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Biological Control of the Pecan Weevil, *Curculio caryae* (Coleoptera: Curculionidae), with Entomopathogenic Nematodes

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Abstract: *Steinernema carpocapsae* (Weiser) strain A11, *S. feltiae* (Filipjev) strain SN, and *Heterorhabditis bacteriophora* Poinar strains HP88 and Georgia were tested for their efficacy as biological control agents of the pecan weevil, *Curculio caryae* (Horn), in pecan orchard soil-profile containers under greenhouse conditions. Percentage *C. caryae* parasitism by *S. carpocapsae* and *H. bacteriophora* strain HP88 and Georgia was consistently poor when applied either prior to or following *C. caryae* entry into the soil, suggesting that these nematode species and (or) their enterobacteria are poor biological control agents of weevil larvae. Soil taken 21 days following application of *S. carpocapsae* or *H. bacteriophora* strain HP88 induced a low rate of infection of *Galleria mellonella* larvae, whereas soil that had been similarly treated with *H. bacteriophora* strain Georgia induced a moderate rate of infection. Percentage *C. caryae* parasitism by *S. feltiae* was consistently low when applied following *C. caryae* entry into the soil and was inconsistent when applied as a barrier prior to entry of weevil larvae into the soil. Soil taken 21 days following application of *S. feltiae* induced a high rate of infection of *G. mellonella* larvae.

Key words: biological control, *Curculio caryae*, entomopathogenic nematode, heterorhabditid, *Heterorhabditis bacteriophora*, pecan weevil, *Steinernema carpocapsae*, *Steinernema feltiae*, steinernematid.

The pecan weevil, *Curculio caryae* (Horn), is a major insect pest of pecan, *Carya illinoensis* (Wang.) K. Koch. *Curculio caryae* is distributed across the pecan belt from the Carolinas to New Mexico (5) and causes two types of damage. Prior to shell hardening, feeding punctures by adult weevils cause premature nut drop. After shell hardening and initiation of the dough stage, larvae destroy the kernel (1).

There are four periods in the life cycle of pecan weevils during which they are potentially vulnerable to control: (i) as adults emerging from the soil in late summer and early fall; (ii) as free-living adults while feeding and ovipositing in the nut; (iii) as larvae exiting the nut and burrowing into the soil; and (iv) as larvae, pupae, or adults, collectively, during their 2–3 year subterranean period.

In commercial orchards, control relies exclusively on the use of chemical insecticides. However, there is a need to develop alternative methods of control that do not lead to secondary pest resurgence or adversely affect beneficial insect species, and that are environmentally acceptable.

Steinernematid and heterorhabditid nematodes have been shown to infect over 200 insect species from several orders, including nine species in five genera of the Curculionidae (9). Third-stage infective juveniles (IJ) contain cells of the mutualistic enterobacteria *Xenorhabdus* spp. in their intestines. The IJ penetrate the host's natural openings, enter the haemocoel, then release the bacteria, which multiply rapidly and kill the host by septicemia, generally within 48 hours.

In field trials, the nematode *Steinernema carpocapsae* (Weiser) strain DD-136 (10) caused 59.6% mortality of pecan weevil larvae after 20 days (14). Harp and Van Cleave (4) reported 20% parasitism of pecan weevil fourth instar larvae and pupae by *Steinernema* sp. in one group of laboratory specimens. Ring et al. (11) screened two nematode species (*S. carpocapsae* strains A11 and Mexican and *Heterorhabditis* sp.) against pecan weevil under labora-

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tory conditions and reported 80, 86, and 75% larval mortality, respectively, after 28–35 days. In 1986, W. L. Tedders recovered *H. bacteriophora* Poinar Georgia strain from dead, reddish-colored weevil larvae found in a pecan orchard at Byron, Georgia, but Nyczepir et al. (8) reported this species was weakly pathogenic to *C. caryae* in laboratory tests.

