Improved Control of *Curculio caryae* (Coleoptera: Curculionidae) through Multi-Stage Pre-Emergence Applications of *Steinernema carpocapsae*[1]

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Abstract The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecan in North America. Entomopathogenic nematodes have potential as alternative control agents for *C. caryae*. In prior studies, when single applications of entomopathogenic nematodes were applied during adult weevil emergence, only moderate efficacy was observed. The objective of this study was to determine the compounded impact of multistage nematode applications on *C. caryae* mortality over a 2-year period. Experiments were conducted in a pecan orchard in Byron, GA. In the fall of 2007, freshly-emerged *C. caryae* larvae were placed in pots under the tree canopy. The nematode, *Steinernema carpocapsae* (Weiser), was applied 3 times in spring through fall of 2008 (targeting *C. caryae* larvae) and 3 times during the spring and summer of 2009 (primarily targeting adults). The percentage of surviving *C. caryae* was determined in the fall of 2008 and 2009, approximately 1 and 2 years after larvae emerged. In 2008 (1 year postemergence), the number of surviving *C. caryae* was significantly less in treated pots (3.75%) compared with untreated pots (7.38%). In 2009 (2 years postemergence), the number of surviving *C. caryae* was reduced further and was significantly less in treated pots (0.5%) compared with untreated pots (2.63%). When corrected for natural mortality, after 2 years the nematode treatments provided 81% control. These results indicate promise for reducing the weevil below economic levels through repeated multistage applications of *S. carpocapsae*. In future research, the approach will be tested on an orchard scale, and nematode application rates and timing will be optimized.

Key Words biological control, entomopathogenic nematode, pecan weevil

Pecan, *Carya illinoinensis* Koch, is an important nut crop in North America (Wood 2003). The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans throughout the southeastern US, OK, KS, and parts of TX (Mizell 1985). Adults emerge from soil in late July to August to feed on and oviposit in the developing nuts (Harris 1985). Larvae develop within the nut, and fourth instars drop to the ground where they burrow to a depth of 8 - 25 cm, form a soil cell, and overwinter. The following year, approx. 90% of larvae pupate and spend the next 9 months in the soil cell as adults (Harris 1985). The remaining 10% of the population spend an additional year in the soil as larvae and emerge as adults in the third year (Harris 1985). Thus, *C. caryae*'s life cycle is usually 2 and sometimes 3 years (Harris 1985). The majority of *C. caryae* adults

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emerge from soil over a 4 - 6 week period usually beginning in midAugust (Harris 1976); larvae emerge from nuts over several months in the autumn and early winter.

Current recommendations for *C. caryae* control consist mainly of above-ground applications of chemical insecticides (e.g., carbaryl or pyrethroids) targeting adults in the canopy (Hudson et al. 2011). Application of chemical insecticides is recommended every 7 - 10 d during peak *C. caryae* emergence (Hudson et al. 2011). Due to problems associated with aphid and mite resurgence that often result from chemical applications (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is desirable. Soil applications of entomopathogenic nematodes may provide an alternative control measure for suppression of *C. caryae* larvae or adults (Shapiro-Ilan 2003; Shapiro-Ilan et al. 2006a).

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are biological control agents that can be used to suppress a variety of economically important insect pests including a number of weevil species (Shapiro-Ilan et al. 2002a, Grewal et al. 2005). These nematodes have a mutualistic symbiosis 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 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 (Poinar 1990). The nematodes molt and complete up to 3 generations within the host after which IJs exit the cadaver to find new hosts (Kaya and Gaugler 1993).

Efficacy in entomopathogenic nematode applications depends on matching the appropriate nematode species to the specific target pests (Shapiro-Ilan et al. 2002a). A broad screening of virulence to *C. caryae* larvae, in which 15 nematode strains representing 9 species were tested under laboratory conditions, indicated relatively low to moderate suppression among all treatments (Shapiro-Ilan 2001a). In contrast to the larvae, laboratory studies indicated that adult *C. caryae* are highly susceptible to entomopathogenic nematode infection, particularly to *Steinernema carpocapsae* (Weiser) (Shapiro-Ilan 2001b, 2003, Shapiro-Ilan et al. 2003). Thus, initial field trials were directed toward adult *C. caryae* (Shapiro-Ilan et al. 2006a).

