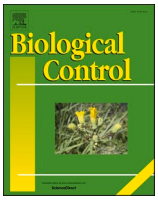
Biological Control 202 (2025) 105705

Contents lists available at ScienceDirect

Biological Control

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



Evaluation of persistent versus commercial nematode strains for management of *Curculio caryae* (Horn) and other weevils in pecan

Eddie K. Slusher [a,e], Elson Shields [b,f], Will Harges [c], Jermaine D. Perier [d], David Shapiro-Ilan [a,*]

[a] USDA-ARS Southeastern Fruit and Tree Nut Research Station, Byron, GA, USA
[b] Department of Entomology, Cornell University, Ithaca, NY, USA
[c] Strawberry Creek Ranch, Porter, OK, USA
[d] Department of Entomology, University of Georgia, Tifton, GA, USA
[e] Texas A&M AgriLife Research and Extension Center, Stephenville, TX, USA
[f] Persistent Biocontrol, Austin, TX, USA

HIGHLIGHTS

- Pecan weevil is a major pecan pest.
- Entomopathogenic nematodes are effective against pecan weevil.
- Entomopathogenic nematodes can be costly to apply and need frequent reapplication.
- Commercial nematode suppression of pecan weevils carried over into 2023.
- Commercial nematodes were more effective than putative persistent nematode strains.

ARTICLE INFO

Keywords:
Biological control
Fuller rose beetle
Steinernema carpocapsae
Steinernema feltiae
Two-banded Japanese weevil

ABSTRACT

Pecan weevil is a key pest of pecans. In addition, weevils such as Fuller rose beetle (FRB) and two-banded Japanese weevil (TJW) feed on pecan foliage and roots. Entomopathogenic nematodes (EPNs) have previously been shown to be effective tools for pecan weevil management. However, EPNs need frequent reapplication. Thus, there is a need to develop persistent strains of EPNs that can be applied less frequently and at lower rates. In this study, we compared two persistent strains of EPNs, NY01′ (*Steinernema carpocapsae* Weiser) and NY04′ (*Steinernema feltiae* Filipjev), against two commercial EPN strains, ScAll (*S. carpocapsae*) and SfSn (*S. feltiae*), in the lab and field. For the field study, the suppressive ability of each pair of EPNs on pecan weevil, FRB, and TJW was compared alongside a water only control. EPNs were only applied in the first year of the study (2022) and insect populations were monitored in 2022 and 2023. For the field study in Georgia, significantly fewer TJW were caught in trees treated with either nematode type in both study years. For the field study in Oklahoma, significantly fewer pecan weevils were caught in trees treated with commercial nematodes compared to the persistent nematodes and control in both study years. In lab trials, there was a lack of consistency in survival of the four strains. The results of this study indicate that commercial nematodes can have substantial carryover across two field seasons and can be applied at a significantly lower rate and still provide pest suppression.

1. Introduction

Pecan weevil (*Curculio caryae* Horn) is a key pest of pecans (Shapiro-Ilan and Gardner 2012; Shapiro-Ilan et al. 2017). Adults emerge from the soil in late summer and fall (late July – September) and feed on the kernel of developing nuts; females then lay eggs in the nuts. Pecan weevil larvae develop within the nut until they reach their fourth instar, after which they drop to the ground and overwinter in the soil. Soil-dwelling stages spend anywhere between two–three years in the soil (Usually 90 % of the population stays in the soil for 2 years, while the remaining 10 % emerge in year 3) before adults emerge to start the cycle again (Boethel and Eikenbary 1979).

* Corresponding author.
E-mail addresses: eddie.slusher@ag.tamu.edu (E.K. Slusher), eshields@persistentbiocontrol.com (E. Shields), willh@stonewoodok.com (W. Harges), jermaine.perier@uga.edu (J.D. Perier), david.shapiro@usda.gov (D. Shapiro-Ilan).

https://doi.org/10.1016/j.biocontrol.2025.105705
Received 21 November 2024; Received in revised form 17 January 2025; Accepted 20 January 2025
Available online 22 January 2025

In addition to pecan weevil, other species of weevil may be found in pecan orchards. Some examples include the Fuller rose beetle (*Naupactus cervinus* Boheman) (Tedders and Woods 1994; Bloem et al. 2002) and the two-banded Japanese weevil (*Pseudocneorhinus bifasciatus* (Roelofs) (Coleoptera: Curculionidae). Both species consist of populations of parthenogenic females that are widespread on many species of crops (King 1958; Maier 1983; Bloem et al. 2002). In both species, larvae are root-feeding, while the adults will feed on the foliage, buds, and blossoms of their hosts (King 1958; Maier 1983). Although feeding on pecans does occur, the effect of these weevils on yield is thus far unknown.

Previous research has indicated that entomopathogenic nematodes (EPNs) are virulent to pecan weevils (Shapiro-Ilan and Gardner 2012; Shapiro-Ilan et al. 2017). High levels of control using the nematode *Steinernema carpocapsae* Weiser have been reported (Shapiro-Ilan and Gardner 2012; Shapiro-Ilan et al. 2017). However, EPNs can be costly to apply to some crops. Moreover, populations of EPNs can be reduced before, during, and after application, e.g., with 40–80% loss often occurring within a few hours of post-application due to dehydration or desiccation from ultraviolet light exposure (Smits 1996), or simply due to the delivery method, such as irrigation lines (Ulu and Erdoğan, 2023; White, 1927). Those that survive and settle into the soil often experience a reduction in population at a rate of 5–10 % per day due to predation or desiccation, however, this can vary due to strain and environment (Smits 1996). For example, *S. carpocapsae* was reported to last 5 weeks in a cornfield (Warshaw 1992), while *S. feltiae* lasted up to 7 weeks in a turkey house (Geden et al. 1987). In addition, evaluation of *Heterorhabditis bacteriophora* Poinar, *H. megidis* Poinar, Jackson, & Klein, and *S. feltiae* Filipjev in maize found that all three species only persisted in the soil for 2–5 months (Kurtz et al. 2007, Ulu and Erdoğan, 2023).

