Parasitism of entomophilic nematodes on the sugarcane rootstalk borer, *Diaprepes abbreviatus* (L.) (Coleoptera:Curculionidae), larvae[1]

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ABSTRACT

Ten greenhouse experiments tested the effectiveness of the entomophilic nematodes *Steinernema feltiae* Filipjev, *S. glaseri* Weiser, and *S. bibionis* Bobien against larvae of the sugarcane rootstalk borer, *Diaprepes abbreviatus* (L.). *S. feltiae* caused mortality to 12- to 16-week-old *D. abbreviatus* larvae at population densities of 1,000 to 3,000 nematodes/300 cm^3 of soil. Four hundred *S. glaseri* or *S. bibionis* also caused significant mortality of the grubs. An increase of 4,000 to 40,000 *S. glaseri*/300 cm^3 of soil increased mortality of the grubs. *S. bibionis* infectivity decreased with time, and high rates of mortality of the infective juveniles were recorded under laboratory conditions. When grubs were placed in petri dishes in direct contact with the nematodes before placing the infected grubs in soil, the mortality was independent of the density or nematode species used. These results demonstrated the effectiveness of the entomophilic nematodes and their potential for biocontrol of *D. abbreviatus*.

RESUMEN

Parasitismo de nematodos entomofílicos, al gorgojo de la raíz de la caña de azúcar

Se realizaron 10 experimentos de invernadero para investigar la eficacia de los nematodos entomofílicos *Steinernema feltiae, S. glaseri* y *S. bibionis* para combatir larvas de 14 a 16 semanas de edad de *Diaprepes abbreviatus*. *S. feltiae* causó mortalidad en densidades poblacionales de 1,000 a 3,000 nematodos/300 $cm.^3$ de suelo. La densidad de 400 *S. glaseri* o *S. bibionis* causó mortalidad significativa de las larvas del insecto. Un aumento en la densidad poblacional de 4,000 a 40,000 *S. glaseri* 300 $cm.^3$ de suelo aumentó la mortalidad de las larvas. Se observó que *S. bibionis* fue altamente susceptible a cambios en temperatura ambiente y su infectividad se redujo marcadamente con el tiempo. Cuando las larvas de *D. abbreviatus* se expusieron en placas de petri al contacto directo de los nematodos, la mortalidad fue independiente de la densidad o la especie de nematodos que se usara. Los resultados demuestran que los nematodos entomofílicos son una alternative potencial para combatir las larvas de *D. abbreviatus*,

INTRODUCTION

The sugarcane rootstalk borer (SRB), *Diaprepes abbreviatus* (Coleoptera:Curculionidae), is considered the most economically important insect

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pest of Puerto Rico and the West Indies (1,6,12,13) in crops such as sugarcane, ornamentals, starchy crops, forest and fruit trees. The grub, attacking the root of these plants, reduces yield and in some instances causes death of plants. In Puerto Rico the insect is abundant throughout the year, both on the north and south coasts of the island. SRB was also found infesting citrus trees in the state of Florida in 1964 (3,4,16).

The weevil deposits egg masses between plant leaves. Hatched larvae drop to the ground and, boring tunnels ,feed on the rootstalk of plants, (11). Most larval growth occurs during the first 3 to 4 months or 8th instar, the stage in which it causes the greatest damage (2,12). Wolcott (12,14,15) reported the efficacy of the insecticide Aldrin for control of white grubs, including SRB. After the banning of this product in 1973 by the Environmental Protection Agency (EPA), all the efforts to find a good substitute have failed. Effective insecticides are not yet available for the control of this pest.

A survey was conducted in 1977-78 by the Agricultural Experiment Station of the University of Puerto Rico to collect data from sugarcane farms affected by this insect. Forty-six percent of all farms were infested, with an estimated loss of $27.7 million in 70,000 acres (5). No estimated loss data in other crops are available, although the damage done by this insect is known.

