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Research

Insect–Microbial Interactions

Control of Pecan Weevil With Microbial Biopesticides

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Abstract

The pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae), is a key pest of pecans *Carya illinoinensis* ([Wangenh.] K. Koch) (Fagales: Juglandaceae). Control recommendations rely on broad spectrum chemical insecticides. Due to regulatory and environmental concerns, effective alternatives for *C. caryae* control must be sought for pecan production in conventional and organic systems. We explored the use of microbial biopesticides for control of *C. caryae* in Georgia pecan orchards. Three experiments were conducted. The first investigated an integrated microbial control approach in an organic system at two locations. Three microbial agents, Grandevo (based on byproducts of the bacterium *Chromobacterium subtsugae* Martin, Gundersen-Rindal, Blackburn & Buyer), the entomopathogenic nematode *Steinernema carpocapsae* (Weiser), and entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, were applied to each treatment plot (0.6 ha) at different times during the season. A second experiment compared the effects of *S. carpocapsae* and *B. bassiana* applied as single treatments relative to application of both agents (at different times); survival of *C. caryae* was assessed approximately 11 mo after larvae were added to pots sunk in an organic pecan orchard. In a conventional orchard (with 1.0 ha plots), the third experiment compared Grandevo applications to a commonly used regime of chemical insecticides (carbaryl alternated with a pyrethroid). All experiments were repeated in consecutive years. The combined pest management tactic (experiment 1) reduced *C. caryae* infestation relative to non-treated control plots in both locations in 2014 and one of the two locations in 2015 (the other location had less than 1% infestation). In experiment 2, no differences among combined microbial treatments, single-applied microbial treatments or different numbers of application were observed, yet all microbial treatments reduced *C. caryae* survival relative to the control. In the third experiment, both Grandevo and standard chemical insecticide applications resulted in lower weevil infestation than the control (both years) and there was no difference between the insecticide treatments in 2014 although the chemical insecticide regime had slightly lower infestation in 2015. These results provide evidence that microbial biopesticides can substantially reduce pecan weevil infestations in organic and nonorganic systems.

Key words: *Beauveria bassiana*, biological control, *Chromobacterium subtsugae*, *Curculio caryae*, organic

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] (Fagales: Juglandaceae) is an economically important North American nut crop (Wood 2003). The pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae), is a key pecan pest affecting orchard nutmeat yield and quality throughout the Southeastern United States, and portions of Texas and Oklahoma (Payne and Dutcher 1985). Commercial pecan growers do not have any set tolerance levels for *C. caryae* damage (once the weevil establishes in the orchard the population can increase rapidly over subsequent years). These insects have a 2- or 3-yr life cycle (Harris 1985) with most adult weevils emerging from soil beneath trees from late July through September to feed on and oviposit in, the kernel of developing fruit (Harris 1985). Larval development is completed within the nutmeat of the ripening nut. Fourth instars drop to the ground and burrow to a depth of 8–25 cm, form a soil cell, and over-winter. During the following autumn approximately 90% of larvae pupate and spend the next 9 mo in the soil as adults (Harris 1985). The remaining 10% of the population spend approximately 2 yr in the soil as larvae and emerge as adults in the third year (Harris 1985).

Current control recommendations for *C. caryae* consist primarily of above-ground applications of chemical insecticides (e.g., carbaryl and certain pyrethroids) to suppress adults (Harris

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1999, Wells et al. 2016). When *C. caryae* is detected in the orchard, applications of chemical insecticides are recommended every 7–10 d during peak emergence of *C. caryae* (which is generally ≥6 wk) (Wells et al. 2016). Although these chemical insecticide applications are effective in controlling *C. caryae* in conventionally managed orchards, there is a dearth of knowledge regarding pest management in organic pecan systems. Additionally, due to problems associated with aphid and mite resurgence that often result from chemical insecticide applications that target *C. caryae* (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies in both organic and conventional systems is warranted. Thus, our objective was to explore alternative control strategies for *C. caryae* that could be employed in organic and conventional pecan orchard systems.

Three experiments were conducted to address our objective. In the first experiment (experiment 1) we tested an integrated approach using three microbial biopesticides to control *C. caryae*: the entomopathogenic nematode *Steinernema carpocapsae* (Weiser), the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, and Grandevo (based on byproducts of the bacterium *Chromobacterium subtsugae* Martin, Gundersen-Rindal, Blackburn & Buyer). The microbial agents were chosen based on efficacy indicated in prior studies. Laboratory and small field-plot tests indicated that soil applications of *S. carpocapsae* (Shapiro-Ilan et al. 2001a,b; Shapiro-Ilan and Gardner 2012) and *B. bassiana* (Shapiro-Ilan et al. 2003a, 2008) caused high mortality in ground-dwelling stages of *C. caryae*. Applications of Grandevo, as a curative approach to control adult *C. caryae* in the canopy, have also shown promise in laboratory and small-plot field trials (Shapiro-Ilan et al. 2013). These microbial biopesticides have not, however, been tested in combination in large plot field studies. Furthermore, prior to this research, assessments of these treatments only determined *C. caryae* mortality but did not assess crop protection (i.e., the impact on percentage of weevil infested nuts).