Studies in sweet potato on *S. carpocapsae*, *S. feltiae*, *H. bacteriophora*, and an unknown *Heterorhabditis* sp. found that only the unknown *Heterorhabditis* sp. was recovered at elevated levels $>$ 230 days post-application (Jansson et al. 1993). Thus, in most instances, commercial usage of EPNs has relied on reapplication at regular intervals (e.g., seasonally, or annually) in an inundative approach to biological control (Shapiro-Ilan et al. 2022, 2023).

The development of persistent strains of EPNs that can survive multiple years in the soil and provide multiple years of control is a potentially valuable tool for reducing costs associated with EPN applications (Shields and Testa 2017; Shields et al. 2018). Previous studies have found that EPNs can persist for a long time in the soil with timetables ranging from a year to as long as fifty years across multiple species of EPN (Mráček and David 1986; Gaugler et al. 1992; Klein and Georgis 1992) and in agricultural fields for multiple growing seasons (Shields and Test 2017; Shields et al. 2018, 2021; Shields and Testa 2020).

Certain EPN strains have been reported to exhibit superior persistence in biocontrol applications. For example, *S. carpocapsae* (NY01′ strain), *S. feltiae* (NY04′ strain), and *H. bacteriophora* (Oswego strain) were isolated from soils in New York, USA and reared to maintain their persistence genes. These strains significantly provided multiple years of persistence and suppression for alfalfa snout beetle, *Otiorhynchus ligustici* (L.) (Coleoptera: Curculionidae), black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), whitefringed beetle, *Naupactus* spp. Dejean (Coleoptera: Curculionidae), and wireworms (Coleoptera: Elateridae) (Lauriault et al. 2020; Neumann and Shields 2008; Shields 2021; Shields et al. 2018, 2021; Shields and Testa 2020). However, these studies only compared the effectiveness of the persistent strains among themselves and did not directly compare them to entomopathogenic nematodes that are commercially available strains.

Additionally, the persistent strains indicated above (*S. carpocapsae* NY01′ and *S. feltiae* NY04′) were found to be effective at lower rates than commercially recommended rates. A general recommendation for commercial EPN applications is to apply a minimum of 2.5 billion IJs per ha (Shapiro-Ilan et al. 2022). The persistent strains were reported to be effective at 2–10 times lower than the commercially recommended rates (Shields et al. 2018; Lauriault et al. 2020). Those EPN inoculation rates have been further decreased to 100–150 million IJs per ha in agricultural crops with no negative impact and have been used to successfully inoculate corn fields against corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), across 10,000 ha in the past 2 years (Shields, unpublished).

In this study, we compare the suppression abilities of commercially available *S. carpocapsae* (ScAll strain) and *S. feltiae* (SfSn strain) against persistent counterparts *S. carpocapsae* (NY01′ strain) and *S. feltiae* (NY04′ strain) against pecan weevil, two-banded Japanese weevil, and Fuller rose beetle in two pecan orchards in different pecan growing regions of the United States (Oklahoma and Georgia). The field experiments were conducted using low EPN application rates. In addition, we also evaluated persistence of the two *S. carpocapsae* strains and the two *S. feltiae* strains against each other in the lab.

2. Materials and methods

2.1. Field study

2.1.1. Site Layout

This study was conducted in two pecan orchards: one mixed cultivar orchard located at the USDA-ARS station in Byron, GA (32°39′25.6″ N 83°44′31.7″ W) and one native orchard located in Porter, Oklahoma at the Strawberry Creek Ranch (35°54′25.3″N 95°29′32.7″ W). Plots were arranged in a randomized complete block design. The experimental setup for the Byron site initially consisted of 4 blocks containing three plots (1 plot per treatment) with three trees per plot in 2022. However, due to orchard removal (due to age), only three blocks were assessed in 2023. The Oklahoma site consisted of 4 blocks containing three plots. However, due to higher pecan weevil densities at this site, just two trees per plot were used. To prevent cross-contamination by nematode movement, there were at least two buffer trees between each treatment in every cardinal direction (~36 m from trunk to trunk).

2.1.2. EPN production and application

Two EPN species representing four different strains were evaluated in this experiment including two commercially available strains: *S. carpocapsae* (ScAll strain), *S. feltiae* (SfSn strain) and two persistent strains: *S. carpocapsae* (NY01′) and *S. feltiae* (NY04′). *S. carpocapsae* (All strain), and *S. feltiae* (SN strain) were chosen because they are the most produced strains for those species (Shapiro-Ilan, personal observation). All four nematode strains were cultured in vivo at 25° C in the last instars of commercially obtained *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). SfSn and ScAll used for inoculating *G. mellonella* were obtained from culture collections stored at the USDA-ARS Southeastern Fruit and Tree Nut Research Station Invertebrate Pathology Lab (Byron, GA). NY01′ and NY04′ used for inoculation were obtained from Elson Shield's laboratory at Cornell University (Ithaca, NY).

