreage. For example, if nematodes are applied at the rate used in this study (1,500,000 IJs per tree) then the total number of nematodes required per hectare would be approximately 6-fold less than the minimum recommended rate (2.5×10^9 IJs per ha) if the entire acreage was covered (Shapiro-Ilan et al., 2002). Nonetheless, an economic analysis of the relative costs of the different application methods and the use of Barricade versus irrigation should be implemented.

Acknowledgments

We thank Ryan Burciaga, Leigh Ann Burnell, Stacy Byrd and Kathy Halat, for technical assistance. This study was supported in part by USDA-NIFA-SARE (Sustainable Agriculture Research and Education) Grant No. LS11-241. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the United States Department of Agriculture.

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