Since there are no chemicals with an EPA use permit available for larval control of this pest, a series of laboratory and greenhouse tests was conducted to evaluate three species of the entomophilic nematodes of the genera *Steinernema* as biocontrol agents for SRB. These nematodes attack a wide range of insects and have been tested against various soil-inhabiting pests (7,10). At present, the nematodes are commercially produced as biological control agents against various insect pests.

MATERIALS AND METHODS

Infective juveniles of *Steinernema feltiae*, *S. glaseri*, and *S. bibionis* obtained from Biosis Laboratories, Palo Alto, California, were tested against larvae of SRB in 10 greenhouse experiments. Different population densitities of the nematodes were inoculated into 10 cm diameter plástic pots filled with 300 g of a steam-sterilized soil-sand mixture (3:1), with a 31.2% field capacity.

Four SRB grubs (14 weeks old) were placed into each pot 24 hours after nematode introduction (experiments 1 to 7). Grubs were reared on artificial diet according to Beaver's method (2). Soil moisture was maintained at approximately 60 percent field capacity. Daily temperatures ranged from 20° to 31° C throughout the course of the experiments. Soil pH was 7.4. Potato tubers (one per pot) were used as food for the grubs. Grub mortality was observed 8 days after nematode treatment and all data were submitted to analysis of variance.

Experiments 1 and 2 determined whether densities of *S. feltiae* lower than 4,000 nematodes/300 g soil could control the insect grub. Each treatment was replicated eight times in a complete randomized block design. In experiment 1, each of the three nematode species was poured evenly on the surface of the potted soil and incorporated in the top 0.5 cm at 400, 1,000, 2,000 and 3,000 infective juveniles. *S. glaseri* was used in experiments 3 and 4, whereas *S. bibionis* was used in experiments 5 through 7.

Experiments 8 through 10 evaluated the effect of direct contact of the grubs with the nematodes before introducing the infected grubs into the soil. SRB grubs were exposed to nematode densities of 400, 4,000 and 40,000 nematodes in 15 cm diameter petri dishes. After 18 hours of direct contact, 3 grubs were introduced into pots filled with 300 g of soil and treated as in the previous experiments.

RESULTS

In experiment 1 (table 1) rates of 1,000, 2,000, and 3,000 nematodes per pot were significantly effective in killing the grubs, whereas in test 2 significant mortality was obtained only at 2,000 and 3,000 nematode densities. Highest grub mortality observed was 50%.

Tests 3 and 4 were conducted with *S. glaseri* (table 2). In test 3 all the nematode treatments were effective in killing SRB in contrast with test 4, where effectiveness was found in treatments of 4,000 and 40,000 infective nematodes per pot. Highest mortality observed was 59.2%. Table 3 presents the results of experiments 5, 6, and 7. In experiment 5 *S. bibionis* at all nematode densities was significantly effective as compared with the control. Highest mortality observed was 68.3%. In experiment 6 the 40,000 nematode density was the only treatment statistically superior to the control, causing 22.5% mortality, whereas in experiment 7 no control of the grubs was observed.

TABLE 1.—*Mortality of* Diaprepes abbreviatus *grubs (12 to 16 weeks old) exposed to four population densities of* Steinernema feltiae[1]

Treatment (Nematodes per 300 g soil)	Grub mortality (%)	
	Exp. 1	Exp. 2
400	16.7 b[2]	15.6 c
1,000	37.5 a	21.8 bc
2,000	46.9 a	40.6 ab
3,000	46.9 a	50.0 a
Control	6.3 b	9.4 c

[1]Grubs introduced in soil 24 hours after nematode inoculation.

[2]Values in columns followed by a common letter do not differ significantly at P = 0.05, according to Duncan's multiple range test.

