Control of the larva of the sugarcane rootstalk borer, *Diaprepes abbreviatus* (L), with the entomogenous nematode *Neoaplectana carpocapsae* Weiser[1]

Jessé Román and Wilfredo Figueroa[2]

ABSTRACT

In a series of greenhouse tests to determine the susceptibility of the larval stage of the curculionid *Diaprepes abbreviatus* to the entomogenous nematode *Neoaplectana carpocapsae*, the nematodes controlled the insect larvae. Grubs 1.5 to 3 months old (smaller than one inch long) were less vulnerable to death than older 3- to 4-month-old grubs. The highest grub mortality, 86.66%, was obtained with the nematode density of 40,000 nematodes per pot. When nematode density was increased from 40,000 to 400,000 nematodes per pot grub mortality did not increase. Grubs placed in direct contact with nematodes in petri plates were equally killed, independently of the nematode density used. Direct nematode inoculation through grub's mouth, anus or a combination of mouth and anus or steeped in water containing nematodes did not increase grub mortality beyond that already obtained in the soil. If similar results are obtained in the field, this method of control could be of great value to reduce *D. abbreviatus* populations.

INTRODUCTION

Investigations on the use of entomogenous nematodes, i.e., nematodes that control insects, have been intensified during the last years mainly because of the necessity to find more effective and safer means of control. In spite of this change, the amount of research dedicated to this trend is limited. Investigations have been conducted in Australia, Canada, China, England, France, Italy, New Zealand, the Soviet Union, the United States of America, Central and South America, and some islands in the eastern part of the Caribbean Sea (4, 6).

In Puerto Rico preliminary investigations were started in 1980 with the cooperation of personnel from the United States Department of Agriculture, at Orlando, Florida. A survey was conducted which revealed the almost total absence of neoaplectanid nematodes in the Island. Only one unidentified specimen of *Heterorhabditis* was found near the Dorado area (5). Later a project was started to investigate the effectiveness of these nematodes in controlling the grubs of the curculionid *Diaprepes abbreviatus* (L), an important pest of sugarcane and many other agricultural crops.

The larval stages of *D. abbreviatus* attack the roots and underground portions of sugarcane stems causing great damage and in cases of heavy

[1] Manuscript submitted to Editorial Board April 26, 1984.

[2] Nematologist and Research Assistant, Agricultural Experiment Station, University of Puerto Rico, Mayagüez Campus, Río Picedras, P.R.

infestations, death of the plants. Losses attributed to this insect have been estimated in $27.7 million (3). After aldrin was removed from the market, no other insecticide has proved to be effective in controlling this pest.

Investigations conducted in Florida, USA, by Beavers et al. (2), demonstrated that among the natural enemies attacking the grub of *D. abbreviatus* in citrus orchards, the entomogenous nematodes *Heterorhabditis* sp. Poinar and *Neoaplectana carpocapsae* Weiser were the most important. Thus, it seems that the use of these nematodes has potential to control the insect. Controlled experiments were carried out to determine the action of *N. carpocapsae* species against grubs of *D. abbreviatus.*

MATERIALS AND METHODS

Eight greenhouse tests were conducted to investigate the effect of different *N. carpocapsae* (Mexican strain) population densities in controlling the grub of *D. abbreviatus.* Four of these experiments were conducted with 3- to 4-month-old grubs (about 1 in. long), two with 2- to 3-month-old grubs (about ½ in. long) and two with 1 ½- to 2-month-old grubs (about ¼ in. long). One laboratory experiment was conducted to determine the effect of inoculating the nematodes into the grub's body through different points of entry.

For the greenhouse tests, nematodes reared in wax moth larvae, *Galleria mellonella* L, were pippetted at different densities (tables 1 to 4) into 10 cm diameter plastic pots filled with 250 cm^3 of steam-sterilized 3:1 soil-sand mixture. Three *Diaprepes* grubs, reared on artificial diet following the method of Beavers (1) or in a sterilized soil-sand mixture (3:1) with potato tubers, were also introduced into each pot. One potato tuber per pot was used as food source for the grubs. Treatments, replicated 10 times, were arranged in random block designs. Observations on grub mortality were made 8 days after inoculation and data subjected to analysis of variance.

