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Measuring field efficacy of *Steinernema feltiae* and *Steinernema riobrave* for suppression of plum curculio, *Conotrachelus nenuphar*, larvae

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Abstract

The plum curculio, *Conotrachelus nenuphar*, is a major pest of pome and stone fruit. Our objective was to determine the ability of *Steinernema feltiae* and *Steinernema riobrave* to control *C. nenuphar* larvae in soil. Nematodes were applied at 100 infective juveniles/cm^2 in peach orchards at two locations (Byron, GA and Quincy, FL) in 2002 and 2003. Treatments were compared to an untreated control and, in Byron only, application of imidacloprid. Two methods of evaluation were used to test efficacy; in Byron, 50 (2002) or 100 (2003) larvae were buried in plots 3 days prior to treatment applications, whereas in Quincy, larvae entered the soil from 100 naturally infested peach fruits placed in each plot prior to treatment. Treatment efficacy was based on average number of adults emerged/plot (captured in wire cone cages). In all trials (regardless of method), *S. riobrave* was the only treatment that caused a significant reduction in weevil emergence. *S. riobrave* applications resulted in greater than 97% *C. nenuphar* control in two out of three trials, and 77.5% control in the fourth trial. The lower level of control in the fourth trial was likely due to a prolonged period of larval exit from infested fruit into soil and lower temperatures during this period. Timing applications in accordance with pest phenology and environmental conditions appear to be critical in achieving high levels of efficacy. The observed high levels of *C. nenuphar* control by *S. riobrave* are intriguing and justify further studies.
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Keywords: Biological control; *Conotrachelus nenuphar*; Entomopathogenic nematode; Peach; *Steinernema*

1. Introduction

The plum curculio, *Conotrachelus nenuphar* (Herbst), is a major pest of pome and stone fruit in North America (Racette et al., 1992). Adult weevils enter orchards from overwintering sites in the spring, feed, and oviposit in fruit. Attacked fruit aborts or is deformed rendering it non-saleable. Larvae continue to develop in fallen fruit, exit as fourth instars, and burrow into the soil (1–8 cm) to pupate (Racette et al., 1992). After emergence, adults feed on fruit and migrate to litter surrounding the orchard to overwinter (Olthof and Hagley, 1993; Racette et al., 1992). In the southern United States, an additional generation may occur on many peach cultivars prior to overwintering (Horton et al., 2003).

Current control recommendations for *C. nenuphar* consist solely of above-ground applications of chemical insecticides to suppress adults (Horton et al., 2003; Olthof and Hagley, 1993). Due to environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic nematodes are one of the potential control options (Shapiro-Ilan et al., 2002a).

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are obligate parasites of insects (Poinar, 1990). These nematodes have a mutualistic relationship with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990). Infective juveniles (IJs), the only free-living stage, enter hosts through

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natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, 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 search out new hosts (Kaya and Gaugler, 1993).

Entomopathogenic nematodes can effectively control a variety of economically important insect pests including a number of weevil species (Klein, 1990; Shapiro-Ilan et al., 2002b). Due to the nematode's sensitivity to desiccation and ultraviolet (UV) radiation, applications to soil or cryptic habitats tend to be most efficacious (Kaya and Gaugler, 1993). Fourth instar, pupae, and adult *C. nenuphar* occur in or on the soil (Racette et al., 1992), and are therefore potential targets.

Research on use of entomopathogenic nematodes to suppress *C. nenuphar*, however, has been limited. Initial studies indicated two species, *Steinernema feltiae* (Filipjev), and *Steinernema carpocapsae* (Weiser), to be pathogenic to *C. nenuphar* larvae in the laboratory (Olthof and Hagley, 1993; Tedders et al., 1982). To determine which nematode might be most efficacious under field conditions, Shapiro-Ilan et al. (2002a) compared the virulence of six steinernematid and heterorhabditid species to *C. nenuphar* larvae and adults in the laboratory and concluded that *Steinernema riobrave* Cabanillas Poinar and Raulston and *S. carpocapsae* showed the greatest promise for adult control, whereas *S. feltiae* and *S. riobrave* showed the most promise for larval control. In the only field study reported, *S. carpocapsae*, applied to suppress adult *C. nenuphar*, failed to provide acceptable levels of fruit protection (Bèlair et al., 1998); this failing, however, was likely due to exposure of the nematodes to UV radiation and desiccating conditions because the applications were made above-ground without any protective formulations (Bèlair et al., 1998). No field tests have previously been reported using entomopathogenic nematodes in soil applications to control *C. nenuphar*. The objective of this study was to determine efficacy of applying *S. riobrave* and *S. feltiae* to soil for suppression of *C. nenuphar* larvae under field conditions. We were also interested in determining the importance of timing of nematode application (based on pest phenology and environmental conditions) on resultant efficacy.