Infective juveniles (IJs) were collected from cadavers using a White trap (White 1927, Hazir et al. 2022) system modified for mass production (Shapiro-Ilan et al. 2023). This modified system involved placing *G. mellonella* larvae that were previously infected with one of the four strains of EPNs onto a small, inverted plastic container (5.2 L, 29.2 cm x 18.7 cm x 15.2 cm, Sterilite Corporation, Townsend, MA, USA) lined with moistened paper towel. This plastic container was then placed inside a larger plastic container (30 L, 60 cm x 41.6 cm x 16.5 cm, Sterilite Corporation, Townsend, MA, USA) filled with 10 L of water which the IJs would migrate to upon emergence. IJs were collected from the water every day to prevent high mortality due to lack of oxygen. All emerged IJs were stored at 14° C until use and were used no later than 2 weeks post emergence.

Treatments were applied in 2022. The area of application was a circle with a 4 m radius (50.24 m^2 area) around each pecan tree within the plot. Treatments consisted of commercial strains, persistent strains, or a water-only control. Commercial strains (SfSn and ScAll) were

combined into a 1:1 mixture prior to application. The same was done for the two persistent strains (NY04′ and NY01′). The reason for mixing the strains was based on a recommendation to do so for the persistent strains to enhance efficacy (Shields et al. 2021) (so the same was done with the commercial strains).

Originally the goal was to apply the EPNs at a rate of ~ 12,500 IJs/m^2 per strain within the 4 m radius area for each strain pair's (ScAll + SfSn or NY01′ + NY04′) assigned trees (e.g. ~ 12,500 IJs/m^2 ScAll + ~12,500 IJs/m^2 SfSn for commercial EPN treated trees). However, due to the difficulty of mass rearing some of the strains, two separate applications were applied at each location. The first application, consisting of ~ 125,000 IJs per/strain/tree, was applied on June 9th, 2022, and June 15th, 2022, at the Byron and Porter sites, respectively. The second application, consisting of ~ 500,000 IJs per/strain/tree, was applied on August 16th, 2022, and August 25th, 2022, at Byron and Porter, respectively. Ultimately, Nematodes were applied at a rate of 625,000 IJs per/strain/ treated tree (~12,440 IJs/m^2 per treated tree for each strain tested).

For application, nematodes were mixed in (~7 L) of water and applied evenly around the treatment area using 7-liter plastic watering cans (Novelty Manufacturing, Lancaster, PA, USA). For the control trees, an equal amount of only water was applied. Irrigation was maintained in the Byron site immediately after application and several days afterward, to allow time for nematode establishment; irrigation was not available at the Oklahoma site. No applications were made in 2023, so this process was not repeated. Rather, the persistence of the nematode treatment effects from 2022 was assessed.

2.1.3. Weevil collection

For Georgia, all weevils (pecan weevil, Fuller rose beetle, and two-banded Japanese weevil) were collected from August to October 2022 and May to November 2023. For Oklahoma, weevils (pecan weevil only) were collected from August to October during both years of the study. The early collection time in the second year in Georgia was done to get a better representation of the Fuller rose weevil and two-banded weevil, which arrived earlier in the field season than the pecan weevil. To capture emerging adult weevils, circle traps consisting of wire mesh (1.5-mm pore size) with a 44-cm wide open area were placed ~ 1 m above the soil surface on the sides of each treated tree (Shapiro-Ilan et al. 2017). The number of traps placed on each tree was determined according to the diameter of each tree's trunk. Traps were placed so that the entire circumference of the tree was covered. Traps were checked every other day and the total number of weevils emerging for each species among treatment plots was compared to quantify the number of weevils emerging in each treatment.

2.2. Lab study

2.2.1. Experimental design

Entomopathogenic nematode persistence was assessed in the laboratory based on methods described in Shapiro-Ilan et al. 2006. Experimental units consisted of 30 ml plastic cups (Comfy Package, Brooklyn, NY, USA) containing five grams of oven-dried sand (Quikrete® Premium Play Sand, Atlanta, GA, USA) at 8 % moisture. Approximately 5,000 IJs per strain were applied in 0.5 ml of tap water with five replicates per strain for each assessment period (1, 7, 14, 21, 28, 42, 56 days). To prevent desiccation due to the sand drying out during the assessment period, cups were placed in 49.2-liter plastic trash bags with a glass flask containing 100 ml of water and stored at 25° C.

2.2.2. EPN assessment

For each assessment period, sand from each sample was individually emptied into a glass beaker. For the first trial, samples were dumped into 300 ml of water. During the second trial, this amount was reduced to 100 ml of water to increase nematode recovery. After dumping, 4 ml of water was used to rinse any remaining sand/nematodes from each sample cup. This brought the total volume of the beaker up to 304 ml (first trial) and 104 ml (second trial). The sand suspension was then spun using a magnetic stir bar at 300 RPM to decant IJs from the sand. For each suspension, 2 ml of water was pipetted onto a 60 x 15 mm grided Petri dish (Corning Incorporated, Corning, USA) and placed under a Meiji RZ stereo microscope (Meiji Techno Co. Ltd., Hicksville, USA). The number of live and dead nematodes was then counted. IJs were considered alive if they were moving or if they responded to probing with a small needle. Two counts were recorded for each sample (4 ml total). To prevent cross-contamination between samples, both the glass beaker and Petri dish were rinsed multiple times with water between samples.

2.3. Analysis

All data were analyzed using the GLIMMIX procedure with a repeated measures design (Distribution: Gaussian, Link: Identity) using SAS 9.4 statistical software assuming a normal distribution. For the field study, statistical analysis was performed to compare the number of captured weevils among the treatment groups using tree, treatment, and tree*treatment as fixed effects. To meet the assumption of normality, the Georgia data was transformed using the square root function. The Oklahoma data was transformed using the log + 1 function.

