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Effect of Soil Moisture and a Surfactant on Entomopathogenic Nematode Suppression of the Pecan Weevil, *Curculio caryae*

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Abstract: Our overall goal was to investigate several aspects of pecan weevil, *Curculio caryae*, suppression with entomopathogenic nematodes. Specifically, our objectives were to: 1) determine optimum moisture levels for larval suppression, 2) determine suppression of adult *C. caryae* under field conditions, and 3) measure the effects of a surfactant on nematode efficacy. In the laboratory, virulence of *Heterorhabditis megidis* (UK211) and *Steinernema carpocapsae* (All) were tested in a loamy sand at gravimetric water contents of negative 0.01, 0.06, 0.3, 1.0, and 15 bars. *Curculio caryae* larval survival decreased as moisture levels increased. The nematode effect was most pronounced at −0.06 bars. At −0.01 bars, larval survival was ≤5% regardless of nematode presence, thus indicating that intense irrigation alone might reduce *C. caryae* populations. Overall, our results indicated no effect of a surfactant (Kinetic) on *C. caryae* suppression with entomopathogenic nematodes. In a greenhouse test, *C. caryae* larval survival was lower in all nematode treatments compared with the control, yet survival was lower in *S. carpocapsae* (Italian) and *S. riobrave* (7–12) treatments than in *S. carpocapsae* (Agriotos), *S. carpocapsae* (Mexican), and *S. riobrave* (355) treatments (survival was reduced to approximately 20% in the *S. riobrave* [7–12] treatment). A mixture of *S. riobrave* strains resulted in intermediate larval survival. In field experiments conducted over two consecutive years, *S. riobrave* (7–12) applications resulted in no observable control, and, although *S. carpocapsae* (Italian) provided some suppression, treatment effects were generally only detectable one day after treatment. Nematode strains possessing both high levels of virulence and a greater ability to withstand environmental conditions in the field need to be developed and tested.

Key words: Biological control, *Curculio caryae*, entomopathogenic nematode, field trial, *Heterorhabditis*, pecan weevil, surfactant, soil moisture, *Steinernema*.

Pecan (*Carya illinoensis*) is an important nut crop in North America (Wood, 2003). The pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae), is a key pest of pecan (Payne and Dutcher, 1985). Adults emerge from soil in late July to August to feed on and oviposit in nuts (Harris, 1985). Larvae develop within the nut, and fourth instars drop to the ground where they burrow to a depth of 8 to 25 cm, form a soil cell, and overwinter. The following year, approximately 90% of larvae pupate and spend the next nine months in the soil cell as adults (Harris, 1985). The remaining 10% of the larval 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 two and sometimes three years (Harris, 1985). The bulk of *C. caryae* adults emerge from soil over a four to six week period usually beginning in mid-August (Harris, 1976); larvae emerge from nuts over several months in the autumn and early winter (Boethel and Eikenbary, 1979; Harris and Ring, 1979).

Control recommendations for *C. caryae* currently consist of foliar applications of chemical insecticides (e.g., carbaryl) to kill the adults (Harris, 1999; Hudson et al., 2006). Due to environmental and regulatory concerns, research toward developing alternative control strategies is warranted. Soil applications of entomopathogenic nematodes may provide an alternative control measure for suppression of *C. caryae* larvae or adults (Shapiro-Ilan, 2003).

Entomopathogenic nematodes are obligate parasites in the families Steinernematidae and Heterorhabditidae. Entomopathogenic nematodes kill insects with the aid of a mutualistic bacterium, which is carried in their intestine (*Xenorhabdus* spp. and *Photorhabdus* spp. are associated with *Steinernema* spp. and *Heterorhabditis* spp., respectively) (Poinar, 1990). The nematodes complete two to three generations within the host, after which free-living infective juveniles (IJ) emerge to seek new hosts (Poinar, 1990). More than 50 species of entomopathogenic nematodes have been described (Qiu et al., 2005; Sturhan et al., 2005; Adams et al., 2006).

Successful use of entomopathogenic nematodes in soil applications depends on various biotic and abiotic factors. A number of these factors can be controlled and optimized to improve efficacy (Shapiro-Ilan et al., 2006a). For example, a biotic factor that can enhance efficacy is the choice of nematode species or strain in relation to the target host (Shapiro-Ilan et al., 2002, 2006a). In terms of abiotic factors, application under favorable soil moisture levels is critical (Georgis and Gaugler, 1991; Kaya and Gaugler, 1993). Additionally, efficacy can be enhanced through improved formulation or addition of adjuvants (Wright et al., 2005; Shapiro-Ilan et al., 2006a).

Although several previous studies have indicated that entomopathogenic nematodes can contribute to population reduction in *C. caryae* (Nyczepir et al., 1992; Smith et al., 1993; Shapiro-Ilan, 2003), research toward optimizing efficacy and determining the potential to

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incorporate the approach into a viable pest management program is limited. In field trials directed at *C. caryae* larval control, only three nematode species have been tested thus far (Nyczepir et al., 1992; Smith et al., 1993), and results indicated relatively low levels of suppression when standard application rates (e.g., 25–75 IJ/cm^2) were used. A broader screening of virulence to *C. caryae* larvae, in which 15 nematode strains representing nine species were tested under laboratory conditions, also indicated relatively low to moderate suppression (Shapiro-Ilan, 2001a). We anticipate that new nematode strains and optimization of soil conditions may yield higher levels of efficacy. 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), yet no field tests have been conducted targeting *C. caryae* adults with nematodes. Our overall goal in this study was to investigate several aspects of *C. caryae* suppression using entomopathogenic nematodes. Specifically our objectives were to: 1) conduct laboratory studies to determine optimum moisture levels for control (focusing on larval suppression), 2) determine efficacy vs. *C. caryae* adults under field conditions, and 3) measure the effects of a surfactant on *C. caryae* suppression (vs. larvae and adults). Surfactants are one group of adjuvants that can enhance nematode efficacy (Wright et al., 2005), e.g., Schroeder and Sieburth (1997) observed increased nematode suppression of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), with the addition of surfactants.

