Biological Control

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Efficacy of *Steinernema carpocapsae* plus fire gel applied as a single spray for control of the
lesser peachtree borer, *Synanthedon pictipes*

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ABSTRACT

The efficacy of aboveground applications of entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) can be severely limited by the nematode's susceptibility to UV radiation and desiccation. The lesser peachtree borer, *Synanthedon pictipes*, is a major pest of stone fruit; larvae attack trees aboveground by tunneling into the trunk and scaffold limbs. In previous research, *S. carpocapsae*, caused high levels of *S. pictipes* mortality when a sprayable fire gel (Barricade®) was applied on top of the nematode application as a protectant. One drawback to the approach is that two applications must be made (first nematodes are applied followed by the fire gel); furthermore, the previous experiments did not compare nematode application to the existing standard chemical insecticide. Therefore, the objectives of this study were to 1) determine if a diluted rate of fire gel can protect nematodes when applied as a single spray, and 2) compare the efficacy of nematode applications with the chemical insecticide, chlorpyrifos. The experiment was conducted in a peach orchard in Quincy, Florida in 2013 and 2014. Treatments included: 1) chlorpyrifos, 2) *S. carpocapsae* applied in aqueous suspension only or 3) with a full rate (approximately 4% applied separately) or 4) 2% Barricade® (applied with nematodes in a single spray), and 5) a non-treated control. The treatments were applied post-harvest (in the fall) to *S. pictipes*-infested bark wounds; *S. pictipes* survival was assessed 8 (2013) or 14 (2014) d post-application. In 2013, chlorpyrifos and nematodes with Barricade® at 2% or the full rate reduced *S. pictipes* survival relative to the non-treated control and nematodes without Barricade®. In 2014, nematodes applied with 2% Barricade® was the only treatment that reduced *S. pictipes* survival. We conclude that *S. carpocapsae* and Barricade® can be

1 applied as a single spray, and in our experiments the treatment was at least as effective as the
2 chemical standard.

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4 *Keywords:* Biological control; Entomopathogenic nematode; Formulation; Gel; Peach;
5 *Steinernema carpocapsae*; *Synanthedon pictipes*
6

1. Introduction

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are biological control agents that can be used to control a variety of economically important insect pests (Shapiro-Ilan et al., 2002; Grewal et al., 2005). However, the efficacy of aboveground applications using EPNs can be limited due to harmful effects of ultraviolet radiation or desiccation (Shapiro-Ilan et al., 2002). Conceivably, improved formulations may improve efficacy of aboveground applications of EPNs by protecting the nematodes from harmful environmental conditions (Glazer et al., 1992; Baur et al., 1997; Head et al., 2004; Schroer and Ehlers, 2005; Shapiro-Ilan et al., 2006). We have been studying improved use of EPNs for aboveground applications against the lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) (Lepidoptera: Sesiidae).

Synanthedon pictipes is an important pest of peach, *Prunus persica* (L.) Batsch, and other *Prunus* spp in the eastern United States (Johnson et al., 2005). In general, two generations of *S. pictipes* occur per year. In the southeastern US, adult emergence typically begins in March and peaks in April and May, and a second adult emergence peak occurs between July and September. Adult moths lay eggs on the trunk and scaffold limbs usually in cracks in the tree's bark or near injured areas (Bobb, 1959; Johnson et al., 2005; Cottrell et al., 2008). Larvae bore into the inner bark and cambium where they feed and develop. Larvae, including most instars, overwinter in the tunnels. Damage from larval feeding reduces tree vigor and in heavy infestations frequently leads to loss of scaffold limbs and/or premature loss of trees and orchard productivity (Johnson et al., 2005).

Current control recommendations for *S. pictipes* depend on intensive use of chemical insecticides. For instance, recommendations in Georgia and South Carolina, the key peach producing states in the southeastern US, call for multiple applications annually that specifically target *S. pictipes* at different stages of the crop's phenology; chlorpyrifos is recommended as the most effective chemical of choice (Johnson et al., 2005; Horton et al., 2015). Thus, due to costs, along with regulatory and environmental concerns associated with such chemical usage (Coppel and Mertins, 1977; National Research Council, 1989; Cohen, 2000), development of alternative strategies is warranted. EPNs are one possible alternative tactic for *S. pictipes* control (Shapiro-Ilan and Cottrell, 2006; Lacey and Shapiro-Ilan, 2008).

