N O T E

Virulence of Entomopathogenic Nematodes to Pecan Weevil (Coleoptera: Curculionidae) Adults[1]

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The pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae), is an important pest of pecans in much of the eastern pecan growing region of the United States (Payne and Dutcher 1985. Pp. 103-116, *In* W. W. Neel (ed.) Pecan weevil: research perspective. Quail Ridge Press, MS). These insects have a two to three year life-cycle, most of which is spent below the soil surface. Adult weevils emerge from soil primarily from mid-August to mid-September and cause damage by feeding on and ovipositing in developing nuts; larvae then drop to the soil surface primarily October and November and burrow down to form a cell 8 to 25 cm below where the life-cycle will be completed (Harris 1985. Pp. 51-58, *In* W. W. Neel (ed.): Pecan weevil: research perspective. Quail Ridge Press, MS). It is during the above-ground stages (emerging adults to dropping larvae) that *C. caryae* is most susceptible to pest control measures, which currently consist solely of foliar chemical insecticide sprays against adults (e.g., carbaryl) (Payne and Dutcher 1985. Pp. 103-116, *In* W. W. Neel (ed.) Pecan weevil: research perspective. Quail Ridge Press, MS). Due to ecological and regulatory concerns, research towards development of non-chemical control alternatives is warranted.

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) are obligate parasites of insects and potent biological control agents (Kaya and Gaugler 1993. Annu. Rev. Entomol. 38: 181-206). These nematodes can be highly effective in controlling certain weevil larvae such as the diaprepes root weevil, *Diaprepes abbreviatus,* (L.) (Bullock et al. 1999. Florida. Entomol. 82: 1-7; Duncan and McCoy 1996. Environ. Entomol. 25: 174-178) and the black vine weevil, *Otiorhynchus sulcatus* (F.) (Schirocki and Hague 1997. Ann Appl. Biol. 18: 46-47; Shanks and Agudelo-Silva 1990. J. Econ. Entomol. 83: 107-110). Prior to the research reported herein, the only stage of *C. caryae* tested for susceptibility to entomopathogenic nematodes was

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fourth instar, and the level of virulence detected was poor to moderate (Nyczepir et al. 1992. J. Invertebr. Pathol. 60: 104-106; Shapiro-Ilan 2001. J. Econ. Entomol., 94: 7-13; Smith et al. 1993. J. Nematol. 25: 78-82). The goal of this research was to determine the virulence of entomopathogenic nematodes towards adult *C. caryae.* Specific objectives were to: (1) compare the virulence of several entomopathogenic nematode species, (2) determine if the nematode with the highest virulence could maintain a high level of infectivity when the exposure period to the insect is relatively short, and (3) determine reproductive capacity of entomopathogenic nematodes in *C. caryae* adults.

Virulence towards adult *C. caryae* was compared among *H. bacteriophora* Poinar (Hb and Oswego strains), *S. carpocapsae* (Weiser) (All strain), *S. feltiae* (Filipjev) (SN strain), and *S. riobrave* Cabanillas, Poinar, and Raulston (355 strain) using procedures described by Shapiro et al. (1999. J. Econ. Entomol. 92: 1086-1092). Nematodes were cultured in the last instar of *Galleria mellonella* (L.) according to procedures described by Woodring and Kaya (1988. Southern Cooperative Series Bulletin 331, Arkansas Agric. Exp. Sta., Fayetteville) and held at 13°C for up to 5 wks before being used in experiments. Adult *C. caryae* were collected after emergence in pecan orchards (Byron, GA) using Circle traps (Mulder et al. 1997. Oklahoma Extension Facts F-7190, Pp. 1-8). Experimental units were 30-ml lidded plastic cups (3 to 4 cm i.d., 3.5 cm deep) filled with soil (sand:silt:clay = 84:10:6, pH = 6.1, organic matter = 2.8%, final moisture level = field capacity, i.e., 14%). Each cup contained one adult *C. caryae* (collected within the previous 24 h) and one slice of apple (approximately, 1 ± 0.4 g) as a food and moisture source for the weevil. Five hundred infective juvenile (IJ) nematodes were pipetted onto the soil surface of each cup (controls received only water), which were then incubated at 25°C for 4 d before *C. caryae* mortality was recorded. There were three replicates of 10 cups per treatment, and the experiment was repeated three times (each treatment was included in at least two trials). After the first trial (which indicated high virulence in *S. carpocapsae*), an additional treatment was added where weevils were exposed to *S. carpocapsae* (500 IJs per cup) for 15 min and then transferred to cups without nematodes. The cups were then incubated for 4 d as described previously. Percentage mortality was corrected for control mortality using Abbott's formula (Abbott 1925. J. Econ. Entomol. 18: 265-267), arcsine transformed, and analyzed with analysis of variance and Duncan's multiple range test (SAS 1985. Version 5 ed. SAS Institute, Cary, NC). The number of IJs produced per infected *C. caryae* was determined in 5 cadavers per nematode species using the White trap method (White 1927. Science 66: 302-303), and differences among treatments were tested with analysis of variance (SAS 1985. Version 5 ed. SAS Institute, Cary, NC). All nematodes except *H. bacteriophora,* Hb strain, were evaluated for reproductive capacity (because this species was already represented by the Oswego strain).

Steinernema carpocapsae caused an average (±SE) of 99 ± 1% control in adult *C. caryae* (after 4 d exposure), which was significantly greater than mortality caused by all other nematode treatments (F = 11.28; df = 5,28; P = 0.0001) (Fig. 1). The nematodes, *H. bacteriophora* (Hb strain) and *S. riobrave* caused greater *C. caryae* control than *S. feltiae.* The trial effect was not significant, nor was the interaction between trial and treatment (F = 0.4; df = 3, 28; P = 0.76, and F = 0.71; df = 5, 28; P = 0.62, respectively); therefore, data were combined among trials.