The choices for specific treatments to address our objectives were based on previous literature. Nematodes used in our experiments included *S. carpocapsae, Heterorhabditis megidis* Poinar, Jackson and Klein, and *Steinernema riobrave* Cabanillas, Poinar, & Raultson; relative to other nematodes tested thus far, these nematodes have shown superior virulence to *C. caryae* adults or larvae (Shapiro-Ilan, 2001a, 2001b, 2003; Shapiro-Ilan et al., 2003). To measure surfactant effects on nematode efficacy, we chose Kinetic, because Schroeder and Sieburth (1997) found this product to be among the most active in enhancing nematode suppression of *D. abbreviatus* in potted citrus.

Materials and Methods

Insect and nematode cultures: *Curculio caryae* cannot be continuously cultured in the laboratory, therefore, for laboratory and greenhouse experiments, fourth instars were collected from infested nuts on the USDA-ARS Research Station, Byron, GA. Larvae were stored in autoclaved soil at 25°C for 2 wk (to remove diseased individuals), and remaining larvae stored up to 4 mon in sterile soil at 4 to 10°C prior to experimentation (Shapiro-Ilan, 2001a). Nematodes used in laboratory and greenhouse experiments were cultured in last instar greater wax moth, *Galleria mellonella* (L.), based on procedures described by Kaya and Stock (1997). The *G. mellonella* were obtained from Webster's Waxie Ranch (Webster, WI). Nematodes used in field experiments were produced in *G. mellonella* by BioControl Systems (Greendale, IN). After harvest, nematodes were stored at 13°C for less than 2 wk before being used in experiments.

Determination of soil moisture effects: A laboratory experiment was conducted to determine and optimize moisture effects on suppression of *C. caraye* larvae with entomopathogenic nematodes. The nematodes used in the experiment were *H. megidis* (UK211 strain) and *S. carpocapsae* (All strain). Virulence was evaluated based on procedures described by Shapiro-Ilan (2001a). Experimental units were plastic cups (473 ml, 9.5-cm diam.; Fabri Kal Corp, Kalamazoo, MI) filled with oven-dried (at 70°C) soil from a USDA-ARS pecan orchard (Byron, GA) and containing one *C. caryae* larva each. The soil was a loamy sand (84% sand, 10% silt, 6% clay; 2.8% organic matter; pH = 6.1). The factorial experiment included five soil moisture levels and three nematode treatments (the two nematodes and a water-only control). Final soil moisture levels were 23.6%, 18.5%, 11.5%, 10.8%, and 5.1 %, which correspond to gravimetric water contents of negative 0.01, 0.06, 0.3, 1.0, and 15 bars, respectively, as determined by pressure extractor methods (Tempe cells from 0.01 to 1.0 bar and a pressure plate extractor for 15 bars) as described by Klute (1986). Our goal was to observe nematode activity in a range of soil moistures from wilting point (–15 bars) to near saturation (–0.01 bars), including intermediate levels such as field capacity (–0.3 bars). The soil for each treatment was mixed thoroughly in a plastic bag with an appropriate amount of water (i.e., the amount of water for that treatment minus the amount of water in which the nematodes were added). The cups were first filled to approximately 1/3 deep with the moistened soil, the insect larva was added, and the cup was then filled, leaving about 0.5 cm space on top. Nematodes were applied in 0.5 ml water to the soil surface (25 IJ/cm^2). Cups were then pooled by replicate (block) and stored at 25°C in large plastic bags with moist paper towels. Weevil survival was determined 10 d after treatment. Each treatment combination was replicated three times with 10 cups per replicate (450 cups total), and the entire experiment was repeated once in time (two trials).

Susceptibility of C. caryae larvae to nematodes and a surfactant: The effects of the organo-silicone surfactant Kinetic (Helena Chemical Company, Collierville, TN) on the ability of various entomopathogenic nematode strains to suppress *C. caryae* larvae were tested in the greenhouse. The nematodes used in this experiment were three strains of *S. carpocapsae* (Agriotos, Italian, and Mexican), two strains of *S. riobrave* (355 and 7–12),

and a mixture of four *S. riobrave* strains (355, 5, 3–8b, and 7–12). Stuart et al. (2004) reported that a mixture of *S. riobrave* strains caused higher mortality in *D. abbreviatus* than a number of individual strains applied at the same rate. Inclusion of a mixed strain treatment in our experiment was also aimed at determining whether the superior efficacy might be observed against *C. caryae*.

Prior to the greenhouse test, toxicity of the surfactant to nematodes was assessed in the laboratory based on procedures described by Schroeder and Sieburth (1997). Ten milliliter mixtures of 0%, 0.025%, 0.05%, 0.1%, 0.2% and 2.0% Kinetic plus 500 IJ of the six nematode treatments were added to 50-mm-diam. petri dishes. Viability of 50 randomly chosen nematodes per dish was assessed after 24 and 48 hr incubation at 25°C; nematodes were considered to be alive if they responded to probing with a needle. There were five replicates of each nematode treatment × surfactant combination.

