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Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores[1]

N. Simões,[2] C. Laumond,[3] and E. Bonifassi[3]

Abstract: *Steinernema carpocapsae* (Breton strain), *S. glaseri,* and *Heterorhabditis bacteriophora* were evaluated for their potential to control immature stages of the Japanese beetle, *Popillia japonica,* on Terceira Island (the Azores). In bioassays carried out at temperatures higher than 15 C, *S. glaseri* and *H. bacteriophora* caused 100% mortality of larvae, whereas *S. carpocapsae* caused 56% larval mortality. At temperatures slightly below 15 C, only *S. glaseri* remained effective. In field plots, in September, *S. glaseri* and *S. carpocapsae* reduced larval populations by 91% and 44%, respectively, when applied at the rate of 10^6 nematodes/m^2. In April, *S. glaseri* caused 31% reduction in numbers of larvae, but *S. carpocapsae* was ineffective. In colder months (November–February) neither steinernematids nor *H. bacteriophora* reduced larval populations. Increasing the application rate from 10^6 to 5×10^6 infective stage *S. glaseri* per m^2 increased efficacy from 63% to 79% mortality.

Key words: Biological control, entomogenous nematode, *Heterorhabditis bacteriophora,* nematode, *Popillia japonica, Steinernema carpocapsae, S. glaseri.*

The Japanese beetle, *Popillia japonica* Newman, is an important insect pest of lawns and pastures in the immature stages, and of field and garden crops when adult. This pest was first found on Terceira Island (the Azores), in the early 1970s. By 1986 the insect had spread throughout most of the island (about 40,000 ha), and the population increases every year (N. Simões and A. Martins, unpubl.).

In the United States, where this pest was first found in 1916 (7), an entomogenous nematode, *Steinernema glaseri* Steiner, was found parasitizing *P. japonica* grubs in New Jersey and has been successfully used in field experiments against the beetle in pastures (10). *Steinernema glaseri* has also been used in New Zealand for the control of scarab grubs (12) and in the United States against numerous insects (21).

With the isolation of *S. carpocapsae* Weiser in Czechoslovakia and in the United States in 1955, field experiments against Coleoptera in turf generated new interest, and the nematodes were tested in Australia and the United States (11,14,15, 21). *Steinernema carpocapsae* (DD-136 strain) proved to be effective against a large number of soil insects (8,18,22), including some important coleopterous pests of rape (2). *Steinernema carpocapsae* (Breton strain) isolated from larvae of the black vine weevil, *Otiorhynchus sulcatus* Fabr., is also highly effective against other rape pests (C. Laumond, unpubl.). *Heterorhabditis bacteriophora* Poinar has also provided control of Coleoptera (9). This paper summarizes observations on the efficacy of *S. carpocapsae* (Breton strain), *S. glaseri,* and *H. bacteriophora* (NC1 strain) against *P. japonica* on Terceira Island (the Azores).

Materials and Methods

Nematode culture: Steinernema glaseri was obtained from Dr. G. O. Poinar, Jr. (University of California, Berkeley), and *H. bacteriophora* (NC1 strain) was obtained from Biosys, Palo Alto, California. The two species were cultured in laboratory-reared last instar larvae of the greater wax moth, *Galleria mellonella* (L.) (6). *Steinernema carpocapsae* was mass produced in vitro at the INRA, Antibes, France, using a three-dimensional method (3,4).

Nematodes were stored in distilled water at 9 C in 200- × 20-mm plastic petri dishes at 2×10^6 infectives/dish for ≤2 months.

Assays in pots: Bioassays were conducted on Terceira Island in plastic pots with 2 kg of sterilized soil sown with rye (*Secale cere-*

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[2] Departamento de Biologia, Universidade dos Azores, 9502 Ponta Delgada, Azores, Portugal.

[3] INRA, Laboratoire de Biologie des Invertébrés, 123, Bd. Francis Meilland, BP. 2078, 06606 Antibes, France.