A suspension of nematodes consisting of 66,000 nematodes in 100 ml of water was prepared in the laboratory. Grubs were directly inoculated with a drop of the suspension, which contained approximately 66 nematodes, through mouth, mouth-anus, and anus with a fine syringe. Also a treatment consisting of an immersion of grubs for 3 min in the suspension of nematodes was added. Control grubs were inoculated with one drop of distilled water through the anus. Grubs were placed in 15 ml plastic cups covered with a cardboard lid. Treatments, replicated 10 times, were arranged in a random block design. Observations were made 8 days after treatment and data analyzed statistically.

RESULTS AND DISCUSSION

Tables 1 to 3 summarize the results of greenhouse studies to determine the effect of different densities of *N. carpocapsae* in the control of different stages of the grub of *D. abbreviatus*. Table 1 presents data on mortality of 3- to 4-month-old grubs. In test 1 (table 1) all nematode densities used, i.e., 400, 4,000, and 40,000 nematodes per pot, were significantly effective in killing the grubs when compared with the control. No significant differences were obtained between the following

TABLE 1.—*Mortality of 3- to 4-month-old grubs of* Diaprepes abbreviatus *exposed to various concentrations of* Neoaplectana carpocapsae

Treatment (nematodes/pot)	Average grub mortality (%)			
	Test 1	Test 2[1]	Test 3	Test 4
400	38.33 b[2]	66.66 a	18.33 b	16.66 bc
4,000	55.00 ab	61.66 a	61.66 a	33.33 b
40,000	73.33 a	76.66 a	76.66 a	86.66 a
400,000			80.00 a	76.66 a
Control	3.33 c	0.00 b	3.33 b	13.33 c

[1] Grubs exposed to nematode treatment in petri plates for 16 hr prior to pot inoculation.
[2] Values in columns followed by a common letter do not differ significantly at P = 0.05.

TABLE 2.—*Mortality of 1½- to 2-month-old grubs of* Diaprepes abbreviatus *exposed to various concentrations of* Neoaplectana carpocapsae

Treatment (nematodes/pot)	Average grub mortality (%)	
	Test 5	Test 6
400	13.33 a[1]	5.00 bc
4,000	20.00 a	26.66 ab
40,000	35.00 a	35.00 a
Control	15.33 a	0.00 c

[1] Values in columns followed by a common letter do not differ significantly at P = 0.05.

treatments: 400 and 4,000 or between the 4,000 and 40,000 nematodes per pot. Highest grub mortality observed was 73.33%.

Table 1 shows data from a second test similar to the first test, except that in the second, grubs were placed in petri plates containing nematodes for 16 h before inoculation into pots. It shows that all nematode treatments were equally effective in killing the grubs, but significantly different from the control. These data seem to indicate that when grubs are previously exposed to nematode infection, the mortality will be the same regardless of the nematode density used. Highest percent mortality was 76.66% which was close to that obtained in the first test, both with 40,000 nematodes per pot.

Table 1 presents data from tests 3 and 4, in which an additional nematode density treatment (400,000 nematodes per pot) was included. It can be observed that in both tests there were no significant differences in mortality between the lower nematode density treatment (400 nematodes per pot) and the control. Also, no significant differences in effectiveness were obtained between the two highest nematode density treatments (40,000 and 400,000 nematodes per pot). Nevertheless, effectiveness of treatments with 4,000, 40,000, and 400,000 nematodes per pot were significantly better than the control. The data seem to indicate that, under the conditions in which the experiments were conducted, a higher grub mortality cannot be obtained with densities over 40,000 nematodes per pot. The highest grub mortality, 86.66%, was obtained in test 4 with the 40,000 nematodes per pot.

TABLE 3.—*Mortality of 2- to 3-month-old grubs of* Diaprepes abbreviatus *exposed to various concentrations of* Neoaplectana corpocapsae

Treatment (nematodes/pot)	Average grub mortality (%)	
	Test 7	Test 8
400	31.66 ab[1]	10.00 b
4,000	40.00 a	38.33 a
40,000	55.00 a	38.33 a
Control	3.33 b	10.00 b

[1] Values in columns followed by a common letter do not differ significantly at P = 0.05.