The greenhouse experiment consisted of a factorial with the six nematode treatments plus a water-only control and three surfactant levels, i.e., 0%, 0.2%, and 2.0% Kinetic. Experimental units consisted of plastic buckets (11.5–14-cm-diam., 15-cm depth) with approximately 50 1-mm holes punched through the bottom for drainage. Soil (the same as in the laboratory-moisture test described above) was initially filled to 5-cm depth in each bucket and packed uniformly with a wooden pestle (this was to simulate the compacted subsurface soil in an orchard), 10 *C. caryae* larvae were added, and the soil was then filled to a 13-cm depth. The insects were left to burrow into the soil for 1 wk prior to treatment applications. Nematode-surfactant combinations were applied by pipette in 10 ml water; the pipette was then rinsed once with an additional 10 ml, which was also applied to the soil surface. Nematodes were applied at a rate of 19,905 IJ/bucket (approximately 150 IJ/cm^2). Soil moisture was maintained at field capacity or slightly above by irrigating regularly (approximately every other day). Two weeks after treatment application, *C. caryae* survival was assessed. The experiment contained five buckets for each treatment combination (21 × 5 = 105 pots total), which were arranged on benches by replicate (block), and the entire experiment was repeated once (i.e., two trials).

Susceptibility of C. caryae adults to nematodes and a surfactant: Experiments were conducted to determine field efficacy of entomopathogenic nematodes in suppressing *C. caryae* adults; the effect of adding a surfactant (Kinetic) on nematode activity was tested simultaneously. The nematodes used in the experiments were *S. carpocapsae* (Italian) and *S. riobrave* (7–12). These strains were chosen because the species *S. carpocapsae* and *S. riobrave* have shown relatively high virulence toward *C. caryae* adults; the Italian strain was among the most virulent *S. carpocapsae* in laboratory tests against *C. caryae*, and *S. riobrave* (7–12) and *S. carpocapsae* (Italian) caused the highest larval mortality in our greenhouse experiment (Shapiro-Ilan et al., 2003; see results section).

Field experiments were conducted in a 25-yr-old mixed variety ('Desirable', 'Cape Fear', 'Cheyenne', and 'Stuart') pecan orchard, with 12.5 m^2 tree spacing, located on the USDA-ARS research station in Byron, GA. Plots were arranged in a randomized complete block design. Each row constituted a block, and each plot consisted of a single tree. To increase distance between plots, every other tree was used as a plot within a row. Treatments included the two nematodes or a water-only control plus three levels of surfactant (0%, 0.2%, or 2%), and there were six replicates per treatment.

Treatment applications were made 16 August 2004, and 25 August 2005. Treatments, i.e., appropriate combinations of nematodes, surfactant, or controls, were mixed together and applied in a total of 15 liters of water, which was split into two watering cans and spread evenly over a circular area extending 4 m from the trunk (approximately equal to the tree's drip-line). Nematodes were applied at a rate of 100 nematodes/cm^2. Irrigation was applied to the area of application via micro-jet sprinklers prior to application (approximately 10 liters/plot, just to wet the ground). Approximately 20 liters of water was applied (via micro-jet) immediately after application and every 2 to 3 d thereafter as needed to maintain moist soil throughout the sampling period.

Sampling and assessment of treatment effects was based on procedures described by Shapiro-Ilan et al. (2004, 2007). Adult *C. caryae* were collected in Circle trunk traps attached to pecan tree trunks (Mulder et al., 2003). This is a passive trap that captures naturally emerging weevils as they crawl up the trunk or fly to the lower portion of the trunk; Raney and Eikenbary (1968) estimated that 84% of emerging adult *C. caryae* fly or crawl to the main stem of the tree. The traps were made of wire mesh (1.5-mm pore size) with an open area (approximately 44-cm wide) facing toward the soil and tapering up to a removable top. Traps were placed on the trunk low to the ground (30 cm above the soil surface) to maximize capture of *C. caryae* that crawled to the trunk, i.e., *C. caryae* that flew directly to the trunk or canopy were unlikely to be caught. The top of the trap (the removable one-way cone portion that actually captures the weevils) was placed on the trap approximately 24 hr prior to each collection (sample date). *Curculio caryae* adults were collected in traps 1, 8 and 15 d after treatment. To avoid contamination of nematodes across plots, we placed plastic bags over our shoes (held with rubber bands) just prior to entering plots and removed the bags upon exiting.

On each day that *C. caryae* adults were trapped in the field, the insects captured in each trap top were placed in separate plastic bags and brought to the laboratory to estimate levels of nematode infection. All *C. caryae*

adults were placed individually in 30-ml plastic cups (3–4 cm i.d., 3.5-cm deep) with a 3-cm cotton wick moistened with approximately 2.1 ml of tap water plus a small apple slice (approximately 1 cm × 0.5 cm) for food. Cups were placed in plastic boxes (28 × 15 × 9.5-cm deep) organized by block and incubated in darkness at 25°C. After 3 d (2004) or 4 to 5 d (2005) of incubation, the percentage *C. caryae* survival per plot was determined.

Statistical analyses: Treatment effects in all experiments were analyzed through ANOVA ($\alpha = 0.05$) in a factorial analysis (SAS Software, version 9.1, 2001, SAS Institute, Cary, NC). If no interaction between main effects (moisture × nematode effects in the laboratory, surfactant × nematode in the greenhouse and field studies) was detected, then only the main effects were considered and simple effects were combined; if a significant interaction between main effects was detected, then the analysis was split and made at each level of the main effects (Steel and Torrie, 1980). Note that only significant interactions (and their statistics) are reported in the Results section. If the *F* test was significant, treatment differences were further elucidated through the LSD test (SAS Software, version 9.1, 2001, SAS Institute, Cary, NC). Prior to analysis, percentage data (survival) were arcsine of square root transformed (Steel and Torrie, 1980); untransformed means are presented in figures. The relationship between soil moisture and *C. caryae* survival in each treatment (*H. megidis, S. carpocapsae* and control) was also analyzed by linear regression.