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ale) seeds. Each pot was artificially infested with five field-collected third instar larvae of *P. japonica,* individually placed in 5-cm-d holes. Two days later, a 10-ml suspension of 2.2×10^4 infective nematodes was applied to the soil surface (10^6 nematodes/m^2). Control pots were treated with 10 ml of distilled water. Pots were held outdoors, protected from direct sunlight and rain. To prevent desiccation, 10 ml of distilled water was added to each pot every 5 days.

Nematode effectiveness was monitored 3 weeks following treatment by collecting the larvae in each pot. All larvae were dissected to determine the presence of nematodes. Final data consisted of the number of nonparasitized larvae found per pot.

Three assays consisting, for each of the three species, of 10 nematode-treated pots and 10 untreated (control) pots, in a randomized complete block design, were conducted during 1985 (September, October, and December).

Field experiments: Sixteen field trials were conducted on Terceira Island in pastures at different altitudes during a complete life cycle of the Japanese beetle, from August 1985 to May 1986: three in September, four in October, four in December, one in February, three in April, and one in May.

The experiments were carried out in 2- × 6-m plots, selected for high density and regular distribution of Japanese beetle larvae. Before selection of the plots, eight random soil samples, 30 × 30 × 15 cm (Japanese beetle grubs in the Azores do not burrow deeper), were taken to estimate the density and the pattern of distribution of the grubs. Soils were classified as a loam with a particle size distribution of 50.7% sand, 31.8% silt, and 17.5% clay. The pH ranged from 6.8 to 7.2. On each of the chosen plots, 10 PVC cylinders (17 cm diameter × 15 cm high) per treatment were completely inserted in the soil. Treatments were assigned using a randomized complete block design.

In each treated cylinder, a 10-ml suspension of 2.2×10^4 infective nematodes was applied with a pipet (10^6 nematodes/m^2). In the May assay, two treatment doses were used: 10^6 and 5×10^6 nematodes/m^2. Ten milliliters of distilled water were applied to the control cylinders.

Nematode effectiveness was monitored 5 weeks following treatment by collecting the soil contained in each cylinder for examination of live and dead *P. japonica* larvae and pupae. All immatures found were dissected to determine the presence of nematodes. Final data consisted of the number of nonparasitized larvae and pupae found per cylinder.

The Breton strain of *S. carpocapsae* and *S. glaseri* were assayed in all experiments. *Heterorhabditis bacteriophora* was only available in sufficient quantity for the experiments conducted in October and December.

Data were subjected to a two-way analysis of variance (ANOVA); means were separated by Duncan's multiple-range test (5). Mean percentage of mortality was calculated using Abbott's formula (1) and compared after arcsin transformation.

Results

Assays in pots: In September, when the potted soil mean temperature was 23 C, the three nematode species reduced ($P \leq 0.05$) the number of *P. japonica* larvae: 100% reduction for *S. glaseri* and *H. bacteriophora* and 56% for *S. carpocapsae* (Table 1). In October and December, with mean soil temperature in pots decreasing from 18 C to 15 C, *S. glaseri* caused 100% and 92% mortality ($P \leq 0.05$), respectively. Under the same conditions, *H. bacteriophora* and *S. carpocapsae* caused no reduction in numbers of larvae.

Field experiments: In September (experiments C1–C3), with a mean soil temperature of 21 C, *S. glaseri* caused 90, 50, and 64% and *S. carpocapsae* caused 37, 24, and 42% reduction in larval populations, respectively (Table 2). All three instars of *P. japonica,* 1% first, 46% second, and 53%

TABLE 1. Average (± SD) number of nonparasitized *Popillia japonica* larvae after exposure to *Steinernema carpocapsae* (Breton strain), *S. glaseri,* and *Heterorhabditis bacteriophora* in pot tests ($N = 50$).