The three microbial agents were applied at different times. The rationale was to apply microbial agents best suited to control *C. caryae* prior to adult emergence, during adult emergence, and later when larvae are dropping to the soil (which would prevent the subsequent generation's damage). *S. carpocapsae* was applied in the spring and early summer prior to *C. caryae* emergence because the nematodes can penetrate into the insect's pupal cell and kill larvae or adults (Shapiro-Ilan 2001a,b); in contrast, *B. bassiana* is repelled by the soil cell due to antibiotic effects (Shapiro-Ilan and Mizell 2015). Grandevo was applied to the canopy during adult weevil emergence. Grandevo is the only microbial biopesticide that has shown significant promise as a canopy spray (Shapiro-Ilan et al. 2013). *B. bassiana* was applied in the late summer-early fall, which is toward the end of the period of adult emergence and into the period that larvae are dropping to the soil; the idea was to create a "barrier" of fungus in the soil that the weevils would pass through and become infected.

Our hypothesis for experiment 1 was that the combined use of all three biopesticides would result in significant reduction in crop damage due to *C. caryae*. Subsequently, additional studies could be used to refine and optimize the application of these biopesticides. To that end, a second experiment (experiment 2) was conducted to determine if it is advantageous to apply both *B. bassiana* and *S. carpocapsae* to the soil relative to when each is applied alone.

It is important to both conventional and organic pecan growers to know how biopesticide options compare in efficacy to chemical insecticides that are currently recommended for control of *C. caryae*. Thus, in our third experiment (experiment 3), we compared canopy sprays of Grandevo to canopy sprays of a conventional spray program that growers are using (carbaryl and pyrethroids).

Materials and Methods

Microbial Biopesticides

B. bassiana (GHA strain) was obtained as Mycotrol-O (ES formulation, active ingredient 11.3%) from BioWorks (Victor, NY). Grandevo (WP, active ingredient 30%) was obtained from Marrone Bio Innovations (Davis, CA). *S. carpocapsae* (All strain) was obtained from e-nema GmbH (Schwentinental, Germany) for experiment 1, and grown in vivo using *Galleria mellonella* based on procedures described by Shapiro-Ilan et al. (2016a) for experiment 2. Note that in previous field experiments, *S. carpocapsae* obtained from the commercial source (e-nema, GmbH) and lab-grown *S. carpocapsae* did not differ significantly in control of the peachtree borer, *Synanthedon exitiosa* (Shapiro-Ilan et al., 2016b).

Experiment 1: Integrated Use of Microbial Biopesticides

The experiment was conducted at two locations, a commercial pecan orchard (Cleveland Farms, Fort Valley, GA) and in pecan orchards at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory (Byron, GA). The experiment was conducted in 2014 and repeated in 2015. Experimental plots at the Cleveland Farms location were certified organic and those used in this experiment in Byron, Georgia are managed organically (no chemical pesticides or fertilizers were used in the plots for at least 10 yr prior to the experiment). The Cleveland Farm's plots consisted of trees approximately 40–45 yr old (cultivar Elliott) with a spacing of 16 m × 22 m. The USDA plots consisted of trees approximately 70 yr old (a cultivar mix of Stuart and Schley) with 18 m × 18 m spacing. The experiments were randomized complete block designs with four blocks at the Fort Valley (Cleveland) location and three blocks at the Byron (USDA) location. Plot size was approximately 0.6 hectares and were a minimum of 18 m apart.

Two treatments were implemented: an integrated microbial pest management regime versus a no-pest management control. The pest management regime consisted of the three different microbial biopesticides applied at different times during the season (*S. carpocapsae*, Grandevo, and *B. bassiana*). *S. carpocapsae* was applied to soil surrounding the dripline of the tree (approximately 5–6 m radius) on 10 June 2014 at a rate of 1 million infective juvenile nematodes per m^2 in the first trial, and at 250,000 infective juveniles per m^2 on 25 June 2015 in the second trial. Rates of 250,000 to 1 million per m^2 have previously been used against *C. caryae* and various other soil-dwelling insect pests (Shapiro-Ilan and Gardner 2012, Shapiro-Ilan et al. 2016c). Grandevo was applied three times to the canopy during the weevil emergence period (at 2-wk intervals beginning 7 August 2014 and 17 September 2015) at 3.36 kg per hectare, which is the highest recommended label rate. *B. bassiana* was applied to soil around the dripline of the tree on 28 August 2014 and 15 September 2015 at 3×10^{12} conidia per tree (approximately 2.3×10^{10} per m^2); the rate was based on previous experiments that indicated efficacy (Shapiro-Ilan et al. 2008). Soil treatments were applied using a boom sprayer (Polaris Sportsman 400 AWD ATV with a Fimco 45 gallon tank and 12 volt 3.8 GPM pump, and an 8 foot boom with 7 spray nozzles, and screens removed), and the canopy spray treatments were applied using an airblast sprayer (Durand Wayland 1000 gallon Model 3210) with an approximate volume of 935 liters per hectare.