2. Materials and methods

2.1. Nematodes and insects

For each experiment, nematodes *S. feltiae* (SN strain), and *S. riobrave* (original 355 strain) were reared in parallel on last instar *Galleria mellonella* (L.) at 25 °C according to procedures described in Kaya and Stock (1997). *G. mellonella* larvae were obtained from Webster's Waxie Ranch (Webster, WI). Following harvest, nematodes were aerated and stored at 13 °C for less than 2 weeks before experimentation.

2.2. Byron, Georgia experiments

Nematode efficacy in suppression of *C. nenuphar* was evaluated in 2002 and 2003 in a 24-year-old mixed variety peach orchard at the USDA-ARS research farm in Byron, Georgia (soil was a loamy sand). Plots (0.66 m^2) were located within the row equidistant between trees that were spaced approximately 5.5 m apart; there was no ground cover within the plots. The location of plots in 2002 and 2003 (although in the same orchard) were separated by 27.4 m. Three days prior to treatment applications, *C. nenuphar* larvae, obtained from naturally infested peaches or plums (Shapiro-Ilan et al., 2002a), were placed approximately 5 cm below the soil surface within the plots' perimeters. To mimic natural conditions, larvae were then covered with soil and a layer of peaches (from the same location within the orchard). In 2002, 50 larvae were placed in each plot, whereas twice that amount was used in each plot in 2003. We increased the number of larvae buried in 2003 because of a low percentage emergence observed in 2002. It should be noted, however, that the density of larvae used per unit area in both trials was very high relative to well-managed commercial orchards (D.L.H., personal observation). Nematodes (*S. feltiae* and *S. riobrave*) were applied at a rate of 100 infective juveniles/cm^2 to soil (with peaches on surface) in each plot. For comparison, imidacloprid (Admire, Bayer CropScience LP, Research Triangle Park, NC) was also applied according to the labeled rate of 1754 ml/ha. All treatments (and a non-treated check) were applied by watering can in 2 liters of water. Treatments were applied on May 23, 2002 and May 8, 2003. The experiment was organized in a randomized block design with five replicates per treatment. Cone traps made of aluminum screening (hole size 0.03 cm diameter, and dimensions of 109 cm bottom diameter tapering to 5 cm diameter, height 91.4 cm) fitted with boll weevil traps on top (Boethel et al., 1976; Duncan et al., 2001) were placed over each plot and secured around the edges with potting soil. The plots were watered equally (ca. 2 liters) every 2–3 days unless rain was considered sufficient to make irrigation unnecessary. Once adult weevils were observed to begin emerging, the number of weevils in each trap was recorded every 2–3 days in 2002 and everyday in 2003, until zero emergence was recorded on three consecutive sample dates. To gauge pertinent temperatures prior to application (when larvae would be developing in fruit)

and after application (i.e., during the expected active period of the nematodes [Shapiro-Ilan, 2002b]), daily maximum and minimum temperatures (air and soil at 10 cm depth) were recorded 2 weeks before application and 3 weeks thereafter.

Due to low percentage of *C. nenuphar* larvae emerging as adults in untreated plots in Byron, 2002 (<20%), the question was raised whether the orchard might contain high densities or uneven distributions of endemic nematode populations or other pathogens in sufficient numbers to cause significant suppression. Therefore, pretreatment sampling was conducted in the Byron, 2003 plots. Seven days before treatments were applied, four soil cores (ca. 50 ml each) were taken from each plot and placed in a 150 mm petri dish with five *C. nenuphar* larvae. Larval mortality was assessed after 7 days incubation at 25 °C.

