Isolates of entomogenous nematodes *Heterorhabditis* spp. and mortality of larvae of *Galleria mellonella, Cylas formicarius, Euscepes postfasciatus* and *Cosmopolites sordidus*[1]

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ABSTRACT

The effect of several isolates of entomogenous nematodes, *Heterohabditis* spp., from Puerto Rico was tested under laboratory conditions against larvae of *Galleria mellonella, Cylas formicarius, Euscepes postfasciatus,* and *Cosmopolites sordidus*. Five isolates demonstrated their capacity to kill the insect larvae. Isolate 2 was considered highly effective against all insects tested; isolates 14 and 27 caused high mortality rates to larvae of *G. mellonella, E. postfasciatus* and *C. sordidus*. Isolate 17 was effective against larvae of *C. sordidus*; isolate 18 against *E. postfasciatus*. The rest of the isolates tested varied greatly in their ability to kill the larvae of the insects used.

RESUMEN

Aislados de nematodos entomógenos, *Heterorhabditis* spp., y la mortalidad de larvas de los insectos *Galleria mellonella* (L.), *Cylas formicarius* (Fabricius), *Euscepes postfasciatus* (Fairmaire) y *Cosmopolites sordidus* (Germar).

Se estudió en el laboratorio el efecto de varios aislados de Puerto Rico de nematodos entomógenos, *Heterorhabditis* spp., sobre larvas de *Galleria mellonella, Cylas formicarius, Euscepes postfasciatus* y *Cosmopolites sordidus*. Cinco aislados demostraron su capacidad para matar las larvas de los insectos. El aislado 2 fue altamente efectivo contra las larvas de todos los insectos estudiados; el 14 y el 27 les causaron alta mortalidad a las larvas de *G. mellonella, E. postfasciatus* y *C. sordidus*. El aislado 17 fue efectivo contra *C. sordidus*; el 18 contra *E. postfasciatus*. Los otros aislados probados variaron altamente en su eficacia contra las larvas de los insectos examinados.

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INTRODUCTION

In current agricultural programs, chemical products are the most practical means of control for many pests affecting crops of economic importance. However, a great majority of the insects that are serious constraints for selected commodities spend their larval stages within the stem, underground parts of plants and in the soil, rendering chemical control useless or of limited effectiveness. This problem calls for management strategies directed toward the biological control of the concealed stages of many insects.

In recent years, the subject of biological control has received greater attention because of the necessity of finding suitable and effective techniques to reduce insect populations with minimum hazards to the environment and to human and animal life. Biological control is a potential alternative in integrated pest management programs and low-input cropping systems. Its implementation requires low physical input, minimizes insect resistance to chemicals and provides long-lasting control of pests.

One suitable tool in these efforts seems to be the use of entomopathogenic nematodes. The efficacy of nematodes of the Steinernematidae and Heterorhabditidae families has been demonstrated against a wide variety of insects that inhabit the soil, bore the root and stem or attack the foliage (2, 4, 9, 10, 14, 15, 16). These nematodes are known not only to be highly effective and specific for insect control but also safe for the environment and for human and animal life, and they can be easily mass produced (3, 5, 6, 7, 11, 12, 15).

The use of commercially reared specimens of these nematodes, without considering their habitat, can present intrinsic problems of behavior and erratic effectiveness under different environmental conditions. Therefore, the highest efficacy under tropical conditions can be expected from isolates endemic to these latitudes. Several isolates from Puerto Rico of the genus *Heterorhabditis* have been reared in the laboratory to be used against insects from tropical soils.

Laboratoty tests were performed to determine the efficacy of the above mentioned isolates in controlling three important soil insect pests: the sweet potato weevil, *Cylas formicarius* (Fabricius), the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire), and the banana corm weevil, *Cosmopolites sordidus* (Germar). The isolates were also tested against the greater wax moth, *Galleria mellonella* (L.), which is a highly suitable host for nematode reproduction. Results of these tests are here presented and discussed.