Treatment effects were assessed after harvest by determining the percentage of nuts infested with pecan weevil. Nuts were harvested between November and January each year. At least 100 nuts were randomly sampled per plot (range of 100 to 200). Only sample trees that were buffered by at least one other tree on each side within that treatment were used (i.e., trees on the edge of the plot were not sampled). Nuts with obvious *C. caryae* exit holes (Hudson 2007) were counted as infested. The remaining nuts were cracked and checked for the presence of *C. caryae* larvae.

Experiment 2: Small Plot Test Comparing Soil Applied Microbial Agents

The experiment was conducted in plastic buckets based on procedures described by Shapiro-Ilan and Gardner (2012) in an organically managed pecan orchard at the USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory. The orchard was a mix of cultivars of Desirable, Stuart, Cheyenne and Cape Fear (this is a separate organic orchard block from those utilized in experiment 1). The trees were approximately 27 yr old and spaced at 12.2 m × 12.2 m. The buckets (27.94 cm diameter × 30.48 cm height) had 160 mm holes drilled into the sides and a lid to allow for aeration. On 21 November 2014, 125 *C. caryae* larvae, collected according to (Shapiro-Ilan 2001), were placed into each bucket, which had been sunk into the ground approximately 3 cm below the soil surface and approximately 2 m from a tree trunk under the tree canopy. Any larvae that did not burrow down into the soil after 1 d were replaced. A lid was placed on the buckets, and they were covered with soil.

The experiment had a randomized complete block design with five treatments (including the control) and six blocks (6 replicates per treatment, thus, 5 treatments × 6 replicates = 30 trees total). The blocks were ordered by row going from north to south. Each bucket was placed in a randomly chosen cardinal direction. Each treatment application was separated from the next by a buffer tree.

The four biopesticide treatments varied in the microbial agent(s) added and or timing of application: 1) "Bb", *B. bassiana* applied twice, 1 wk prior and 1 d prior to introduction of *C. caryae* larvae at 1×10^{10} conidia per m^2; thus, *B. bassiana* was applied as a barrier treatment to the larvae entering soil, 2) "ScLow", *S. carpocapsae* applied once at 250,000 infective juveniles per m^2 (Shapiro and Gardner 2012) approximately 10 mo after larval introduction (applied 1 October 2015), 3) "ScHigh" applied twice (21 May 2015 and 1 October 2015) with each application at 1 million infective juveniles per m^2, 4) "Bb+Sc", a combination of Bb and ScHigh, and 5) an untreated control (*C. caryae* larvae and water only). All treatments were applied by distributing a 20 ml aqueous suspension from a plastic beaker evenly onto the soil surface.

Treatment effects were assessed approximately 11 mo after *C. caryae* larvae had been added to the soil. Assessments were made by replicate 22 to 24 October 2015 (labor requirements negated the possibility of assessment on a single day). Soil from each bucket was placed in a large plastic bin and the percentage of surviving *C. caryae* was determined. The experiment was repeated with 175 larvae (collected in the fall of 2015) and assessed 4 to 6 November 2016 (hence two complete trials were conducted).

Experiment 3: Comparison of Biopesticide and Chemical Insecticide Canopy Sprays

The experiment was conducted in four mature pecan orchards at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory (Byron, Georgia). These orchard blocks, unlike those utilized in experiments 1 and 2, was not managed organically. The exact age of the trees in these mixed cultivar orchards is unknown but estimates are between 90 to 100 yr. Tree spacing varies by orchard ranging from 27 m to 18 m. The experiment was arranged in a randomized complete block design. Each orchard was a block that contained three treatments applied to approximately 1 ha plots.

The three treatments were: 1) Grandevo applied four times at a rate of 3.36 kg per hectare, 2) a chemical insecticide regime consisting of carbaryl (Loveland Products, Loveland CO, active ingredient 43%) at 9.35 liters per hectare alternated bifentrhin (Fanfare ES, Adama USA, Raleigh, NC, active ingredient 22.6%) at 0.7 liters per hectare with two sprays of each (four total), and 3) an untreated control. Sulfoxaflor (Closer, Dow Agrosciences, Indianapolis, IN, active ingredient 21.8%) at 0.201 liters per hectare was also applied with chemical insecticide regime to reduce aphid populations. Applications were made using airblast sprayer as described in experiment 1 with a total volume of 935 liters per hectare. The experiment was conducted in 2014 and repeated in 2015; applications were made between 13 August and 18 September. After all applications were completed, treatment effects were assessed using a hydraulic lift (JLG Lift Model 600A) to sample 100 nuts per tree from the canopy of trees within the plots and determining percentage *C. caryae* infestation.