In other weevil species, adults were found to have low susceptibility, or to be less susceptible than larval stages to nematode infection, e.g., the fuller rose beetle,

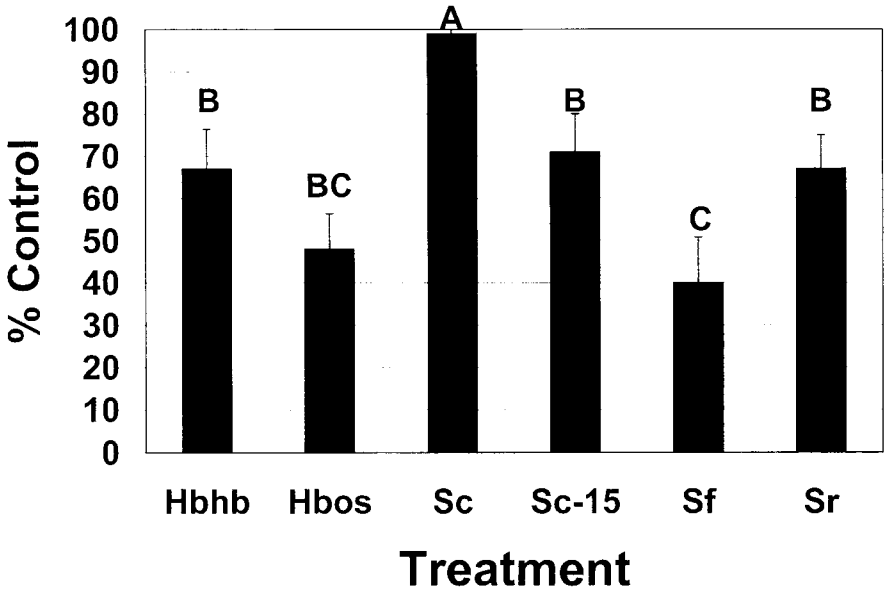

Fig. 1. Percentage control of adult *Curculio caryae* (after correction with Abbott's formula) with entomopathogenic nematodes. Hbhb, *Heterorhabditis bacteriophora* (Hb); Hbos, *H. bacteriophora* (Oswego); Sc, *Steinernema carpocapsae* (All); Sf, *S. feltiae* (SN); Sr, *S. riobrave* (355). Weevils were exposed to nematodes continually for four days except for Sc-15, for which exposure time was 15 minutes. Letters above bars indicate statistical differences among treatments (Duncan's test $\propto$ = 0.05).

Asynonychus godmani Crotch (Morse and Lindegren 1996. Florida. Entomol. 79: 373-384), the sweetpotato weevil, *Cylas formicarius,* (F.) (Mannion and Jansson 1992. J. Econ. Entomol. 85: 1642-1650), and the West Indian sugarcane weevil, *Metamasius hemipterus* (Oliver) (Giblin-Davis et al. 1996. J. Entomol. Sci. 31: 240-251).

Entomopathogenic nematodes also have been reported to be more virulent to immature stages than adults in various other insects outside of Curculionidae such as the American cockroach, *Periplaneta americana* (L.), (Zervos and Webster. 1989. Can. J. Zool. 67: 1609-1611), and the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Geden et al. 1985. J. Entomol. Science 20: 331-339). In contrast, we found the adult stage of *C. caryae* to be highly susceptible to *S. carpocapsae.* In a previous study conducted under essentially identical experimental parameters (e.g., the same soil, cups, and nematode application-rate, etc.), mortality of fourth-instar *C. caryae* caused by *S. carpocapsae* was less than 50%, which was not significantly different from control mortality (Shapiro-Ilan 2001. J. Econ. Entomol., 94: 7-13). Although the studies on larvae and adults were not conducted simultaneously, it is clear that adult *C. caryae* are more susceptible to *S. carpocapsae* than fourth-instar larvae.

One approach to controlling *C. caryae* adults may be to apply entomopathogenic

nematodes in a narrow (perhaps 1 to 2 m) band around each pecan tree. The weevils that crawl to the tree trunk would then be infected as they pass the area of application. If nematodes were applied as such, the cost of application would be reduced relative to treating a broader area (e.g., the entire orchard). However if this approach were used, weevils would have to be infected with nematodes after only a relatively short exposure time. The data indicate that *S. carpocapsae* has the potential to infect *C. caryae* and cause >70% mortality after 15 minutes of exposure (Fig. 1). *Steinernema carpocapsae* is a good candidate for this "banding" application approach because the nematode has an ambushing foraging strategy and will remain near the soil surface when applied there (Lewis et al. 1992. Parasitology 105: 309-319; Moyle and Kaya 1981. J. Nematol. 13: 295-300).

All nematodes tested reproduced successfully in adult *C. caryae.* Numbers of IJs produced per insect were ($\overline{X}$ ± SE) 51,700 ± 19462, 20773 ± 9435, 5820 ± 5264, and 28342 ± 5861 for *H. bacteriophora* (Oswego), *S. carpocapsae, S. feltiae,* and *S. riobrave,* respectively. No significant differences were detected in the number of IJs produced per insect ($F = 2.8$; df = 3,15; $P = 0.076$). Considering the F and P values, and the high level of variation around treatment means, it is likely that significant differences would have been detected had more replicates been incorporated. The reproductive capacity and high virulence of *S. carpocapsae* in *C. caryae* adults, indicates some potential for recycling and autodissemination (Lacey et al. 1995. Biocontr. Sci. Technol. 5: 121-130; Timper et al. 1988. Environ. Entomol. 17: 546-550). Future research will investigate the ability of entomopathogenic nematodes to control *C. caryae* under field conditions.

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