In the laboratory and greenhouse experiments, the repeated experiments were combined and trials were considered a block. Data from each year's field trial were analyzed separately. Treatment effects in each year's field experiment were analyzed for each sampling date as well as over the entire period (15 d). In the analyses covering the entire experimental period, a conservative error term (treatment × block) was used to test for treatment effects (Cochran and Cox, 1957).

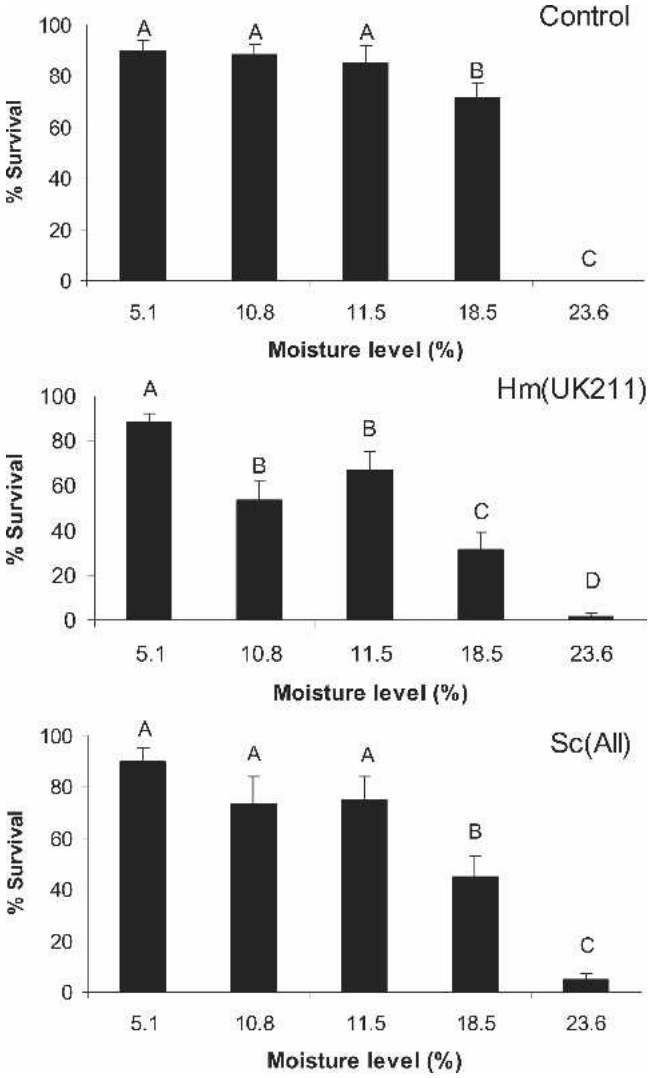

FIG. 1. Survival of *Curculio caryae* larvae in the laboratory at various soil moisture percentages following application of *Heterorhabditis megidis* (UK211 strain), *Steinernema carpocapsae* (All), or an untreated (water-only) control. Hm = *H. megidis*, Sc = *S. carpocapsae*. Nematodes were applied at a rate of 25 IJ/cm^2. Different letters above bars indicate statistical differences within each treatment ($P \leq 0.05$, based on LSD tests).

RESULTS

Determination of soil moisture effects: A significant interaction between moisture and nematode effects was detected ($F = 3.01$; df = 8,60; $P = 0.007$). Therefore, moisture effects were analyzed for each nematode treatment, and nematode effects were compared at each moisture level. Moisture effects were detected in the control and both nematode treatments ($F = 51.9$; df = 4,24; $P < 0.0001$, $F = 24.0$; df = 4,24; $P < 0.0001$, and $F = 23.9$; df = 4,24; $P < 0.0001$, for the control, *H. megidis*, and *S. carpocapsae*, respectively). *Curculio caryae* larval survival tended to decrease as moisture levels increased (Table 1; Fig. 1). Lower survival was observed at 18.5% than at 5.1%, 10.8%, and 11.5% in both treatments and the control, and for *H. megidis* the highest survival was detected in 5.1% moisture (Fig. 1).

Nematode treatment effects were detected at the intermediate moisture levels 10.8%, 11.5%, and 18.5%, but not at the extremes of 5.1% and 23.6% ($F = 0.28$; df

TABLE 1. Regression statistics for survival of *Curculio caryae* larvae (Y-axis) at differing soil moisture levels (X-axis) following application of *Heterorhabditis megidis* (UK211 strain), *Steinernema carpocapsae* (All), or an untreated (water-only) control.[a]

Treatment	Variable	Estimate	SE	*t*	*P*	R^2
Control	Slope	–.067	0.009	–7.26	<0.001	0.65
	Intercept	1.90	0.14	13.53	<0.001	
H. megidis	Slope	–0.062	0.006	–9.53	<0.001	0.76
	Intercept	1.59	0.10	15.98	<0.001	
S. carpocapsae	Slope	–.062	0.008	–7.80	<0.001	0.69
	Intercept	1.76	0.12	14.35	<0.001	

[a] Nematodes were applied at a rate of 25 IJ/cm^2.

= 2,14; $P = 0.76$, $F = 7.68$; df = 2,14; $P = 0.006$, $F = 4.01$; df = 2,14; $P = 0.042$, $F = 9.26$; df = 2,14; $P = 0.0027$, and $F = 2.33$; df = 2,14; $P = 0.136$, for 5.1%, 10.8%, 11.5%, 18.5%, and 23.6% moisture, respectively) (Fig. 2). At 10.8% moisture, *C. caryae* larval survival was lower in the *H. megidis* treatment than both the control and the *S. carpocapsae* treatment. At 11.5% moisture, larval survival was lower in the *H. megidis* treatment than the control, and the *S. carpocapsae* treatment was intermediate (Fig. 2). At 18.5% moisture, no difference was detected between the two nematode treatments; however, both reduced survival relative to the control (Fig. 2). Larval survival at the highest moisture level was ≤5% regardless in the treatments as well as the control (Fig. 2).