For the lab study, each sample was extrapolated to figure out the total amount of nematodes in each cup by multiplying the dilution factor (based on the amount of water in which each sample was placed) (trial 1 = Average # of live nematodes per sample*(304/2); trial 2 = Average # of live nematodes per sample*(104/2). Percentage survival was calculated by dividing the average number of live nematodes collected on each sampling day by the average number of live nematodes collected on day 1 (Based off methodology in Shapiro-Ilan et al. 2006). Due to a significant interaction between strain and trail, each trial was analyzed separately. For the model, strain, day, and stain*day were used as fixed effects. Lab data were transformed using the log + 1 function. Means were separated using the Tukey-Kramer test at a 0.05 significance level.

3. Results

3.1. Field study

3.1.1. Fuller rose beetle

Fuller rose beetle was only captured at the Byron site. Across both years of the study, no significant differences were detected amongst the commercial and persistent EPN strains (2022: $F = 2.59$, $DF = 2, 632$, $P = 0.0759$; 2023: $F = 2.60$, $DF = 2, 1696$, $P = 0.0748$; Fig. 1A).

3.1.2. Two-banded Japanese weevil

Two-banded Japanese weevil was only captured at the Byron site. In both 2022 and 2023, significantly lower weevil numbers were captured in the trees treated with IJs compared to the control trees (2022: $F = 5.52$, $DF = 2, 632$, $P = 0.0042$; 2023: $F = 8.51$, $DF = 2, 1696$, $P = 0.0002$, Fig. 1B).

3.1.3. Pecan weevil

At the Byron site, there was no significant difference in pecan weevil numbers in 2022 for any treatment ($F = 1.69$, $DF = 2, 632$, $P = 0.1850$, Fig. 1C). In 2023, significantly more pecan weevils were captured in trees treated with persistent strain compared to trees treated with commercial nematodes or the control trees ($F = 7.21$, $DF = 2, 1696$, $P = 0.0008$, Fig. 1C).

At the Oklahoma site, in 2022 and 2023, significantly fewer pecan weevils were captured in trees treated with commercial nematodes than in trees treated with persistent nematodes or the control (2022: $F = 11.10$, $DF = 2, 355$, $P < .0001$; 2023: $F = 17.61$, $DF = 2, 175$, $P < .0001$ Fig. 1D).

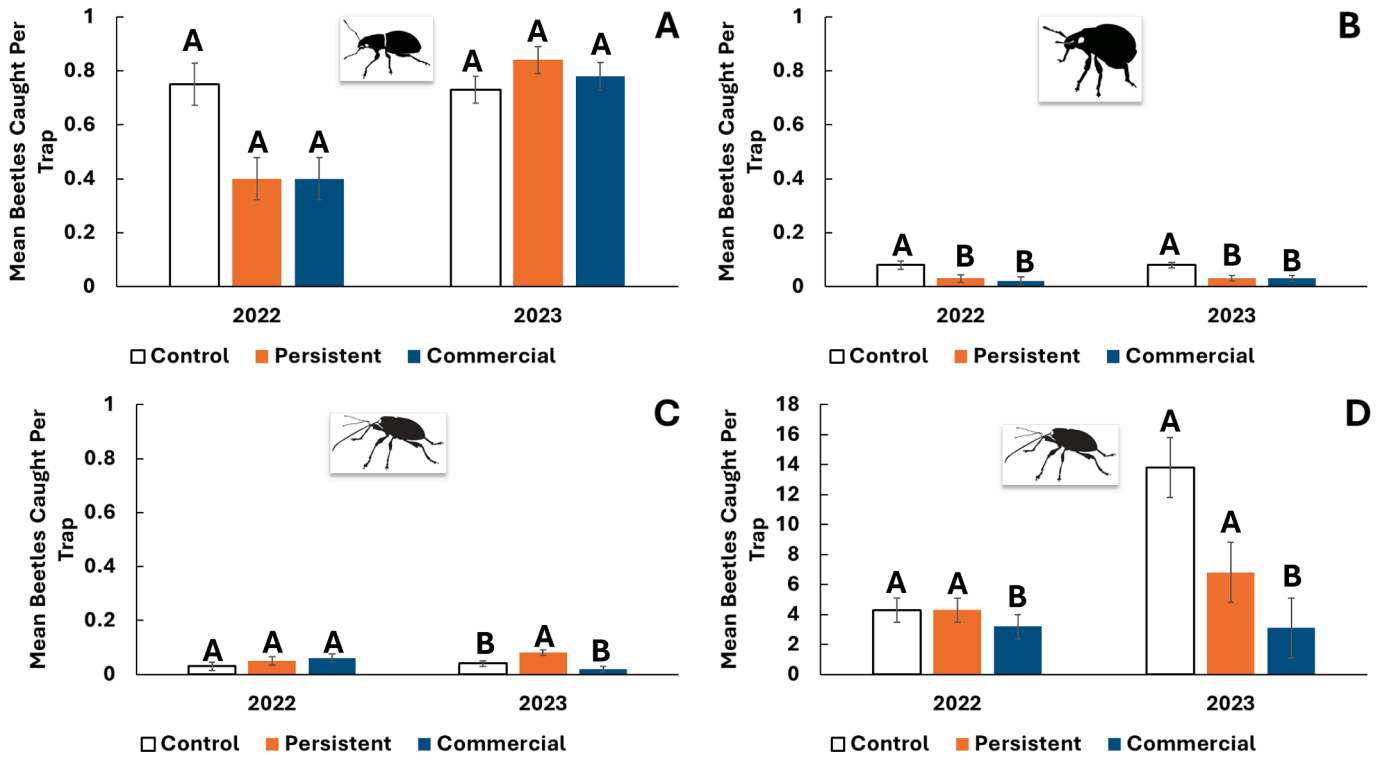


Fig. 1. Mean number of beetles caught per trap per year for A) Fuller rose beetle, B) Two-banded Japanese weevil, C) Pecan weevil (**Georgia,** GA), and D) Pecan weevil (**Oklahoma,** OK). Persistent or commercial strains of entomopathogenic nematodes**, at a ratio of 1:1,** were applied in 2022 only; the same plots were assessed in 2022 and 2023. Years were analyzed separately. Differing letters above bars designate a significant difference at α = 0.05.

