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Efficacy of *Steinernema carpocapsae* for control of the lesser peachtree borer, *Synanthedon pictipes*: Improved aboveground suppression with a novel gel application

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ABSTRACT

Safe and effective tactics are needed for control of the lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson), which is a major pest of stone fruits (*Prunus* spp) in eastern North America. Virulence of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser), to *S. pictipes* has been demonstrated in the laboratory. However, achieving field efficacy has been difficult because *S. pictipes* attacks the tree aboveground where nematodes are subjected to damaging environmental conditions, e.g., UV radiation and desiccation. We investigated the potential of various formulations to improve the efficacy of aboveground applications. First, we screened five potential adjuvants at 2%, 20%, and 40% concentrations in water for toxicity to *S. carpocapsae* in the laboratory: Anti-Stress, Moisturin®, Nu-Film®, Shatter-Proof, and Transfilm®. In general, the adjuvants did not adversely affect nematode survival except at the highest rate. Subsequently, Shatter-Proof was tested in field trials in 2008 and 2009. *S. carpocapsae* was applied alone or with Shatter-Proof to peach limbs pre-infested with *S. pictipes* larvae. Furthermore, the experiments included the following treatments: *S. carpocapsae* followed by a post-application covering of latex paint, moistened diaper, or a gel spray (Barricade®). Controls of water-only, or water plus Shatter-Proof, Barricade®, or paint (without nematodes) were also included. The nematodes-only treatment failed to reduce *S. pictipes* survival relative to the water-only control in either year. Additionally, the nematode treatments with Shatter-Proof or paint did not differ from nematodes-only, water-only or their respective control treatments without nematodes. The diaper treatment with nematodes showed some potential as an efficacy enhancer (e.g., insect survival was reduced relative to nematodes-only in one year). In contrast, in both years, nematodes plus Barricade® reduced *S. pictipes* relative to the controls and the nematodes-only treatment; survival in the Barricade® treatment was 30% and 0% in 2008 and 2009, respectively. We conclude that nematode treatments followed by application of a sprayable gel such as Barricade® can enhance control of *S. pictipes* and possibly other aboveground pests as well.

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1. Introduction

The lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) (Lepidoptera: Sesiidae) 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 central Georgia adult moth emergence can occur in January and February, but more typically first brood emergence begins in March and peaks in April and May, and the second brood's emergence peaks between July and September. Adult moths lay eggs on the trunk and scaffold limbs usually in cracks in the tree's bark and often in the crotch 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. Second generation larvae overwinter in the tunnels. Damage from larval feeding reduces tree vigor and in heavy infestations often 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 example, recommendations in Georgia and South Carolina, the key peach producing states in the southeastern US, call for multiple applications annually that

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specifically target *S. pictipes* at different stages of the crop's phenology (Horton et al., 2009; Johnson et al., 2005). These *S. pictipes*-specific insecticide applications have improved control of this key southeastern peach pest, though control has not returned to levels experienced prior to regulatory changes made in the early 1990s. Therefore, the cost, along with regulatory and environmental concerns associated with such chemical usage (Coppel and Mertins, 1977; National Research Council, 1989; Cohen, 2000), warrant development of alternative strategies. Entomopathogenic nematodes are one possible alternative tactic for *S. pictipes* control (Shapiro-Ilan and Cottrell, 2006; Lacey and Shapiro-Ilan, 2008).

Entomopathogenic nematodes 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), including a variety of sesiid borers (Miller and Bedding, 1982; Deseö and Miller, 1985; Kaya and Brown, 1986; Cossentine et al., 1990; Nachtigall and Dickler, 1992; Williams et al., 2002; Shapiro-Ilan et al., 2009). In laboratory studies, Shapiro-Ilan and Cottrell (2006) reported that several steinernematids caused high levels of mortality in *S. pictipes* whereas heterorhabditids were less virulent. Given that *S. carpocapsae* (Weiser) caused numerically higher mortality (Shapiro-Ilan and Cottrell, 2006) and *S. carpocapsae* has proved to be highly effective in controlling the closely related peachtree borer, *Synanthedon exitiosa* (Say) (Cottrell and Shapiro-Ilan, 2006; Shapiro-Ilan et al., 2009), we initiated studies to determine the ability of *S. carpocapsae* to control *S. pictipes* under field conditions.