Laboratory studies indicate that several EPN species are highly virulent to *S. pictipes*, especially *Steinernema carpocapsae* (Weiser) (Shapiro-Ilan and Cottrell, 2006). Nevertheless, field studies indicate that aboveground applications with *S. carpocapsae* fail to cause significant *S. pictipes* mortality, presumably due to UV radiation or desiccation effects on the nematodes (Shapiro-Ilan et al., 2010). However, in earlier research we discovered that, *S. carpocapsae*, can cause high levels of *S. pictipes* mortality when a sprayable fire gel (Barricade®) is applied as a protectant on top of the nematode application. One drawback to the approach is that two applications must be made (first the nematodes then the fire gel on top); the Barricade®, when applied at its full rate (approximately 4%) is too viscous to go through normal agricultural spray equipment and therefore requires that the nematodes to be applied separately. The requirement of a dual application reduces the ease-of-handling and attractiveness of the approach to growers. We hypothesized that a lower concentration of Barricade®, when combined with EPNs, could be applied as a single application using standard spray equipment, and the combination would still be effective for control of *S. pictipes*. Thus, our first objective was to test this hypothesis.

1 Additionally, in prior research we did not compare nematode applications to chemical insecticide
2 treatments. Therefore, the second objective of this study was to compare the efficacy of
3 nematode applications with chlorpyrifos.

4 5 **2. Materials and Methods**

6 7 *2.1. Field site and nematode cultures*

8
9 The experiments were conducted in Quincy, Florida, at the University of Florida, North
10 Florida Research and Education Center. Peaches (16-year-old University of Florida test variety
11 M2-6 trees) were planted with a 4.5 x 6 m spacing in a fine sandy loam soil. The experiment was
12 conducted in 2013 and repeated in 2014.

13 Commercially produced nematodes, *S. carpocapsae* (All strain) were used in experiments.
14 In 2013, nematodes were obtained from Becker Underwood (Ames, IA) and in 2014 from E-
15 Nema (Schwentinental, Germany). Infective juvenile nematodes (IJs) were stored at 13 °C for <
16 2 weeks before use. Nematode viability was $\geq 95\%$ in all experiments.

17 18 *2.2. Field experiments*

19
20 Four treatments and a non-treated control were included in the experiments. Three
21 nematodes treatments, which were applied in aqueous suspension, included nematodes with a
22 full rate of Barricade® (approximately 4%), nematodes with 2% Barricade®, and nematodes
23 without Barricade®. The chemical standard, chlorpyrifos was also applied as Nufos® 4E

(44.9% a.i. Cheminova, Inc., Research Triangle Park, NC) in 2013, and Lorsban® Advanced Insecticide (40.2% a.i., Dow Agrosiences, Indianapolis, IN) in 2014. A non-treated control was included. In our previous research (Shapiro-Ilan et al., 2010), we observed that Barricade® applied alone, or an application of water only, did not produce significant effects on *S. pictipes* survival; therefore we did not include these additional controls (water-only or Barricade®-only) in the current experiments.

Treatments were applied to wounds infested with *S. pictipes*. Infested wounds were identified and marked prior to treatment application (Johnson et al., 2005). Approximately 20 ml of suspension was applied to each wound. For nematode applications, one million IJs were applied to each wound (in a suspension of 50,000 IJs per ml). Chlorpyrifos was applied at a recommended field rate (approximately 7015 ml per hectare). All treatments were applied as a single spray using a 7.6 liter handheld pump sprayer (Ortho/Scotts company, Marysville, OH), except the Barricade® full rate was applied separately from the nematode suspension (applied immediately after as a cover spray) using a 94.6 liter electric sprayer (“Dependable 12-Volt Standard 25 gal Sprayer,” Fimco Industries, Dakota Dunes, SD).

Treatments were applied on November 5, 2013 and October 14, 2014. Experiments were arranged in randomized complete block designs. In 2013 there were 4 blocks of 3 wounds per treatment, and in 2014 there were 6 blocks of 6 wounds per treatment. All wounds were either on the same tree or two adjacent trees, and there was a minimum of a two-tree buffer between each treatment. Treatment effects were determined by assessing the number of surviving *S. pictipes* in wounds (Shapiro-Ilan et al., 2010) 8 or 14 d post-application in 2013 and 2014, respectively; the bark over each wound was peeled back, the wound was searched, and the number of live or dead larvae was recorded.