2.3. Quincy, Florida experiments

Nematode efficacy for *C. nenuphar* larval control was also evaluated in Quincy, Florida in 2002 and 2003 at the University of Florida, North Florida Research and Education Center. Plots (0.39 m^2) were located in the rows equidistant between peach trees (7-year-old University of Florida test variety M2-6 trees with 4.5×6 m spacing in a fine sandy loam soil); there was no ground cover within the plots. Plot areas in 2002 and 2003 were separated by 5 m. Rather than burying larvae (as in Byron), a method that more closely simulates natural conditions was chosen. One hundred peaches that had fallen from trees in the experimental site and were infested with *C. nenuphar* (indicated by the distinctive crescent shaped scars on the fruit [Racette et al., 1992]) were placed within each plot and larvae were allowed to emerge naturally. In order to estimate the number of larvae emerging in each plot, 110 (2002) or 100 (2003) infested peaches were also placed in each of five Berlese funnels and larval exit was recorded approximately every 2–3 days until no larvae were recorded on two consecutive sample dates. The Berlese funnels were placed in the field under the peach trees adjacent to the cone cage plots about 25 cm above the ground. Treatments (five replicates of each in a completely randomized design) were applied 10 days (2002) and 2 (2003) days after fruit was placed in the plots, i.e., on May 10, 2002 and April 11, 2003. An earlier application date was chosen in 2003 because, unlike the previous year, it was clear that there would be a shortage of peaches available for testing at a later date (based on fruit set, which was already evident). Treatments included *S. riobrave* and *S. feltiae* plus a non-treated check (imidacloprid was not evaluated in the Florida trials) and were applied in the same manner and rates as in the Byron, Georgia trials. Irrigation, emergence monitoring (via cone cages described by Duncan et al., 2001 and dimensions of 70.5 cm bottom diameter), and temperature recording were also as described for the Byron trials. Additionally, in 2003, to determine whether any treatment effects persisted from the previous year, infested fruit and cone cages were also placed on 2002 plots, and number of emerging weevils was recorded in 2003 as previously described.

2.4. Data analysis

The average total number of weevils emerged per plot was analyzed for treatment effects through analysis of variance and mean separation was elucidated through Tukey's multiple range test (SAS, 2001). Percentage control (relative to the number of weevils emerged in the non-treated check) was calculated using Abbott's formula (Abbott, 1925). *T* tests (SAS, 2001) were used to detect between year differences in temperatures just prior to and during the application periods.

3. Results

3.1. Byron, Georgia experiments

In trials conducted during 2002 and 2003, *S. riobrave* was the only treatment that caused a significant reduction in *C. nenuphar* emergence relative to the untreated check ($F = 4.1$; $df = 3.16$; $P = 0.025$, and $F = 80.0$; $df = 3.16$; $P < 0.0001$, for 2002 and 2003, respectively) (Figs. 1A and B). In 2002, weevil emergence from imidacloprid-treated plots was significantly greater than emergence from *S. riobrave* treatments, whereas emergence from *S. feltiae*-treated plots was not different from either of the other treatments (Fig. 1A). In 2003, no significant differences in emergence were detected among chemical or nematode treatments (Fig. 1B). In both years, based on Abbott's formula, *S. riobrave* applications essentially resulted in complete suppression of *C. nenuphar* emergence (100 and 99.5% control in 2002 and 2003, respectively) (Figs. 1A and B). No larval mortality was observed in pretreatment soil sampling (conducted in 2003).

Adult weevil emergence patterns were similar in 2002 and 2003; more than 90% of the weevils that emerged were captured by 26 days post-treatment (Fig. 2A). In untreated checks, an average (SEM) of 16.8 (6.2) and 41.6 (9.3)% of the buried larvae emerged as adults in 2002 and 2003, respectively. Average daily minimum air and maximum soil temperatures were higher in 2002 than 2003 ($P = 0.0001$ for both tests), whereas maximum air temperatures were lower in 2002 than 2003 ($P = 0.047$), and minimum soil temperatures did not differ between years ($P = 0.26$) (Table 1).