Experiment	Treatment	Average no. (± SD) of nonparasitized grubs†	Percentage reduction‡
September	Control	3.4 ± 1.2 a	
	S. carpocapsae	1.5 ± 1.3 b	56
	S. glaseri	0.0 ± 0.0 c	100
	H. bacteriophora	0.0 ± 0.0 c	100
October	Control	2.4 ± 1.2 a	
	S. carpocapsae	2.5 ± 1.2 a	0
	S. glaseri	0.0 ± 0.0 b	100
	H. bacteriophora	2.1 ± 0.6 a	0
December	Control	2.0 ± 1.0 a	
	S. carpocapsae	1.4 ± 0.9 a	0
	S. glaseri	0.2 ± 0.1 b	92
	H. bacteriophora	2.3 ± 1.0 a	0

† Means followed by the same letter are not significantly different ($P = 0.05$, Duncan's multiple-range test).
‡ Percentage reduction corrected by Abbott's formula.

third, were similarly distributed in the three field plots.

In experiments conducted in October (C4–C7) and December (C8–C11), second and third instar larvae were present, and mean soil temperatures ranged from 13.7 C to 15.1 C. The three nematode species caused no reduction in larval numbers.

Similar results were obtained in February (C12), with the two steinernematids against second and third instar larvae, when mean soil temperature reached 12.4 C, the lowest in the year.

In April (C13–C15), in the assay conducted at a mean soil temperature of 15 C (150 m altitude), treatments with *S. glaseri* and *S. carpocapsae* resulted in 26 and 13% reduction in larval populations, respectively, whereas in the treatments at mean soil temperatures of 14.5 and 14 C (250 and 300 m altitude, respectively) no reduction was observed. In the three assays, second and third instar larvae were present in similar proportions.

In the May experiment (C16), with mean soil temperature of 16 C, *S. glaseri* and *S. carpocapsae* caused 63 and 39% overall mortality of larvae and pupae, respectively. When *S. glaseri* was used at a rate of 5 million infectives per square meter (a fivefold increase of the usual rate), the combined mortality of larvae and pupae increased ($P \leq 0.05$) from 63 to 79% (Table 3).

DISCUSSION

In the Azores, *P. japonica* larvae remain active in the upper layers of soil from September of one year to May of the following year (19,26). However, the relatively low soil temperatures recorded from October to March (under 15 C) proved to play a limiting role in the effectiveness of all three nematode species tested under field conditions. This finding agrees with the results of field experiments conducted with *Steinernema glaseri* and *Heterorhabditis bacteriophora* against *Costelytra zealandica* (13), *Otiorhynchus* sp. (17,23), and other Coleoptera on rape (2).

The finding that all three nematode species were ineffective under field conditions from October to December, while *S. glaseri* was effective at similar temperatures in pot experiments in October, is difficult to explain. It demonstrates, however, that the efficacy of entomopathogenic nematodes is best evaluated through field trials that embody a broader range of influencing factors than are usually incorporated into bioassays.

Under warmer conditions, *S. glaseri* proved to be more effective than *S. carpo-*

TABLE 2. Average number (± SD) of nonparasitized *Popillia japonica* larvae and pupae in soil contained in cylinders treated with *Steinernema carpocapsae* (Breton strain), *S. glaseri,* and *Heterorhabditis bacteriophora.*

