Journal of Nematology 23(2):205–209. 1991.

Morphological Observations of *Pratylenchus penetrans* from Celery and Strawberry in Southern Ontario

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Abstract: *Pratylenchus penetrans* was obtained from Premier strawberry in Norfolk County and the Niagara Peninsula and from celery in the latter area. Host affected the dimensions of *P. penetrans* to a greater extent than geographical area in Ontario. Adults of *P. penetrans* from southern Ontario tended to be smaller than those reported elsewhere. The presence of three lip annules was consistent in the seven populations studied, although in some specimens one of the annules did not entirely encompass the head. Crenations around the tail tip of females of *P. penetrans* was common in the populations studied. Not all of the morphological characters were proportional in size to length of the females of *P. penetrans*.

Key words: *Apium graveolens*, celery, *Fragaria chiloensis*, lesion nematode, morphology, *Pratylenchus penetrans*, strawberry.

In southern Ontario, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven is involved in disease complexes including tobacco brown root rot (6), peach replant problems (5), strawberry root rot (12), and celery rusty root (11). During investigations of the distribution, host–parasite relationships, and control of this nematode, many specimens were killed, fixed, mounted, and measured. To establish the extent of morphological variation in populations of this species in Ontario, Canada, this paper presents the dimensions of females of *P. penetrans* from a single field in each of seven farms in the Niagara Peninsula and Norfolk County and of developing juveniles from a greenhouse culture of celery (*Apium graveolens* var. *dulce* Pers. cv. Utah 15).

MATERIALS AND METHODS

Soil and root samples were collected from 1-year-old strawberry (*Fragaria chiloensis* Duchesne cv. Premier) from two sites in the Niagara Peninsula (Abbs and Kasharawich) and four sites from Norfolk County in southern Ontario (Chary, Culver, Premock, and Watt). In addition one celery site (Thorpe) was sampled in the Niagara Peninsula. Nematodes were extracted from soil samples by the Cobb sieving and decanting method (2,10) and from washed root samples by incubating in 473-ml glass jars for 2 weeks at 17 C (14). A 0.1% solution of streptomycin sulfate was used in the jars to suppress growth of bacteria and fungi.

To kill extracted nematodes, 10-ml suspensions were placed in test tubes (2.5 × 20 cm) and warmed slowly in a water bath to 60 C and held for 2 minutes. After cooling to 21 C, an equal volume of 6% formalin solution was added to each suspension to fix the nematodes.

Ten preserved *P. penetrans* females were selected at random from each suspension and mounted in clear lactophenol solution on a slide. Cover slips were supported by glass rods of slightly greater diameter than the largest nematode and sealed with glyceel. Morphological characters of 10 female nematodes were measured with a calibrated micrometer eyepiece in a compound microscope. The tails of females from each population were drawn to scale with a camera lucida. Data on morphological characters from each population were subjected to an analysis of variance, and LSD were calculated at the 5% level.

Juvenile measurements were made from a single population of *P. penetrans* originating from a strawberry planting in the Niagara Peninsula. This population was maintained on celery in Vineland silt loam (61% sand, 28% silt, 11% clay) in plastic tubs (46 × 46 × 27 cm) kept in a greenhouse at 22–24 C for 5 years. Roots from

Received for publication 15 February 1990.
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randomly selected celery plants were washed and placed in a mistifier to extract *P. penetrans.* Nematodes were killed, fixed, and mounted on slides as described. Morphological characters of juveniles (J2, J3, J4) and females were measured on 38 specimens of each life stage as described. Correlation coefficients were calculated for the morphological characters measured, treating all juvenile stages as a group.

Results and Discussion

The mean length of 10 *P. penetrans* females from each of the seven sites ranged from 417 to 477 μm (Table 1). The longest females were from celery. The length of females from the six strawberry sites ranged from 417 to 438 μm. The other measured morphological characters varied in direct proportion to the length of the female.

The ratios for females from strawberry plantings in Norfolk County were lower than those for females from plantings in the Niagara Peninsula, whereas the b and the c ratios were similar for females from plantings in both areas (Table 1).

The shape of the stylet knobs ranged from broadly rounded to indented anteriorly. Indented stylet knobs were most common.

The number of annules between the anus and the tail tip of females varied from 20 to 26 (Table 1). The greatest number of tail annules occurred on some of the shortest females. Tail tips were basically conical, sloping dorsally, some were truncate or clavate (8). Female tail termini were usually smooth, but 18% of tail termini had one or more crenations occurring primarily dorsally (8). The tail terminus crenations were usually coarser than body annules (8).

Juvenile nematodes from celery in greenhouse tub cultures resembled the adults in shape but not in size. The sex and life stage of each juvenile was judged from the degree of development of the reproductive system. Mean lengths were 225 μm, 306 μm, and 408 μm for J2, J3, and J4 females, respectively, and 370 μm for J4 males (Table 2). The difference in the width of female J2 and J3 was 22% but only 4% between J4 females and males (Table 2).