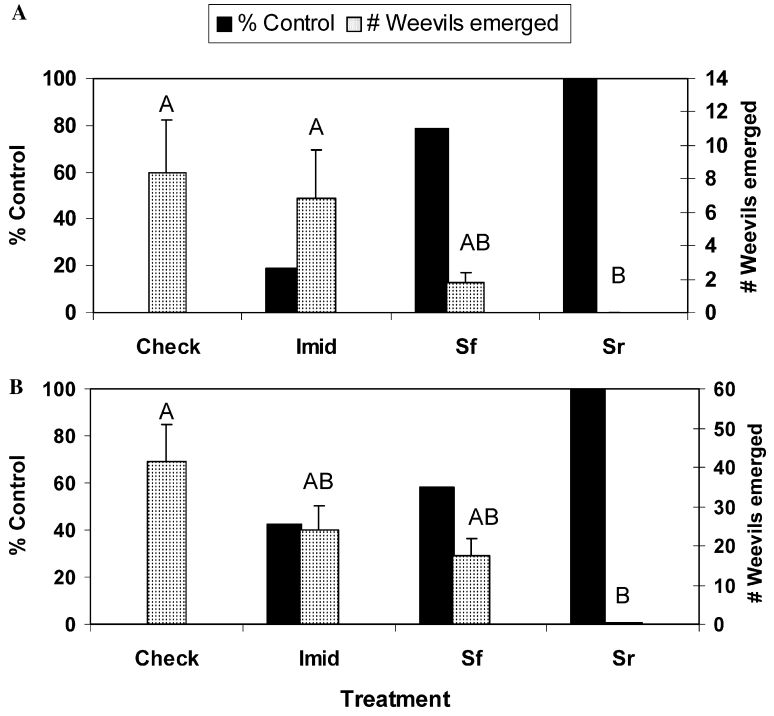

Fig. 1. Mean (SEM) emergence of adult *Conotrachelus nenuphar* from larvae, and percentage control (according to Abbott's formula), after exposure to entomopathogenic nematodes (100 infective juveniles/cm^2) or imidacloprid (0.175×10^{-2} μl/cm^2) in a peach orchard in Byron, Georgia 2002 (A) and 2003 (B). Check, water only; Imid, imidacloprid; Sf, *Steinernema feltiae*; and Sr, *S. riobrave*. Different letters above bars indicate statistically significant differences in emergence (Tukey's test, $\alpha = 0.05$).

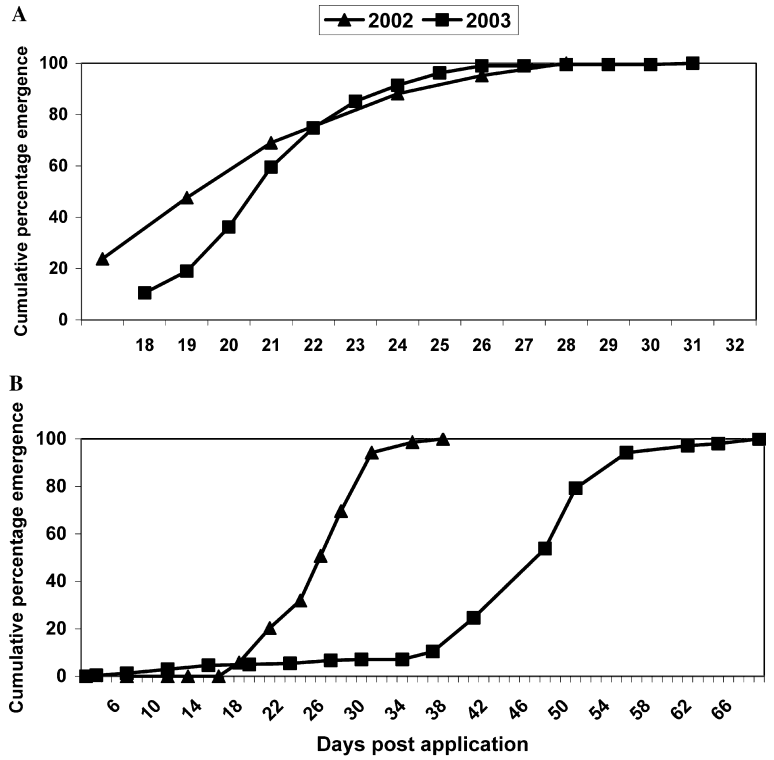

Fig. 2. Cumulative percentage emergence of *Conotrachelus nenuphar* adults from larvae buried in Byron, Georgia (A) or from larval infested fruit in Quincy, Florida (B). A total of 42, 208, 69, and 240 weevils emerged (from five replicate plots) in Byron 2002, Byron 2003, Quincy 2002, and Quincy 2003, respectively.