This study was done to investigate the potential of the nematodes *S. carpocapsae* strain A11, *S. feltiae* (Filipjev) (10) strain SN, and *H. bacteriophora* strains HP88 and Georgia as biological control agents for the pecan weevil.

Materials and Methods

Simulated soil-profile containers: Norfolk fine sandy loam soil was taken from a pecan orchard at the USDA-ARS Research Laboratory in Byron, Georgia. This soil is deep, friable, and well drained and has developed from thick beds of acid sandy loam of the Coastal Plain uplands. Soil pH was 6.45 for the top soil and 5.80 for the underlying clay. Top soil and clay were ground separately in a soil shredder. Kraft board plant containers (7.6 cm wide × 7.6 cm long × 22.9 cm deep) were packed with a 10.2-cm clay soil base, then filled with a 7.6-cm topsoil layer, simulating the natural pecan orchard soil profile at Byron, Georgia. Water (ca. 40 ml) was added to each soil profile container to ensure a hardpan at the topsoil–clay base interface. This hardened clay-base soil is required for the normal formation of soil cells by *C. caryae* larvae. Soil moisture averaged approximately 14.6 and 18.4%, respectively, in the topsoil and clay.

Insects: Weevil-infested pecans were collected from an orchard at the USDA-ARS Research Laboratory in Byron, Georgia, during late September and early October. Nuts were taken to the laboratory, where they were placed onto wooden racks with 1.27-cm mesh hardware cloth bottoms. After larvae emerged from the nuts and passed through the hardware cloth bottom, they were funneled into collecting containers lined with moist paper towels. Larvae were collected daily and used in experiments on the same day they were collected.

Nematodes: Steinernema carpocapsae strain A11, *S. feltiae* strain SN, and *H. bacteriophora* strain HP88 and Georgia were obtained from BIOSYS, Palo Alto, California. The latter strain had been originally isolated from *C. caryae* larvae in Byron, Georgia, and sent to BIOSYS for culturing. Steinernematids were cultured by liquid fermentation technology, in which the media consisted of a yeast source, vegetable oil, and a source of protein (2). Heterorhabditids were reared on the greater wax moth larvae, *Galleria mellonella* (L.). Pretest nematode infectivity 72 hours following exposure of one *G. mellonella* larvae to one infective stage nematode ranged from ca. 51.0% to 56.3% parasitism (7).

Experimental protocol: Two experiments were designed to simulate potential methods of application of the nematodes under field conditions: (i) barrier method—Nematodes were applied to the soil surface 2 hours prior to the introduction of *C. caryae* larvae to evaluate the ability of the nematodes to parasitize *C. caryae* larvae as the latter entered the soil; and (ii) search method—Nematodes were applied to the soil surface 5 hours following the introduction of *C. caryae* larvae to evaluate the ability of the nematodes to locate and parasitize *C. caryae* larvae after the latter had burrowed into the soil.

In each experiment, a suspension of IJ of each nematode was applied to the soil surface in 40 ml distilled water. In the 1989 tests, *S. carpocapsae, S. feltiae,* and *H. bacteriophora* HP88 were evaluated at rates of 31, 46, and 62 IJ/cm^2 surface area of soil. In the 1990 tests, *S. carpocapsae* and *S. feltiae* were evaluated at rates of 31, 62, and 78 IJ/cm^2 surface area of soil; *H. bacteriophora* Georgia was evaluated only at 78 IJ/cm^2 due to a shortage of this nematode. The recommended application rate for commercial products is 2.5×10^9/ha or 24.8 IJ/cm^2 (3).