Statistical Analyses

For experiments 1 and 3, proportions infested nuts among treatments were analyzed. Weevil survival (proportion surviving) was analyzed for experiment 2. Based on the inspection of residual plots, all data were analyzed by logistic regression (SAS Software 2002, Warton and Hui 2011). Subsequently, for tests involving three or more treatments, differences were elucidated through the lsmeans procedure with a Tukey adjustment. All analyses were performed using SAS V9.4 (SAS Software 2002).

Results

Experiment 1: Integrated Use of Microbial Biopesticides

In 2014, the combined microbial biopesticide regime resulted in lower infestation of *C. caryae* compared with the untreated control at the Cleveland Farm location ($\chi^2 = 4.92$; df = 1; $P = 0.027$) and at the USDA-ARS location ($\chi^2 = 11.24$; df = 1; $P = 0.0008$); (Fig. 1). At the Cleveland Farm, percentage weevil infestation was less than 1.5% in treated and non-treated plots, whereas in USDA-ARS location infestation was 16% ± 2.10 (mean ± SEM) in the treated plots and 27% ± 2.65 in the control plots.

In 2015, no significant treatment effects were detected at the Cleveland Farm location ($\chi^2 = 1.68$; df = 1; $P = 0.20$); the percentage of nuts infested with *C. caryae* was 0.43% ± 0.43 in non-treated plots and 0% in treated plots (Fig. 2). A significant treatment effect was observed at the USDA-ARS location in 2015 ($\chi^2 = 23.02$; df = 1; $P < 0.0001$) (treated plots had infestation of 7.6% ± 5.2 and non-treated plots 17.2% ±14.5%) (Fig. 2).

Experiment 2: Small Plot Test Comparing Soil Applied Microbial Agents

In the first trial (applications made in the fall of 2014 and assessed the following year), survival of *C. caryae* was lower in all biopesticide treatments relative to the control, and there were no differences among treatments ($\chi^2 = 49.90$; df = 4; $P < 0.0001$) (Fig. 3). Weevil survival in the control was 4.67% ± 0.73 and <1.7% in all treatments (Fig. 3).

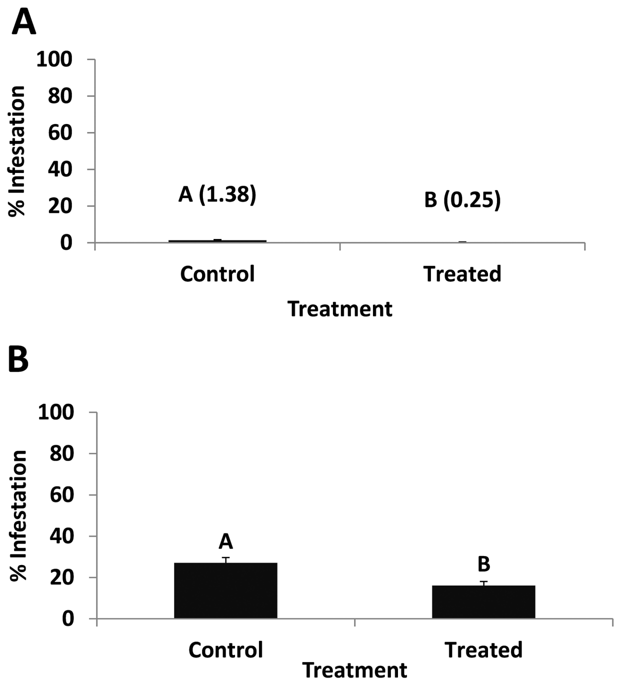

Fig. 1. Percentage *Curculio caryae* infestation following sequential application of microbial-based pesticides *Beauveria bassiana, Chromobacterium subtsugae*, and *Steinernema carpocapsae* (=Treated) versus a non-treated control. The experiment was conducted in orchards of the Cleveland Farm, Fort Valley, Georgia (A) and USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory (B) in 2014. Different letters above bars indicate statistical differences (analysis using logistic regression and means (lsmeans) separation by Tukey's HSD [α = 0.05]).

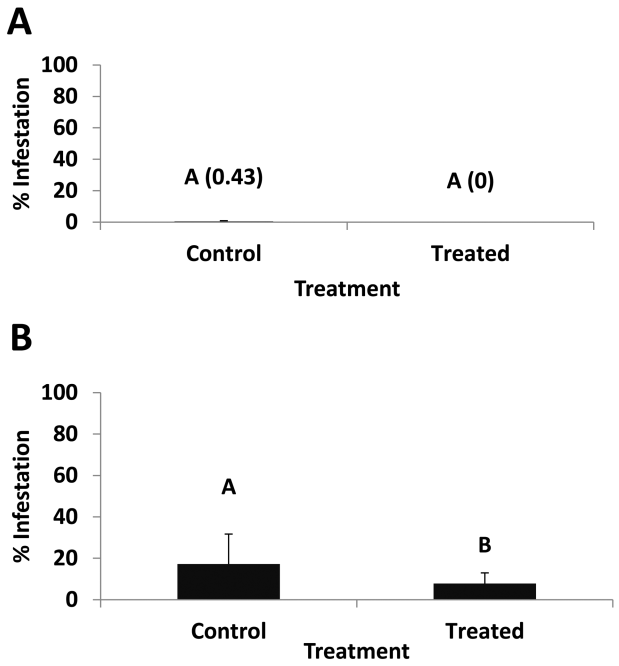

Fig. 2. Percentage *Curculio caryae* infestation following sequential application of microbial-based pesticides *Beauveria bassiana, Chromobacterium subtsugae*, and *Steinernema carpocapsae* (=Treated) versus a non-treated control. The experiment was conducted in orchards of the Cleveland Farm, Fort Valley, Georgia (A) and USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory (B) in 2015. Different letters above bars indicate statistical differences (analysis using logistic regression and means (lsmeans) separation by Tukey's HSD [α = 0.05]).