Table 1
Average (SEM) daily maximum (max) and minimum (min) air and soil temperatures in Byron, Georgia and Quincy, Florida[a]

Location, year	Air Max	Air Min	Soil Max	Soil Min
Byron, 2002	28.43 (0.97)b	19.47 (0.40)a	28.99 (0.37)a	21.04 (0.59)a
Byron, 2003	30.71 (0.56)a	15.57 (0.56)b	25.42 (0.23)b	21.74 (0.19)a
Quincy, 2002	30.53 (0.62)a	16.31 (0.78)a	33.12 (0.48)a	23.63 (0.65)a
Quincy, 2003	25.80 (1.06)b	11.69 (1.04)b	26.30 (1.03)b	18.21 (0.78)b

[a] Temperatures were recorded during the following periods: May 9–June 13 (Byron, 2002), May 1–June 5 (Byron, 2003), April 26–May 31 (Quincy, 2002), and March 28–May 2 (Quincy, 2003). Soil temperatures were recorded at a depth of 10 cm. Within each column (and within each location) different letters following means between years indicate statistical significance (T test, $\alpha = 0.05$).

3.2. Quincy, Florida experiments

Results from trials conducted in Quincy were similar to those conducted in Byron in that *S. riobrave* was the only treatment that caused a significant reduction in *C. nenuphar* emergence relative to the untreated check ($F = 25.6$; $df = 2.12$; $P < 0.0001$, and $F = 33.1$; $df = 2.12$; $P = 0.0014$, for 2002 and 2003, respectively) (Figs. 3A and B). In both years, emergence from *S. riobrave*-treated plots was lower than emergence from *S. feltiae*-treated plots (Figs. 3A and B). In the 2002 Quincy trial, *S. riobrave* caused 97.1% control, whereas 77.5% control was observed in 2003 (Figs. 3A and B). In 2003, no significant treatment effects (persisting from the previous year) were detected in weevil emergence among cages placed on plots treated in 2002 ($F = 7.5$; $df = 2.12$; $P = 0.35$); mean numbers (SEM) of emerged *C. nenuphar* per plot were 37.2 (10.1), 47.0 (9.2), and 56.2 (7.7) for the control, *S. feltiae*, and *S. riobrave* treatments, respectively.

Adult emergence patterns differed between years. In 2002 more than 90% of the captured weevils emerged by 30 days after nematode application, whereas a similar percentage of emergence did not take place until 56 days post-application in 2003 (Fig. 2B). Larval exit from infested fruit (captured in Berlese funnels) was also relatively prolonged in 2003 compared with 2002. In 2002, larvae began exiting fruit 9 days prior, and completed exiting 2 days prior to treatment application (Fig. 4). In 2003, larval exiting did not begin until 4 days after nematode application and did not cease until more than 30 days post-application (Fig. 4). Berlese funnel captures indicated that an average (SEM) of 0.90 (0.23) and 1.47 (0.27) larvae emerged per infested peach in 2002 and 2003, respectively. If we assume similar numbers of larvae per fruit exited into the experimental plots, then we may estimate that the resulting percentage adult emergence in untreated plots ([adults captured in cone traps/expected number of larvae per plot] × 100) would be 15.3 (2.6) and 32.6 (5.8) in 2002 and 2003, respec-

