Laboratory Bioassays and Field Trials of Entomogenous Nematodes for Control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Citrus[1]

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ABSTRACT Laboratory, greenhouse, and field tests were made in Florida to determine the potential of the entomogenous nematodes, *Neoaplectana glaseri* Steiner or *Neoaplectana carpocapsae* Weiser (Mexican strain), as biological control agents for the root weevil, *Diaprepes abbreviatus* (L.). In the laboratory, when nematodes were applied to the soil surface at rates of 25, 250, and 2,500 per cm², mortality of 3-mo-old *D. abbreviatus* larvae ranged from 24 to 88%. *N. glaseri* and *N. carpocapsae* had no significant effect on neonate larvae or pupae. In the greenhouse, when *N. glaseri* or *N. carpocapsae* were introduced to *D. abbreviatus* infested citrus seedlings, larval populations were significantly ($P < 0.05$) reduced. In field tests, when nematodes were applied at 250 per cm², mortality of 3-mo-old *D. abbreviatus* larvae was 35 and 65% for *N. glaseri* and *N. carpocapsae*, respectively. In laboratory, greenhouse, and field tests, *N. carpocapsae* (Mexican strain) was significantly ($P < 0.05$) superior to *N. glaseri* as a biocontrol agent for *D. abbreviatus* larvae.

KEY WORDS *Diaprepes abbreviatus*, *Neoaplectana carpocapsae*, *Neoaplectana glaseri*, entomogenous nematodes

A ROOT WEEVIL, *Diaprepes abbreviatus* (L.), is indigenous to the West Indies and it is now established in the United States (Woodruff 1964). The "vaquita," as *D. abbreviatus* is referred to in the West Indies, is an important pest of citrus, sugarcane, and other agricultural crops. Under natural conditions, the adult oviposits between two leaves; first instars drop to the ground, burrow into soil, and feed on roots until they emerge as adults (ca. 1 yr) (Wolcott 1936). In citrus, the larvae feeding on roots can kill the tree (Schroeder & Sutton 1977).

Entomogenous nematodes are promising biological control agents for a broad range of soil-inhabiting insect species (Poinar 1971). In a survey of Florida citrus grove and ornamental nursery soils, two entomogenous nematodes, *Neoaplectana carpocapsae* Weiser and *Heterorhabditis* sp., were found to be infectious to *D. abbreviatus* larvae (Beavers et al. 1983). Diaz & Hernandez (1978) reported the use of *N. carpocapsae* for control of the citrus root weevil, *Pachnaeus litus* (Germar), on bagged citrus trees in Cuba. Laumond et al. (1979) determined that *D. abbreviatus* adults were hosts of *N. carpocapsae* in a host-range study in the laboratory. Roman & Figueroa (1985), in greenhouse tests with *N. carpocapsae*, reported 86% mortality of *D. abbreviatus* large larvae (3- to 4-mo-old weevil grubs) in Puerto Rico. Greenhouse tests of Beavers (1984) showed that *Neoaplectana glaseri* Steiner had potential as a biological control agent for *D. abbreviatus*. Entomogenous nematodes also have been reported to reduce populations of several other root weevil species (Harlan et al. 1971, Burman et al. 1979). The experiments described here were conducted to evaluate pathogenicity of *N. glaseri* and *N. carpocapsae* to larvae of *D. abbreviatus* under laboratory, greenhouse, and field conditions. Field tests were conducted in an infested citrus grove in Florida with the objective of evaluating entomogenous nematodes as biological control agents for citrus-root-feeding weevils.

Materials and Methods

The citrus rootstock used in the greenhouse tests was a hybrid *Poncirus trifoliata* (L.) Osbeck × *Citrus grandis* (L.) Osbeck. Seedlings with an average diameter of 8.89 mm at a point 10 cm above the soil surface were grown in individual clay pots (25 cm diameter by 25 cm deep) in a medium consisting of 1:1 (vol/vol) Florida peat and masonry sand. The soil was amended with 4.1 kg dolomite, and 5.9 kg 14-14-14 Osmocote/m³ and was at a pH of 5.5. The plants were maintained on raised benches in a greenhouse under ambient light and temperatures (June–October 1985).

D. abbreviatus neonate larvae were obtained from eggs oviposited by field-collected adults maintained in the laboratory. Large larvae were obtained from the laboratory colony reared on artificial diet medium (Beavers 1983). The weight of

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the 3-mo-old larvae was 620.5 ± 9.5 mg (mean ± SEM). *D. abbreviatus* pupae were obtained by placing large larvae in individual vials in a soil mix of three parts peat, one part sand, one part pine bark, and one part cypress shavings (vol/vol/vol/vol). The moisture in the soil was 30% (wt/wt) and pupal cells were not disturbed.