Susceptibility of C. caryae larvae to nematodes and a surfactant: Survival of *C. caryae* larvae was lower in all nematode treatments compared with the control, yet differences in virulence were detected among the nematode strains ($F = 10.21$; df = 6,187; $P < 0.0001$) (Fig. 3). *Curculio caryae* survival was lower in *S. carpocapsae* (Italian) and *S. riobrave* (7–12) treatments than in *S. carpocapsae* (Agriotos), *S. carpocapsae* (Mexican), and *S. riobrave* (355) treatments (Fig. 3). Survival following the application of a mixture of *S. riobrave* strains (*S. riobrave* [Mix]) was not different from that of *S. carpocapsae* (Italian), *S. riobrave* (7–12) or *S. carpocapsae* (Mexican), but was lower than survival from *S. carpocapsae* (Agriotos) and *S. riobrave* (355) treatments (Fig. 3). The surfactant (Kinetic) had no effect on weevil survival ($F = 0.35$; df = 2,187; $P = 0.703$); mean ± SE percentage survival was 39.7 ± 3.6, 38.6 ± 3.6, and 36.4 ± 3.5, for 0%, 0.2%, and 2% surfactant, respectively.

Susceptibility of C. caryae adults to nematodes and a surfactant: When nematode effects were averaged over all sample dates, *C. caryae* survival was lower in the *S. carpocapsae* (Italian) treatment than in the control or *S.*

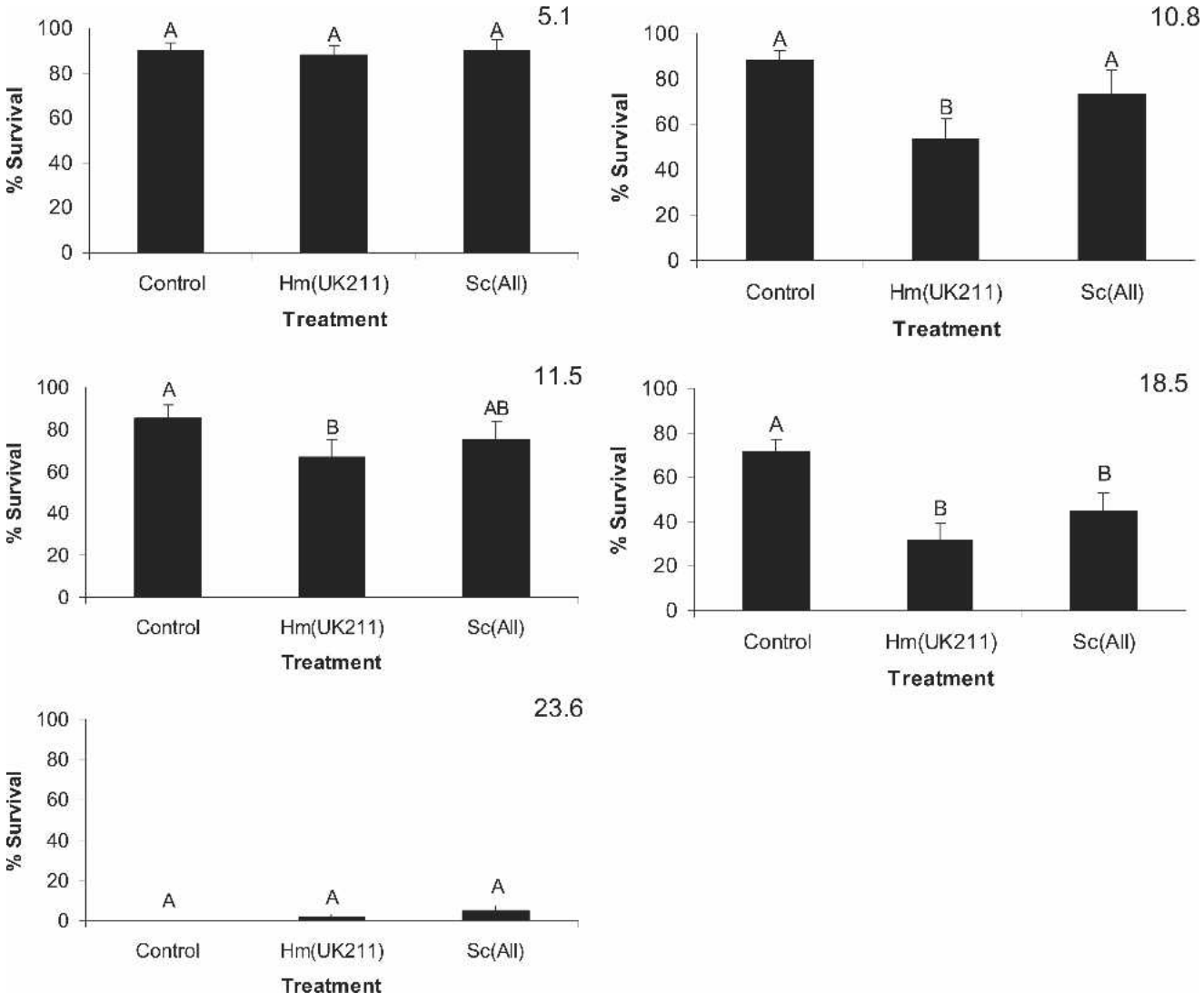

FIG. 2. Nematode effects on *Curculio caryae* larval survival in a laboratory study conducted at various soil moisture levels (5.1% to 23.6%), which are denoted in the upper right hand corner. Hm(UK211) = *Heterorhabditis megidis* (UK211 strain), Sc(All) = *Steinernema carpocapsae* (All), control = water only. Nematodes were applied at a rate of 25 IJ/cm^2. Different letters above bars indicate statistical differences within each moisture level ($P \leq 0.05$, based on LSD tests).