The efficacy of aboveground applications using entomopathogenic nematodes can be limited due to harmful effects of ultraviolet radiation and desiccation (Gaugler and Boush, 1978; Begley, 1990; Shapiro-Ilan et al., 2006). Nonetheless, a number of studies indicate aboveground applications of entomopathogenic nematodes can result in high levels of control for a variety of pests (Arthurs et al., 2004) including several *Synanthedon* spp. (Miller and Bedding, 1982; Deseö and Miller, 1985; Kaya and Brown, 1986; Nachtigall and Dickler, 1992). In the case of *S. pictipes*, however, our initial studies indicated that aboveground field applications with *S. carpocapsae* failed to cause significant *S. pictipes* mortality (unpublished data).

Conceivably, improved formulations or application techniques may improve efficacy of aboveground applications with entomopathogenic nematodes (Shapiro-Ilan et al., 2006; Lacey and Shapiro-Ilan, 2008). For example, addition of anti-desiccants or other adjuvants have been reported to provide improved aboveground control of various foliar pests including the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Glazer et al., 1992; Baur et al., 1997; Head et al., 2004; Schroer and Ehlers, 2005). Compared with foliar applications, relatively little attention has been devoted to improvement of entomopathogenic nematode formulation for application to borer pests. Miller and Bedding (1982) tested the potential of a wetting agent, Arlatone® in combination with *Steinernema feltiae* (Filipjev) for control of the currant borer, *Synanthedon tipuliformis* (Clerck), but reported no effect of the adjuvant compared with nematodes applied alone. Our objective was to test different formulations for improved control of *S. pictipes*. Initially, we screened five potential adjuvants for toxicity to *S. carpocapsae* in the laboratory. Subsequently, in field trials, we compared nematodes applied alone with nematodes plus one of the adjuvants that was deemed promising in the laboratory; additionally, we tested nematode applications followed by several post-application cover treatments (intended to protect the nematodes).

2. Materials and methods

2.1. Nematodes and insects

The nematodes used in this study, *S. carpocapsae* (All strain) were cultured at 25 °C in commercially obtained last instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) according to procedures described by Kaya and Stock (1997). After harvesting, infective juvenile nematodes (IJs) were stored at 13 °C for <2 weeks before use. Nematode viability was $\geqslant$95% in all experiments.

A laboratory colony of *S. pictipes* was maintained at the USDA, ARS, SEFTNRL in Byron, GA on green thinning apples (based on Cottrell et al., 2008) with periodic introduction of field-collected larvae into the colony. This colony served as the source of *S. pictipes* eggs used in field studies for the current study. After emergence, adults were housed in a 122 × 56 × 60 cm screen cage and provided water and 10% honey water in separate 275 ml plastic containers. Adults mated in this cage and females oviposited on a section of a peach limb that had been wrapped in cheesecloth. Eggs were collected from the cheesecloth.

2.2. Toxicity of adjuvants to S. carpocapsae

Toxicity of adjuvants was tested in the laboratory. Experimental arenas consisted of 100 mm Petri dishes lined with filter paper (Whatman No. 1). Approximately 5000 IJs were applied to the filter paper in 1 ml of tap water containing 2%, 20%, or 40% of the following five adjuvants: Anti-Stress 2000, Moisturin, Nu-Film-17®, Shatterproof, and Transfilm® (Table 1). A control consisted of nematodes applied in tap water only. Dishes were stored in plastic crispers with wet paper towel (to maintain high relative humidity) at 25 °C. After 7 d the nematodes were rinsed into another Petri dish using a squeeze wash bottle (containing tap water) and the percentage IJ survival was determined based on movement response when probed with a dissecting needle (Kaya and Stock, 1997). A minimum of 30 IJs were counted per dish. The experiment was organized as a completely randomized design. There were three replicates of each treatment and control, and the entire experiment was repeated (two trials).

