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Host range of *Hemicycliophora poranga* and its pathogenicity on tomato

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Summary – *Hemicycliophora poranga* was extracted from soil around *Musa* sp. in California, USA. The nematode was cultured on tomato cv. Big Boy. After 31 days at 25 °C, final nematode populations and reproductive indices increased on cabbage, cowpea, bean and okra, and similar increases in final nematode populations were obtained on lettuce and tomato cv. VF 145-B 7879, after 72 days. Pepper, onion and cucumber sustained low to zero nematode populations and were considered non-hosts. Two-week-old tomato cv. Big Boy plants inoculated with 0, 100, 500, 1000, 5000, 10 000, and 20 000 nematodes resulted in significantly lower shoot lengths and root weights at Pi 5000 and Pi 20 000 than at Pi 0 and Pi 10 000, 44 days after inoculation. After 28 days, with eight-week-old tomato cv. Big Boy plants, only root weights were lower at Pi 1000 than all other Pi levels, except Pi 5000. In both cases, nematode populations increased significantly at all Pi levels, except Pi 0 ($P < 0.05$). *Hemicycliophora poranga* fed primarily on lateral root primordia, and occasionally on primary root tips. Feeding resulted in the formation of small galls. During feeding, a polysaccharide plug and feeding tube were observed around the stylet and at the feeding site, respectively.

Résumé – ***Pathogénie de* Hemicycliophora poranga *envers la tomate et d'autres plantes tests*** – *Hemicycliophora poranga*, extrait de la rhizosphère de *Musa* sp., en Californie, USA, s'élève facilement sur la tomate cv. Big Boy. Les expériences concernant la gamme d'hôtes et les niveaux d'inoculum ont été conduites dans une enceinte à conditions contrôlées. Après 31 jours à 25 °C, la population finale (Pf) des nématodes et les taux de reproduction correspondent à quatre à cinq fois la valeur de l'inoculum (Pi) sur le chou, *Vigna unguiculata*, le haricot et le gombo. Des accroissements de Pf similaires ont été observés après 72 jours sur la laitue et la tomate var. VF 145-B 7879. Pour le piment, l'oignon et le concombre, aucun nématode, ou de très faibles populations sont présentes; ces plantes sont donc considérées comme non hôtes. Des tomates cv. Big Boy âgées de 2 semaines ont été inoculées avec 0, 100, 500, 1000, 5000, 10 000 et 20 000 nématodes : 44 jours après l'inoculation, la longueur et le poids des racines étaient plus faibles pour les Pi 5000 et 20 000 que pour les Pi 0 et 10 000. Dans le cas de tomates cv. Big Boy. âgées de 8 semaines, après 28 jours, seul le poids des racines était plus faible à Pi 1000 qu'à tous les autres niveaux de Pi, excepté Pi 5000. Dans les deux cas, la population s'accroît quelle que soit la Pi, à l'exception de Pi O. *H. poranga* se nourrit essentiellement sur les primordiums des racines latérales et, occasionnellement, à l'extrémité des racines. La prise de nourriture provoque la formation de petites galles. Pendant cette prise de nourriture, un bouchon de polysaccharides et un tube nutritionnel ont été observés, respectivement autour du stylet et à l'endroit de l'insertion de celui-ci.

Key-words : *Hemicycliophora,* tomato, pathogenicity, Nématodes.

The genus *Hemicycliophora* contains over 100 species (Raski & Luc, 1987) distributed throughout the world. Very little is known about the biology and pathogenicity of most species. Much of what is known of the biology and pathogenicity of the genus is attributed to documented studies of a few species. These species primarily include those which induce galls on the roots of their host plants, e.g. *Hemicycliophora arenaria* Raski, 1958 and *H. similis* Thorne, 1955 (Van Gundy & Rakham, 1961). *Hemicycliophora nudata* Colbran, 1963, also a gall-forming species is no longer a member of the genus but has been transferred to the genus *Caloosia* (Colbran, 1963; Brzeski, 1974). Sofrygina (1972) originally described the species *H. salicis* and reported that it fed on spherical, knob-like root swellings, very much like *H. arenaria.* Plant symptoms, produced in response to nematode feeding, are not known for most *Hemicycliophora* species. However, we do know that not all species induce gall formation. *Hemicycliophora typica* de Man, 1921 caused stubby-root symptoms on carrots in sandy soil, while only slight or no symptoms were observed on other hosts which supported increased populations of the species (Kuiper, 1959). Zacheo *et al.* (1987) observed slight swellings, but no gall formation, on rice

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roots due to *H. typica*. Stunted growth and aberrant root development was attributed mainly to *H. conida* Thorne, 1955 in the Netherlands (Kuiper, 1977; Spaull & Mewton, 1982). There is then, the possibility for a range of plant symptoms to be induced by different *Hemicycliophora* spp.

