A Survey of Puerto Rican Soils for Entomogenous Nematodes Which Attack *Diaprepes abbreviatus* (L.) (Coleoptera:Curculionidae)[1,2]

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ABSTRACT

A survey was conducted to determine the presence of entomogenous nematodes which might parasitize *Diaprepes abbreviatus* (L.) larvae in Puerto Rican soils. One larva (2.3%) was parasitized with *Heterorhabditis* sp. Poinar when 4-month-old larvae were placed in the soil at eight different sites throughout the Island. Soil samples, taken from sugarcane fields and pasture lands in five geographical regions during July and September 1980, and January and April 1981, and inoculated with *D. abbreviatus* larvae did not reveal entomogenous nematodes. In the laboratory, when *Neoaplectana carpocapsae* Weiser was introduced into sterile soil from these regions, 40% of the exposed *D. abbreviatus* larvae became infected. We believe this is the first report of the entomogenous nematode, *Heterorhabditis* sp., occurring in Puerto Rico.

INTRODUCTION

Diaprepes abbreviatus (L.), the so-called surgarcane rootstalk borer weevil or "vaquita," is now considered to be the most important pest of sugarcane in Puerto Rico (7); other cultivated host plants such as *Citrus* sp. and pigeon pea, *Cajanus indicus*, are also seriously attacked. The weevil eggs are deposited in masses between sugarcane blades. Hatchling larvae drop to the ground where they feed on the root systems during their younger larval stages, and as they mature, they bore into the subterranean portions of the cane stems. In heavily infested areas, portions of newly planted sugarcane fields are destroyed; late replanting is necessary. In addition, maturing cane stools dry and brown early; the result is premature harvest or death of the plant. The adult weevils emerge after 1–2 years (8). In 1979–80, the gross income from sugarcane production in Puerto Rico was $56 million (3), an amount which represents an important role in the Island's economy. Although the total land devoted to sugarcane production is currently being reduced, attempts are being made to increase yields by intensifying production technology (6).

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An important aspect in this effort is to develop new control techniques for *D. abbreviatus*, since losses attributed to this insect during 1978–79 were estimated to be $27.7 million (4). So far, no new insecticides evaluated since aldrin was removed from the market have proved to be effective in controlling this insect.

In a recent 2-year study to determine the natural enemies attacking the subterranean stages of *D. abbreviatus* in Florida (2), the entomogenous nematodes, *Heterorhabditis* sp. Poinar (probably *H. heliothidis*) and *Neoaplectana carpocapsae* Weiser were found to be the primary parasites recovered when *D. abbreviatus* larvae were introduced into the soil. The results of their study indicated that the parasitization rate was high from May through November, peaking at 70% in July 1980. *D. abbreviatus* larvae were inoculated into soil samples taken from 55 groves located throughout Florida's citrus growing region; in 47% of the groves either *Heterorhabditis* sp. and/or *Neoaplectana* sp were present.

Since these entomogenous nematodes are not known to occur in Puerto Rico but are widespread in Florida and show potential for biological control of *D. abbreviatus*, we conducted a soil survey in Puerto Rico to determine whether nematode parasites of *D. abbreviatus* larvae were present.

MATERIALS AND METHODS

Two survey techniques were used. In the first, 10 laboratory-reared 4-month-old *D. abbreviatus* larvae (1) obtained from the USDA Citrus Root Weevil Laboratory, Orlando, Florida, were caged in individual 7.5 × 12.5 cm wirescreen cages (324 mesh/cm^2) and placed 30 cm deep in sugarcane fields at eight locations October 29, 1979 (fig. 1a). The cages were recovered after 2 weeks and the larvae examined for nematode infection. In the second test, soil samples were collected from sugarcane fields and pasturelands in five geographical zones of Puerto Rico: north, south, east, west and central (fig. 1b). Samples were taken from five areas within each geographic zone and brought to the laboratory. Soil samples were taken at each site at four different times: July and September 1980, and January and April 1981. Samples were analyzed for pH and for percent sand, clay, and silt content. In the laboratory, each sample was thoroughly mixed and placed in 1-kg aliquots in cylindrical cardboard containers with three replications. These were inoculated with five *D. abbreviatus* larvae (375 larvae/test) and stored at 23–26° C for 2 to 3 weeks. The soil was adequately moistened throughout the duration of each test. At the end of the incubation period, each sample was carefully screened and larvae examined. All larvae recovered were cut into 2 or 3 pieces, and placed on a 10-mesh screen on top of Baermann funnels filled