Examination of the development of the gonad and the state of molting of individual nematodes showed that the length of the various stages of *P. penetrans* overlapped. This was most noticeable between the J3 and J4 adults. For example, some J3 females were 360–380 μm long, whereas some J4 females were only 315–330 μm long.

The J2 head region was anatomically complete, with three annules externally and a conspicuous cephalic framework internally. The head increased 19% in height from J2 to J4 females and 24% from J2 to J4 males (Table 2). The J2 esophageal region was also anatomically complete; its length increased 23% between J3 and J4 females and 33% between J3 and J4 males. The posterior ventral esophageal lobe and the procorpus increased in length more than the metacorpus and isthmus.

Thirty-five of the thirty-eight correlations made with the dimensions of 11 morphological characters were significant (Table 3). Of these, three of the correlations exceeded 0.900: body length vs. gut length, body length vs. length of reproductive system, and esophagus length vs. length of posterior esophageal gland. Conversely, there was no correlation between body length or body width with length of head, nor between stylet length and tail length.

The host induced a greater effect on the dimensions of *P. penetrans* than did the limited geographical area. Body lengths of females from celery were greater than those from strawberry. Length of females from strawberry in Norfolk County were more variable than those from strawberry in the Niagara Peninsula.

The presence of three head annules was consistent in all populations, although on some specimens one of the three annules would not entirely encompass the head.

The stylet lengths of juveniles, males, and females were shorter than those reported in other studies (7,8). Possibly the shorter length may be caused by the host

TABLE 1. Morphological characters of *Pratylenchus penetrans* females from strawberry and celery from seven grower sites.

Character	Strawberry						Celery	LSD 5%
	Norfolk County				Niagara Peninsula			
	Chary	Culver	Premock	Watt	Abbs	Kasharawich	Thorpe	
Body length	430.5 ± 22.3	423.3 ± 27.1	417.0 ± 23.9	438.8 ± 13.9	438.8 ± 15.8	438.7 ± 20.8	477.7 ± 25.4	28.3
Vulva to anterior	338.8 ± 19.4	335.1 ± 21.9	334.7 ± 20.0	347.4 ± 15.7	346.8 ± 14.3	353.4 ± 19.5	378.4 ± 23.1	24.9
Width at vulva	18.5 ± 1.4	17.2 ± 0.7	17.2 ± 2.5	18.4 ± 2.3	16.9 ± 0.8	15.0 ± 1.3	18.0 ± 1.9	1.9
Vulva to tail tip	91.7 ± 8.0	88.2 ± 6.9	71.8 ± 2.7	91.4 ± 3.1	92.0 ± 7.1	85.3 ± 4.0	99.3 ± 7.0	11.5
Tail length	21.4 ± 1.1	22.5 ± 1.8	19.7 ± 2.0	21.8 ± 1.5	22.1 ± 2.3	22.2 ± 1.5	24.0 ± 4.0	2.4
Tail width at anus	10.5 ± 1.0	9.7 ± 0.8	9.3 ± 0.7	10.8 ± 1.0	10.9 ± 0.8	9.5 ± 0.9	13.2 ± 2.3	1.5
a ratio	23.7	24.7	22.5	24.4	26.2	29.6	26.5	1.3
b ratio	7.1	7.0	6.4	7.3	6.8	—	7.4	n.s.
c ratio	20.4	18.9	21.5	20.1	20.0	20.1	19.9	n.s.
No. annules, anus to tail tip	22.9 ± 1.8	26.4 ±.1.9	21.3 ± 1.3	23.6 ± 2.3	21.9 ± 1.8	20.7 ± 1.8	21.6 ± 2.4	2.5
Stylet length	14.6 ± 1.0	14.6 ± 0.7	—	14.2 ± 0.7	—	—	—	—
Esophagus length	49.4 ± 2.8	48.3 ± 2.6	—	47.4 ± 4.6	52.0 ± 3.6	—	52.3 ± 5.0	2.1
Metacorpus length	11.3 ± 1.1	10.8 ± 0.9	9.6 ± 1.1	10.4 ± 0.8	9.7 ± 0.8	—	9.7 ± 1.6	0.78
Metacorpus width	8.3 ± 0.6	7.4 ± 0.5	6.6 ± 0.6	8.2 ± 0.7	7.1 ± 0.5	—	7.9 ± 0.7	0.74
Esophageal basal lobe	44.4 ± 6.7	32.0 ± 3.8	—	41.1 ± 6.1	—	—	—	7.6

Measurements in μm unless noted otherwised.
All values are means ± SE of 10 specimens.
Blanks in the table indicate insufficient data; n.s. = not significant ($P \leq 0.05$).

TABLE 2. Morphological characters of *Pratylenchus penetrans* juveniles from celery grown in a greenhouse.