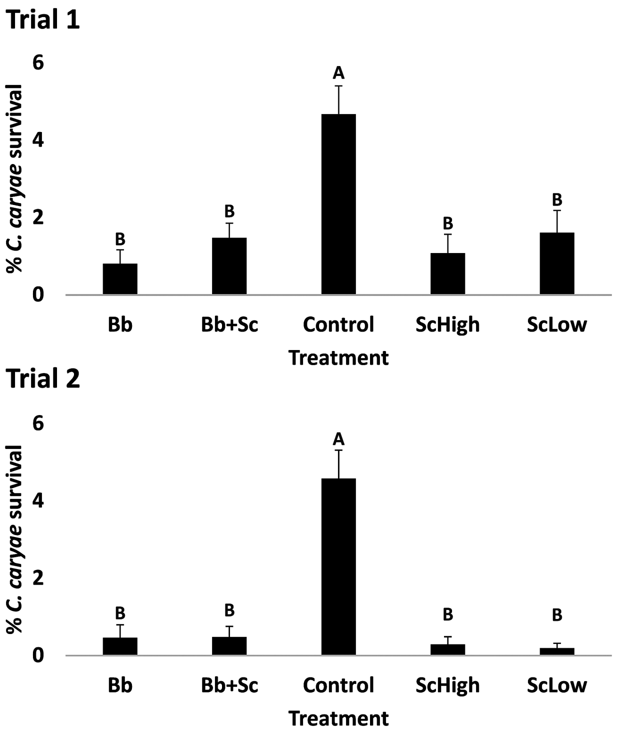

Fig. 3. Percentage survival of *Curculio caryae* 11 mo after 125 (Trial 1) or 175 (Trial 2) larvae were added to plastic pots sunk in a pecan orchard and exposed to different microbial biopesticide treatments. Trial 1 was initiated in the fall of 2014 and Trial 2 was initiated in the fall of 2015. Bb = *Beauveria bassiana* (two applications); Bb+Sc = *B. bassiana* and *Steinernema carpocapsae* (two applications each); ScHigh = two applications of *S. carpocapsae*; ScLow = one application of *S. carpocapsae,* Control = untreated. Different letters above bars indicate statistical differences (analysis using logistic regression and means (lsmeans) separation by Tukey's HSD [α = 0.05]).

The second trial results were similar to the first; *C. caryae* survival was lower in all biopesticide treatments relative to the control, and there were no differences among them (χ^2 = 33.46; df = 4; $P < 0.0001$) (Fig. 3). Weevil survival in the control was 4.57% ± 0.74 and <0.5% in all treatments (Fig. 3).

Experiment 3: Comparison of Biopesticide and Chemical Insecticide Canopy Sprays

In 2014, both the chemical insecticide regime and Grandevo treatment resulted in a reduction in percentage *C. caryae* infestation relative to the control, and the two treatments were not different from each other (χ^2 = 87.46; df = 2; $P < 0.0001$) (Fig. 4). Percentage infestation was 48.25% ± 16.4, 24.0% ± 7.10 and 23.30% ± 8.3 in the control, Grandevo treated, and chemical insecticide treated plots, respectively. In 2015, both treatments (chemical insecticides and Grandevo) caused a reduction in infestation of *C. caryae* relative to the control (13.1% ± 5.1), yet the percentage infestation in the chemical insecticide treatment (0%) was lower compared with the Grandevo treatment (1.8% ± 0.78) (χ^2 = 71.91; df = 2; $P < 0.0001$) (Fig. 4).

Discussion

The integrated approach using multiple biopesticides was successful in reducing damage caused by *C. caryae*. To our knowledge, this is the first report of application of microbial biopesticides, or any