2.3. Field experiments

In 2008 and 2009, experiments were conducted to determine formulation effects on the ability of *S. carpocapsae* to control *S. pict-*

Table 1
Adjuvants and post-application covers used in experiments.

Name	Material	Source	Common use
Anti-stress 2000	Carbon chain polymers with an acrylic base	Polymer Ag Inc., Fresno, CA	Foliar spray
Barricade®	NPE-Free gel	Barricade International, Inc. Hobe Sound FL	Fire retardant gel, protection of property
Huggies®, size 2	Sodium polyacrylate	Kimberly-Clark, Neenah, WI	Infant diaper
Olympic FastHide®	White latex paint semi-gloss	PPG Architectural Pittsburgh, PA	Surface covering (paint)
Moisturin	Bicyclic oxazolidines	GSI Horticultural, Bend, OR	Foliar anti-transpirant plant coating
Nu-Film-17®	di-1-p-Menthene	Miller Chemical and Fertilizer Corporation, Hanover, PA	Spreader/sticker
Shatter-Proof	Acrylic resin	Polymer Ag Inc., Fresno, CA	Preservative for fresh and dried flowers
Transfilm®	Polymeric terpenes, oxidized polyethylene	PBI/Gordon Corporation, Kansas City, MO	Anti-transpirant and sticker

ipes in peaches. The experiments were conducted in an orchard on the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA. The orchard consisted of a 9-year-old mixed variety peach orchard with spacing of 5.5 × 6.1 m; soil was a loamy sand.

Trees were artificially infested with *S. pictipes*. On each tree, on four separate scaffold limbs, an incision of approximately 5 cm was cut into the outer bark. The bark was peeled back and at least 30 *S. pictipes* eggs were placed under the bark. A band of cheesecloth around the limb covered the incision closing the wound and holding the bark in place and protected the eggs. Nematodes were applied approximately 1 month later, when *S. pictipes* were approximately 5th and 6th instars. Not all wounds successfully sustained *S. pictipes* infestations. Therefore, three to 5 days prior to nematode application, cheese cloth was removed and each wound was examined for active infestation by checking for fresh frass (Johnson et al., 2005). Only trees with at least one actively infested wound were included in the experiment.

A variety of nematode formulation treatments was tested (9 treatments and controls were included). Among the adjuvants that had been tested in the laboratory, Shatter-Proof (at the labeled rate of 12.5%) combined with nematodes in an aqueous suspension was chosen for inclusion in the field study because overall it caused numerically the lowest nematode mortality (see Section 3). Thus, *S. carpocapsae* was applied alone or with Shatter-Proof. Additionally, the experiments included the following treatments: *S. carpocapsae* followed by a post-application covering of latex paint, moistened disposable diaper, or a biodegradable gel (Table 1). All post-application covers were applied to the *S. pictipes* wounds immediately after nematodes or water had been sprayed. Controls of water-only, or water plus Shatter-Proof (12.5%), Barricade® gel, or paint (without nematodes) were also included. Each diaper was moistened with 400 ml of tap water and fastened around a tree limb thereby covering the *S. pictipes* wound. Diapers used to cover nematodes on wounds had shown promise in earlier studies (unpublished data) and thus provided impetus for testing other post-applications covers. We did not include a diaper treatment without nematodes because we had already established in prior research that a diaper applied alone would not affect *S. pictipes* survival (unpublished data). The Barricade® was applied with a 94.6 l electric sprayer ("Dependable 12-Volt Standard 25 gal Sprayer," Fimco Industries, Dakota Dunes, SD). A coat of latex paint was applied with a paint brush to cover the wound.

