Characterization and field application of *Heterorhabditis bacteriophora* strain HP88 (Heterorhabditidae : Rhabditida)

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SUMMARY

Strain HP88 of *Heterorhabditis bacteriophora* from western United States is described and compared to the type strain (HB1) from Australia. Using a dorsal and two smaller subventral terminal cuticular processes, the infective stages of HP88 were able to bore through the external cuticle of worker termites. The HP88 strain of *H. bacteriophora* could equal or surpass the control provided by chemical insecticides in field trials against larvae of the Japanese beetle *(Popillia japonica).*

RÉSUMÉ

Caractérisation et application au champ de la souche HP88 de Heterorhabditis bacteriophora *(Heterorhabditidae : Rhabditida)*

La souche HP88 de *Heterorhabditis bacteriophora* provenant de l'ouest des États-Unis est décrite et comparée à la souche type (HB1) originaire d'Australie. Grâce à des procès cuticulaires terminaux — un dorsal, deux subventraux — les stades infestants de la souche HP88 peuvent forer la cuticule externe des termites ouvriers. Lors d'essais au champ pour lutter contre les larves de *Popillia japonica,* la souche HP88 de *H. bacteriophora* a montré une action égale ou supérieure à celle des insecticides chimiques.

The insect-parasitic nematode, *Heterorhabditis bacteriophora* was first described from a noctuid moth larva (*Heliothis punctigera* Ha11) at Brecon, South Australia (Poinar, 1976). It was subsequently designated as strain HB1, thereby separating it from strain C8406 from Tainan, China and strain V16 from Geelong, Victoria, Australia (Akhurst, 1987). We now describe a new strain, HP88, which originated from the United States, thus showing the wide distribution of this species. This strain has been mass produced and used successfully in field trials against several insect pests in North America (Georgis & Poinar, 1989).

Materials and methods

The HP88 strain was isolated from infected June beetle larvae (*Phyllophaga* sp.; Scarabaeidae : Coleoptera) which had been sent to the senior author on September 20th, 1982 from the vicinity of Logan, Utah. The nematodes were then maintained on larvae of the wax moth, *Galleria mellonella* L. For greenhouse and field trials, nematodes were supplied by Biosys (Palo Alto, CA) where they were produced *in vitro.*

For the descriptive portion of the paper, the nematodes were grown in *Galleria* larvae. Infected insects were held at 22 °C and dissected on days 4 and 5 to recover the first generation hermaphroditic females, and on days 7 and 8 to obtain the males and second-generation amphimictic females. Infective stage juveniles were examined after being held for 3 months in water at 12 °C.

All nematodes were killed in hot (55 °C) Ringer's, fixed in TAF and processed to glycerin for measurements.

For studies on host penetration, small blocks of 1.0 % water agar were used. In each of ten agar blocks (1 cm long by 0.5 cm wide by 0.5 cm deep) a small groove was made which could contain the body of a worker western subterranean termite, *Reticulitermes hesperus* Banks. About a hundred infective stage nematodes were added (in a small drop of water) to each groove and the termite was covered with liquid agar. Observations and photographs were made with a Nikon Optiphot microscope fitted for differential interference contrast.

Host preference studies were performed in the laboratory using 9 cm Petri dishes lined with two filter papers. To each dish were added 50 infective stage HP88 in 2 ml of water. Ten individuals of each of thirteen insect species were added to each dish (with five replications). Mortality was recorded 72 h after the nematodes and hosts were placed together and maintained at 22 °C.

In the greenhouse tests, potting soil (moisture content about 26 %, based on volumetric measurements) was added to 3.78 liters (= 1 gallon) sized pots (without plants). In the first trial, ten, third and fourth stage black vine weevil larvae *(Otiorhynchus sulcatus)* were placed 10 cm below the soil surface. In the second trial, the same number of larvae were placed 20 cm below the soil surface. In both trials, the larvae were enclosed in a mesh screen cage (8 cm in diameter and 1 cm high) to restrict their movements. To the surface of each pot were added 25 000 infective stage HP88 in 200 ml water. For comparative purposes, the same number of *Steinernema carpocapsae.* All strain and *S. bibionis* SN strain were added to separate pots with weevil larvae. Controls contained only insect larvae in potted soil. All trials were replicated four times and the evaluation was made 10 days after the application.