3.2. Lab study

Due to a significant interaction between trail and strain, trials were analyzed individually. When comparing the strains overall in trial 1, The persistent *S. feltiae* strain had significantly higher survival than the *S. feltiae* commercial strain. No other significant differences were found among the other 3 strains (F = 4.26, DF = 3, 72, $P = 0.0079$; Fig. 2). For trial 2, the commercial strain of *S. feltiae* had significantly higher survival than the other three strains. In addition, the commercial *S. carpocapsae* strain had significantly higher survival than the persistent *S. feltiae* (F = 43.56, DF = 3, 72, $P < .0001$; Fig. 2).

Significant differences were also found when comparing the survival within each sampling date in trial 2 but not in trial 1. On the 7-day sampling date in trial 2, the persistent *S. carpocapsae* strain had significantly lower survival than the other three strains (F = 19.12, DF = 3, 16, $P < .0001$; Fig. 3). On sampling day 28 in trial 2, the persistent *S. feltiae* had significantly lower survival than the other three strains (F = 12.50, DF = 3, 16, $P = 0.0002$; Fig. 3). On day 56 in trail 2, commercial *S. feltiae* had significantly higher survival than the other three strains. In addition, the persistent *S. carpocapsae* strain had higher survival than the persistent *S. feltiae* strain (F = 119.44, DF = 3, 16, $P < .0001$; Fig. 3).

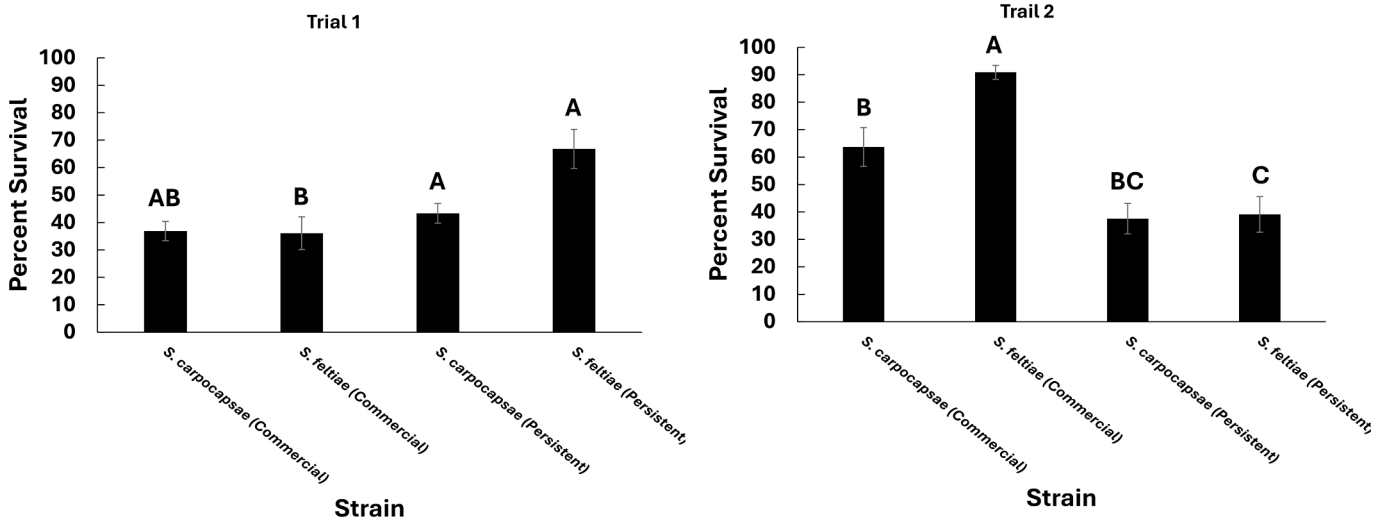


Fig. 2. Mean survival per cup for the lab test comparing overall survival of all four strains in trial 1 and trial 2 sampled at 7, 14, 21, 28, 42, and 56 days post inoculation. Differing letters designate a mean significant difference at α = 0.05.

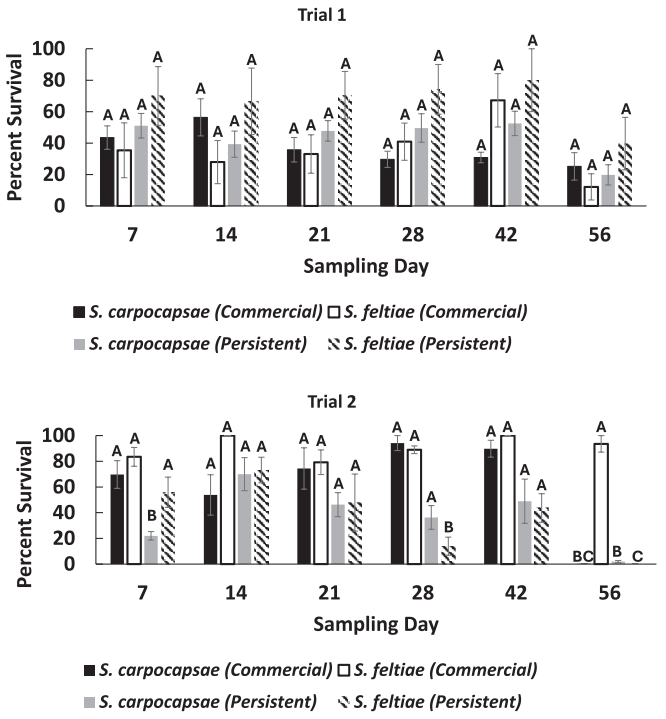


Fig. 3. Mean survival per cup for the lab test comparing all four strains at different sampling days in trial 1 and trial 2. Differing letters designate a mean significant difference at $\alpha = 0.05$.

