Growth Response of Three Vegetables to Smooth- and Crenate-Tailed Females of Three Species of *Pratylenchus*[1]

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Abstract: The effect of morphological variants of females of *Pratylenchus penetrans, P. neglectus,* and *P. crenatus* on the growth of three vegetables was studied. Variants were characterized by having either a smooth or crenate tail terminus. Pea was inoculated with variants of *P. penetrans,* one female per seedling, and grown at light intensities ranging from 1,350 to 21,600 lux in a series of five experiments. Only crenate-tailed females of *P. penetrans* suppressed the growth of pea and only when pea was grown at 3,900 lux. Radish was inoculated with morphological variants of *P. penetrans, P. neglectus,* and *P. crenatus,* four females per seedling, and grown at 3,900 lux in two experiments. Again, only crenate-tailed females of *P. penetrans* inhibited growth. The two variants of *P. penetrans* had a similar infectivity, greater than that of the other two species of *Pratylenchus.* Only crenate-tailed *P. penetrans* reproduced on radish. Onion was inoculated with variants of *P. penetrans* and *P. crenatus,* four females per seedling, and grown at 14 C at 12,900 lux. Again, only crenate-tailed *P. penetrans* inhibited growth. The variants of *P. penetrans* had a similar infectivity, greater than that of *P. crenatus.* Neither species reproduced on onion at low temperatures. *Key Words:* Pea, radish, onion, light intensity.

Morphological variations in the tail terminus of females of *Pratylenchus penetrans* Cobb were described by Tarté and Mai (5) in populations from several geographic sources and from different hosts. The variations were of two types, smooth-tailed and crenate-tailed. Crenate-tailed females have termini with two or more annules that occur at any point around the terminus but more commonly toward the dorsal side. In a study of the sex expression of *P. penetrans,* both smooth- and crenate-tailed females produced smooth- and crenated-tailed progeny (6). Tarté and Mai (6) also noted that Wando pea seedlings inoculated singly with crenate-tailed females were stunted after several weeks whereas seedlings inoculated with smooth-tailed females were not (4). This paper details initial experiments with Wando pea and subsequent experiments with other hosts that demonstrate that smooth- and crenate-tailed *P. penetrans* are pathotypes.

MATERIALS AND METHODS

General procedures: Experiments were performed with three species of vegetables (pea, *Pisum sativum* L. cv. Wando; radish, *Raphanus sativus* L. cv. Early Scarlet Globe; and onion, *Allium cepa* L. cv. Topaz) and three species of *Pratylenchus* [*P. penetrans* (Cobb), *P. neglectus* (Rensch), and *P. crenatus* Loof]. *P. penetrans* and *P. neglectus,* from New York state, each originated from a single gravid female. The former was reared on alfalfa callus tissue and the latter on rye in a greenhouse. *P. crenatus,* from the province of Prince Edward Island in Canada, was stored at 4 C in heavily infested soil until required.

Since the variation in the tail terminus occurs in the females, only females were used in the inoculum. For inspection, females were mounted singly on 20 × 20-mm coverslips, inverted on a deepwell slide, and examined at 700× magnification. The coverslip was held in place with two streaks of petroleum jelly, one on each side of the well. Egg white, the mounting medium used initially, was replaced by a 5% cellulose solution because albumen was not consistent in quality and dried too quickly. Both media restricted nematode movement enough to critically observe the tail terminus, although only when the nematode was in a lateral position. If tail crenation was in doubt the nematode was discarded. Selecting sufficient nematodes for an experiment took five days, during which the nematodes were stored at 7 C.

All seed were surface-sterilized in a 0.6% soln. of sodium hypochlorite, washed in sterile water, and germinated at 20–25 C.

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Coarse seed was germinated in dishes of sterile moist silica sand, and fine seed was germinated in inverted dishes of sterile water agar.

Seedlings were inoculated in dishes of sterile water agar. The root was placed in a drop of water containing the appropriate number of nematodes, covered with sterile silica sand, and incubated at 20 C for 72 h to allow the nematodes to enter the roots. Uninoculated seedlings were treated in the same manner but without nematodes in the drop of water. After the 72-h postinoculation period the seedlings were planted in sterile soil in clay pots.