Table 2 presents the information from tests 5 and 6 where 1 ½- to 2-month-old grubs were used. In test 5 there were no significant differences between the treatments. An unusually high mortality was observed in the control pots (15.33%). No explanation has been found for these results.

In test 6 no significant differences were observed between the lowest nematode density (400 nematodes per pot) and the control. The densities of 4,000 and 40,000 nematodes per pot were statistically similar but statistically more effective than the control. Highest mortality in the latter two tests was 35%; obtained with the highest nematode density of 40,000 nematodes per pot.

Table 3 presents data of tests 7 and 8 conducted with 2- to 3-month-old grubs. Results of both tests were very similar. No significant differences in mortality were obtained between the lower density of 400 nematodes per pot and the control. Treatments of 4,000 and 40,000 nematodes per pot were equally significant but better than the control. The highest grub mortality (55%) was obtained in test 7 with the 40,000 nematodes per pot.

Table 4 presents the data obtained from test 9. Nematodes inoculated

through the mouth or through the mouth and anus did not significantly increase grub mortality when compared with the control. However, an anal inoculation alone and immersion of the grub for 3 minutes in water containing nematodes did increase significantly the grub mortality over that obtained in the control. The two former treatments were statistically similar.

The results of these studies indicate that *N. carpocapsae* controls the larvae of *D. abbreviatus* under greenhouse conditions. If similar results are obtained in the field, the method could have great value in reducing the larval population of *Diaprepes* in the soil.

TABLE 4.—*Mortality of 3- to 4-month-old grubs of* Diaprepes abbreviatus *after body inoculations with* Neoaplectana carpocapsae *and grub immersion in water containing nematodes*

Treatment	Average grub mortality (%)
	Test 9
Oral inoculation[1]	20.00 ab[4]
Oral-Anal Inoculation[1]	30.00 ab
Anal Inoculation[1]	40.00 a
Grub Immersion (3 min)[2]	50.00 a
Control[3]	0.00 b

[1] 66 nematodes/drop of water.

[2] Immersion in 100 ml of water containing 66,000 nematodes.

[3] Anal inoculation of one drop of distilled water.

[4] Values in columns followed by a common letter do not differ significantly at P = 0.05.

RESUMEN

Se hizo una serie de pruebas en el invernadero para determinar la susceptibilidad de la larva del curculiónido *Diaprepes abbreviatus* al nematodo entomófago *Neoaplectana carpocapsae*. Los resultados demostraron que el nematodo controló la larva del insecto. Los gusanos de 1 ½ a 3 meses de edad (menos de una pulgada de largo) fueron menos vulnerables que los de 3 a 4 meses de edad o de mayor tamaño. La mayor mortalidad, 86.66%, se obtuvo con 40,000 nematodos por tiesto. El aumento de la densidad de 40,000 a 400,000 nematodos por tiesto no aumentó la tasa de mortalidad de los gusanos. Las larvas en contacto directo con nematodos en placas de Petri murieron en cantidades iguales, independientemente de la densidad de los nematodos. El inocular los gusanos con nematodos por la boca, el ano o la combinación de boca y ano y el sumergir los gusanos en agua con nematodos tampoco aumentó la tasa de mortalidad. El inocular nematodos entomófagos pudiera ser exitoso, de encontrarse resultados similares, para controlar las poblaciones de *D. abbriaviatus* en el campo.

LITERATURE CITED

1. Beavers, J. B., 1982. Biology of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) reared on an artificial diet, Fla. Entomol. 65: 263–69.
2. McCoy, C. W. and Kaplan, D. T., 1983. Natural enemies of the subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida, Environ. Entomol. 12: 840–43.
3. Estación Experimental Agrícola, Univ. de P. Rico, 1979. Informe Anual 1978–79, pág. 4.
4. Gaugler, R., 1981. Biological control potential of neoaplectanid nematodes, J. Nematol. 13: 241–49.
5. Román, J. and Beavers, J. B., 1983. A survey of Puerto Rican soils for entomogenous nematodes which attack *Diaprepes abbreviatus* (L) (Coleoptera: Curculionidae), J. Agric. Univ. P.R. 67: 311–16.
6. Vassink, H., 1983. Entomopathogenic nematodes in Latin America, OTAN Newsletter 15 (2): 49–52.