Characters	Females			Male (J4)
	J2	J3	J4	
Body length	224.8 (191.4–270.0)	306.4 (255.0–378.0)	407.9 (315.0–509.6)	369.9 (335.7–463.6)
Width at vulva	11.3 (9.0–11.3)	14.4 (10.5–21.8)	19.2 (15.0–23.8)	19.9 (16.7–34.3)
Head height	2.2 (2.0–2.5)	2.3 (2.0–3.2)	2.7 (2.3–3.2)	2.9 (2.0–3.9)
Stylet	11.9 (11.0–12.0)	12.7 (12.0–14.3)	13.4 (11.9–14.3)	12.9 (11.8–14.7)
Esophagus	44.4 (37.5–52.5)	52.5 (42.0–58.5)	57.6 (49.2–66.0)	66.6 (59.8–73.5)
Intestine length	151.4 (121.9–189.0)	222.3 (182.2–281.3)	310.7 (228.0–398.0)	281.2 (252.7–318.3)
Tail length	15.2 (12.8–19.5)	19.2 (15.0–27.0)	23.9 (20.7–29.0)	22.1 (19.6–26.5)
Reproductive system	13.9 (10.5–18.0)	28.3 (15.0–88.5)	104.1 (47.6–187.3)	96.3 (44.1–240.1)
Procorpus length	21.7 (16.8–29.1)	25.3 (18.7–31.2)	29.0 (23.6–33.0)	27.0 (22.5–29.4)
Metacorpus length	10.5 (8.4–15.6)	10.5 (8.4–12.5)	13.1 (10.1–17.8)	12.3 (10.8–13.7)
Isthmus length	13.6 (10.8–18.7)	15.0 (9.6–20.9)	15.6 (12.0–21.3)	14.5 (7.8–19.6)
Esophageal basal lobe	26.6 (21.6–44.4)	36.2 (27.6–50.4)	46.8 (29.0–56.4)	42.6 (28.4–56.8)

All values are means of 10 specimens (range in μm).

cultivar and the more severe climate in northern Ontario.

In most populations studied, it was common (18%) for the tail tip of female *P. penetrans* to be partially crenated. Partially crenate-tailed females have been found in populations from strawberry in Maryland (9), orchard grass (3), and alfalfa callus cultures in New York (8) and North Carolina (7). The number of crenate-tailed females in a population is influenced by the host and the environment (8). In this study celery and strawberry were not as effective as cabbage (*Brassica oleracea* var. *capitata* L.) and pea (*Pisum sativum* L.) in inducing crenate-tailed females. However, whether crenation of tail termini is genetically determined is still unknown.

Body lengths of *P. penetrans* juveniles from greenhouse celery cultures were similar, but adults from southern Ontario were much smaller than those from *Cryptomeria japonica* (4), alfalfa and pea in New York (8), and strawberry in Maryland (9). The size differences of the females may be due to nutrition (1,8,13), environment, or both.

Large differences in the development from J2 to J4 of *P. penetrans* on celery occurred in the intestine and the reproductive system which increased in length by 110% and 300%, respectively. These increases were highly correlated with increased body length. The length of the J2 intestine was independent of the reproductive system because there was no cellular increase in the germinal primordium. In J3 and J4, however, the elongation of the reproductive system was concomitant

TABLE 3. Correlation coefficients ($r_{0.001} = 0.401$) for 11 morphological characters of *Pratylenchus penetrans* juveniles.

	Bl	Bw	Hl	Sl	El	Il	Tl	Rs	Pl	Ml	P
Body length (Bl)	1.0										
Body width (Bw)	0.857	1.0									
Head length (Hl)	0.061	0.397	1.0								
Stylet length (Sl)	0.775	0.721	0.719	1.0							
Esophagus length (El)	0.838	0.720	0.606	0.708	1.0						
Intestine length (Il)	0.998	0.854	0.546	0.767	0.813	1.0					
Tail length (Tl)	0.899	0.640	0.508	0.176	0.744	0.891	1.0				
Reproductive system (Rs)	0.905	0.833	0.533	0.442	0.740	0.907	0.789	1.0			
Procorpus length (Pl)	—†	—	—	0.614	0.814	—	—	—	1.0		
Metacorpus length (Ml)	—	—	—	0.501	0.713	—	—	—	0.518	1.0	
Posterior gland (P)	—	—	—	0.648	0.938	—	—	—	0.664	0.566	1.0

Juvenile stages J2, J3, and J4 are treated as a group.
† Not determined.

with the elongation of the intestinal region of the body and resulted in high correlation coefficients. Elongation of the intestinal region of the body was great enough that there was no reflexing of the anterior end of the ovary. In the esophageal region of females, the greatest change was an increase of 80% in the length of the posterior lobe.

The range of morphological variation discussed here is similar to that in other studies (7–9). Crenation of the tail tip was one of the more conspicuous variations. Although variant populations differed, depending upon host and geographical location, measurements of all specimens were within the limits of those for *P. penetrans.*

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