The initial approach was to apply *S. carpocapsae* during the time of adult *C. caryae* emergence (Shapiro-Ilan et al. 2006a). Limited suppression of *C. caryae* adults was observed, but the effect was insufficient and short-lived, i.e., approx. 60% mortality was observed and only within the first week postapplication (Shapiro-Ilan et al. 2006a). Therefore, it was clear that improved efficacy would be needed prior to implementation of this microbial control tactic.

Conceivably, the previous field trials (Shapiro-Ilan et al. 2006a) failed to achieve high levels of efficacy because a single exposure to nematodes during adult-stage *C. caryae* is insufficient; multiple or prolonged exposure may be required. Therefore, we hypothesized that the cumulative effects of multiple soil applications to the larvae (during the first year of the weevil's life-cycle) and to the adults thereafter (e.g., in the second year of the life-cycle) will cause high levels of *C. caryae* mortality prior to emergence. This approach takes advantage of the long, 2 - 3 year period, that *C. caryae* spends in the soil. Thus, the objective of the experiment was to determine if multistage preemergence applications of *S. carpocapsae* during the weevil's life cycle would result in significant suppression.

Materials and Methods

Research to determine the efficacy of nematodes in multistage applications versus *C. caryae* was conducted in a pecan orchard located at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory (SEFTNRL) in Byron, GA. The orchard is approx.1.5 ha and contains mixed cultivars (Desirable, Stuart, Cheyenne, and Cape Fear) that are 27 years old and spaced at 12.2 m. Experiments were conducted in plastic pots (25.4 cm diam × 48 cm deep) filled with soil (loamy sand) from the orchard. The pots were buried so that the soil surface in the pots was level with the orchard floor. A metal screen (18 × 16 mesh) was placed on the bottom of each pot to prevent *C. caryae* escape and deter entry by predators (e.g., ants or spiders). Four pots, arranged in the 4 cardinal directions, were placed around 8 trees approx. 2.5 m from the trunk (32 pots total).

Fourth-instar *C. caryae* were collected from infested nuts on the USDA-ARS Research Station, Byron, GA, in the fall of 2007. Larvae were stored in autoclaved soil at 25°C for 2 wk (to remove diseased individuals) (Shapiro-Ilan 2001a), and then 100 larvae were placed in each pot. A physical barrier (Tree-Tanglefoot, The Tanglefoot Company, Grand Rapids, MI) was placed on the top 2 cm of the perimeter of each pot to deter predator arthropods (e.g., ants and spiders) and a screen on top of the soil within the pot to prevent bird predation.

The pots surrounding half the trees received nematode treatments, whereas the pots surrounding the other half received only tap water (control). Additionally, half of the pots within each plot (2 pots per tree) were designated for measuring *C. caryae* survival approx. 1 year after nematode application, and the other half of the pots were designated for measuring *C. caryae* survival after 2 years. The experiment was arranged in a completely randomized block design with 4 replicates.

Steinernema carpocapsae (All strain) was applied to pots at a rate of 200 IJs per cm^2 in 2008 and 2009. The nematodes were cultured at 25°C in commercially obtained last-instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) according to procedures described by Shapiro-Ilan et al. (2002b). After harvesting, IJs were stored at 13°C for < 2 wks before use. Nematodes were suspended in 50 ml tap water and poured evenly onto the soil-surface inside the pots. Approximately 2.5 cm of soil from the area surrounding the pot was then added to the soil-surface in each of the treated pots to protect the nematodes from UV radiation. The control pots were handled in the same manner except only 50 ml of water was applied. Subsequently, approximately 2 cm of additional water was added to each pot. In 2008, *S. carpocapsae* was applied 3 times (2 June, 8 August, and 3 September). After each nematode application, pots received additional water (approx. 2 cm) 3 times per week for up to 2 weeks posttreatment. In the fall of 2008 (31 October), 2 pots from each tree (north and south side) were evaluated through destructive sampling. The soil from each pot was emptied into large plastic bins and the percentage of surviving *C. caryae* was determined.

Three applications were made to the remaining pots in 2009 (6 May, 5 June, 22 June). Application procedures were the same as those described for 2008. Also, similar to the procedures described for 2008, pots were destructively sampled to determine *C. caryae* survival on 26 June 2009.

A separate experiment was conducted to determine the effect of *C. caryae* density in pots. The information on density effects was intended to verify that the number of weevils per pot in the *S. carpocapsae* experiment was appropriate, and the information

could assist in optimizing future experiments that measure *C. caryae* in the field. Additionally, results could provide insight on how population density impacts *C. caryae* survival in nature. The experiment was conducted in a pecan orchard in SEFTNRL in Byron, GA. The orchard consists of Stuart variety trees that are approx. 70 years old and spaced 18.3 m apart. *Curculio caryae* larvae were collected and added to buried pots (17 December 2009) as described above. Four treatments consisted of 25, 50, 100 or 500 larvae per pot. The percentage of surviving *C. caryae* was determined on 30 September 2010 according to procedures described above. The experiment was arranged in a completely randomized design with 3 replicates per treatment.