4. Discussion

The results of this study indicate that the persistent strains of *S. carpocapsae* and *S. feltiae* had no significant advantage in persistence over their commercial counterparts in the field and lab. Previous studies in multiple cropping systems such as corn, strawberries, and sweet potatoes had shown that persistent strain nematodes reduce crop damage from pests over multiple years (Shields et al. 2018, 2021; Shields and Testa 2020). However, the persistent strains utilized in this study did not significantly reduce pecan weevil captures in 2022 or 2023 at both locations whereas commercial strains reduced pecan weevils in Oklahoma in both years. It should be noted that this comparison was only made over two functional field seasons. Longer persistence and phased infectivity with these strains, as seen with other reports and species of weevils, may inhibit pecan weevil populations in subsequent field seasons (Shields et al. 2009; Shields and Testa 2017, 2020). Additionally, the EPNs were applied at extremely lower rates (~125 million IJs per ha) compared to the norm (2.5 billion IJs per ha, Shapiro-Ilan et al. 2022); higher rates would likely have shown higher levels of efficacy.

Adult pecan weevil emerges from the soil at irregular intervals with a small portion (~10%) of weevils spending as long as 3 years before emergence (Harris and Neel 1985). This may indicate that we may not see the true effects of nematode suppression for a few more years. Previous studies on NY01′ and NY04′ found that NY01′ maintained a population in 8–13 % of soil cores, while NY04′ maintained a population in 21–32 % of soil cores for up to 6 years post application (Shields et al. 2018). In addition, other factors such as the presence of alternative hosts and soil conditions, may have also played a role in the results we saw. For Georgia, the low pecan weevil numbers we saw in this study reflect current population trends throughout the state.

We also did not observe any consistent advantages in persistence across the two trials of our lab study. While the persistent *S. feltiae* outperformed commercial *S. feltiae* in the overall model for trial 1, this was not consistent in trial 2. However, this study looked at the survival of a single generation of nematodes over the course of the study. Previous research has indicated that the persistence of persistent nematodes lies in establishing the strains in the soil at low rates and allowing them to recycle and build up their populations (Shields et al. 2018; Shields and Testa 2020; Shields et al. 2021). Our lab study did not take recycling potential into account.

Future research could examine which of the two species applied to the field (*S. carpocapsae* or *S. feltiae*) are present in subsequent years and which EPN strain is most responsible for the results observed in this study. In a previous study with a 50/50 mixture of persistent nematodes (NY01′ and NY04′) in alfalfa fields, *S. feltiae* was found in 20–30 % of samples recovered and had the greatest effect on pest suppression. Meanwhile. *S. carpocapsae* was not recovered at all (Lauriault et al. 2020). The application of multiple EPN species can have its advantages. For example, *S. carpocapsae* often stays at around 5–7 cm into the soil profile and has limited dispersal (Ferguson et al. 1995). Meanwhile, *S. feltiae* prefers to be deeper in the soil and is more mobile (Neumann and Shields 2006). Thus, it has been argued that the advantages of using these two species provide better coverage of the treated area.

We also examined mortality in the root-feeding weevils collected in this study. We did not see any significant effects on Fuller rose beetle control in either year of our study. This contrasted with previous research showing great reduction (80 %) by S. *carpocapsae* on Fuller rose beetle in citrus compared to water-treated control trees in the second-year post-application. This could have been attributed to our rate (12,500 IJs/m^2) which was lower than the lowest rate (500,000 IJs/cm^2) applied in Morse and Lindegren 1996. Regardless, there is previous evidence that entomopathogenic nematodes can successfully suppress pecan weevil and Fuller rose beetle (Perier et al. 2024; Shapiro-Ilan et al. 2017; Mores and Lindegren 1996). Thus, there is potential that by treating pecan weevil with entomopathogenic nematodes, growers can also potentially target other minor pests as well. We also observed a significant reduction in two-banded Japanese weevil in both years of the study. Previous research in peach found that application of EPNs significantly reduced the number of emerging adult root-feeding weevils including two-banded Japanese weevils (Wong et al. 2022). However, in our study, two-banded Japanese weevil was caught in exceptionally small numbers across both collecting periods, thus making it hard to draw conclusions on our treatments having any effect. Both root-feeding weevils are known to be major agriculture pests in other crops such as citrus and avocado and are potential pests on peach (Ebeling and Pence 1952; Mores and Lindegren 1996; Cottrell and Horton 2013). However, their economic effects on pecans are poorly understood. Future studies will be needed to analyze potential effects of root-feeding weevil feeding on pecan production.