Nematodes were applied on October 2, 2008 and June 29, 2009. The nematodes were applied directly to each *S. pictipes* wound using a 7.6 l handheld pump sprayer (Ortho/Scotts company, Marysville, OH). The application rate per wound was 50,000 IJs per ml sprayed to run-off, which constituted approximately 20 ml (hence approximately 1 million IJs per wound). Percentage surviving larvae per wound was assessed 5 d post-treatment in 2008 and 7 d post-treatment in 2009; the bark over each wound was peeled back, the wound was searched, and the number of live or dead larvae was recorded. Daily maximum and minimum ambient temperature, relative humidity, and precipitation were recorded from a nearby weather station at the USDA, ARS, SEFTNRL from the time of nematode application until treatment evaluation. The experiments were arranged in a randomized block design with four blocks per treatment i.e., each block contained one tree for each treatment resulting in 36 trees total.

2.4. Statistical analyses

In the laboratory experiment, toxicity of adjuvants at different rates was analyzed with ANOVA. If a significant difference ($P \leqslant 0.05$) was detected, then treatment differences relative to the no-adjuvant control were elucidated through Fisher's protected LSD test (SAS, 2002). Additionally, with the goal of determining the overall least toxic adjuvant, a factorial analysis was applied to the laboratory data comparing across rates (for overall adjuvant effects), and also across adjuvant (for overall rate effects) using ANOVA and LSD. Data from both laboratory trials (repeated in time) were combined, and variation among trials was accounted for as a block effect (two-way ANOVA).

In the field experiments, treatment effects were analyzed with ANOVA; if the *F*-test was significant ($P \leqslant 0.05$) then differences were elucidated through Fisher's protected LSD test (SAS, 2002). Percentage survival (for all experiments) was arcsine transformed prior to analysis (Southwood, 1978; SAS, 2002). Non-transformed means are presented.

3. Results

3.1. Toxicity of adjuvants to S. carpocapsae

At 7 d post-treatment differences in nematode survival were detected among adjuvants and the control ($F = 29.77$; df = 15, 79; $P = 0.04$) (Fig. 1). At the 40% rate, nematode survival was less than the control in all adjuvants except Anti-Stress (Fig. 1). At the 2% and 20% rates, however, no differences in nematode survival were detected relative to the control except survival was reduced in the Transfilm® treatment at 20% (Fig. 1).

In a factorial analysis of the adjuvant-toxicity data, no interactions between main effects (adjuvant and rate) were detected ($F = 0.73$; df = 8, 74; $P = 0.67$). Therefore, main effects were analyzed individually across levels (Cochran and Cox, 1957). When combined across rate, no differences were detected among adjuvants ($F = 0.73$; df = 8, 74; $P = 0.74$) (Fig. 2A). Nematode survival was greater than 92% in all treatments, and numerically the highest survival was observed in Shatter-Proof (Fig. 2A). When combined across adjuvant, the rate effect was significant ($F = 0.73$; df = 8, 74; $P = 0.003$) (Fig. 2B). The 40% rate was different from the 2% and 20% rates (which were not different from each other) (Fig. 2B).

3.2. Field experiments

In the 2008 field trial, differences in *S. pictipes* survival were detected among treatments and controls ($F = 5.32$; df = 8, 67; $P < 0.0001$) (Fig. 3). Nematodes applied without adjuvant or with