Treatment effects were determined through ANOVA (SAS 2002) by comparing the average percentage of surviving *C. caryae* in each pot. Percentages were arcsine transformed prior to analysis (Southwood 1978, SAS 2002). Nontransformed means are presented.

Results and Discussion

Our results indicate that multistage preemergence applications of *S. carpocapsae* caused substantial suppression of *C. caryae* during a 2-yr period (Figs. 1, 2). In the first year of the experiment (approx. 1 year after larvae emerged), *C. caryae* survival was lower in *S. carpocapsae*-treated pots relative to untreated pots ((Fig. 1) ($F = 6.52$; df = 1, 11; $P = 0.027$). In the second year of the experiment, *C. caryae* survival was depressed further than the previous year and was lower in treated pots relative to untreated pots; *C. caryae* survival in treated pots was less than 1% (Fig. 2) ($F = 4.84$; df = 1, 11; $P = 0.050$).

In contrast to this study, in previous studies attempts were made to control emerging adult populations of *C. caryae* with entomopathogenic nematodes, and results

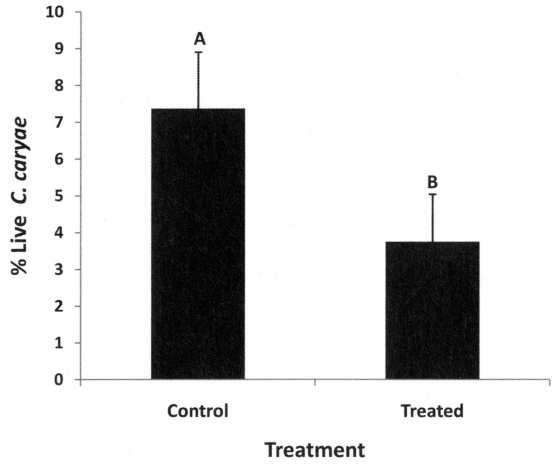

Fig. 1. Percentage survival of *C. caryae* in pots after 1 year in a Byron, GA pecan orchard. Control = water only, Treated = *Steinernema carpocapsae*. Bars with different letters indicate a statistical difference ($P \leq 0.05$).

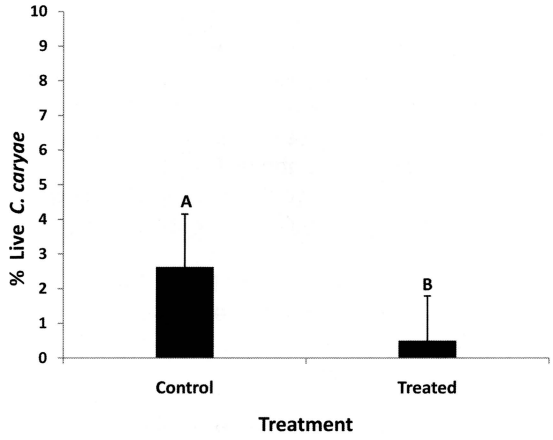

Fig. 2. Percentage survival of *C. caryae* in pots after 2 years in a Byron, GA pecan orchard. Control = water only, Treated = *Steinernema carpocapsae*. Bars with different letters indicate a statistical difference ($P \leq 0.05$).

indicated only poor or moderate suppression (Shapiro-Ilan et al. 2006a). Additionally, single nematode applications targeting *C. caryae* larvae in a protected (greenhouse) environment did not reach the levels of suppression observed in this study, e.g., less than 30% *C. caryae* larval mortality was observed by Nyczepir et al. (1992) and Smith et al. (1993). Our hypothesis that multiple applications prior to *C. caryae* emergence would cause high levels of suppression was supported.

No treatment effects were observed in the experiment measuring density effects on *C. caryae* survival within pots (Fig. 3) ($F = 1.20$; df = 3, 8; $P = 0.369$). Percentage survival ranged from 2.4% in the 25 weevils-per-pot treatment to 7.3% in the 50 weevils-per-pot treatment. These results indicate that the number of weevils we used per pot in the *S. carpocapsae* experiment was suitable, and that the percentage of surviving *C. caryae* would not have been significantly affected had we chose otherwise. Conceivably, these results might also apply to a lack of density effects on natural *C. caryae* in the orchard (outside of pots). Other abiotic (e.g., moisture, temperature) or biotic (e.g., endemic pathogen load) factors may be more important, yet additional research is required to confirm this possibility.