Nematodes used were *N. glaseri* and *N. carpocapsae* (Mexican strain) and were obtained from Biosis (Palo Alto, Calif.). The stock colony was maintained on water-soaked sponges at 10°C. Nematodes were checked for viability before application.

Laboratory Study. Bioassay chambers, tubes 10 cm high by 5 cm in diameter, with a screen bottom, were filled with sterile sand. The moisture content was 9% (wt/wt), the optimum for neonate larvae penetration (Jones & Schroeder 1983). Nematodes were added to the sand at 25, 250, and 2,500 per cm^2, 10 replications for each rate and nematode species. Bioassay chambers were maintained at 27 ± 2°C under natural photoperiod. One hundred neonate *D. abbreviatus* larvae were added to the surface of the sand in each chamber. The chamber was placed over water, larvae moved through the sand, penetrated the screen, and were recovered from the water. Counts were made after 14 d.

To evaluate pathogenicity of nematodes to large larvae, individual larvae were placed in 4-cm-diameter cups, filled with 29 ml each of Lakeland-type sandy soil (thermic-coated typic quartzipsamments) with a moisture content of 7% (wt/wt). The sand was treated with nematodes at 25, 250, and 2,500/cm^2. Cups were arranged in groups of 10 replicated five times per rate per species of nematode, held at 27 ± 2°C for 2 wk, and then larvae were examined. Infection was determined by dissecting dead larvae to determine presence or absence of nematodes. Data were analyzed by analysis of variance (ANOVA) and means were separated by Duncan's (1955) new multiple range test.

Pupae were evaluated for nematode infection with methods described for large larvae. For this life stage, the nematodes were added to the sand at 250 per cm^2 after the pupal cell was formed.

Greenhouse Study. One hundred citrus seedlings were infested with *D. abbreviatus* by adding 100 neonate larvae to each 10-liter pot. Greenhouse temperatures ranged from 26 to 30°C. After 2 mo, nematodes were placed 5 cm below the soil surface at rates of 25, 250, and 2,500 nematodes per cm^2, 20 replicates per rate. One month after addition of the nematodes, the seedlings were removed from the pot and the weight and number of larvae per seedling determined. Data were subjected to ANOVA. Means were separated where appropriate by Duncan's new multiple range test.

Field Study. Cylindrical wire screen cages (0.32-cm mesh) 15 cm in diameter by 16 cm high were filled with Lakeland-type sand obtained from the test site. Five 3-mo-old *D. abbreviatus* larvae were placed in each cage and the cage buried 15 cm below the soil surface in a citrus grove. The soil above each cage was treated with one of the two species of nematodes at 250 nematodes per cm^2 or 44,150 per cage site. There were 10 replications per treatment and 10 checks. Twenty-one days after treatment, the cages were removed and the larvae recovered. Dead larvae were dissected to determine presence or absence of nematodes. The test was conducted in September to coincide with a period of large larvae under Florida conditions.

Results and Discussion

Laboratory Study. The presence of the entomogenous nematodes, *N. glaseri* or *N. carpocapsae*, in the sand had no apparent effect on survival of *D. abbreviatus* neonate larvae. Overall, 71 ± 6% (mean ± SEM) of the neonate larvae moved through the nematode-treated sand compared with a mean of 64 ± 8% recovered from the untreated check. Application of nematodes to the soil to prevent neonate larvae from reaching the root area would have little or no effect on the larval population.

Mortality of 3-mo-old larvae was significantly ($P > 0.05$) greater for *N. carpocapsae* compared with *N. glaseri* for the three rates of nematodes applied (Table 1). Susceptible larvae died within 48 h and living larvae remained active in the sand for 2 wk with little increase in mortality. A minimal lethal dose to susceptible larvae was not determined.

Pupal mortality was not significantly different ($P < 0.05$) from check for *N. glaseri* or *N. carpocapsae*. Nematode-infected pupae were, however, recovered from treated sand (ca. 2%), indicating that pupae are slightly susceptible to nematode attack.

Greenhouse Study. At the time nematodes were added to the citrus seedlings, it was determined by examining 20 seedlings that the *D. abbreviatus* larvae had attained a weight of 277.71 ± 7.7 mg (mean ± SEM) and the number of developing larvae per seedling was 15.5 ± 3.1. Four weeks later when the remaining seedlings were examined, larval weight was 353.3 ± 17.6 mg and the number of larvae per seedling was significantly reduced ($P < 0.05$) in the nematode treatments (Table 2). *N. carpocapsae* had a significantly ($P < 0.05$) greater effect on the larval population compared with *N. glaseri* for the three dosage levels. The test was terminated when larvae were 3 mo old because the epidermal layer of the roots was completely devoured to the soil surface. The total effect of nematodes would probably have been greater if larvae had been able to develop to full size (ca. 650.0 mg) and were exposed to the nematodes for the 3 additional mo.