Experimental containers were placed on benches in an air-conditioned greenhouse maintained at 22–25 C. Nematode strains

and rates and untreated controls (untreated controls received 40 ml distilled water) were arranged in a completely randomized design. Each container was given five *C. caryae* larvae. The experiments were terminated after 21 days and larval mortality was recorded. As an indicator of IJ survivorship and ability to penetrate insect larvae and (or) cause infection, soil taken 21 days following nematode application was evaluated for its ability to induce *G. mellonella* larval mortality by a common soil bioassay method (6,15). In this bioassay, 50 g of soil from individual soil-profile containers that had been used for the *C. caryae* infections (two containers per nematode strain in 1989; four in 1990) were placed in each of 10 petri dishes with 10 *G. mellonella* larvae per dish. The bioassay was maintained at 22 ± 2 C for 5 days, after which dead larvae were dissected to determine the presence of nematodes. Data were subjected to an analysis of variance and means were separated by the Waller-Duncan procedure (13).

Results

No significant differences in percentage *C. caryae* parasitism between nematode dosage rates for any of the three nematode species tested occurred in either experiment in 1989 or 1990. Therefore, dosage rate data were pooled by nematode species for comparison among nematode species.

In 1989, results from the barrier method experiment indicated that percentage larval parasitism was significantly greater in *S. carpocapsae*-treated containers (11.7%) than in the untreated controls (3.0%) (Table 1). However, percentage parasitism in the *S. feltiae*-(7.6%) and *H. bacteriophora* HP88-(0.3%) treated containers was not significantly different from that in the untreated controls. Results from the search method experiment indicated that percentage larval parasitism was significantly greater in the *S. feltiae*-treated containers (9.4%) than in the untreated controls (1.1%), whereas percent parasitism in the *S. carpocapsae*-(2.7%) and *H. bacteriophora*-HP88 (1.3%) treated containers was not significantly different from that in the untreated controls. Parasitism of weevil larvae in the control containers was due to parasitism by *Heterorhabditis* spp. naturally present in the pecan orchard soil, as indicated by the red color of these parasitized larvae (10). Soil recovered from soil-profile containers at the termination of the experiments induced rates of parasitism in *G. mellonella* larvae of 11.0% (*S. carpocapsae*), 96.5% (*S. feltiae*), and 0.0% (*H. bacteriophora* HP88) (Table 2).

In 1990, results from the barrier method experiment showed that percentage larval parasitism was significantly greater in the *S. feltiae*-(27.4%) treated containers than in *S. carpocapsae* (12.9%) treated and untreated controls (9.2%) (Table 3). However, percentage parasitism in the *H. bacteriophora* Georgia-(21.6%) and *S. carpocapsae*-(12.9%) treated containers was not significantly different from that in the

Table 1. Percentage parasitism of *Curculio caryae* larvae by *Steinernema carpocapsae, S. feltiae,* and *Heterorhabditis bacteriophora* HP88 in simulated soil-profile plant containers: 1989.

	Experiment 1 (barrier method)			Experiment 2 (search method)		
Nematode species	*N*†	% Live‡	% Parasitism§	*N*†	% Live‡	% Parasitism§
S. carpocapsae	90	85.7 b	11.7 a	90	93.9 a	2.7 b
S. feltiae	90	90.0 ab	7.6 ab	90	88.9 b	9.4 a
H. bacteriophora	90	98.9 a	0.3 c	90	95.8 a	1.3 b
Control	30	94.0 a	3.0 bc	30	96.9 a	1.1 b

Means followed by the same letters are not significantly different at $P \leq 0.05$ (Waller-Duncan procedure).

† Number of replicates, with 30 containers/nematode dosage rate. Total *C. caryae* larvae recovered in the *S. carpocapsae, S. feltiae, H. bacteriophora* HP88, and control treatments were 414, 428, 420, and 135, respectively, in Experiment 1, and 408, 375, 373, and 138, respectively, in Experiment 2.

‡ Number of live larvae recovered as a percentage of total larvae recovered from containers 21 days after treatment.

§ Number of dead larvae from which nematodes were recovered as a percentage of total larvae recovered from containers 21 days after treatment.

TABLE 2. Posttest *Galleria mellonella* larval mortality (21 days) resulting from infection by *Steinernema carpocapsae, S. feltiae, Heterorhabditis bacteriophora* HP88 and Georgia in 1989 and 1990.