Weather data were recorded for the period of nematode application until assessment of *S. pictipes* survival. Specifically, average daily mean, minimum, and maximum temperatures were recorded as well as relative humidity and precipitation. The weather station (from which data were obtained) is located on the University of Florida, North Florida Research and Education Center less than 0.6 km from the peach orchard used in the experiments.

2.3. Statistical analyses

Treatment effects were analyzed with ANOVA based on average number of surviving *S. pictipes* per wound; if the F-test was significant ($P \leq 0.05$) then differences were elucidated through the Student–Newman–Keuls’ test (SAS, 2002). The average number of live *S. pictipes* was square-root transformed prior to analysis (Southwood, 1978; SAS, 2002). Non-transformed means ($\pm$ SE) are presented. Additionally, Abbott’s formula (Abbott, 1925) was used to estimate the percentage suppression of treatments relative to the non-treated control.

3. Results

Treatment effects were observed in 2013 ($F=4.06$; $df = 4, 52$; $P = 0.0061$) (Fig. 1). Compared with the non-treated control, lower *S. pictipes* survival was observed in wounds receiving nematodes with Barricade® (2% or full rate) or chlorpyrifos treatments (Fig. 1). In contrast, *S. pictipes* survival in wounds receiving nematodes without Barricade® was not different from the control. No difference between the nematode + Barricade® treatments and chlorpyrifos treatments were detected (Fig. 1) and all three caused lower *S. pictipes* survival

1 compared with the nematode treatment applied without Barricade®. Abbott's formula indicated
 2 55% control in the nematodes plus Barricade® full rate treatment, to 68% control in the
 3 nematodes plus 2% Barricade® treatment (Fig. 1).

4 In 2014, treatment effects were also observed, but the *S. carpocapsae* application with 2%
 5 Barricade® was the only treatment that resulted in lower *S. pictipes* survival compared with the
 6 non-treated control ($F=3.37$; $df = 4, 90$; $P = 0.013$) (Fig. 2). The numbers of surviving *S. pictipes*
 7 in the other treatments (*S. carpocapsae* with a full rate of Barricade® or without Barricade® and
 8 chlorpyrifos) were intermediate between the number in the nematode treatment with 2%
 9 Barricade® and the control (Fig. 2). The nematode plus 2% Barricade® treatment cause 76%
 10 control according to Abbott's formula (Fig. 2).

11 Weather data during the experimental periods (from nematode application to treatment
 12 assessment) are displayed in Table 1. Temperatures, relative humidity and precipitation were
 13 higher in 2014 than 2013.

15 4. Discussion

17 We hypothesized that a diluted concentration of Barricade® (2%) when combined with *S.*
 18 *carpocapsae* could be applied as a single effective spray for control of *S. pictipes*. This
 19 hypothesis was supported; the 2% Barricade®+nematode treatment was equally effective as the
 20 full gel rate. Thus, similar to observations made on the full rate of Barricade® in our earlier
 21 study (Shapiro-Ilan et al., 2010), it appears that the single spray approach also provides adequate
 22 protection from environmental extremes such as desiccation & UV (because superior control was
 23 observed relative to nematodes without any protective formulation). Additionally, *S. pictipes*

1 suppression in both of the Barricade®+nematode treatments was not different from the chemical
2 standard application (which addresses our second objective). In fact, arguably the 2%
3 application was the most effective treatment because it was the only one that caused significant
4 *S. pictipes* control relative to the non-treated control in both years of the experiment.

5 It is unclear why 2% Barricade®+nematodes was the only treatment to cause significant
6 control in 2014, whereas all treatments except the nematodes without Barricade® caused
7 suppression in 2013. It is unlikely that differences in weather during the experimental periods
8 caused the reduced number of significant treatments in 2014 relative to 2013. Given that the
9 optimum temperature for *S. carpocapsae* (All strain) infection is 25 °C (Grewal et al., 1994),
10 temperatures in 2014 were more conducive to this nematode than in 2013. The average higher
11 relative humidity in 2014 would also favor infection (Shapiro-Ilan et al., 2012). Conceivably, the
12 higher level of precipitation in 2014 could have washed off the treatments from the *S. pictipes*
13 wounds thereby reducing efficacy. However, this seems improbable because the numerical ratio
14 of control:treatment was higher for all 2014 treatments compared to 2013 (see Abbott's formula
15 results Figs. 1 & 2), and one would expect the opposite if nematodes or chlorpyrifos were
16 washed off due to precipitation. Most likely, the reason for the discrepancy is due to a lower
17 population of *S. pictipes* in the second year of the experiment. The mean number of *S. pictipes*
18 per wound in the non-treated limbs was more than 2.5 times higher in 2013 than 2014; therefore,
19 there was more room for suppression in the first year of the experiment.