MATERIALS AND METHODS

Eighty-four soil samples collected from different localities throughout Puerto Rico were properly labeled, brought to the laboratory, thoroughly

mixed and placed in 325-cm³ plastic containers filled to capacity. Ten *Galleria* larvae, reared in the laboratory using the media described by Poinar (17), were introduced into each container for the recovery of entomogenous nematodes. Samples were kept at room temperature and 50% field capacity humidity. After 5 days, dead insects were taken out of the containers and placed in White's traps to collect emerging infective juveniles (IJ) (18). Specimens were collected every 3 days, washed twice with a 0.1% formalin solution and twice with distilled water.

Washed specimens were placed in Erlenmayer flasks (350 ml containing approximately 25 ml of distilled water and kept in an incubator at 15°C. Nineteen nematode isolates were stored for further studies. All isolates were identified as belonging to the genus *Heterorhabditis* according to Poinar (16).

Four laboratory tests were conducted. The first test was performed utilizing *G. mellonella* larvae to determine the pathogenicity of the isolates. Ten late instar larvae were placed in each plastic Petri plate (6.0 cm diameter) lined with a double layer of filter paper (5.5 cm diameter). Sixteen isolates were used in the first test with *G. mellonella*. For each one, 5 ml of distilled water containing 250 IJ per plate (25 IJ/larva) were poured evenly over the filter paper. Five milliliters of water was added to the check plates. Each isolate (treatment) was replicated five times in a completely randomized block design. Plates were kept at room temperature. *Galleria* larva mortality was recorded at 24, 48, and 72 h.

The second test was performed with the sweet potato weevil *Cylas formicarius*. Field-collected adult weevils were placed in 30-cm³ wire cages containing sweet potato storage roots as a source of food and oviposition site. Cages were brought to the laboratory and kept at room temperature. After 30 days, larvae were removed from the roots and placed in plastic Petri plates (6.0 cm diameter) with filter paper as described above. Six late instar larvae were placed in each plate. In addition, a piece of root (1x3x3 cm) superficially disinfected with 10% Clorox solution for 3 minutes and washed with distilled water, was introduced into each plate as a source of food and shelter for the larvae. Each one of 14 isolates (all isolates except 5 and 16) was poured evenly over the filter paper in the plates at population densities of 120 nematodes per plate (20 IJ/larva) as described for test 1. Five milliliters of water was added to the check plates. Each treatment was replicated five times in a completely randomized block design. Plates were kept at room temperature and larval mortality was recorded as for test 1.

The third test was conducted with the West Indian sweet potato weevil, *Euscepes postfasciatus*. Adult weevils were collected from the field and methods were similar to those for the second test. In this test, contrary to the second, 10 late instar larvae and a nematode density of 200 nematodes per plate (20 IJ/larva) were used for testing 16 isolates.

Test four was performed with the banana corm weevil, *C. sordidus*. Sixth or seventh instar larvae were removed from field-grown banana plants. Larvae were placed into 1-cm tunnels made with a cork borer in healthy banana corms with the outermost tissue peeled off. Tunnel apertures were plugged with a small piece of the bored tissue and the corms transported to the laboratory, where the larvae were taken out. In the laboratory, banana corm cylinders (7x5 cm) were prepared. Two tunnels (3x1 cm) were bored per cylinder. These were placed in 14-cm-diameter Petri plates lined with a double layer of filter paper. One *C. sordidus* larva was introduced into each tunnel. For each of the 16 isolates, 5 ml of water containing 100 nematodes per plate (50 IJ/larva) was poured evenly over each corm cylinder. Five milliliters of water was poured over the check corm cylinders. Each treatment was replicated five times in a completely randomized block design. Plates were left uncovered at room temperature and larval mortality was recorded as in test one. In all four trials dead larvae of each insect species were placed in White's traps for recovery of IJ. All data were statistically analyzed.

RESULTS

Tables 1 to 4 present results of the four tests. Table 1 presents the mortality of *G. mellonella* caused by the 16 *Heterorhabditis* isolates.