Field Study. The number of large larvae recovered from the nematode-treated soil was significantly ($P < 0.05$) reduced compared with the

Table 1. Mortality of 3-mo-old *D. abbr* in sand treated with *N. glaseri* or *N. carp* laboratory

Nematode dosage (no./cm^2 in cup, diam 4 cm)	*N. glaseri*		*N. c*
	% killed by nematodes (mean ± SEM)	% dead unknown	% kill by nema (mean SEM
25	24 ± 4.1a	8	40.0 ±
250	33 ± 6.2a	0	42.5 ±
2,500	51 ± 5.1a	12	88.3 ±
0	0	4	0

Percentages in rows followed by the same le icantly different ($P < 0.05$; Duncan's [1955] n test).

check. We recovered 20, 12, and 5 from *N. glaseri*, *N. carpocapsae*, spectively. Mortality attributed to 35 and 65% for *N. glaseri* and N respectively. Both species of entom todes evaluated under field conditi tial as biological control agents for *D. abbreviatus*.

In the laboratory, greenhouse, a *N. carpocapsae* (Mexican strain) ($P < 0.05$) superior to *N. glaseri* control agent for *D. abbreviatus*. T *D. abbreviatus* as a host for entom todes is apparent. Laboratory rese biological activity of nematode str to develop a virulent biological needed.

Additional field trials are requ use of nematodes as a significant agent for larvae of *D. abbreviatus* under Florida conditions. With tode pathogenicity and cultural nematode survival, i.e., grove ir genous nematodes could provide

Table 2. The effect of *N. glaseri* on larval populations of *D. abbreviat* citrus seedlings in the greenhouse

Nematode dosage (no./cm^2 in pot, diam 24 cm)	*N. glaseri*	
	No. larvae recovered/ seedling (mean ± SEM)	% population reduction
25	10.5 ± 2.1	32a
250	13.6 ± 1.3	12a
2,500	10.3 ± 2.1	34a
0	15.5 ± 5.2	

Percentages in rows followed by the s icantly different ($P < 0.05$; Duncan's [1 test).

Table 1. **Mortality of 3-mo-old *D. abbreviatus* larvae in sand treated with *N. glaseri* or *N. carpocapsae* in the laboratory**

Nematode dosage (no./cm² in cup, diam 4 cm)	*N. glaseri*		*N. carpocapsae*	
	% killed by nematodes (mean ± SEM)	% dead unknown	% killed by nematodes (mean ± SEM)	% dead unknown
25	24 ± 4.1a	8	40.0 ± 6.7b	4
250	33 ± 6.2a	0	42.5 ± 8.2b	8
2500	51 ± 5.1a	12	88.3 ± 5.0b	4
0	0	4	0	5

Percentages in rows followed by the same letter are not significantly different ($P < 0.05$, Duncan's [1955] new multiple range test).

check. We recovered 20, 12, and 50% live larvae from *N. glaseri*, *N. carpocapsae*, and check, respectively. Mortality attributed to nematodes was 35 and 65% for *N. glaseri* and *N. carpocapsae*, respectively. Both species of entomogenous nematodes evaluated under field conditions have potential as biological control agents for large larvae of *D. abbreviatus*.

In the laboratory, greenhouse, and field studies, *N. carpocapsae* (Mexican strain) was significantly ($P < 0.05$) superior to *N. glaseri* as a biological control agent for *D. abbreviatus*. The suitability of *D. abbreviatus* as a host for entomogenous nematodes is apparent. Laboratory research to evaluate biological activity of nematode strains and species, to develop a virulent biological control agent, is needed.

Additional field trials are required to validate use of nematodes as a significant biological control agent for larvae of *D. abbreviatus* feeding on citrus under Florida conditions. With increased nematode pathogenicity and cultural practices to favor nematode survival, i.e., grove irrigation, entomogenous nematodes could provide an economically feasible (ca. 0.01 cents per million) (Bedding 1984) and environmentally acceptable method for the management of root weevil infestations in Florida and the Caribbean basin.

Table 2. **The effect of *N. glaseri* and *N. carpocapsae* on larval populations of *D. abbreviatus* feeding on potted citrus seedlings in the greenhouse**

Nematode dosage (no./cm² in pot, diam 24 cm)	*N. glaseri*		*N. carpocapsae*	
	No. larvae recovered/ seedling (mean ± SEM)	% population reduction	No. larvae recovered/ seedling (mean ± SEM)	% population reduction
25	10.5 ± 2.1	32a	8.6 ± 1.1	48b
250	13.6 ± 1.3	12a	6.8 ± 2.1	56b
2,500	10.3 ± 2.1	34a	1.2 ± 1.4	92b
0	15.5 ± 5.2		—	

Percentages in rows followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] new multiple range test).

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