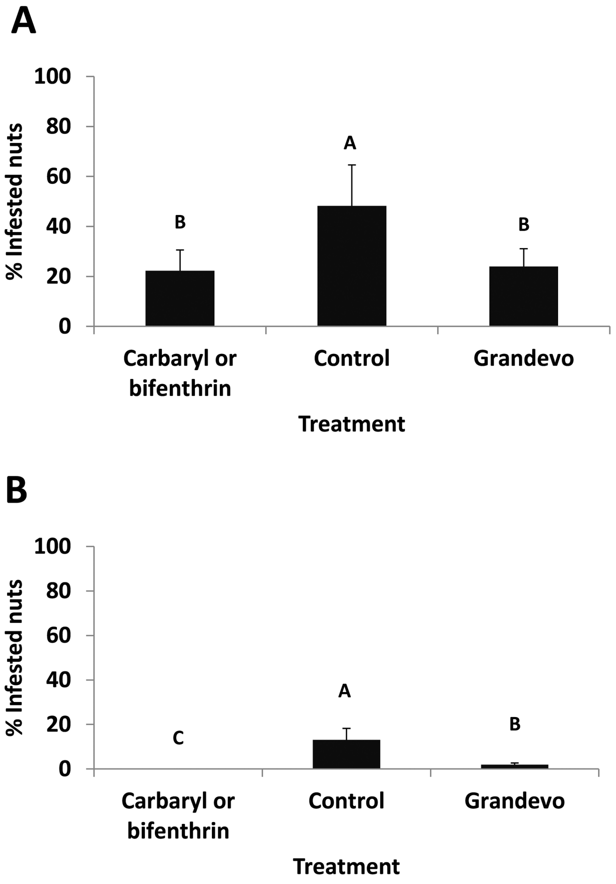

Fig. 4. Percentage *Curculio caryae* infestation following application of alternating carbaryl or bifenthrin, Grandevo (*Chromobacterium subtsugae*) in 2014 (A) or 2015 (B). Control = untreated. Different letters above bars indicate statistical differences (logistic regression and lsmeans with a Tukey adjustment).

organically labeled product, reducing damage caused by *C. caryae* in field trials (all prior experiments only measured weevil mortality). The particular microbial agents chosen were based on prior literature indicating efficacy of the biopesticides when applied alone in laboratory or small plot trials (Shapiro-Ilan 2001a,b; Shapiro-Ilan and Gardner 2012; Shapiro-Ilan et al. 2013). The concept was to use the combined effect of microbial control agents to obtain a cumulative impact on the *C. caryae* population. Indeed, cumulative application of *S. carpocapsae* applied repeatedly to a single cohort of *C. caryae* over 2 yr (Shapiro-Ilan and Gardner 2012) resulted in substantially higher levels of control compared with a single application (Shapiro-Ilan et al. 2006). The goal in the current study was also to apply the different treatments to stages of the weevil that are most vulnerable to the particular microbial agent applied. Thus, *S. carpocapsae* was applied to soil prior to *C. caryae* emergence because the nematode can penetrate the soil cell and is virulent to both larvae and adult weevils (particularly the adults) (Shapiro-Ilan 2001a,b; Shapiro-Ilan et al. 2003b). Grandevo was applied as a curative agent to the canopy during weevil emergence; application of the nematodes or fungi to the canopy would not be feasible due to potential environmental degradation (e.g., due to UV radiation or desiccation) and cost (Shapiro-Ilan et al. 2012). *B. bassiana* was applied as a barrier to larvae that dropped from nuts to enter the soil (thus preventing the next generation). Conceivably, *B. bassiana* could also be applied during weevil emergence to infect those adults that go to the canopy. The drawback is that the fungus can take 7 d to kill *C. caryae* (Shapiro-Ilan et al. 2004a, 2008) thus some damage to nuts would be expected. However the weevils have a pre-oviposition period of about 6.5 d (Criswell et al. 1975) so at least the bulk of egg-laying may be prevented thereby also reducing the following generation's impact in the orchard.

Once it has been established that this integrated approach using three biopesticides can effectively control *C. caryae*, the next step is to optimize the regime by determining whether efficacy can be retained while reducing the number or types of applications. Thus, experiment 2 was conducted to compare single or combined applications of the soil treatments. Results indicated no advantage in applying *B. bassiana* or *S. carpocapsae* alone or in combination, and the rate of *S. carpocapsae* or number of applications did not affect the outcome. Previous field studies assessing effects on entomopathogenic nematode efficacy against curculionid pests have shown positive relationships with rate of application (McCoy et al. 2000, Harvey et al. 2012), whereas others did not (Morse and Lindegren 1996). It is possible that had we assessed the soil in the buckets for survival of *C. caryae* after 2 yr (the minimum full life-cycle of *C. caryae*) instead of 1 yr, we might have observed some differences among treatments. However, weevil survival in the treated soil was already very low after 1 yr, especially in the second trial (less than 0.5%) making discernible differences among treatments less likely. The high level of natural mortality observed in the *C. caryae* populations was not surprising as it has been recorded previously (Harris et al. 1981, Neel and Sikorowski 1985, Shapiro-Ilan and Gardner 2012). At this point, our results suggest that a single application of one microbial agent to the soil (nematodes or fungus) would be sufficient to reduce substantially *C. caryae* populations. These results need to be confirmed on larger plot studies that also measure crop damage.