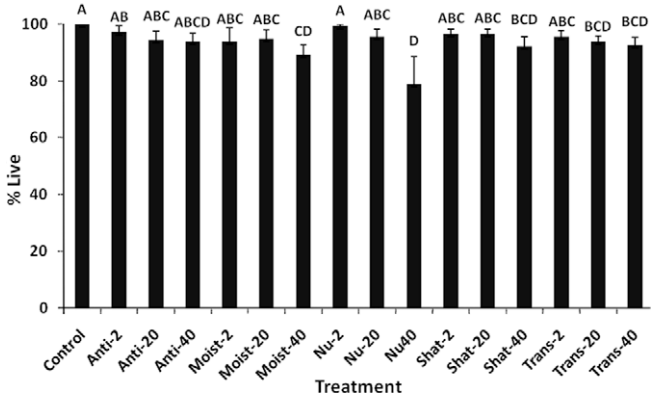

Fig. 1. Mean percentage survival (±SE) of *Steinernema carpocapsae* infective juveniles following 7 d exposure to various adjuvants at different rates. Anti = Anti-Stress 2000; Moist = Moisturin; Nu = Nu-Film-17®; Shat = Shatter-Proof; Trans = Transfilm®; Control = water-only. Adjuvants were mixed with tap water at 2%, 20%, and 40% (designated after each adjuvant abbreviation by −2, −20, and −40, respectively). Different letters above bars indicate statistical differences ($P \leqslant 0.05$, LSD).

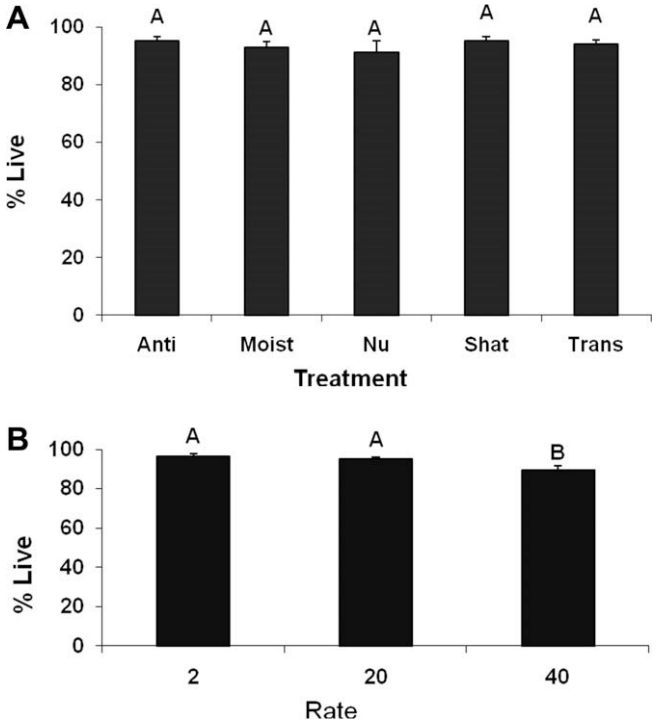

Fig. 2. Mean percentage survival (±SE) of *Steinernema carpocapsae* infective juveniles following 7 d exposure to various adjuvants combined across rates (A), and across adjuvants (B). Anti = Anti-Stress 2000; Moist = Moisturin; Nu = Nu-Film-17®; Shat = Shatter-Proof; Trans = Transfilm®. Adjuvants were mixed with tap water at 2%, 20%, and 40%. Different letters above bars indicate statistical differences ($P \leqslant 0.05$, LSD).