Our results indicate that both commercial nematodes can serve as an effective tool for pecan weevil management. In addition, commercial nematodes can potentially provide management for multiple seasons, as substantial carryover was observed in Oklahoma in 2023. Given the lower rate (~125 million IJs per ha vs. 2.5 billion per ha, a 20-fold difference) there is potential to lower the number of EPN applications for pecan weevil management. How this affects the costs associated with applications would require more evaluation but could result in a more affordable approach at a lower rate. Future studies should examine how much recycling of commercial EPNs can be done before reapplication. Data was only collected over two growing seasons, so it is not clear if the commercial strains of EPNs will continue to be present and suppress pest populations in the coming seasons. Given the evidence in previous literature (Gaugler et al. 1992; Klein and Georgis 1992; Jansson et al. 1993), it seems possible that the nematodes applied in this study could persist for several years. Koppenhöfer and Sousa (2024) recently replicated the methods in Shields and Testa 2017 to develop persistent strains of *S. carpocapsae* and *H. bacteriophora* and applied them to turfgrass. Follow up sampling found evidence of suppression of numerous turfgrass pests across three field seasons. Future research will explore optimization of rate application to determine the most cost-effective rate for growers that could still meet pest management needs.

CRediT authorship contribution statement

Eddie K. Slusher: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elson Shields:** Writing – review & editing, Validation, Resources, Conceptualization. **Will Harges:** Writing – review & editing, Resources, Investigation, Conceptualization. **Jermaine D. Perier:** Writing – review & editing, Software, Formal analysis. **David Shapiro-Ilan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Funding

This work was supported by The Georgia Agricultural Commodity Commission for Pecans (GACCP) Research Grant (FY23).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the technical assistance of Ashley Allgary (USDA/UGA), Stacy Byrd (USDA), Tony Testa (Cornell University), Domonique White (USDA), Darius Ruff (USDA), Seleah Starks (USDA), and Janyah Robinson (USDA). We would also like to thank Quentin Read (USDA) for assistance with statistics. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Georgia or the United States Department of Agriculture.

References

Bloem, S., Mizell III, R.F. , O'Brien, C.W., 2002. Old traps for new weevils: new records for curculionids (Coleoptera: Curculionidae), brentids (Coleoptera: Brentidae) and anthribids (Coleoptera: Anthribidae) from Jefferson Co., Florida. Fla. Entomol., 85 (4):632-644. doi: 10.1653/0015-4040(2002)085[0632:OTFNWN]2.0.CO;2.

Boethel, D.J., Eikenbary, R.D., 1979. Status of pest management programs for the pecan weevil. In: *Pest Management Programs for Deciduous Tree Fruits and Nuts*. Springer, US, Boston, MA, pp. 81–119.

Cottrell, T.E., Horton, D.L., 2013. Emergence of root-feeding weevils (Coleoptera: Curculionidae) in Central Georgia peach orchards. J. Entomol. Sci. 48 (3), 184–194. https://doi.org/10.18474/0749-8004-48.3.184.

Ebeling, W., Pence, R.J., 1952. Pests of the avocado. California Avocado Society Yearbook 37, 113–133.

Gaugler, R., Campbell, J.F., Selvan, S., Lewis, E.E., 1992. Large-scale inoculative releases of the entomopathogenic nematode *Steinernema glaseri*: assessment 50 years later. Biol. Control 2 (3), 181–187. https://doi.org/10.1016/1049-9644(92)90057-K.

Harris, M.K., Neel, W.W., 1985. Pecan phenology and pecan weevil biology and management. In: *Pecan weevil: research perspective*. Quail Ridge Press, Brandon, MS, pp. 51–58.

Hazir, S., Kaya, H., Touray, M., Cimen, H., Shapiro-Ilan, D.I., 2022. Basic laboratory and field manual for conducting research with the entomopathogenic nematodes, Steinernema and Heterorhabditis, and their bacterial symbionts. Turk. J. Zool. 46 (4), 305–350. https://doi.org/10.55730/1300-0179.3085.

Jansson, R.K., Lecrone, S.H., Gaugler, R., 1993. Field efficacy and persistence of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) for control of sweetpotato weevil (Coleoptera: Apionidae) in southern Florida. J. Econ. Entomol. 86 (4), 1055–1063. https://doi.org/10.1093/jee/86.4.1055.

King, J.R., 1958. Occurrence, distribution, and control of Fuller's rose beetle in Florida citrus groves. Proceedings of the Florida State Horticultural Society 71, 146–152.

Klein, M.G., Georgis, R., 1992. Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. J. Econ. Entomol. 85 (3), 727–730. https://doi.org/10.1093/jee/85.3.727.

Koppenhöfer, A.M., Sousa, A.L., 2024. Long-term suppression of turfgrass insect pests with native persistent entomopathogenic nematodes. J. Invertebr. Pathol. 204, 108123. https://doi.org/10.1016/j.jip.2024.108123.

Kurtz, B., Toepfer, S., Ehlers, R.U., Kuhlmann, U., 2007. Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. J. Appl. Entomol. 131 (6), 420–425. https://doi.org/10.1111/j.1439-0418.2007.01202.x.

Lauriault, L.M., Shields, E.J., Testa, A.M., Porter, R.P., 2020. Persistence of select introduced entomopathogenic nematodes in the US southwest as potential biological control for whitefringed beetle in alfalfa. Southwest. Entomolog. 45 (1), 41–50. https://doi.org/10.3958/059.045.0104.

Maier, C.T., 1983. Influence of host plants on the reproductive success of the parthenogenetic two-banded Japanese weevil, *Callirhopalus bifasciatus* (Roelofs) (Coleoptera: Curculionidae). Environ. Entomol. 12 (4), 1197–1203. https://doi.org/10.1093/ee/12.4.1197.

Morse, J.G., Lindegren, J.E., 1996. Suppression of fuller rose beetle (Coleoptera: Curculionidae) on citrus with *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Fla. Entomol. 79 (3), 373–384.