An alternative to applying the entomopathogens at different times (as we did) would have been to apply them simultaneously. Field application of certain combined microbials (such as entomopathogenic nematodes and fungi) against curculionid pests have been reported to exhibit synergistic efficacy (Ansari et al. 2006). In the case of *C. caryae*, however, a laboratory study indicated that simultaneous application of *B. bassiana* and *S. carpocapsae* resulted in additive or antagonistic interactions (Shapiro-Ilan et al. 2004b). Therefore, we did not expect an advantage from simultaneous application and chose to apply the entomopathogens separately.

Grandevo was applied as a canopy spray in an integrated approach (in sequence with the other biopesticides) as well as a stand-alone product. Furthermore, as a stand-alone, the biopesticide was compared with a standard synthetic chemical insecticide regime typical of current grower usage. Results indicated Grandevo alone not only controlled *C. caryae*, but provided control similar to the synthetic chemical insecticide (no difference between treatments was observed the first year and less than 2% difference in infestation in the second year). There is a dearth of prior literature on Grandevo applied as a stand-alone product in field trials, especially against coleopteran pests. Balusu and Fadamiro (2012) reported some efficacy against the yellow-margined leaf beetle, *Microtheca ochroloma* (Stål; Coleoptera: Chrysomelidae) but only against the larval stage. Our results confirm and expand upon known efficacy of Grandevo against *C. caryae* previously observed in small plot field trials (Shapiro-Ilan et al. 2013).

Although we demonstrated the efficacy of biopesticide-based approaches to control *C. caryae*, cost efficiency of the methods has yet to be evaluated. The cost of using all three biopesticides as an integrated strategy at the full rates would likely be cost prohibitive. However, Grandevo could conceivably be used as a curative for weevils in the canopy, and soil applications (of nematodes and/or fungi) could be applied to hot spots in orchards where weevils may be especially concentrated. For additional cost benefits, it would be useful to determine if lower rates of Grandevo (e.g., at 66% or 33%

of rates we used) may also be effective. Furthermore, given that laboratory research indicated Grandevo is toxic to the black pecan aphid, *Melanocallis caryaefoliae* (Davis; Homoptera: Aphididae; another serious pest of pecans (Shapiro-Ilan et al. 2013), yet has little impact certain natural enemies (Ray and Hoy 2014), including aphid predators (unpublished data), additional benefits are likely from Grandevo applications. In contrast, the current, recommended spray regimes using certain broad spectrum chemical insecticides to control *C. caryae* tend to flare aphid and mite pests and reduce populations of natural enemies (Dutcher and Payne 1985, Wells 2016). Preliminary trials indicate the potential for Grandevo to be used at lower rates and control pecan aphids while conserving natural enemies; specifically, in the first year of a 2-yr field trial, neonicotinoid insecticides reduced populations of lady beetles (Coleoptera: Coccinellidae) and green lacewings (Neuroptera: Chrysopidae), whereas Grandevo did not reduce these predator population, but did reduce aphid populations (unpublished data). Thus, additional research is needed to optimize a microbial biopesticide strategy for maximum control of *C. caryae*, while minimizing cost. Once the microbial approaches for controlling *C. caryae* are optimized, a full cost-benefit analysis can be conducted for using the biopesticides as stand-alone products or integrated with each other, or with conventional tactics.

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References Cited

Ansari, M. A., F. A. Shah, L. Tirry, and M. Moens. 2006. Field trials against Hoplia philanthus (Coleoptera: Scarabaeidae) with a combination of an entomopathogenic nematode and the fungus Metarhizium anisopliae CLO 53. Biol. Control 39: 453–459.

Balusu, R. R., and H. Y. Fadamiro. 2012. Evaluation of organically acceptable insecticides as stand-alone treatments and in rotation for managing yellow-margined leaf beetle, *Microtheca ochroloma* (Coleoptera: Chrysomelidae), in organic crucifer production. Pest Manag. Sci. 68: 573–579.

Criswell, J. T., D. J. Boethel, R. D. Morrison, and R. D. Eikenbary. 1975. Longevity, puncturing, and ovipositional activities by the pecan weevil on three cultivars of pecans. J. Econ. Entomol. 68: 173–177.

Dutcher, J. D., and J. A. Payne. 1985. The impact of pecan weevil control strategies on non-target arthropods, pp. 39–50. *In* W. W. Neel (ed.), Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

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

Harris, M. K. 1999. Pecan weevil management considerations, pp. 66–73. *In* B. E. McCraw, E. H. Dean, and B. W. Wood, (eds.), Pecan industry: current situation and future challenges, third national pecan workshop proceedings, April 1998. USDA, Agricultural Research Service, Washington DC.

Harris, M. K., J. A. Jackson and D. R. Ring. 1981. Calculating a static economic threshold and estimating economic losses for pecan weevil. Southwest. Entomol. 6: 165–173.

Harvey, C. D., K. M. Alameen, and C. T. Griffin. 2012. The impact of entomopathogenic nematodes on a non-target, service-providing longhorn beetle is limited by targeted application when controlling forestry pest Hylobius abietis. Biol. Control 62: 173–182.

Hudson, W. 2007. Insects and mites associated with pecans, pp. 137–170. *In* L. Wells (ed.), Southeastern pecan growers handbook. University of Georgia Extension Service, Bulletin No. 1327, Athens, GA.