Hemicycliophora poranga was first reported from Brazil (Monteiro & Lordello, 1978) infesting the rhizosphere of cabbage. Since then, it has only been reported from Argentina (Chavez, 1983). In 1991, *H. poranga* was extracted from the rhizosphere of *Musa* sp. growing in a nursery in Alameda, California, USA. The nematode was easily cultured on potted tomato plants, and induced the formation of small root galls. Because it was possible to obtain large populations of the nematode species, differential inoculum and test host trials were conducted in a growth chamber to study nematode reproduction and influence of its feeding on different plants. The results are discussed in this paper.

Materials and methods

Hemicycliophora poranga extracted from the rhizosphere of *Musa* sp. were maintained on *Lycopersicon esculentum* cv. Big Boy at 25 ± 1 °C in a growth chamber.

Host trials

In the first experiment, nine vegetable plants were tested : cowpea (*Vigna unguiculata* cv. California Blackeye No. 46), tomato (*Lycopersicon esculentum* cv. VF 145-B 7879), cabbage (*Brassica oleracea* var. *capitata* cv. Hybrid Grandslam), garden bean (*Phaseolus vulgaris* cv. Evergreen), pepper (*Capsicum annuum* cv. Jupiter), onion (*Allium cepa* cv. Yellow Sweet Spanish), lettuce (*Lactuca sativa* cv. Parris Island COS MT), cucumber (*Cucumis sativus* cv. Market More), and okra (*Abelmoschus esculentus* cv. Clemson Spineless). Vegetable seeds were germinated in sterilized sand in 10-cm pots in a greenhouse, then transferred to an Envirotol® growth chamber set at 25 ± 1°C day and night, 12 h fluorescent light and 12 h dark. Three to 4-week-old plants were inoculated with 100 nematodes/pot, and the pots were arranged randomly in the growth chamber. Each treatment was replicated five times. The plants were fertilized two or three times during the experiment. The experiment was terminated after 31 days. At that time, plant roots were examined for galls, and nematodes were extracted by sugar centrifugation (Jenkins, 1964).

The experiment was repeated for a longer period with those plants that had maintained similar and low nematode populations for 31 days. Cabbage, earlier proven to be a good host, was included for comparison. The experiment was terminated after 72 days and the plants were processed as before. Final nematode counts were obtained and nematode reproductive indices (Pf/Pi = final nematode population/initial nematode population) were determined. To compare nematode reproduction and reproductive indices on different plants, data were subjected to statistical analyses of variance and the means were separated by Duncan's multiple range test.

Effect of initial population densities

Two-week-old tomato cv. Big Boy seedlings in 10-cm pots containing sterile sand were inoculated with initial nematode densities of Pi 0, 100, 500, 1000, 5000, 10 000, and 20 000. The plants were arranged randomly in the growth chamber maintained at 25 ± 1 °C day and night, 12 h light and 12 h dark, for 44 days.

The experiment was repeated using 8-week-old tomato plants inoculated with all Pi levels except Pi 20 000, and held for 28 days. At the end of both experiments, shoot length, fresh root weight, final nematode populations and reproductive indices were determined. Data were subjected to statistical analyses of variance, and the means were separated by Duncan's multiple range and Fisher's least significant difference tests.

Histological sectioning and staining to observe nematode-root gall attachment

Fresh root galls with nematodes attached were carefully excised from the root system and rinsed with sterile water. The galls and attached nematodes were then fixed in 3 % glutaraldehyde for 12 h in vacuum, rinsed in 0.01 M phosphate buffer, post-fixed in 2 % osmium tetroxide for 1 h, rinsed again in phosphate buffer, then stained in 2 % aqueous uranyl acetate for 30 min, dehydrated in a alcohol series to absolute alcohol and embedded in Spurr's medium (Spurr, 1969).

Sections, 1 μm thick, were cut with a Reichert Om U3, diamond knife microtome and stained for total carbohydrates by the periodic acid-Schiff's reaction (PAS). Mounted sections were placed in 0.5 % periodic acid solution in distilled water for 30 min at room temperature and washed in running water for 10 min. Sections were then stained in Schiff's reagent for 30 min at room temperature, rinsed in tap water for 10-20 s, placed in 2 % sodium bisulfite for 1-2 min, washed in tap water for 5-10 min, and mounted in Permount®. Microscope observations and photographs were taken at 1250 × magnification.