TABLE 1.—*Effect of different isolates of* Heterorhabditis *spp. on the mortality of* Galleria mellonella *at three intervals (Test 1)*

Isolate Num.	Mortality (%)		
	24h	48h	72h
PRF-2	78 ab[1]	80 ab	92 a
" 5	14 ef	32 de	46 de
" 8	32 cd	42 de	46 de
" 14	66 b	76 ab	88 ab
" 16	20 de	26 e	38 e
" 17	18 de	26 e	38 e
" 18	42 c	50 cd	66 cd
" 19	34 cd	42 de	50 de
" 22 A	66 b	74 b	86 ab
" 22 B	32 cd	42 de	52 de
" 27	84 a	88 a	94 a
" 38	22 de	36 de	46 de
" 54	70 b	82 ab	88 ab
" 60	28 cd	36 de	44 de
" 62	30 cd	38 de	44 de
" 63	60 b	66 bc	72 bc
Check	6 f	8 f	10 f

[1]Means in columns followed by the same letter do not differ significantly when P = 0.05.

TABLE 2.—*Effect of different isolates of* Heterorhabditis *spp. on the mortality of* Cylas formicarius *at three intervals (Test 2)*

Isolate Num.	Mortality (%)		
	24h	48h	72h
PRF-2	74 a[1]	93 a	99 a
" 8	15 bcd	18 bc	50 bc
" 14	3 d	32 bc	56 bc
" 17	3 d	18 bc	31 cd
" 18	19 bcd	43 b	71 b
" 19	10 bcd	26 bc	40 bcd
" 22 A	28 b	46 b	59 bc
" 22 B	29 b	53 b	70 b
" 27	29 b	32 bc	56 bc
" 38	10 bcd	26 bc	56 bc
" 54	22 bc	32 bc	42 bcd
" 60	6 bcd	18 bc	42 bcd
" 62	13 bcd	32 bc	69 b
" 63	22 bc	32 bc	50 bc
Check	4 d	8 c	20 d

[1]Means in columns followed by the same letter do not differ signicantly when P=0.05.

TABLE 3.—*Effect of different isolates of* Heterorhabditis *spp. on the mortality of* Euscepes postfasciatus *at three intervals (Test 3)*

Isolate Num.	Mortality (%)		
	24h	48h	72h
PRF-2	32 cd[1]	44 ab	62 ab
" 5	22 cde	28 bc	36 cd
" 8	38 bc	42 ab	48 bc
" 14	54 ab	68 a	76 a
" 16	8 f	14 c	22 d
" 17	22 cde	32 abc	38 cd
" 18	62 a	66 a	70 a
" 19	14 def	18 c	24 d
" 22 A	13 def	20 c	32 cd
" 22 B	22 cde	30 abc	36 cd
" 27	60 a	62 a	70 a
" 38	24 cde	30 abc	34 cd
" 54	32 cde	54 a	64 ab
" 60	8 f	16 c	24 d
" 62	11 ef	26 bc	34 cd
" 63	13 def	32 abc	32 cd
Check	14 def	18 c	24 d

[1]Means in columns followed by the same letter do not differ signicantly when P=0.05.

TABLE 4.—*Effect of different isolates of* Heterorhabditis *spp. on the mortality of* Cosmopolites sordidus *at three intervals (Test 4)*

Isolate Num.	Mortality (%)		
	24h	48h	72h
PRF-2	45 ab[1]	55 b	65 ab
" 5	0 d	17 d	30 d
" 8	1 dc	17 d	25 d
" 14	65 a	70 ab	83 a
" 16	0 d	0 f	17 f
" 17	55 ab	83 a	83 a
" 18	35 b	45 c	45 c
" 19	1 dc	10 de	10 de
" 22 A	10 c	10 de	17 de
" 22 B	4 dc	17 d	35 d
" 27	40 ab	50 bc	55 bc
" 38	4 dc	17 d	25 d
" 54	45 ab	55 bc	65 ab
" 60	0 d	1 ef	10 ef
" 62	40 ab	50 bc	55 bc
" 63	1 dc	10 de	25 de
Check	0.5 d	0.5 f	8 f

[1]Means in columns followed by the same letter do not differ signicantly when P = 0.05.