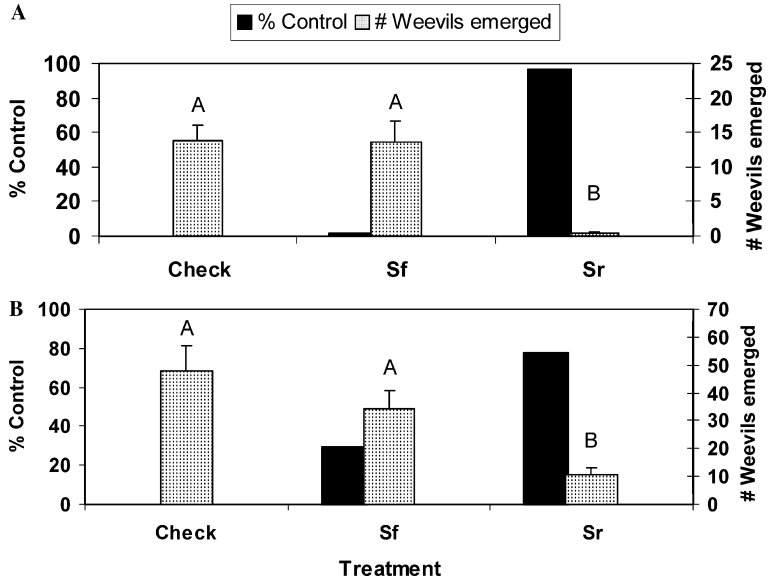

Fig. 3. Mean (SEM) emergence of adult *Conotrachelus nenuphar* adults from larval infested peaches, and percentage control (according to Abbott's formula), after exposure to entomopathogenic nematodes (100 infective juveniles/cm^2) in a peach orchard in Quincy, Florida 2002 (A) and 2003 (B). Check, water only; Sf, *Steinernema feltiae*; and Sr, *S. riobrave*. Different letters above bars indicate statistically significant differences in emergence (Tukey's test, $\alpha = 0.05$).

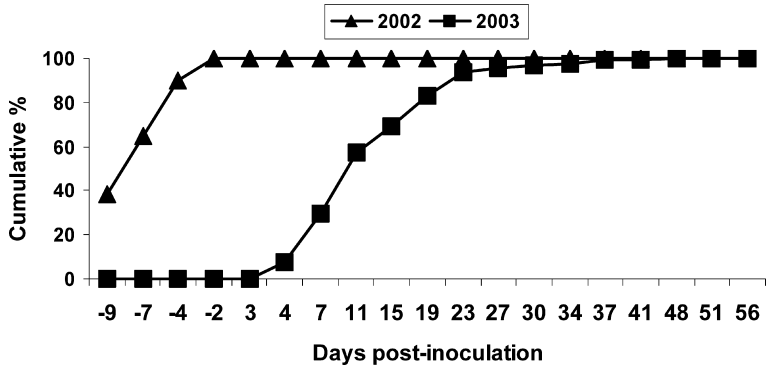

Fig. 4. Cumulative percentage of *Conotrachelus nenuphar* larvae exiting infested peaches relative to days post-application of entomopathogenic nematodes. Infested peaches (110 in 2002 and 100 in 2003) were collected in Quincy, Florida and placed in Berlese funnels to monitor larval exit. A total of 497 and 736 weevils exited (from five replicates) in 2002 and 2003, respectively.

tively. Average daily maximum and minimum air and soil temperatures were consistently higher in 2002 than 2003 ($P = 0.0004$, 0.0008 for air maximum and minimum, respectively, and 0.0001 for both soil temperature tests) (Table 1).

4. Discussion

Applications of *S. riobrave* resulted in greater than 97% *C. nenuphar* control in three out of four trials. In one trial, a lower level of control was observed in *S. riobrave* plots (77.5% in Quincy, FL, 2003). Most likely, the lower level of control observed in Quincy 2003 was due to the earlier application time, and associated phenological or temperature effects. Starting the test earlier in 2003 apparently resulted in prolonging larval exit from fruit by more than 2 weeks relative to larval exit in 2002. The delayed emergence was likely associated with lower air temperatures in 2003 relative to 2002. Similarly, a study conducted in Fort Valley, Georgia, indicated the average time for larvae that hatched in fruit in April to reach maturity and exit was 21.5 days as compared with 13.3 days for larvae hatching in May (Snapp, 1930). Thus, by the time some of the larvae entered the soil in 2003, a portion of the remaining nematodes may have been present without a host for 2 weeks or more. As the number of days in soil without a host increases, efficacy of *S. riobrave* can be expected to decrease; indeed, efficacious populations of applied *S. riobrave* were observed to persist in the soil for only 2–3 weeks (Duncan and McCoy, 1996; McCoy et al., 2000).

Additionally, lower soil temperatures in Quincy, 2003 might have reduced *S. riobrave* infectivity (ability to invade the host) and virulence (disease producing power) when larvae were encountered (Grewal et al., 1994). We believe that if *S. riobrave* had been applied at a later time in Quincy, 2003, when soil and air temperatures were more favorable, then the level of control would have been more similar to the other three trials. Our observations indicate that timing of nematode application (based on pest phenology and environmental conditions) is critical to achieving high levels of *C. nenuphar* control. Other studies support the importance of these factors in using entomopathogenic nematodes for suppression of other insects (Shapiro-Ilan et al., 2002b).