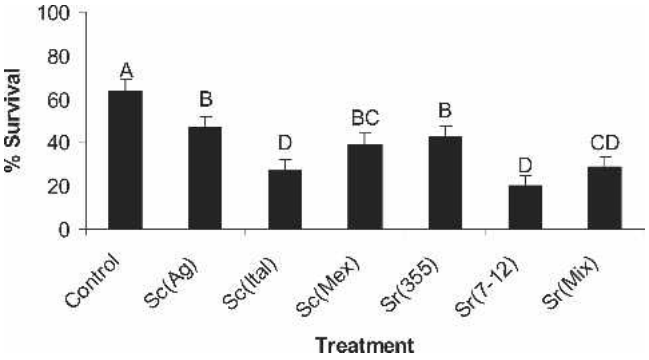

FIG. 3. Survival of *Curculio caryae* larvae in a greenhouse study following application of entomopathogenic nematodes. Sc = *Steinernema carpocapsae* (Agriotos, Italian, and Mexican strains), Sr = *Steinernema riobrave* (355, and 7–12 strains), mix = a mixture of several *S. riobrave* strains (355, 5, 3–8b, and 7–12), control = water only. Treatment effects are averaged over presence and absence of a surfactant, Kinetic at 0, 0.2 and 2.0%. Nematodes were applied at a rate of 150 IJ/cm^2. Different letters above bars indicate statistical differences ($P \leq 0.05$, based on LSD tests).

riobrave treatment in 2004 ($F = 5.61$; df = 2,10; $P = 0.023$), and, in 2005, survival in the *S. carpocapsae* (Italian) treatment was lower than in the control with intermediate survival in the *S. riobrave* treatment ($F = 4.18$; df = 2,10; $P = 0.048$) (Fig. 4). No surfactant effect was detected in 2004 or 2005 ($F = 1.18$; df = 1,10; $P = 0.30$ and $F = 0.23$; df = 1,10; $P = 0.64$, for 2004 and 2005, respectively); mean ± SE percentage weevil survival for absence and presence of surfactant (respectively) was 63.1 ± 7.7 and 73.1 ± 6.0 in 2004, and 73.9 ± 4.6 and 75.9 ± 4.5 in 2005. A total of 204 weevils were captured in 2004 and 405 in 2005.

In 2004 and 2005, *S. carpocapsae* (Italian) caused lower *C. caryae* survival than the control and *S. riobrave* (7–12) (between which no difference was detected) 1 d post-treatment ($F = 4.96$; df = 2,19; $P = 0.019$ and $F = 6.26$; df = 2,27; $P = 0.006$, for 2004 and 2005, respectively) (Fig. 5), but no differences in nematode effect were detected on subsequent sampling dates ($F = 0.18$; df = 2,10; $P = 0.385$ and $F = 2.24$; df = 2,19; $P = 0.224$ for d 8 and d 15 in 2004, and $F = 0.73$; df = 2,23; $P = 0.494$ and $F = 0.22$; df = 2,20; $P = 0.805$ for d 8 and d 15 in 2005) (Fig. 5). A surfactant effect was detected on d 15 in 2004 ($F = 6.57$; df = 1,19; $P = 0.019$); mean ± SE percentage weevil survival was 44.8 ± 11.5 and 82.0 ± 10.2 for the absence and presence of surfactant, respectively. No other surfactant effects were detected on any sampling dates ($F = 0.21$; df = 1,19; $P = 0.652$ and $F = 0.4$; df = 1,10; $P = 0.543$ for d 1 and d 8 in 2004, and $F = 0$; df = 1,27; $P = 0.961$, $F = 0$; df = 1,23; $P = 0.969$, and $F = 0.20$; df = 1,20; $P = 0.660$ for d 1, d 8 and d 15 in 2005); mean ± SE percentage weevil survival over these sample

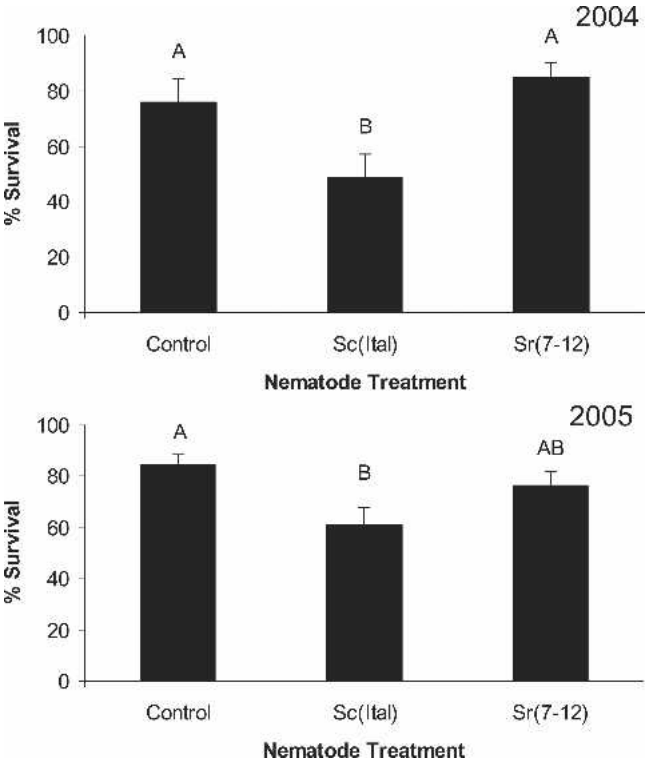

FIG. 4. *Curculio caryae* adult survival in field experiments (2004 and 2005) following entomopathogenic nematode applications. Survival is averaged over a 15 d sampling period and across presence and absence of the surfactant, Kinetic (2.0%). Sc(Ital) = *Steinernema carpocapsae* (Italian strain), Sr(7–12) = *S. riobrave* (7–12), control = water only. Nematodes were applied at a rate of 100 IJ/cm^2. Different letters above bars indicate statistical differences ($P \leq 0.05$, based on LSD tests).