Field effectiveness of HP88 was compared with two insecticides (Bendiocarb and Trichlorfon) in an experiment to control larvae of the Japanese beetle, *Popillia japonica* Newman, in a golf course at Sunbury, Ohio on August 20, 1987. The fairway was planted with Kentucky bluegrass *(Poa pratensis)* (30 %), bentgrass (*Agrostis* sp.) (25 %) and annual bluegrass *(Poa annua)* (45 %). The experimental design was a randomized complete block with five replicates; individual plots were 3 meters square. The nematodes were applied to the surface of the turf at the rate of 2.5 and 5 billion/ha (= 1 and 2 billion/acre) in 568 liters (= 150 gallons) of water. They were applied using a Coz sprayer with four 800 µm Tee jet nozzles mounted on a 2 meter boom operating at 32 psi. Immediately after treatment, the experimental area was irrigated with 1.3 cm of water. Thereafter the area was irrigated with 0.7-1.3 cm of water each evening unless rain had fallen during the day. This amount of irrigation was a regular managerial practice for the golf course.

Insecticides were applied in granular form with a precalibrated Gandy 2.5 spreader. The Japanese beetle grubs were mainly in the top 3 cm of soil with 80 % in the third instar and 20 % in the second instar.

Evaluations were made on October 2 (42 days after treatment) by taking four 930 cm^2 (= 1 ft^2) samples of turf from each plot and counting the number of healthy grubs. At the time of application, soil temperature at 3 cm depth was 22 °C (73 °F) and soil moisture 26 %. At the time of sampling, soil temperature at 3 cm depth was 18 °C (64 °F) and soil moisture was 28 %. The soil was a silty loam with 9 % organic matter and a pH of 4.3.

Data was analyzed by ANOVA for a randomized complete block design (Ryan, Joiner & Ryan, 1985) and mean separations were based on Duncan's (1955) multiple range test (P = 0.05).

Heterorhabditis bacteriophora Poinar, strain HP88

MEASUREMENTS AND DESCRIPTION

Adults (general) : Head truncate to slightly rounded; six distinct lips surrounding the mouth opening; each lip bears an inner labial papilla; at the base of each subdorsal and sublateral lips are two additional papillae representing an outer labial papilla and a cephalic papilla; base of each lateral lip contains an elliptical amphidial opening and an outer labial papilla; stoma mostly collapsed, cheilorhabdions lining the non-collapsed portion; two additional segments in the collapsed portion may represent separate pro and mesorhabdions or fused pro and mesorhabdions and separate metarhabdions; additional characters similar to those of HB1 described earlier (Poinar, 1976).

Hermaphroditic female (n = 15). (Fig. 1 F) : Length = 3.9 (3.3-4.4) mm; greatest diameter = 185 (144-216) µm; length of portion of stoma = 9.5 (6.0-15.0) µm; width of stoma opening = 16 (12-21) µm; distance from anterior end to nerve ring = 132 (114-159) µm; distance from anterior end to excretory pore = 205 (174-240) µm; distance from anterior end to base of pharynx = 184 (171-207) µm, length of tail = 81 (69-90) µm; body diameter at anus = 49 (36-60) µm; V = 43 (35-48). Vulva functional and protruding slightly (6-9) µm from body contour; anus located on protrusion.

Amphimictic female (n = 15). (Figs. 1 A, B, D) : Length = 1.4 (1.0-1.7) mm; greatest diameter = 91 (63-113) µm; length of noncollapsed portion of stoma = 3.8 (3.2-4.5) µm; width of stoma opening = 7.6 (6.4-9.6) µm; distance from anterior end to nerve ring = 83 (72-88) µm; distance from anterior end to excretory pore = 130 (177-149) µm; distance anterior end head to base of pharynx = 123 (112-136) µm; length of tail = 71 (66-80) µm; body diameter at anus = 24 (19-26) µm; V = 48 (43-50). Vulva nonfunctional, not protruding from body contour and often surrounded by a mating deposit; anus not located on anal protrusion but usually just anterior to anal protrusion.