Steinernema feltiae failed to suppress *C. nenuphar* emergence. In contrast to this study, *S. feltiae* was found to be highly virulent to *C. nenuphar* larvae in comparison with five other entomopathogenic nematode species under laboratory conditions (Shapiro-Ilan et al., 2002a). Perhaps *S. feltiae*, a relatively cold-adapted nematode (Grewal et al., 1994) failed to control *C. nenuphar* in the field due to above-optimum temperatures. Yet, *S. feltiae* did not cause significant *C. nenuphar* suppression in the Quincy, 2003 trial when temperatures were lower and closer to the nematode's optimum activity level. The reasons that *S. feltiae* failed to control *C. nenuphar* are unclear, but the results reinforce that laboratory results cannot necessarily be extended to field conditions.

In other studies imidacloprid showed efficacy for control of certain curculionids, such as *Diaprepes abbreviatus* (L.) (Quintela and McCoy, 1997), *Otiorhynchus sulcatus* (F.) (Gill et al., 2001), and the iris borer, *Macronoctua onusta* Grote (Gill and Raupp, 1997). Imidacloprid has also been highly efficacious in suppression of white grubs (Coleoptera: Scarabaeidae), but only when applied for control of early instars (Mannion et al., 2001; Potter, 1998). Perhaps the relative low susceptibility of later instars observed in other insects (Mannion et al., 2001; Potter, 1998) may explain imidacloprid's lack of efficacy observed in this study. Alternatively, imidacloprid may simply have low toxicity toward *C. nenuphar* larvae regardless of instar.

Estimates of adult emergence of *C. nenuphar* from larvae in untreated plots were below 50% throughout the study. These estimates, however, are well within the

range of previously reported emergence data (Shapiro-Ilan et al., 2002a; Snapp, 1930). The causes of low percentage emergence are not clear but may have been partially due to other natural enemies (Racette et al., 1992; Snapp, 1930), or a tendency of the *C. nenuphar* larvae themselves to be frail and prone to natural mortality (Shapiro-Ilan et al., 2002a).

The ability of *S. riobrave* to cause high levels of *C. nenuphar* control is intriguing; these levels of field efficacy are not encountered often (Shapiro-Ilan et al., 2002b). The observed level of *C. nenuphar* control is greater or equal to levels of control observed in field studies for control of other weevil pests or other soil-dwelling coleopterans that are currently commercial targets for entomopathogenic nematodes (Klein, 1990; Shapiro-Ilan et al., 2002b). Both methods of evaluation used in this study resulted in essentially the same conclusions; *S. riobrave* caused relatively high levels of control both when larvae were placed in soil prior to application, and under conditions that may be considered more natural, i.e., when larvae exited fruit. Furthermore, it should be noted that *S. riobrave* was capable of causing the observed *C. nenuphar* suppression despite the high densities of *C. nenuphar* in our experiments compared with what might be observed in commercially managed (or unmanaged) orchards (D.L.H., personal observation). The nematode treatments, however, did not appear to provide any residual control in the season following application, which is not unusual for entomopathogenic nematode applications in orchard systems (Lacey and Shapiro-Ilan, 2003; Shapiro-Ilan et al., 2002b).

Due to the biology of *C. nenuphar* and economic tolerance levels for this pest, however, incorporating larval control with *S. riobrave* into an efficacious management strategy is a challenging prospect. Primary *C. nenuphar* damage to pome fruits is caused by adult weevils entering the orchards from overwintering sites (Racette et al., 1992), and currently little or no *C. nenuphar* damage is tolerated in southeastern commercial peach and plum orchards. Nonetheless, control of larvae may be beneficial to organic growers or local marketers and homeowners that do no rely heavily on chemical insecticides and might tolerate higher levels of damage. Additionally, if current chemical insecticides used for adult control are removed from usage due to regulatory pressure (see Cohen, 2000), and replacement control options are not as effective, then supplemental control with entomopathogenic nematodes may be valuable. However, prior to any recommendations, the ability of *S. riobrave* to control *C. nenuphar* larvae in wider commercial-scale applications will need to be tested. Additionally, future studies are required to determine if entomopathogenic nematodes can be efficacious in suppressing *C. nenuphar* adults under field conditions.

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