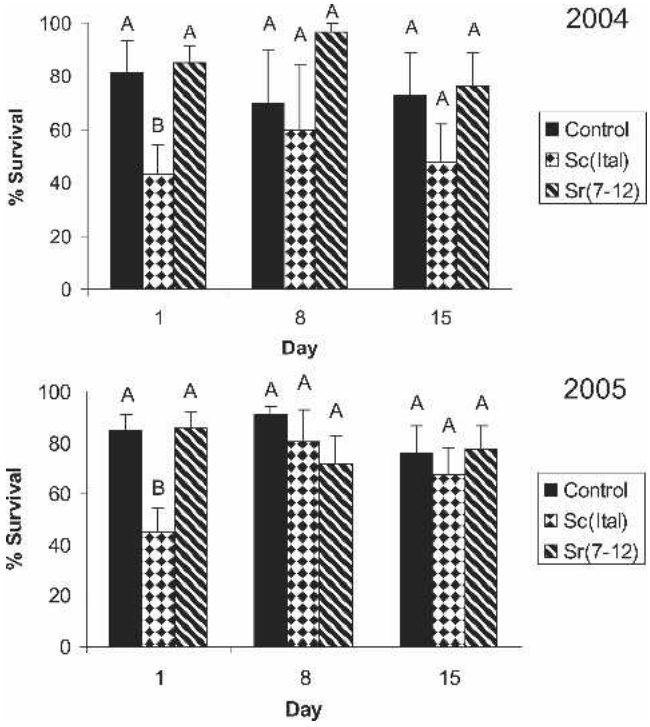

FIG. 5. *Curculio caryae* adult survival in field experiments (2004 and 2005) 1, 8, and 15 d after treatment with entomopathogenic nematodes. Survival is averaged over presence and absence of the surfactant Kinetic (2.0%). Sc(Ital) = *Steinernema carpocapsae* (Italian strain), Sr(7–12) = *S. riobrave* (7–12), control = water only. Nematodes were applied at a rate of 100 IJ/cm^2. Different letters above bars indicate statistical differences ($P \leq 0.05$, based on LSD tests).

dates ranged from 83.3 ± 16.7 on d 8 (in a treatment without surfactant) to 66.1 ± 9.1 on d 1 (in a treatment with surfactant).

Discussion

Soil moisture is a key factor affecting efficacy of entomopathogenic nematode applications (Kaya and Gaugler, 1993; Shapiro-Ilan et al., 2006a). As soil dries, nematode infectivity and survival are severely curtailed (Kaya, 1990; Koppenhöfer et al., 1995); indeed, we did not detect any nematode effect in the lowest moisture level tested. The nematode effect (difference between control and treatment) was most pronounced at 18.5% (–0.06 bars) moisture for soil used in this study. Mortality of *C. caryae* larvae observed in nematode treatments at 18.5 % soil moisture in this study was also higher than mortality observed in earlier studies that used drier soil, e.g., *H. megidis* and *S. carpocapsae* caused no more than 50% mortality when using the same soil at 14% moisture (Shapiro-Ilan, 2001a). Conceivably, the optimum soil moisture level we observed for larval control is also applicable for adult *C. caryae* control, but this will have to be tested in future research.

At the highest soil moisture level tested (23.6%, –0.01 bars), >95% *C. caryae* larval mortality was observed regardless of nematode presence. Due to the apparent sensitivity of *C. caryae* larvae to high moisture levels, it may be possible to control the weevil through intense irrigation. Irrigation or flooding has been considered previously for *C. caryae* control (Nickels and Pierce, 1947), but to our knowledge no replicated studies have been conducted. The potential for over-irrigation to harm the tree would have to be considered if a flooding approach were implemented; however, it should be noted that the pecan tree is native to areas along the Mississippi river and its tributaries (which are known to flood), and flood irrigation is one of the methods currently used in commercial pecan production, particularly in the Southwestern US (Herrera and Sammis, 2000; Worley, 2003). The irrigation approach for *C. caryae* will be explored in future studies.

Because of the extremely high level of control mortality, we could not measure a nematode effect at the 23.6% moisture level. Thus, we do not know whether or not nematode performance was impaired at this moisture level. Although we did not observe any signs of nematode infection at 23.6% (data not shown), e.g., typical color changes in the insect cadavers (Kaya and Stock, 1997), it is still not clear if the nematodes were incapable of infecting or if the insects died before the nematodes had a chance to infect. However, we know that relatively high levels of moisture can deter or prevent nematode movement, survival, and pathogenicity (Wallace, 1958; Kaya, 1990; Kung et al., 1991). A number of studies have reported decreases in nematode virulence and/or survival in soils that are too dry as well as too wet, with optimum nematode activity occurring at intermediate levels (Molyneux and Bedding, 1984; Kung et al., 1991; Koppenhöfer et al., 1995; Grant and Villani, 2003). For example, Koppenhöfer et al. (1995) observed optimum activity of *S. carpocapsae* vs. *G. mellonella* between water potentials of –0.1 bars to –1.0 bars in a sandy loam. The range of activity reported by Koppenhöfer et al. (1995) is wider than the range of activity we observed, but research indicates that optimum activity can vary in different soil types and with nematode strains or species (Molyneux and Bedding, 1984; Koppenhöfer et al., 1995).