Male (n = 15). (Figs 1 E, G) : Length = 687 (567-762) µm; greatest diameter = 43 (36-48) µm; length of noncollapsed portion of stoma = 2.7 (2-3) µm; width of stoma opening = 4.6 (4-5) µm; distance from anterior end to nerve ring = 70 (66-77) µm; distance from anterior end to excretory pore = 113 (105-120) µm, distance from anterior end to base of pharynx = 95 (91-105) µm; reflexion of testis = 78

(63-86) µm; length of tail = 28 (22-30) µm; body diameter at cloacal opening = 18 (12-23) µm; spicules = 39 (38-42) µm; greatest width of spicules 0.6 (0.5-0.7) µm; gubernaculum = 19 (13-21) µm; greatest width of gubernaculum = 0.12 (0.10-0.18) µm; distance from the first genital papilla to the cloacal opening = 43 (37-50) µm; ratio length of gubernaculum/length of spicules = 0.48 (0.33-0.54) µm.

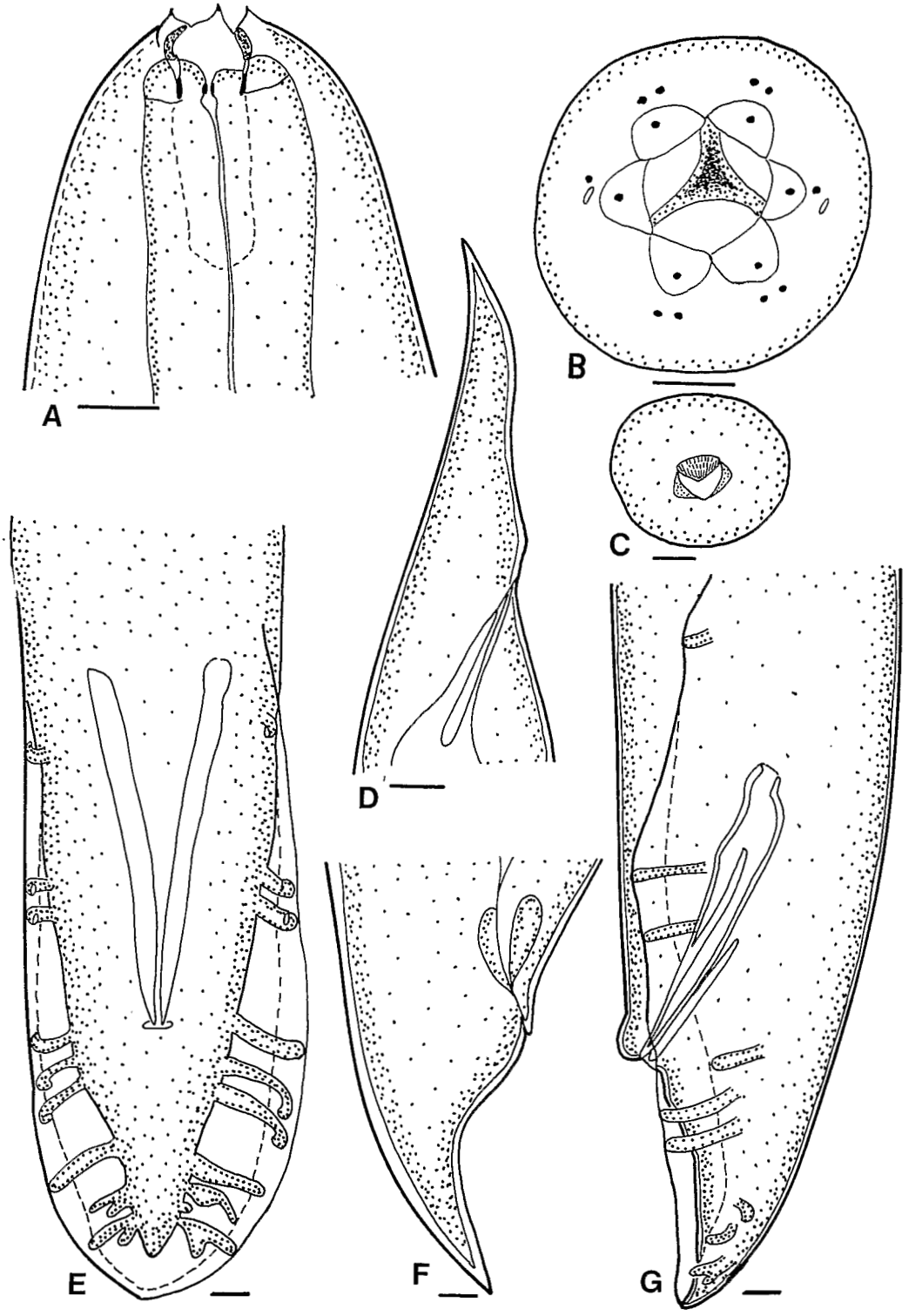

Fig. 1. *Heterorhabditis bacteriophora* strain HP88. A : Amphimictic female, lateral view of mouth region; B : Amphimictic female, en face view; C : Third stage infective juvenile, en face view; D : Amphimictic female, tail, lateral view; E : Male, tail, ventral view; F : Hermaphroditic female, tail, lateral view; G : Male, tail, lateral view. *(Bar equivalent : A, C, D, E, F, G = 10 µm; B = 5 µm.)*