Results

Host trials

Final nematode counts and reproductive indices of *H. poranga* on the different test plants are shown in Table 1.

After 31 days, final nematode populations and reproductive indices increased similarly ($P < 0.05$) four to five times over the initial inoculum on cowpea, bean, okra and cabbage. The remaining test plants supported low nematode populations for 31 days.

Table 1. Comparison of final *Hemicycliophora poranga* populations on different host plants after 31 and 72 days at 25 ± 1 °C (Pi = 100).

Test plants	Final nematode population (Pf)		Reproductive index (Pf/Pi)	
	Experiment		Experiment	
	1 (31 days)	2 (72 days)	1 (31 days)	2 (72 days)
Cowpea	483.4 *a*	–	4.8 *a*	–
Bean	435.0 *a*	–	4.3 *a*	–
Okra	522.6 *a*	–	5.2 *a*	–
Cabbage	397.0 *a*	1365.2 *a*	4.0 *a*	13.7 *a*
Lettuce	120.0 *b*	1325.6 *a*	1.2 *b*	13.3 *a*
Tomato	124.6 *b*	646.8 *b*	1.3 *b*	6.5 *b*
Pepper	53.2 *b*	34.2 *c*	0.6 *b*	0.3 *c*
Onion	34.2 *b*	5.0 *c*	0.4 *b*	0.1 *c*
Cucumber	19.0 *b*	1.2 *c*	0.2 *b*	0.0 *c*

Values are means of five replicates. Means followed by the same letter do not differ (P < 0.05) according to Duncan's multiple-range test.

After 72 days, final nematode population and reproductive index on lettuce was similar to values obtained on cabbage which was shown to be a good host after 31 days. Population values increased on tomato, but were significantly lower than lettuce. Pepper, onion and cucumber did not support the nematode, and were classified as non-hosts.

Effect of initial population densities

Shoot length and root fresh weight were significantly lower ($P < 0.05$) at Pi 5000 and Pi 20 000 than at Pi 0, and significant increases were obtained at Pi 10 000 than at Pi 5000 and Pi 20 000 (Table 2). Final nematode populations increased as initial inocula increased, except at Pi 20 000, which did not differ from Pi 5000 and Pi 10 000 populations. Comparatively, reproductive indices were lower at 20 000 than all remaining Pi levels, and higher at Pi 1 000 than at Pi 100, 10 000 and 20 000. With 8-week old plants, after 28 days growth, no difference in shoot length was observed for all plants ($P < 0.05$). Root weight was lower at Pi 1000 than all other levels except 5000. Final nematode populations increased as initial inocula increased ($P < 0.05$), however, reproduction was significantly lower at Pi 10 000 than at Pi 100 and Pi 500.

Symptoms

Above ground symptoms of nematode infestation were characteristic of an impaired root system. Yellowing, and eventual death of foliage occurred progressing upward from the lowest leaflets. Infested plants were less tolerant to moisture stress than noninfested tomato plants. In heavily infested plants, only the top foliage may remain while the lower plant is defoliated.

Table 2. Effect of different inoculum levels *Hemicycliophora poranga* on growth of two week old tomatoes and final nematode population after 44 days at 25 ± 1 °C.

Pi	Shoot length (cm)	Root fresh weight (g)	Final nematode population ($\sqrt{PF}$)	Pf/Pi
0	22.5 *a*	3.8 *a*	0.0 *a*	0.0 *a*
100	23.2 *a*	3.7 *a*	21.4 *b*	4.9 *b*
500	23.5 *a*	4.5 *a*	50.0 *c*	5.3 *be*
1000	22.4 *ac*	3.1 *ac*	84.6 *d*	7.2 *ce*
5000	19.1 *bc*	2.1 *a*	170.1 *e*	5.8 *bc*
10000	22.5 *a*	4.1 *a*	200.7 *f*	4.1 *b*
20000	16.3 *b*	1.3 *b*	182.0 *ef*	1.7 *a*
LSD (P > 0.05)	3.3	1.5 *a*	19.1	2.1

Values are means of five replicates. Means followed by the same letter do not differ (P < 0.05) according to Duncan's multiple-range test.