Nematode species	Percentage dead *G. mellonella*†			
	1989‡		1990§	
	Mean	Range	Mean	Range
S. carpocapsae	11.0	9–13	5.8	2–10
S. feltiae	96.5	94–99	90.3	75–99
H. bacteriophora HP88	0.0	0–0		
H. bacteriophora Georgia			49.5	19–90

† Percentage dead larvae from which the respective nematode species or strain was recovered after 5 days.

‡ Ten *G. mellonella* larvae in each of twenty 50-g soil samples per nematode strain (total N = 200 *G. mellonella* larvae per nematode strain).

§ Ten *G. mellonella* larvae in each of forty 50-g soil samples per nematode strain (total N = 400 *G. mellonella* larvae per nematode strain).

untreated controls. No significant differences occurred in percentage parasitism among the three nematode species and the untreated controls in the search method experiment. Parasitism of weevil larvae in the control containers was again due to parasitism by *Heterorhabditis* spp. naturally present in the pecan orchard soil, as indicated by the red color of these parasitized larvae (10). Soil recovered from soil-profile containers at the termination of the experiments induced rates of parasitism in *G. mellonella* larvae of 5.8% (*S. carpocapsae*), 90.3% (*S. feltiae*), and 49.5% (*H. bacteriophora* Georgia) (Table 2).

DISCUSSION

Relative to the untreated control, percentage *C. caryae* parasitism by *S. carpocapsae* and *H. bacteriophora* HP88 was consistently poor when applied either prior to or following *C. caryae* larval entry into the soil. These data suggest that both nematode species and (or) their enterobacteria are poor biological control agents of *C. caryae,* providing at best ca. 9% increase in larval parasitism when applied prior to weevil entry into the soil. Additionally, the ability of the soil taken from the soil-profile containers to induce infection in *G. mellonella* larvae was very low 21 days following application with either nematode species. Whether this low capacity of the soil to induce *G. mellonella* infections reflects the absence of nematodes or an inability of nematodes present to infect *G. mellonella* is unknown (6).

Although percentage parasitism of *C. caryae* by *H. bacteriophora* Georgia was ca. 1.7 times that by *S. carpocapsae* when applied before *C. caryae* entry into the soil, its poor performance indicates that this strain is also a poor biological control agent of *C. caryae*. However, soil taken 21 days following application of the Georgia strain induced a moderate rate of infection in *G. mellonella* larvae. These results are not surprising given the facts that the pecan weevil and Georgia strain have likely coevolved and that the pecan weevil has likely developed natural defenses to parasitism by this nematode strain.

Finally, relative to the untreated control, percentage *C. caryae* parasitism by *S. feltiae* was consistently low in the search method experiments. Although percentage parasitism of *C. caryae* by *S. feltiae* was highest in the 1990 barrier method experiment, results were inconsistent (7.6% in 1989 and 27.4% in 1990). Further study of *S. feltiae* is required in order to fully assess its value as a reliable biological control agent. It should also be noted that soil taken 21 days following application of *S. feltiae* induced a high rate of infection of *G. mellonella* larvae.

The discrepancies between the results reported herein and those reported previously for *C. caryae* are likely due to the different experimental methods used, as well as the differential nematode dosage rates. Tedders et al. (14) and Ring et al. (11) used 24-140× and 3× rates, respectively, the highest nematode dosage rate reported here. Although results reported herein are less than desirable from the traditional short-term pest control point of view, it should be noted that nematodes have caused field epizootics in insect species only slightly susceptible under laboratory conditions (12).

TABLE 3. Percentage parasitism of *Curculio caryae* larvae by *Steinernema carpocapsae, S. feltiae,* and *Heterorhabditis bacteriophora* Georgia in simulated soil-profile plant containers: 1990.