Previous laboratory and field tests indicated only limited potential for using entomopathogenic nematodes for control of *C. caryae* larvae (Nyczepir et al., 1992; Smith et al., 1993; Shapiro-Ilan, 2001a, 2003; Shapiro-Ilan et al., 2003). Yet, our greenhouse trials indicated considerably higher levels of larval mortality than previously reported, particularly in the *S. carpocapsae* (Italian) and *S. riobrave* (7–12) treatments. If one applies Abbott's formula (Abbott, 1925), the *S. carpocapsae* (Italian) and *S. riobrave* (7–12) treatments caused approximately 58% and 69% control, respectively, whereas previously reported levels of control in the greenhouse did not exceed 25% using Abbott's formula (Nyczepir et al., 1992; Smith et al., 1993). Possibly, the higher level of nematode virulence observed in our greenhouse study was due to a higher rate of application; 40 IJ/cm^2 or less were applied in previous studies, whereas 150 IJ/cm^2 were used in our greenhouse study. However, despite the high application rates, not all strains exhibited higher virulence. The high virulence levels observed in the greenhouse study may be explained, at least in part, by a high level of innate virulence in the previously untested strains, e.g., *S. riobrave* (7–12). Similar to our study, *S. riobrave* (7–12) exhibited greater virulence to *D. abbreviatus* compared with the original commercialized *S. riobrave* strain (i.e., 355) (Stuart et al., 2004).

Stuart et al. (2004) reported that a mixture of *S. riobrave* strains (in this case 10 strains that included the four used in our mixture) caused higher mortality than *S. riobrave* (355) as well as several other strains, but was not different from the 7–12 strain. Stuart et al. (2004) hypothesized that the genetic diversity contained in the mixture of strains might enhance virulence by providing a greater array of adaptations for overcoming the insect immune system. In this study targeting *C. caryae* larvae, no advantage was observed in using a mixture of strains relative to using one of the more virulent strains (e.g., 7–12) alone.

Overall, our results indicated no surfactant effect on *C. caryae* suppression with entomopathogenic nematodes. In contrast, Schroeder and Sieburth (1997) observed increased suppression of *D. abbreviatus* larvae in soil when *S. riobrave* was combined with several different surfactants, and Kinetic was among those producing

the most pronounced effect. The lack of virulence improvement when applying surfactant-nematode combinations in our study may have been based on soil type effects (Schroeder and Sieburth [1997] used a 3:1 peat:sand mixture) or perhaps use of different host species or nematode strains. Other studies that have applied surfactants to improve nematode efficacy have had mixed results. For example, Richter and Fuxa (1990) observed no effect when adding the surfactant Triton X-100 to *S. carpocapsae* applications targeting the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), whereas Schroer et al. (2005) reported that a mixture of the surfactant Rimulgan and the polymer xanthan facilitated rapid nematode invasion and thus enhanced suppression of the diamondback moth, *Plutella xylostella* (L.).

Our study included the first experiments that measured efficacy of entomopathogenic nematodes in suppressing adult *C. caryae* under field conditions. *Steinernema riobrave* (7–12) applications resulted in no observable control, and, although *S. carpocapsae* (Italian) provided some suppression when averaged over the entire experimental period, treatment effects were generally detectable only one day after treatment. Considering the high levels of adult *C. caryae* mortality observed when *S. carpocapsae* was applied in the laboratory even at rates 10-fold lower than those used in the field (Shapiro-Ilan, 2001b, 2003; Shapiro-Ilan et al., 2003), we expected the nematodes to produce better results than we observed in the field. However, it is well known that results under controlled conditions do not necessarily translate into field conditions where environmental factors can have a substantial impact on nematode efficacy (e.g., Shapiro and McCoy, 2000; Shapiro-Ilan et al., 2006a).

Relative to the results obtained in this study, nematode applications will need to provide greater efficacy and persistence before these organisms can be incorporated into a *C. caryae* management program. One solution may be to screen for nematode strains or species that exhibit greater longevity in soil typical of a pecan orchard. In response, Shapiro-Ilan et al. (2006b) compared 29 strains representing 11 entomopathogenic nematode species for persistence in soil obtained from a pecan orchard in Byron, GA. Among the nematode strains tested, *S. carpocapsae* (Sal strain) and *Steinernema diaprepesi* Nguyen and Duncan exhibited the greatest longevity and thus will be evaluated in future field trials targeting *C. caryae* adults.

It may also be possible to enhance *C. caryae* suppression in the field by creating nematode strains that are both virulent and tolerant of environmental conditions in the field. The Italian strain was found to possess low levels of heat and desiccation tolerance relative to several other *S. carpocapsae* strains, e.g., DD136 (Shapiro-Ilan et al., 2003, 2005). We hypothesize that the lack of heat and desiccation tolerance may have contributed to the Italian strain's poor performance in our field trials. Soil temperatures (e.g., at 5-cm depth) in Byron, GA, during *C. caryae* adult emergence can exceed 30°C (unpublished data), which can reduce infection, establishment, or reproduction of *S. carpocapsae* (Grewal et al., 1994). Additionally, it is conceivable that the nematodes experienced desiccation stress between irrigation events. Therefore, Shapiro-Ilan et al. (2005) generated several novel hybrid and bacterial transfer strains using DD136 and the Italian strain as parent strains. The novel strains exhibited superior heat and desiccation tolerance compared with the Italian strain, but virulence levels were not compromised (virulence in the original DD136 strain was inferior) (Shapiro-Ilan et al., 2005). The next step also will include these novel strains in field trials. Nematode applications resulting in higher levels of adult *C. caryae* control, along with some level of concurrent larval mortality (due to overlapping generations), may result in cumulative suppression that contributes substantially to overall *C. caryae* population reductions.

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