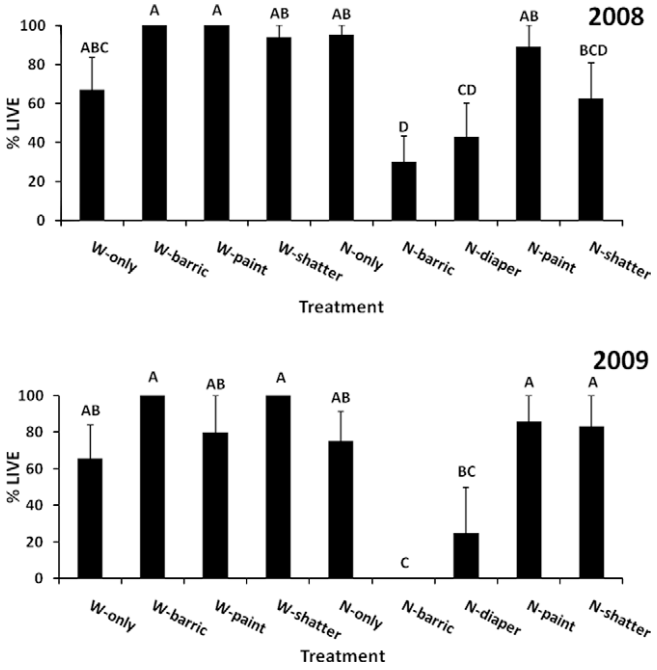

Fig. 3. Mean percentage survival (±SE) of *Synanthedon pictipes* following field applications (2008 and 2009) of *Steinernema carpocapsae* in a peach orchard. W- = water (no nematodes); N- = nematodes applied. Additional adjuvants or post-application cover treatments are indicated by: barric = Barricade®; paint = latex paint; shatter = Shatter-Proof. W-only and N-only indicate treatments without any additional adjuvant or post-application cover. Different letters above bars indicate statistical differences ($P \leqslant 0.05$, LSD).

paint did not affect *S. pictipes* survival relative to the water-only control or the post-application cover controls (paint, Barricade®, or Shatter-Proof without nematodes) (Fig. 3). Shatter-Proof applied with nematodes was also not different from water-only or water plus Shatter-Proof though the treatment did differ from the other post-application cover controls (Barricade® and paint). Survival of *S. pictipes* in the diaper plus nematode treatment did not differ from the water-control, but was lower than all post-application cover controls and the nematodes-only treatment (Fig. 3). In contrast to other nematode treatments, nematodes plus Barricade® caused lower *S. pictipes* survival than all controls; *S. pictipes* survival in the nematodes plus Barricade® was reduced to 30% (Fig. 3). The nematodes plus Barricade® treatment also caused lower survival than the nematodes-only treatment (Fig. 3).

Differences in *S. pictipes* survival were also detected among treatments and controls in 2009 ($F = 2.18$; df = 8, 39; $P = 0.05$) (Fig. 3). Similar to 2008, nematodes plus Barricade® was the only treatment that reduced *S. pictipes* survival relative to all controls, and survival in the nematode plus Barricade® treatment was also lower than in the nematodes-only treatment; 0% survival was detected in the nematode plus Barricade® treatment (Fig. 3). The nematodes applied alone or with paint or Shatter-Proof were not different from any of the controls, and the nematodes plus diaper treatment was only different from two of the post-application cover controls (Fig. 3).

Average (±SD) daily maximum and minimum ambient temperatures during the experimental periods (from nematode application until treatment evaluation) were 28.3 ± 1.8 and 11.7 ± 1.9 °C, respectively in 2008, and 34.7 ± 1.4 and 19.6 ± 1.5 °C, respectively in 2009. During the same periods, average (±SD) relative humidity was 60.1 ± 3.7% in 2008 and 53.8 ± 9.3% in 2009. There was no precipitation during the 2008 field trial. In the 2009 experimental period, two precipitation events were recorded 0.05 cm 6 d post-treatment, and 2.13 cm 7 d post-treatment.

4. Discussion

Applications of *S. carpocapsae* plus the sprayable gel, Barricade®, caused significant reductions in *S. pictipes* in both field trials, whereas the nematodes-only treatment did not affect *S. pictipes* survival. Therefore, our data indicate that the sprayable gel can enhance nematode efficacy in aboveground applications. Based on their moisture holding capacities, gels (e.g., alginates or cross-linked polyacrylamides) have been used as carriers or baits in entomopathogenic nematode formulations for more than two decades (Kaya and Nelsen, 1985; Georgis, 1990; Navon et al., 2002), yet to our knowledge this is the first report of using a sprayable gel as a post-application cover to protect nematodes.

In contrast to the gel, nematodes applied alone or with other adjuvants or post-application cover treatments failed to suppress *S. pictipes* survival relative to the water-only control. This result was unexpected, particularly for the diaper treatment because wrapping *S. pictipes* wounds with diapers after *S. carpocapsae* application had shown efficacy in a prior study (unpublished data). In our field trials, we observed substantial within treatment variation in *S. pictipes* survival, which decreased our ability to detect treatment effects; conceivably, additional replicates may have facilitated additional separation among treatments.