A high level of natural mortality, as indicated by survival in untreated pots, was observed in the *S. carpocapsae* experiment (Figs. 1, 2) as well as the density experiment (Fig. 3). However, based on previous studies, the high levels of mortality were not unexpected (Harris et al. 1981, Neel and Sikorowski 1985). Indeed, even in the laboratory, when *C. caryae* had been surface sterilized and placed on sterile agar with antibiotics, 96.1% mortality was observed within 1 yr at 25°C (Neel and Sikorowski 1985). The majority of mortality was attributed to pathogens that infected upon oviposition or when the larvae were feeding in the nuts (Neel and Sikorowski 1985). A number of agents that are pathogenic to *C. caryae* occur naturally in the environment including bacteria (e.g., *Serratia marcescens*), entomopathogenic nematodes, and fungi (primarily Hypocreales) (Sri Arunotai et al. 1975, Neel and Sikorowski 1985, Nyczepir et al. 1992, Shapiro-Ilan 2003, Shapiro-Ilan et al. 2003).

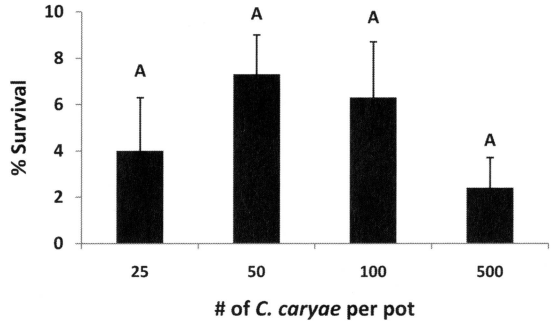

Fig. 3. Percentage survival of *C. caryae* surviving after 1 year in a Byron, GA pecan orchard; initial number of larvae per pot varied from 25 - 500. Bars with different letters indicate a statistical difference ($P \leq 0.05$).

These pathogens likely contributed to the observed control mortality in our study. Nonetheless, despite the high levels of natural mortality, based on Abbott's Eq. (1925), the *S. carpocapsae* treatments still exhibited significant benefits in terms of *C. caryae* control. Specifically, when natural mortality was corrected (Abbott 1925) percentage control was 49% and 81% in 2008 and 2009, respectively.

When microbial control applications fail to achieve the desired pest suppression levels, a number of approaches can be taken to enhance efficacy (Shapiro-Ilan 2003, Lacey and Shapiro-Ilan 2008). One option is improve production, formulation, or application methodologies. In this study, the method of application was improved to increase pest suppression, i.e., by targeting multiple stages. Another option is to improve the biocontrol organism itself, e.g., by utilizing or developing a superior strain or species. Toward improved *C. caryae* control, a hybridization approach was used to develop a *S. carpocapsae* strain that possessed both high levels of virulence and environmental tolerance (Lacey and Shapiro-Ilan 2008). Additionally, Shapiro-Ilan et al. (2006b) screened various entomopathogenic strains for longevity in soil from a pecan orchard, and discovered that *S. carpocapsae* (Sal strain), a strain that is virulent to *C. caryae*, was among the most persistent. In the present study, a commonly used commercial strain of *S. carpocapsae* was used (the All strain). Yet, in future studies, *C. caryae* control might be further augmented by combining the improved application approach with a superior strain.

Curculio caryae can cause devastating losses in pecan. If left unchecked the insect can cause 50 - 90% damage (Cottrell and Wood 2003). Furthermore, based on the insect's fecundity and life-cycle, *C. caryae* has high propensity to increase in the orchard and cause increasingly higher intensities of damage in subsequent years (Harris et al. 1981, Harris 1985, Cottrell and Wood 2003). Therefore, high levels of control are required to protect the crop (Harris et al. 1981). Carbaryl can provide ≥ 95%, whereas some pyrethroids that are used for *C. caryae* control can provide 70 - 95% suppression (Payne and Dutcher 1985). In this study, the level of control observed in pots, with < 1% *C. caryae* surviving, is higher than the biocontrol levels reported previously and may be considered similar to levels provided by chemical insecticides. Additional research is required to expand upon this study and confirm the

level of control on an orchard scale (rather than in pots). Further, the timing, rate, and number of applications required should be optimized.

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