Experiment	Treatment	Average no. (± SD) of nonparasitized grubs†	Percentage reduction‡
September			
C1	Control	7.4 ± 2.3 a	
	S. carpocapsae	4.5 ± 2.3 b	37
	S. glaseri	0.7 ± 0.7 c	90
C2	Control	9.3 ± 3.2 a	
	S. carpocapsae	8.6 ± 2.7 ab	24
	S. glaseri	4.6 ± 3.0 c	50
C3	Control	8.0 ± 1.7 a	
	S. carpocapsae	4.2 ± 1.8 b	42
	S. glaseri	2.8 ± 1.9 c	64
October			
C4	Control	1.6 ± 1.4 a	
	S. carpocapsae	1.7 ± 1.7 a	0
	S. glaseri	2.6 ± 1.6 a	0
	H. bacteriophora	1.8 ± 1.0 a	0
C5	Control	4.7 ± 2.0 a	
	S. carpocapsae	3.9 ± 2.0 a	0
	S. glaseri	5.1 ± 3.2 a	0
	H. bacteriophora	5.7 ± 3.1 a	0
C6	Control	4.6 ± 2.3 a	
	S. carpocapsae	4.7 ± 2.5 a	0
	S. glaseri	3.6 ± 2.0 a	0
	H. bacteriophora	3.3 ± 2.2 a	0
C7	Control	3.9 ± 2.6 a	0
	S. carpocapsae	3.3 ± 1.7 a	0
	S. glaseri	3.3 ± 1.4 a	0
	H. bacteriophora	2.4 ± 1.5 a	0
December			
C8	Control	2.4 ± 1.5 a	
	S. carpocapsae	3.6 ± 2.1 a	0
	S. glaseri	2.9 ± 1.9 a	0
	H. bacteriophora	2.3 ± 1.7 a	0
C9	Control	4.0 ± 1.8 a	
	S. carpocapsae	3.6 ± 1.4 a	0
	S. glaseri	3.9 ± 3.2 a	0
	H. bacteriophora	3.0 ± 2.2 a	0
C10	Control	1.6 ± 1.3 a	
	S. carpocapsae	2.6 ± 1.7 a	0
	S. glaseri	1.6 ± 0.8 a	0
	H. bacteriophora	2.1 ± 1.7 a	0
C11	Control	2.9 ± 1.7 a	
	S. carpocapsae	2.1 ± 2.3 a	0
	S. glaseri	2.3 ± 1.9 a	0
	H. bacteriophora	1.4 ± 1.3 a	0
February			
C12	Control	7.1 ± 4.0 a	
	S. carpocapsae	8.5 ± 3.2 a	0
	S. glaseri	6.0 ± 3.5 a	0
April			
C13	Control	5.3 ± 2.0 a	
	S. carpocapsae	4.7 ± 2.0 ab	13
	S. glaseri	4.0 ± 1.6 b	26
C14	Control	1.5 ± 0.9 a	
	S. carpocapsae	1.8 ± 0.8 a	0
	S. glaseri	1.4 ± 0.7 a	0
C15	Control	4.2 ± 1.5 a	
	S. carpocapsae	4.8 ± 2.0 a	0
	S. glaseri	3.7 ± 1.7 a	0
May			
C16	Control	4.2 ± 1.4 a	
	S. carpocapsae	2.6 ± 1.7 bc	39
	S. glaseri	1.6 ± 1.2 c	63

† Means followed by the same letter are not significantly different ($P = 0.05$, Duncan's multiple-range test).
‡ Percentage reduction corrected by Abbott's formula.

TABLE 3. Percentage reduction of populations of *Popillia japonica* larvae exposed at two different doses of *Steinernema carpocapsae* and *S. glaseri* in field plots.

Dose rate	Percentage reduction†	
	S. carpocapsae	*S. glaseri*
$1 \times 10^6/m^2$	39 a	63 b
$5 \times 10^6/m^2$	38 a	79 c

† Percentage values followed by the same letter are not significantly different ($P = 0.05$, Duncan's multiple-range test). Percentage reduction was corrected by Abbott's formula.

capsae. This may be due to behavioral differences between the two nematode infectives. *Steinernema glaseri* has higher motility and survives longer under natural field conditions than *S. carpocapsae* (9,20,24).

No field assays were conducted with *H. bacteriophora* under warmer conditions, but this nematode was as effective as *S. glaseri* in the pot assays, and both were more effective than *S. carpocapsae.* Similar results have been obtained against *P. japonica* (17) and other Coleoptera (13,23,25,27).

Our assays were mostly directed against third instar larvae, which have the longest feeding period of the various larval instars and live in soil moisture conditions most favorable for nematode activity. However, second instars and pupae were found to be susceptible to parasitism by the nematodes. Thus, although it was not possible to draw conclusions regarding the relative susceptibility of the various stages due to the compounding influence of ambient temperature on infectivity, the nematodes appeared to be effective against a broad range of soil inhabiting stages.

Some reports suggest that increased doses of steinernematid and heterorhabditid infective juveniles do not result in significant increases in host mortality (17,23, 28). However, our preliminary field trials directed against *P. japonica,* using two rates of application, indicated that this was not the case for *S. glaseri* under the conditions that prevailed in the Azores.

Our results suggest that *S. glaseri* may be an important biological control agent against *P. japonica* in the Azores.

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