TABLE 2.—*Mortality of* Diaprepes abbreviatus *grubs (12 to 16 weeks old) exposed to three population densities of* Steinernema glaseri[1]

Treatment (Nematodes per 300 g soil)	Grub mortality (%)	
	Exp. 3	Exp. 4
400	31.7 b[2]	20.8 b
4,000	32.5 b	59.2 a
40,000	55.8 a	5.0 b
Control	10.8 c	5.0 b

[1]Grubs introduced in soil 24 hours after nematode inoculation.

[2]Values in columns followed by a common letter do not differ significantly at P = 0.05, according to Dancan's multiple range test.

In experiments 8 through 10 (table 4) each of the three nematode species caused significant grub mortality regardless of the densities. Highest mortality observed was 90% for *S. glaseri*.

DISCUSSION

Nematode densities of 1,000, 2,000, and 3,000 *S. feltiae* per 300 g of soil were effective in killing the *Diaprepes* grubs (table 1). The former nematode densities are lower than the 4,000 evaluated by Román and Figueroa (8) which proved to be effective for the same nematode. Four hundred *S. feltiae* per pot do not cause mortality of 14- to 16-week-old grubs. It was also demonstrated that *S. glaseri* and *S. bibionis* are effective against SRB at densities as low as 400 nematodes per pot (tables 2 and 3), contrary to *S. feltiae*, which required at least 1,000 nematodes (table 1). The decline in infectivity observed for *S. bibionis* (experiments 6 and 7) may be due to its susceptibility to the high temperatures recorded during the course of the experiments.

In laboratory tests when direct contact between nematodes and grubs occurred before introducing the infected grubs in the soil, mortality was high regardless of density and nematode species used. Román and

TABLE 3.—*Mortality of* Diaprepes abbreviatus *grubs (12 to 16 weeks old) exposed to three population densities of* Steinernema bibionis[1]

Treatment (Nematodes per 300 g soil)	Grub mortality (%)		
	Exp. 5	Exp. 6	Exp. 7
400	56.7 a[2]	7.5 ab	13.3 a
4,000	68.3 a	20.8 ab	25.8 a
40,000	57.7 a	22.5 a	27.5 a
Control	12.5 b	5.0 b	20.0 a

[1]Grubs introduced in soil 24 hours after nematode inoculation.

[2]Values in columns followed by a common letter do not differ significantly at P = 0.05, according to Duncan's multiple range test.

TABLE 4.—*Mortality of* Diaprepes abbreviatus *grubs (12 to 16 weeks old) exposed to three population densities of* Steinernema feltiae, S. glaseri, *and* S. bibionis[1]

Treatment (Nematodes per 300 g soil)	Grub mortality (%)		
	Exp. 8 *S. feltiae*	Exp. 9 *S. glaseri*	Exp. 10 *S. bibionis*
400	65.0 b[2]	90.0 a	72.5 a
4,000	75.0 ab	85.0 a	75.0 a
40,000	87.5 a	80.0 a	82.5 a
Control	22.5 c	20.0 b	25.0 b

[1]Grubs exposed to nematodes in petri dishes for 18 hours before introducing them in soil.
[2]Values in columns followed by a common letter do not differ significantly at P = 0.05, according to Duncan's multiple range test.

Figueroa (8) obtained similar results with *S. feltiae* against SRB. Triggiani et al. (9) observed the same pattern with *S. feltiae* and two different species of insects. These data seem to indicate that if direct contact between nematodes and grubs occurs under soil, grub mortality will always be high.

This study shows that the infective stages of the three species of *Steinernema* are effective in controlling 12- to 16-week-old SRB grubs. It can be concluded that 4,000 *S. feltiae*/300 g of soil are more consistent in causing an approximate 60% mortality than 1,000 to 3,000. Increasing *S. glaseri* rates from 4,000 to 40,000 nematodes per pot increases mortality significantly. Nematodes performed well at 20° to 31° C. Cumulative larval mortality occurred 6 to 8 days after treatment.

The results of these tests suggest that the steinernematid nematodes with no environmental and public health hazards, are a potential control alternative for SRB and probably to other soil-inhabiting insects.

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