Similar to prior studies (Baur et al., 1997; Glazer et al., 1992), the adjuvants with anti-desiccant properties that we tested generally exhibited low toxicity to nematodes. Given its commercial use in holding moisture and providing a protective coating to flowers (http://www.shatter-proof.com), we hypothesized that Shatter-Proof (the adjuvant with the lowest toxicity) mixed with *S. carpocapsae* would result in improved control of *S. pictipes*. This hypoth-

esis was not supported. In previous studies, some anti-desiccants enhanced nematode control in aboveground applications (Glazer et al., 1992; Head et al., 2004), whereas other studies did not show an effect (Miller and Bedding, 1982). Possibly, other adjuvants that were tested in the laboratory would have shown a positive effect on *S. pictipes* control in the field, yet we were limited in the number of treatments that we could feasibly evaluate. Therefore, additional studies are required to compare other adjuvants such as those in our laboratory tests, or used in other studies (e.g., Schroer and Ehlers, 2005) to the use of the sprayable gel, Barricade®.

As far as potential toxicity of the gel or paint covers, in preliminary laboratory experiments (unpublished data) various concentrations of Barricade® did not show toxicity to the nematodes. These findings appear to be supported in our field results in that no indications of toxicity were observed in the gel treatments (indeed they were most effective). In contrast, we could not determine toxicity of the latex paint to nematodes in the lab (it would be too difficult to remove and examine the IJs after exposure). Furthermore, in the field experiments, given that there was no difference among the paint + nematode treatment, nematodes-only treatment, or water-only control, we cannot determine if the paint-nematode treatment failed due to a lack of ability to protect the nematodes from adverse environmental conditions, or due to toxicity of the paint to the nematodes.

In addition to providing significant pest suppression, economic feasibility of the nematode + gel treatment may also be achievable due to the relatively small percentage of the orchard area that would need to be treated. The approach could be directly suitable for small-scale growers who spray their orchards using a handgun wand. For example, if we assume an orchard has an average of one *S. pictipes* wound per tree, or approximately 300 wounds per ha (which would be considered a high level of infestation), then based on the rate of application used in this study, 300 million IJs would be required to treat one ha. 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 *S. pictipes* control would require about 8-fold less than the amount needed relative to applications requiring that the entire acreage be covered. In current commercial pricing, depending on size of order and distributor, the cost per ha (for 300 million IJs) can be approximately $40.00 USD, and the cost of Barricade® gel would likely be less than an additional $15.00 USD per ha.

Consequently, *S. carpocapsae* applications when followed with a sprayable gel that holds and retains moisture, such as Barricade®, may have commercial potential for control of *S. pictipes*. The justification for investigating alternative approaches (such as entomopathogenic nematodes) for *S. pictipes* control is amplified by label restrictions limiting the number of applications per season of efficacious insecticides (Brannen et al., 2005). Conceivably, nematode/gel treatments could be applied as a curative measure in the early spring (i.e., before full foliage when wounds are easily observed) by individually spraying each infested wound.

However, before the nematode/gel approach can be implemented, additional research is needed such as expanded field trials targeting natural *S. pictipes* infestations. Furthermore, additional research is needed to optimize the rate and timing of applications, and the effects of other nematode species, strains, or formulations should be explored as well. It will also be quite important to determine the in-orchard performance of this means of protecting EPNs in above ground applications using air blast sprayers, which are the standard among most commercial fruit producers. Moreover, it will be of interest to determine if single sprays (with the gel and nematodes mixed together) may be effective as opposed to the double application approach (nematodes then gel) that we used in the current study; we used the double application approach to maximize the number of IJs entering the borer galleries and because we did not know how well the nematodes might move out of the gel if the Barricade® and nematodes were combined. Finally, given the enhanced efficacy that we observed for control of *S. pictipes* in this study, the nematode plus sprayable gel approach may also have merit for targeting other borer pests or other insect pests that occur on the tree trunk or limbs, or on other aboveground habitats.

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