Roots of infested plants had small galls (about 2 mm). Single, or clusters of galls were formed at lateral root primordia, and less commonly at primary root tips (Fig. 1). On tomato roots, the surface of galls was usually dark amber in colour, sometimes, bearing cracks.

Nematode attachment

Nematodes fed on lateral root primordia even before the latter had broken through the rootlet. Occasional feeding at the primary root tip was observed, but lateral root primordia were preferred. Stylet probing in non-primordial regions of the root was also observed, but this did not result in feeding. Usually, several juveniles and adults were observed feeding on single galls. The stylet was usually inserted upto 75 % of its length. In several instances, it appeared that the anterior end of the nematode had penetrated the gall. The nematodes were attached to the root galls by an adhesive plug encircling the stylet. The plug gave a strong PAS reaction, and is probably polysaccharide in composition. The amphids, stylet sheath, and dorsal oesophageal gland also gave PAS reactions similar to the plug. A feeding tube, giving a positive periodic acid-Schiff's reaction, was observed at the anterior end of the inserted stylet (Fig. 1 D).

Discussion

Earlier reports of plant hosts of *Hemicycliophora poranga* are few and inconclusive. The nematode has been found in rhizosphere of cabbage (*Brassica oleracea* var. *capitata* cv. Roxo), cauliflower (*Brassica oleracea* var. *botrytis*), bluegrass (*Poa sp.*), Senna (*Cassia aphylla*), and willow (*Salix* sp.) (Monteiro & Lordello, 1978; Chavez 1983). In the present study, cabbage, tomato, broad bean, okra, cowpea and lettuce were determined to be good hosts supporting high nematode populations for 31 and 72 days. The lower final nematode reproduc-