McCoy, C. W., D. I. Shapiro, L. W. Duncan, and K. Nguyen. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of Diaprepes abbreviatus (Coleoptera: Curculionidae). Biol. Control 19: 182–190.

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

Neel, W. W. and P. P. Sikorowski. 1985. Rearing the pecan weevil in the laboratory, Pp. 79–86. *In* W.W. Neel, (ed.), Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Payne, J. A., and J. D. Dutcher. 1985. Pesticide efficacies for the pecan weevil past, present and future, pp. 103–116. *In* W. W. Neel, (ed.), Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Ray, H. A., and M. A. Hoy. 2014. Effects of reduced-risk insecticides on three orchid pests and two predacious natural enemies. Fla. Entomol. 97: 972-928.

SAS Software. 2002. Version 9.4. SAS Institute, Cary, NC.

Shapiro-Ilan, D. I. 2001. Virulence of entomopathogenic nematodes to pecan weevil larvae, *Curculio caryae* (Coleoptera: Curculionidae), in the laboratory. J. Econ. Entomol. 94: 7–13.

Shapiro-Ilan, D. I. 2001b. Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). J. Entomol. Sci. 36: 325–328.

Shapiro-Ilan, D. I., and W. A. Gardner. 2012. Improved control of Curculio caryae (Coleoptera: Curculionidae) through multi-stage pre-emergence applications of Steinernema carpocapsae. J. Entomol. Sci. 47: 27–34.

Shapiro-Ilan, D. I., and R. F. Mizell, 3rd. 2015. An insect pupal cell with antimicrobial properties that suppress an entomopathogenic fungus. J. Invertebr. Pathol. 124: 114–116.

Shapiro-Ilan, D. I., W. A. Gardner, J. R. Fuxa, B. W. Wood, K. B. Nguyen, B. J. Adams, R. A. Humber, and M. J. Hall. 2003a. Survey of entomopathogenic nematodes and fungi endemic to pecan orchards of the Southeastern United States and their virulence to the pecan weevil (Coleoptera: Curculionidae). Environ. Entomol. 32: 187–195.

Shapiro-Ilan, D. I., R. Stuart, and C. W. McCoy. 2003b. Comparison of beneficial traits among strains of the entomopathogenic nematode, Steinernema carpocapsae, for control of Curculio caryae (Coleoptera: Curculionidae). Biol. Control 28: 129–136.

Shapiro-Ilan, D. I., T. E. Cottrell, and W. A. Gardner. 2004a. Trunk perimeter applications of Beauveria bassiana to suppress adult Curculio caryae (Coleoptera: Curculionidae). J. Entomol. Sci. 39: 337–349.

Shapiro-Ilan, D. I., M. Jackson, C. C. Reilly, and M. W. Hotchkiss. 2004b. Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of Curculio caryae (Coleoptera: Curculionidae). Biol.Control 30: 119–126.

Shapiro-Ilan, D. I., T. E. Cottrell, I. Brown, W. A. Gardner, R. K. Hubbard, and B. W. Wood. 2006. Effect of soil moisture and a surfactant on entomopathogenic nematode suppression of the pecan weevil, Curculio caryae. J. Nematol. 38: 474–482.

Shapiro-Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2008. Virulence of Hypocreales fungi to pecan aphids (Hemiptera: Aphididae) in the laboratory. J. Invertebr. Pathol. 99: 312–317.

Shapiro-Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2013. Control of key pecan insect pests using biorational pesticides. J. Econ. Entomol. 106: 257–266.

Shapiro-Ilan, D. I., J. A. Morales-Ramos, and M. G. Rojas. 2016a. In vivo production of entomopathogenic nematodes, pp. 137–158. *In* T. Glare and M. Moran-Diez (eds.), Microbial-based biopesticides: methods and protocols. Human Press, New York.

Shapiro-Ilan, D. I., T. E. Cottrell, R. F. Mizell III, and D. L. Horton. 2016b. Curative control of the peachtree borer using entomopathogenic nematodes. J. Nematol. 48: 170–176.

Shapiro-Ilan, D. I., S. P. Arthurs, and L. A. Lacey. 2016c. Microbial control of arthropod pests of orchards in temperate climates, pp. 253–267. *In* L. A. Lacey (ed.), Microbial agents for control of insect pests: from discovery to commercial development and use. Academic Press, London, UK.

Warton, D. I., and F. K. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology. 92: 3–10.

Wood, B. W. 2003. Pecan production in North America, pp. 1–20. *In* J. D. Dutcher, M. K. Harris, and D. A. Dean (eds.), Integration of chemical and biological insect control in native, seedling, and improved pecan production. Southwest. Entomol. Suppl. No. 27.

Wells, L., T. Brenneman, J. Brock, A. S. Culpepper, W. Hudson, and W. Mitchem. 2016. 2016 commercial pecan spray guide. Bulletin 841. University of Georgia. http://extension.uga.edu/publications.