	Experiment 1 (barrier method)			Experiment 2 (search method)		
Nematode species	*N*†	% Live‡	% Parasitism§	*N*†	% Live‡	% Parasitism§
S. carpocapsae	84	83.0 a	12.9 b	84	83.5 a	10.8 a
S. feltiae	84	69.4 b	27.4 a	84	90.9 a	6.8 a
H. bacteriophora	14	70.0 ab	21.6 ab	14	90.2 a	6.6 a
Control	28	88.6 a	9.2 b	28	88.6 a	5.9 a

Means followed by the same letters are not significantly different at $P \leq 0.05$ (Waller-Duncan procedure).

† Numbers of replicates, with 28 containers/nematode dosage rate for *S. carpocapsae* and *S. feltiae,* and 14 containers/nematode dosage rate for *H. bacteriophora* Georgia. Total *C. caryae* larvae recovered in the *S. carpocapsae, S. feltiae, H. bacteriophora* Georgia and control treatments were 382, 381, 61, and 131, respectively, in Experiment 1 and 324, 299, 56, and 108, respectively, in Experiment 2.

‡ Number of live larvae recovered as a percentage of total larvae recovered from containers 21 days after treatment.

§ Number of dead larvae from which nematodes were recovered as a percentage of total larvae recovered from containers 21 days after treatment.

LITERATURE CITED

1. Boethel, D. J., and R. D. Eikenbary. 1979. Status of pest management programs for the pecan weevil. Pp. 81–150 in D. J. Boethel and R. D. Eikenbary, eds. Pest management programs for deciduous tree fruits and nuts. New York: Plenum Press.

2. Friedman, M. J., S. E. Langston, and S. Pollitt. 1991. Mass production in liquid culture of insect killing nematodes. U.S. Patent No. 5,023,183.

3. Georgis, R., and N. G. M. Hague. 1991. Nematodes as biological insecticides. Pesticide Outlook 2: 29–32.

4. Harp, S. J., and H. W. Van Cleave. 1976. New records of natural enemies of the pecan weevil. Southwestern Entomologist 1:38.

5. Harris, M. K. 1979. Pecan weevil distribution on pecan across the pecan belt. Southern Cooperative Service Bulletin 238, 11 pp.

6. Hominick, W. M., and A. P. Reid. 1990. Perspectives on entomopathogenic nematology. Pp. 327–345 in R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

7. Miller, R. W. 1989. Novel pathogenicity assessment technique for *Steinernema* and *Heterorhabditis* entomopathogenic nematodes. Journal of Nematology 21:574. (Abstr.).

8. Nyczepir, A. P., J. A. Payne, J. J. Hamm, and W. L. Tedders. 1988. *Heterorhabditis heliothidis* (Nematoda: Heterorhabditidae) a parasite of pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae). Journal of Nematology 20:654. (Abstr.).

9. Poinar, G. O., Jr. 1979. Nematodes for biological control of insects. Boca Raton, FL: CRC Press.

10. Poinar, G. O., Jr. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pp. 23–61 in R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

11. Ring, D. R., W. J. Schroeder, A. P. Nyczepir, J. A. Payne, and J. W. Snow. 1988. Nematodes screened against pecan weevil larvae, 1986. Insecticide and Acaricide Tests 13:184.

12. Sexton, S. B., and P. Williams. 1981. A natural occurrence of parasitism of *Graphognathus luecoloma* (Boheman) by the nematode *Heterorhabditis* sp. Journal of Australian Entomological Society 20:253–255.

13. Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach, 2nd ed. New York: McGraw-Hill.

14. Tedders, W. L., D. J. Weaver, and E. J. Wehunt. 1973. Pecan weevil: Suppression of larvae with the fungi *Metarrhizium anisopliae* and *Beauveria bassiana* and the nematode *Neoaplectana dutkyi.* Journal of Economic Entomology 66:723–725.

15. Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: A handbook of biology and techniques. Southern Cooperative Bulletin 331, Arkansas Agricultural Experiment Station, Fayetteville.