with water. After 12 hours, 10 ml of water were drained from the funnels and examined for nematodes.

In a third test to determine whether *N. carpocapsae* was capable of parasitizing *D. abbreviatus* larvae in Puerto Rican soil types, three 1-kg soil samples taken from each of the five geographical zones were steam sterilized, we infested the soil samples with *N. carpocapsae* by placing five nematode-infected bait crickets containing about 375,000 nematodes and four or five *D. abbreviatus* larvae into each container. After an incubation period of 2 weeks, larvae were removed from the soil, placed

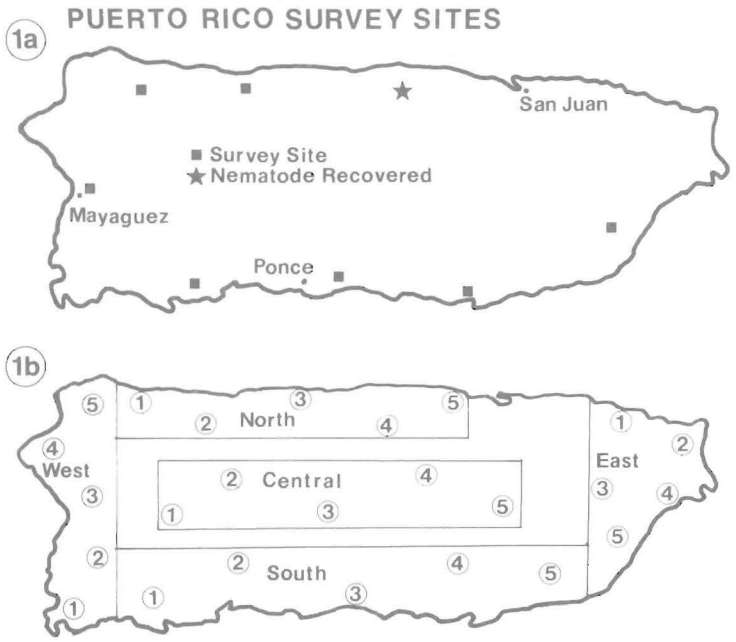

FIG. 1a.—Survey sites for *Diaprepes abbreviatus* larvae placed in individual screen cages. Ten larvae/site. 1b.—Soil sampling sites for laboratory bioassay to determine presence of entomogenous nematodes.

in individual petri dishes on filter paper moistened with a 0.25 NaCl solution, and held to determine nematode emergence.

RESULTS AND DISCUSSION

In the first test, when *D. abbreviatus* larvae were placed in individual screen cages, of the 46 larvae recovered (35 alive, 11 dead), one larva (2.3%) recovered from a sugarcane field approximately 15 km west of

San Juan was infected with *Heterorhabditis* sp. (fig. 1a). No *Neoaplectana* sp. were recovered in this test.

In the second test, when soil samples were infested with *D. abbreviatus* larvae in the laboratory, none of the 1,500 larvae exposed during the 4 sampling periods became infected by either nematode species. Of the total larvae exposed, 67.6% were recovered alive without nematodes, 14.6% were not recovered, probably because of disintegration after death; 7.5% were dead without nematodes; and 10.3% were dead and associated with a number of nematode genera not considered to be entomogenous. These were *Cephalobus* sp., *Aphelenchus* sp., and *Dorylaimus* sp., as well as rhabditids and diplogasterids.