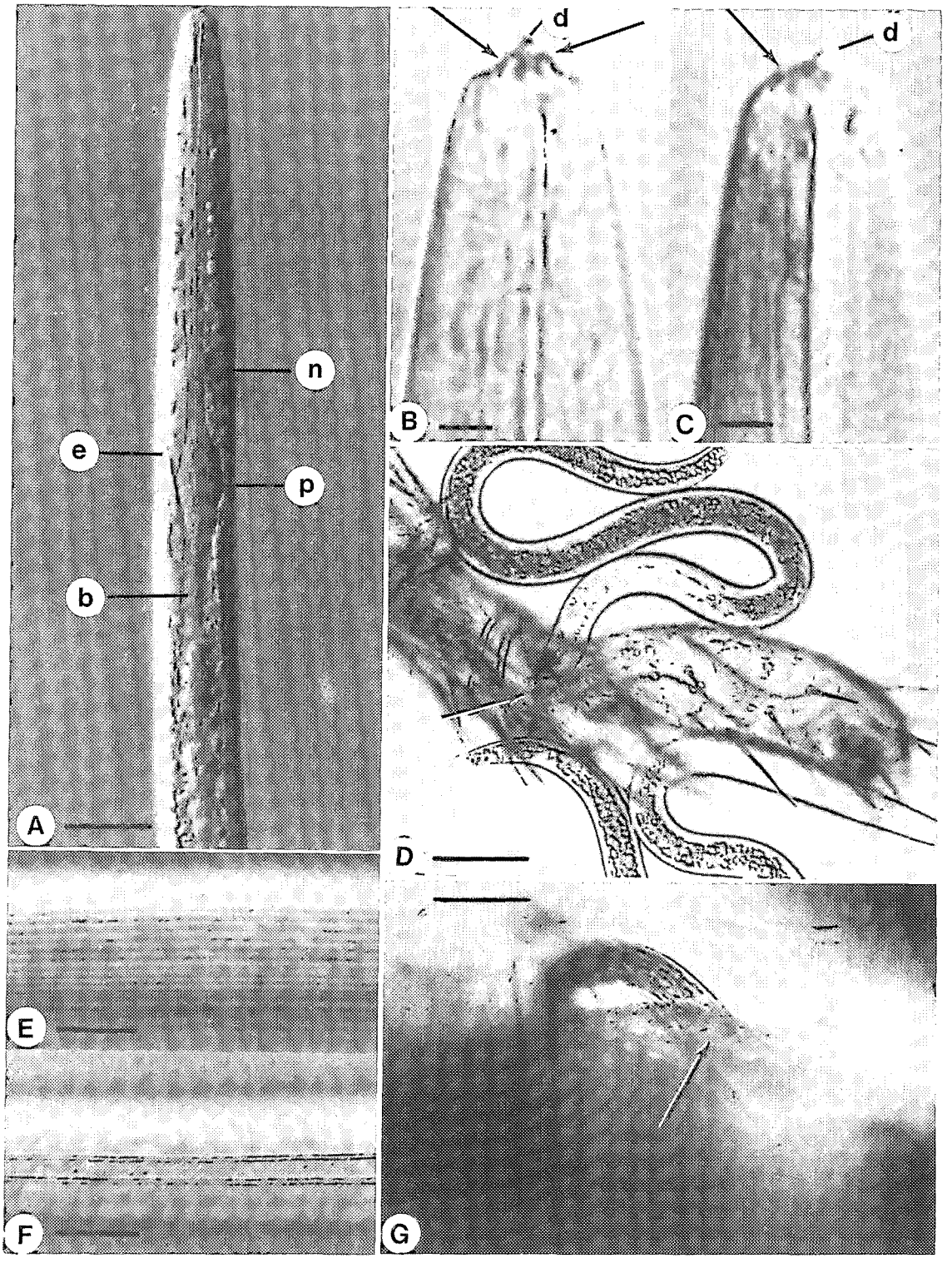

Fig. 2. *Heterorhabditis bacteriophora* strain HP88. A : Third stage infective juvenile (n = nerve ring, e = excretory pore, p = basal pharyngeal bulb, b = bacteria in the intestinal lumen); B : Infective juvenile, head (ventral view) (d = dorsal tooth; arrows = subventral teeth); C : Infective juvenile, head (lateral view) (d = dorsal tooth; arrow = subventral tooth); D : Infective juvenile, exploratory movements prior to penetration (arrow shows head pressed against inter-segmented membrane of termite); E : Infective juvenile, striations on second stage cuticle; F : Infective juvenile, double paired lateral lines on the cuticle; G : Infective stage juvenile passing through the external body cuticle of a termite worker; arrow shows border of insect cuticle. *(Bar equivalent : A = 22 μm; B, C = 2 μm; D, G = 44 μm; E, F = 11 μm).*

Infective 3rd stage juvenile (n = 15). (Figs 1 C, 2) : The following measurements were made on exsheathed third stage juveniles. Length = 538 (480-570) µm; greatest diameter = 20 (18-24) µm; distance from anterior end to nerve ring = 85 (72-93) µm, distance from anterior end to excretory pore = 101 (87-110) µm; distance from anterior end to base of pharynx = 116 (100-125) µm; length of tail = 56 (46-65) µm. Head bearing a dorsal tooth that is elevated 0.5-1.0 µm from the top of a cushion which is roughly 2-3 µm wide at the base; subventral sectors each bearing a smaller tooth only about one-third the length of the dorsal tooth (Figs 2 B, C); left subventral sector may only support a sclerotized plate; cuticle of third stage infective bears two distinct double lateral lines (Fig. 2 F) whereas cuticle of second stage juvenile bears numerous longitudinal striations (Fig. 2 E). The intestinal lumen of the infective stages contain bacterial cells of *Xenorhabdus luminescens* (Fig. 2 A) which are released when the nematode enters the hemocoel of a host.

Voucher specimens

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Biological data

Host penetration : Penetration of the third stage infectives of HP88 was observed with worker termites. The infectives were attracted to the insect and first crawled over its surface, stopping now and then to make more intricate contact with the body surface. When a suitable area was found, the juvenile would flatten itself against the host, then start moving its head back and forth over the termite's cuticle. Preferred areas were intersegmental membranes and smooth areas on the segments. The head would be pushed so hard against the cuticle that it would make an impression. While maintaining this pressure, the infective juvenile would then continuously rasp back and forth until a tear was made. Immediately the nematode would enter the wound and crawl into the body cavity. Soft areas on the body surface (especially around the anus) were most frequently selected as areas to initiate penetration attempts. Even though entry through the cuticle occurred, the majority of nematodes entered the anus and mouth of the termite and penetrated through the alimentary tract. After entering the host, the infectives continued to migrate through the host's body cavity, stopping to rasp at internal tissues. Many found their way into the legs, antennae and mouth palps of the host (Fig. 2 D).