After a 24-h exposure, isolate 27 caused 84% larval mortality. This mortality was statistically similar only to that caused by isolate 2 (78%). At 48 h, 88% of *G. mellonella* larvae had been killed by nematodes from isolate 27. However, there was no statistical difference between the mortality caused by isolate 27 and isolates 2, 14 and 54. Ninety-two and 94% of the larvae were killed by isolates 2 and 27, respectively, after a 72-h exposure. These mortalities were not significantly different from those caused by isolates 14, 22A and 54.

Table 2 presents the mortality of *C. formicarius*. Isolate 2 killed 74, 93 and 98% of *C. formicarius* larvae after 24, 48, and 72 h, respectively. This mortality was significantly different from that caused by all other isolates and the check.

Table 3 presents mortality caused by the *Heterorhabditis* isolates on *E. postfasciatus*. Sixty and 62% larval mortality was caused by isolates 27 and 18, respectively, after a 24-h exposure. This percentage of mortality was different from that of all other treatments.

After 48 h, isolates 14, 18, 27, and 54 had killed 68, 66, 62, and 54% of *E. postfasciatus* larvae, respectively. These mortalities were not different statistically from those caused by isolates 2, 8, 17, 22B, 38, and 63.

Even though after a 72-h exposure larvae killed by isoaltes 14, 18, and 27 were in the range of 70 to 76%, these mortalities were not statistically different from those caused by isolates 2 and 54.

Table 4 presents data on the effect of *Heterorhabditis* isolates on the larvae of *C. sordidus*. Percentage of mortality after a 24-h period caused by nematodes from isolates 2, 14, 17, 54, and 62 ranged from 40 to 65% and did not differ statistically.

After 48 h, isolate 17 caused 83% larval mortality. This mortality was statistically similar only to that caused by isolate 14 (70%).

Observations made at the 72-h period revealed that isolates 14 and 17 caused 83% larval mortality. This percentage of mortality did not differ significantly from that caused by isolates 2 and 54.

DISCUSSION

The results obtained demonstrate that the majority of the *Heterorhabditis* isolates tested varied greatly in their efficacy to kill the larvae of the target insects. Isolate 2 showed the highest degree of efficacy against *C. formicarius*. Similar results have been reported from field experiments by Jansson with the HP88 strain of *H. bacteriophora* against pupae and larvae of this insect (8). Montes and Montejo, under laboratory conditions, obtained mortalilties as high at 96% against *Pachneus litus*, a curculionid of citrus orchards, with *H. heliothidis* (13). Arteaga proved the ability of a Cuban strain of *H. heliothidis* to parasitize different families of insects including the weevils *C. formicarius* and *C. sordidus* (1). The effectiveness of isolate 2 was also expressed against *G. mellonella, E. postfasciatus* and *C. sordidus*.

In *G. mellonella*, a highly susceptible insect species, and in *C. sordidus*, isolate 2 caused high mortality of the larvae as early as 24 h. In *E. postfasciatus*, effectiveness was observed at 48 h.

Isolates 14 and 27 caused high mortality to larvae of *G. mellonella, E. postfasciatus* and *C. sordidus*. However, mortality caused to *C. formicarius* was significantly lower than that caused by isolate 2. Isolate 17 was highly effective against larvae of *C. sordidus*, and isolate 18 against *E. postfasciatus*.

Thus, the data recorded indicate that under laboratory conditions the most promising isolates were 2, 14, 17, 18, and 27. As mentioned above, isolate 2 can be considered highly effective against all four insect larvae tested. The other four had their own specificity. However, the possible efficacy of the rest of the isolates tested, under other experimental conditions and for other insect species, cannot be disregarded.

This study is only preliminary. Additional investigation will be performed under greenhouse and field conditions to further elucidate the

effectiveness of all isolates. Best candidates could be mass reared and incorporated in pest management practices. Presently field investigations are underway in this direction using some of these isolates against the sweet potato weevil, *C. formicarius*.

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