TABLE 1.—*Infection of* **Diaprepes abbreviatus** *larvae by* **Neoaplectana carpocapsae** *in soil types from five geographic regions in Puerto Rico*

Location		Living noninfected larvae	**N. carpocapsae** infected larvae	Dead non-infected	Not recovered	Total
East	1	1	0	3	0	4
	2	2	1	1	0	4
	3[1]	2	1	1	1	5
Central	1	0	1	2	1	4
	2	1	0	3	0	4
	3[1]	1	3	0	1	5
West	1	0	1	3	0	4
	2	3	1	0	0	4
	3[1]	2	1	2	0	5
North	1	0	3	1	0	4
	2	0	2	2	0	4
	3[1]	1	2	2	0	5
South	1	1	3	0	0	4
	2	1	3	0	0	4
	3[1]	0	4	1	0	5
		15	26	21	3	65

[1] Inoculated with five *D. abbreviatus* larvae; all others with four.

In the test to determine whether *N. carpocapsae* could infect *D. abbreviatus* larvae in the Puerto Rican soil types, 40.0% infection was obtained from the 65 larvae exposed (table 1). Infection was obtained in all soil samples except one sample in the eastern and one in the central regions. The greatest infection rate (76.9%) occurred in soils from the southern region, where high populations of *D. abbreviatus* occur.

The soil samples from both regions were highly variable sand (37.8 to 79.8%), clay (12.1 to 30.7%), and silt (8.0 to 37.4%) content; however, the samples from the southern region were of a more uniform sandy texture. The pH of the soil samples ranged from 3.5 to 7.9 and appeared

to have no effect on the ability of *N. carpocapsae* to parasitize the *D. abbreviatus* larvae in the laboratory study.

Although *Heterorhabditis* sp. was recovered from one *D. abbreviatus* larvae placed in the soil, no additional parasitism was found in the 4 subsequent soil samplings. *Neoaplectana carpocapsae* was not detected by either sampling technique; however, it was found to be capable of parasitizing *D. abbreviatus* in the various soil types in the laboratory.

The findings of this study indicate that the natural entomogenous nematode population is very low. This could be due to several factors, such as natural predation by fungi and mites, to toxicity of previously used nematicides, or the *Heterorhabditis* sp. recovered may be a recent introduction with limited ability to disperse. Although little is known about the distribution and occurrence of entomogenous nematodes in insular areas, we believe this is the first report of the occurrence of *Heterorhabditis* sp. in Puerto Rico. Since these entomogenous nematodes are known to have a broad host range (5), and soil is their natural habitat, once established they may become self-perpetuating natural enemies of *D. abbreviatus*. This potential, combined with the recent development of commercial production techniques for these nematodes, justifies further investigation of these organisms for possible biological control of *D. abbreviatus* in Puerto Rico.

RESUMEN

Un reconocimiento para determinar la presencia de nematodos entomófagos que podrían parasitar larvas de la vaquita de la caña de azúcar, *Diaprepes abbreviatus* (L.), se efectuó en diferentes suelos de Puerto Rico. Una larva (2.3%) fue parasitada per *Heterorhabditis* sp. Poinar cuando se colocaron larvas de 4 meses en el suelo en ocho sitios diferentes de la Isla. Muestras de suelo, tomadas de cañaverales y pastizales en cinco zonas geográficas durante julio y septiembre de 1980 y enero y abril de 1981 e inoculadas con larvas de *D. abbreviatus*, no indicaron la presencia de nematodos entomófagos. Cuando *Neoaplectana carpocapsae* Weiser se introdujo en suelo esterilizado a vapor de las diferentes zonas, bajo condiciones de laboratorio, 40% de las larvas de *D. abbreviatus* fueron infectadas. Este parece ser el primer informe de la presencia de *Heterorhabditis* sp. en Puerto Rico. Estudios adicionales sobre el posible uso de estos nematodos en el control biológico de *D. abbreviatus* en Puerto Rico deben ser efectuados.

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