Host selectivity : Although, once inside the hemocoel of an insect, most *Heterorhabditis* spp. will be able to mature and reproduce, not all insects are equally susceptible to these nematodes. There may be various physical and chemical factors which influence the host preference. Table 1 shows that Coleoptera and Lepidoptera appear to be the preferred hosts for this strain.

Table 1

Host preference of *Heterorhabditis bacteriophora* strain HP88.

Order	Insect host	Insect stage	Mortality level*
Orthoptera	*Blatella germanica* (German cockroach)	males	C
Orthoptera	*Scapteriscus vicinus* (Mole cricket)	males	C
Orthoptera	*Periplaneta americana* (American cockroach)	males	D
Orthoptera	*Schistocerca nitens* (Vargant grasshopper)	females	B
Diptera	*Musca domestica* (House fly)	second stage larvae	B
Coleoptera	*Diabrotica* sp. (Southern corn rootworm)	third stage larvae	A
Coleoptera	*Cyclocephalla borealis* (Northern masked chafer)	third stage larvae	A
Coleoptera	*Rhizotrogus majalis* (European chafer)	third stage larvae	A
Coleoptera	*Phyllophaga* sp. (May beetles)	third stage larvae	B
Coleoptera	*Otiorhynchus sulcatus* (Black vine weevil)	pupae	A
Lepidoptera	*Galleria mellonella* (Wax moth)	last stage larvae	A
Lepidoptera	*Manduca quinquemaculata* (Tomato hornworm)	fourth stage larvae	A
Lepidoptera	*Trichoplusia ni* (Cabbage looper)	fouth stage larvae	A

* A = over 80 % mortality; B = 60-80 % mortality, C = 40-60 % mortality; D = 20-40 % mortality.

Experimental results

Greenhouse tests : The results of the greenhouse tests of placing nematodes in soil with black vine weevil larvae showed that HP88 was effective in locating insect larvae, even when the insects had been placed at a depth of 20 cm (Table 2). These results suggest that HP88 has the ability to reach insect larvae in the soil more rapidly than the other two nematode species. This could be related to their small size and host seeking ability.

Field trials : The results obtained by comparing two different dosages of HP88 with standard insecticides for the control of Japanese beetle larvae (Table 3) show that at both dosages (2.5 / 10^9/ha and 7.5 × 10^9/ha) the nematodes can equal the effect of Bendiocarb and surpass the control provided by Trichlorfon.

Table 2

Numbers of surviving black vine weevil larvae *(Otiorhynchus sulcatus)* placed at two depths in soil 10 days after an application of 25 000 nematodes per pot.

Nematode species	Mean number of surviving larvae	
	Insects at 10 cm depth (n = 10)	Insects at 20 cm depth (n = 10)
H. bacteriophora HP88	2.8 ± 0.6 (0.8-6.2)* [72]**	0.5 ± 0.1 (0.1-1.9) [95]
S. carpocapsae ALL	1.0 ± 0.1 (0.6-3.4) [90]	3.8 ± 1.0 (1.7-5.5) [60]
S. bibionis SN	4.8 ± 1.3 (1.6-7.2) [51]	5.3 ± 1.5 (4.7-6.1) [44]
Control	9.8 ± 1.3 (7.3-10.3	9.5 ± 1.6 (7.0-10.5)

* 95 % confidence level; ** % control.

Table 3

Comparison of *H. bacteriophora* HP88 with chemical insecticides against the Japanese beetle, *Popillia japonica*, on a golf course in Sunbury, Ohio (August-October 1987).