20 In addition to the choice of treatments, timing of application can be critical for successful
21 control of *S. pictipes*. Southeastern peach growers generally apply chemical insecticides such as
22 chlorpyrifos in early to middle spring (e.g., March-April) (Johnson et al., 2005; Horton et al.,
23 2015). The application is made as a pre-bloom "barrier" spray primarily targeting eggs and

1 neonates. In this study, the treatments were applied post-harvest and directed to existing
2 infestations. Given that our applications were made at the tail end of the *S. pictipes* egg-laying
3 season, all stages of larvae were likely to be present. It will be of interest in future research to
4 also compare spring-time applications of EPNs and chemical insecticides for *S. pictipes* control.
5 In previous research (Shapiro-Ilan et al., 2010) a June application of *S. carpocapsae* (with
6 average maximum and minimum temperatures during the experimental period at 34.7 °C and
7 19.6 °C, respectively) resulted in zero *S. pictipes* survival. Thus, *S. carpocapsae* applications
8 have been effective for *S. pictipes* suppression under a variety of conditions, and we anticipate
9 equally high levels of control in spring applications. Fall applications, such as those applied in
10 this study, may be particularly beneficial in preventing further damage to the tree from existing
11 infestations, and thereby enhancing tree health and productivity.

12 Poor efficacy in aboveground applications has been a major barrier to expanded commercial
13 expansion using EPNs; the reduced efficacy is associated with EPN sensitivity to UV radiation
14 and desiccation (Arthurs et al., 2004; Shapiro-Ilan et al., 2012). In response, various formulations
15 or adjuvants have been tested to enhance environmental persistence of EPNs following
16 aboveground applications including addition of surfactants and antidesiccants (Glazer et al.,
17 1992; Head et al., 2004; Van Niekerk and Malan, 2015) or a surfactant and polymer (Schroer and
18 Ehlers, 2005). Barricade® is another option that has shown substantial improvement in
19 enhancing efficacy of aboveground applications (Shapiro-Ilan et al., 2010). In this study, we
20 discovered that a single spray of a lower concentration of Barricade® (2%) can significantly
21 enhance efficacy of *S. pictipes* control using EPNs. A comparison of the various formulation
22 and adjuvant approaches is needed. Also, in future research, large scale field trials will be
23 required to confirm our results. Finally, given that multiple applications of EPNs may be

required for certain aboveground pests, including *S. pictipes*, a cost/benefit analysis of the various formulation options is also needed.

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Table 1.

Weather data during the period of *Steinernema carpocapsae* application until assessment of *Synanthedon pictipes* survival^a.

	Daily	Min	Max	RH	Precip
2013	14.96	9.17	21.96	75.22	0
2014	17.68	10.71	25.75	79.27	73.25

^a Average daily mean, minimum and maximum temperatures (°C), average percentage relative humidity (RH), and total precipitation (mm) during the experimental period.

Figure captions

Fig. 1. Mean number of live ($\pm$ SE) of *Synanthedon pictipes* and percentage insect control (based on Abbott's formula), following field applications of *Steinernema carpocapsae* in a peach orchard, 2013. *S. carpocapsae* (Sc) was applied alone ("only") or with Barricade® gel at 2% or the full rate (approximately 4%); a non-treated control and standard chemical insecticide (chlorpyrifos) were also included. Different letters above bars indicate statistical differences ($P \leq 0.05$, Student-Newman-Keuls' test).

Fig. 2. Mean number of live ($\pm$ SE) *Synanthedon pictipes* and percentage insect control (based on Abbott's formula), following field applications of *Steinernema carpocapsae* in a peach orchard, 2014. *S. carpocapsae* (Sc) was applied alone ("only") or with Barricade® gel at 2% or the full rate (approximately 4%); a non-treated control and standard chemical insecticide (chlorpyrifos) were also included. Different letters above bars indicate statistical differences ($P \leq 0.05$, Student-Newman-Keuls' test).

Fig. 1

Fig. 2.