Mráček, Z., David, L., 1986. Preliminary field control of *Cephalcia abietis* L. (Hymenoptera, Pamphilidae) larvae with steinernematid nematodes in Czechoslovakia. J. Appl. Entomol. 102 (1–5), 260–263. https://doi.org/10.1111/j.1439-0418.1986.tb00920.x.

Neumann, G., Shields, E.J., 2006. Interspecific interactions among three entomopathogenic nematodes, *Steinernema carpocapsae* Weiser, *S. Feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar, with different foraging strategies for hosts in multipiece sand columns. Environ. Entomol. 35 (6), 1578–1583. https://doi.org/10.1093/ee/35.6.1578.

Neumann, G., Shields, E.J., 2008. Multiple-species natural enemy approach for biological control of alfalfa snout beetle (Coleoptera: Curculionidae) using entomopathogenic nematodes. J. Econ. Entomol. 101 (5), 1533–1539. https://doi.org/10.1093/jee/101.5.1533.

Perier, J.D., Kaplan, F., Lewis, E.E., Alborn, H., Schliekelman, P., Toews, M.D., Schiller, K.C., Shapiro-Ilan, D.I., 2024. Enhancing entomopathogenic nematode efficacy with Pheromones: A field study targeting the pecan weevil. J. Invertebr. Pathol. 203, 108070.

Shapiro-Ilan, D.I., Cottrell, T.E., Bock, C., Mai, K., Boykin, D., Wells, L., Hudson, W.G., Mizell III, R.F., 2017. Control of pecan weevil with microbial biopesticides. Environ. Entomol. 46 (6), 1299–1304. https://doi.org/10.1093/ee/nvx144.

Shapiro-Ilan, D.I., Stuart, R. J., McCoy, C.W., 2006. A comparison of entomopathogenic nematode longevity in soil under laboratory conditions. J. Nematol. 38(1):119-129. 19259437.

Shapiro-Ilan, D.I., Garrigos Leite, L., Han, R., 2023. Production of entomopathogenic nematodes. In: Morales-Ramos, J., Rojas, G., Shapiro-Ilan, D.I (Eds.), *Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens*, 2nd Edition. Academic Press, Amsterdam, pp. 293–316. https://doi.org/10.1016/B978-0-12-822106-8.00005-1.

Shapiro-Ilan, D.I., Hazir, S., Glazer, I., 2022. *Entomopathogenic nematodes as models for inundative biological control. In: Nematodes as Model Organisms*. CABI, Wallingford, UK, pp. 293–308. https://doi.org/10.1079/9781789248814.0012.

Shapiro-Ilan, D., Gardner, W.A., 2012. Improved control of *Curculio caryae* (Coleoptera: Curculionidae) through multi-stage pre-emergence applications of Steinernema carpocapsae. J. Entomol. Sci. 47 (1), 27–34. https://doi.org/10.18474/0749-8004-47.1.27.

Shields, E., 2021. Biological control of corn rootworm with native NY entomopathogenic nematodes. Progressive Dairy.

Shields, E.J., Testa, A.M., 2020. Multi-year biological control of black vine weevil, *Otiorhynchus sulcatus*, with persistent entomopathogenic nematodes. The Great Lakes Entomologist 53 (2), 119–125. https://doi.org/10.22543/0090-0222.2377.

Shields, E.J., Testa, A.M., Neumann, G., Flanders, K.L., Schroeder, P.C., 2009. Biological control of alfalfa snout beetle with a multi-species application of locally adapted persistent entomopathogenic nematodes: The first success. Am. Entomol. 55 (4), 250–257. https://doi.org/10.1093/ae/55.4.250.

Shields, E.J., Testa, A.M., 2017. Biological control of alfalfa snout beetle with persistent entomopathogenic nematodes: Expanding a single farm's success to an area-wide biological control program. Am. Entomol. 63 (4), 216–223. https://doi.org/10.1093/ae/tmx046.

Shields, E.J., Testa, A.M., O'Neil, W.J., 2018. Long-term persistence of native New York entomopathogenic nematode isolates across crop rotation. J. Econ. Entomol. 111 (6), 2592–2598. https://doi.org/10.1093/jee/toy258.

Shields, E., Testa, A., Rusinek, T., Bornt, C., 2021. Management of Wireworms in Sweet Potatoes with Persistent NY Entomopathogenic Nematodes. The Great Lakes Entomologist 54 (2), 73–80. https://doi.org/10.22543/0090-0222.2401.

Smits, P.H., 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Sci. Tech. 6 (3), 379–388. https://doi.org/10.1080/09583159631352.

Tedders, W.L., Wood, B.W., 1994. A new technique for monitoring pecan weevil emergence (Coleoptera: Curculionidae). J. Entomol. Sci. 29 (1), 18–30. https://doi.org/10.18474/0749-8004-29.1.18.

Ulu, T.C., Erdoğan, H., 2023. Field application of encapsulated entomopathogenic nematodes using a precision planter. Biological. Control 1 (182), 105240. https://doi.org/10.1016/j.biocontrol.2023.105240.

White, G.F., 1927. A method for obtaining infective nematode larvae from cultures. Science 66 (1709), 302–303. https://doi.org/10.1126/science.66.1709.302.b.

Wong, C., Oliveira-Hofman, C., Blaauw, B.R., Chavez, D., Jagdale, G., Mizell III, R.F., Shapiro-Ilan, D., 2022. Using the Nematode, *Steinernema carpocapsae*, to Control Peachtree Borer (*Synanthedon exitiosa*): Optimization of Application Rates and Secondary Benefits in Control of Root-Feeding Weevils. Agronomy 12 (11), 2689. https://doi.org/10.3390/agronomy12112689.