Treatment	Number of living grubs 42 days after treatment*	% Control
HP88 (2.5 / 10^9/ha)	3.5 *a*	79
HP88 (7.5 × 10^9/ha)	2.2 *a*	87
Bendiocarb (4.70 kg [ai]/ha)	3.2 *a*	81
Trichlorfon (8.96 kg [ai]/ha)	6.5 *b*	62
Control	16.9 *c*	—

* Mean of five replicates. Means followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

Discussion

The descriptive studies of HP88 add to our knowledge of the variability found among the species of *Heterorhabditis*. Although this strain is qualitatively similar morphologically to the HB1 (type) strain, quantitative differences occur between the strains. The size of the females can vary considerably and they grow after becoming sexually mature. The amphimictic females of HP88 were smaller than those of HB1 (Poinar, 1976), with a length ranging from 1 050-1 740 µm in comparison to 3 180-3 850 µm in HB1. Similarly, the distance from the head to the excretory pore, nerve ring and pharynx were significantly less in HP88 than in HB1 in the amphimictic females. In addition, the males of HP88 are significantly smaller than those of HB1 (567-762 for HP88 *vs* 780-960 for HB1) yet all other measurements overlap (Poinar, 1976). This indicates that adult size and certain quantitative values cannot be used for specific characters. Nuclear DNA analyses of the genome of HB1 and HP88 using the major sperm protein as a probe showed that both nematode strains were almost identical (in contrast to other species of *Heterorhabditis*) (Jim White, pers. comm.).

Values associated with the infective stages are more stable. Although the values for the total length of the nematode and the length of the tail presented here are shorter than those for HB1, the measurements of HP88 infectives were made without their enclosing cuticles (the cuticles were shed while the nematodes were in storage), whereas the measurements of HB1 infectives included the 2nd stage enclosing cuticle. Since the enclosing cuticle adds roughly 40 µm to the total length and tail length of each individual, there are actually no quantitative (or qualitative) differences between the infectives of the two strains. Thus, it is important to indicate whether the 2nd stage cuticle was present or absent when measurements of infective stages are taken.

The presence of a large dorsal and two smaller subventral terminal teeth on the infective stage is interesting. The presence and arrangement of the subventral teeth may be characteristic of this species since in *H. megadis*, only a dorsal tooth was mentioned (Poinar, 1987) and Wouts (1979) described a " dorsal tooth on a star shaped base, opposite a sclerotized ventral plate " for a New Zealand *Heterorhabditis* population, now considered a separate species. The presence of teeth were not mentioned in the original description of HB1 (Poinar, 1976), probably because they were masked by the enclosing 2nd stage cuticle.

The role of these terminal teeth in HP88 is undoubtedly for entry into an insect host as shown here and earlier by Bedding and Molyneux (1982). Although it is possible for heterorhabditids to enter the body cavity of hosts with thin, intersegmental membranes by penetrating through the cuticle, penetration into the hemocoel through natural openings certainly does occur as emphasized by Mrácek, Hanzal and Kodrík (1988). In such cases, the terminal hooks most certainly assist the nematode in penetrating the alimentary tract and tracheal walls.

Host preference is an important consideration in HP88 and other entomogenous nematodes. Although these nematodes have a wide host range, they have generally adapted to specific hosts in specific soil habitats. Since HP88 was originally recovered from a coleopterous larva, it is not surprising that field trials showed a high mortality level with coleopterous hosts.

Host finding behavior is another variable among strains of insect parasitic rhabditoids. In the present study, the HP88 strain was competitive in locating insects placed 20 cm below the surface of the soil. This rapid host finding ability and the preference for coleopterous larvae were two important factors which con-

tributed to the successful field application of strain HP88 against Japanese beetle larvae. Other factors which contributed to the field success were adequate levels of temperature and moisture to allow movement and infection to occur.

In summary, *H. bacteriophora* strain HP88 has proven to be a highly successful biological control agent. Not only has it shown promise against the Japanese beetle, but it has equaled or bettered the control obtained by insecticides in field tests with the black vine weevil *(Otiorhynchus sulcatus)*, Northern masked chafer *(Cyclocephala borealis)*, European chafer *(Rhizotrogus majalis)* and bluegrass billbug *(Sphenophorus parvalus)* (Georgis & Poinar, 1989).

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