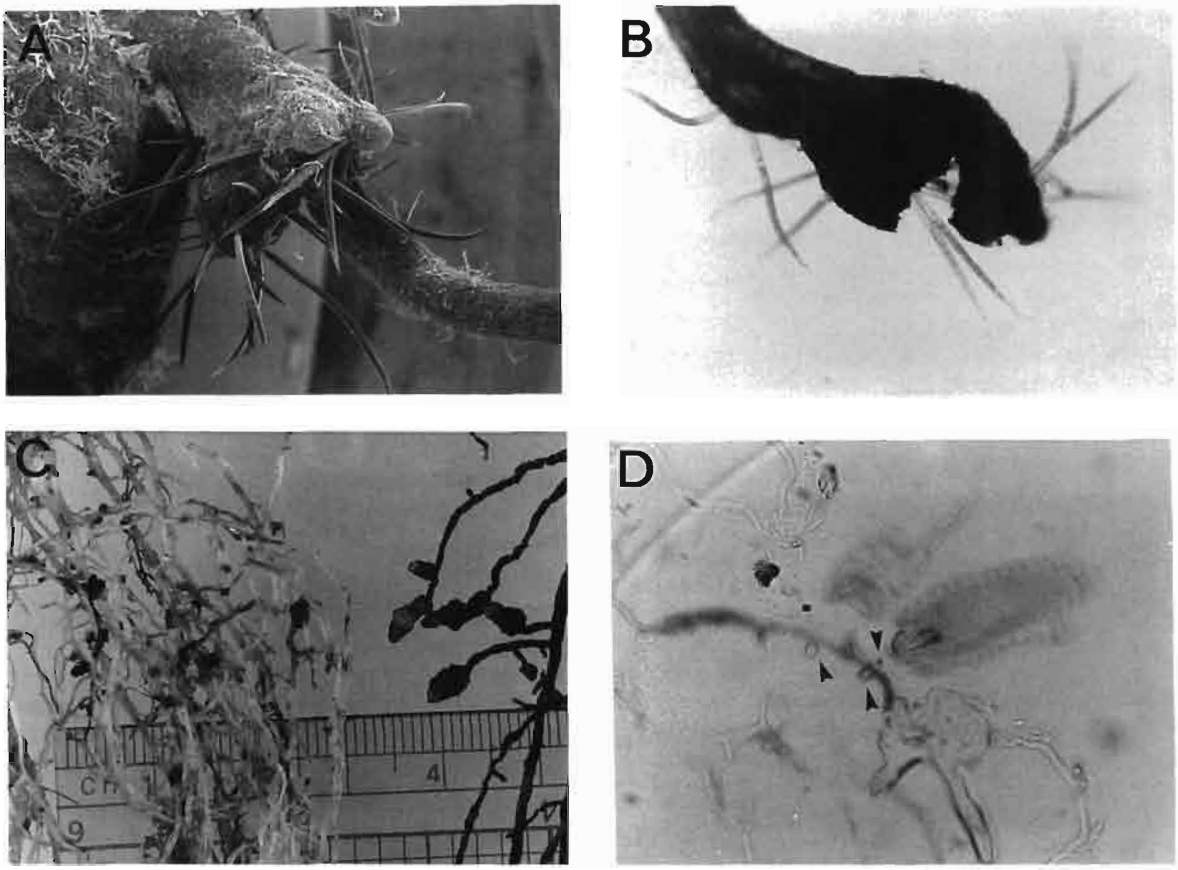

Fig. 1. *Hemicycliophora poranga* parasitizing tomato roots (*Lycopersicon esculentum* cv. Big Boy). A : Scanning electron micrograph of galled lateral root primordium with nematodes attached; Light microscope photograph of galled rootlet with nematodes attached; C : Tomato roots galls induced by *H. poranga* (left) and citrus root galls induced by *H. arenaria* (right); D : Histological section (upper arrow indicates remnant of feeding plug encircling stylet, lower arrows indicate feeding tubes).

tion on tomato (*Lycopersicon esculentum* cv. VF 145B 7879) compared to the other hosts, after 31 and 72 days, is indicative of a differential nematode-host relationship present within the range of hosts. Initial low nematode populations on lettuce after 31 days, were possibly due to a limited food supply because of slow and sparse root growth. In contrast, lettuce plants harvested after 72 days had ample root growth, and supported high nematode populations.

Increase in nematode populations after 28 and 44 days, was possible as long as there was an abundance of food supply and low competition for feeding sites. This also explains why eight-week-old seedlings with root systems denser than those of two-week-old ones supported similar final nematode populations, and greater reproductive indices, in approximately half the time. Differences in plant age/growth prior to nematode inoculations also enabled plants to tolerate greater increases in nematode populations at 28 days than at 44 days. It is possible that the tolerance breaking level for Big Boy tomato plants to *H. poranga* develops at Pi 1000 and/or 5000 after 44 days. However, this can only be proven through further research. After 28 and 44 days, decreases in root weights at Pi 5000 and 1000 were overcome as plants counterbalanced the stress by producing flushes of new root and shoot growth which resulted in increases in plant and nematode values at Pi 10 000. However, with high initial nematode population at Pi 20 000, plant growth and, consequently, final nematode populations were significantly reduced.

Tomato was a good host for other gall-inducing species, as reported for *Hemicycliophora arenaria* on Rutger tomato (Van Gundy & Rackham, 1961). Although reproduction of *H. poranga* on different tomato varieties, including cv. Rutger, was not included in this study the results presented here demonstrate the pathogenic potential of the nematode. Furthermore, the difference in final nematode populations obtained at Pi 100 on tomato cvs, VF 145-B 7879 and Big Boy suggests the presence of a differential host variety response to nematode attack.

Below ground symptoms of *H. poranga* infestation differ from those caused by other sheath nematodes. Root galls induced by the feeding of *H. poranga* are much smaller than the larger, almost spherical galls induced by *H. arenaria, H. similis* and even *Caloosia nudata* (formerly = *H. nudata*). Unlike the latter three species, the primary root tip was not the preferred feeding site for *H. poranga*. Galled lateral root initials and lateral roots were commonly found in all infested plants. Zacheo *et al.*, (1987) also found lateral roots of rice to be the preferred feeding sites for *H. typica*, although only slight swellings were formed in response to nematode feeding. The presence of an adhesive polysaccharide feeding plug has also been reported for the other gall-inducing *Hemicycliophora* spp. (McElroy & Van Gundy, 1968; Kisiel *et al.*, 1971).

The gall-inducing species of *Hemicycliophora* is an example of advanced evolution in nematode ectoparasitism, already discussed by McElroy and Van Gundy (1968), and Kisiel *et al.* (1971) for *H. arenaria* and *H. similis*. *Hemicycliophora poranga* shows a further specialization in its feeding behavior in using a feeding tube. This is a first report of a feeding tube found associated with a gall-inducing *Hemicycliophora* sp. The feeding tube is of polysaccharide composition, possibly formed by the nematode during, and in reaction to feeding. The exact composition and function of a feeding tube is uncertain. Perhaps in addition to the plug, the tube provides stability during nematode feeding and induction of small root galls. Whether the presence of a feeding tube is peculiar to the nematode species, or pertains to other small gall-inducing species of *Hemicycliophora* and members of Criconematidae, would be of evolutionary and behavioral interest.

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