The infectivity of the three species of *Pratylenchus* on radish and onion was determined by flushing out the agar plates after the postinoculation period and counting the females that had not penetrated the roots. Reproductivity was determined at the end of each experiment by placing infected roots in miniature Baermann pans, extracting the nematodes for 2 weeks, and then counting.

A total of 8 experiments were carried out. A randomized block design was used. Data and means were compared in a Duncan's Multiple Range Test. Data from any replicate in a nematode treatment were deleted from the analysis when no nematodes were recovered, unless nematodes were not recovered from all replicates in the treatment.

Pea: Pea was inoculated with *P. penetrans* only, one nematode per seedling, which resulted in three treatments: uninoculated; inoculation with smooth-tailed females; and inoculation with crenate-tailed females. The plants were grown in a sterile loam soil in 10-cm pots, without fertilization. Experiments were carried out at 1,350 (I), 21,600 (II), 8,200 (III), and 3,900 (IV, V) lux in a 12-h photoperiod in a growth room at 21 C. Treatments were replicated 6 to 64 times. Low light intensities were achieved by shading the plants with polypropylene screen. Fresh and dry weights were determined at the conclusion of each experiment.

Radish: Inoculations of radish were with *P. penetrans*, *P. neglectus*, and *P. crenatus* in two experiments; both were carried out at 3,900 lux in a 12-h photoperiod at 21 C. In experiment VI, radish was inoculated with *P. penetrans* and *P. neglectus*, four females per seedling, resulting in four treatments: uninoculated; inoculation with smooth- and crenate-tailed *P. penetrans;* and inoculation with smooth-tailed *P. neglectus*. Treatments were replicated 14 times, and all seedlings were grown in 7.5-cm pots. Infectivity and reproductivity of the two nematodes, and fresh weight of tops and roots were determined. In experiment VII, radish was inoculated with *P. penetrans* and *P. crenatus*, four nematodes per seedling, resulting in five treatments: uninoculated; and inoculation with smooth- and crenate-tailed females of each species. The experimental procedure was identical to the first except that the seedlings were planted in a sterile sandy soil, and dry weight of the tops and roots was determined as well.

Onion: Inoculations of onion were with *P. penetrans* and *P. crenatus* in a single experiment (VIII) carried out at 14 C at 12,900 lux in a 14-h photoperiod. The five treatments were the same as in experiment VII with radish. Seedlings were planted in sterile muck soil in 7.5-cm pots. Infectivity and reproductivity of the two nematodes, and fresh weight of tops and roots, were determined.

RESULTS

Pea: Growth of pea was poor at 1,350 lux (Expt. I) and excellent at 21,600 lux (Expt. II) with no differences among the treatments in the experiments.

At 8,200 lux there was visibly less top growth of pea inoculated with crenate-tailed females of *P. penetrans* than in either of the other treatments. The differences however, were not reflected in either fresh or dry weights of the tops (Table 1, Expt. III).

Expt. IV, the first experiment with pea at 3,900 lux, was terminated after 3 weeks because of infection by *Fusarium* sp. Again, top growth was visibly less with crenate-tailed females than in the other treatments (Fig. 1). Moreover, there was a difference in dry weights of the tops (Table 1, Expt. IV).

In Expt. V, again at 3,900 lux, the total fresh weight of pea was in the following order: crenate-tailed $<$ control $<$ smooth-tailed (Table 1, Expt. V).

TABLE 1. Effect of morphological variants of *Pratylenchus penetrans* on pea grown at different light intensities.

Treatment	Fresh wt. (g)		Dry wt. (mg)	
	Total	Top	Root	Top
Expt. III, 8200 lux, 5 wk				
Control	–	4.65 a[b]	–	623 a
Smooth-tailed[a]	–	4.42 a	–	611 a
Crenate-tailed	–	4.18 a	–	597 a
Expt. IV, 3900 lux, 3 wk				
Control	–	0.91 a	–	67 a
Smooth-tailed	–	0.97 a	–	57 ab
Crenate-tailed	–	0.85 a	–	47 b
Expt. V, 3900 lux, 8 wk				
Control	3.62 b	2.19 b	1.43 a	203 ab
Smooth-tailed	4.31 a	2.74 a	1.57 a	245 a
Crenate-tailed	2.75 c	1.86 bc	0.89 b	161 bc

[a]Each plant inoculated with a single female.
[b]Letters indicate Duncan's multiple-range grouping of treatments that do not differ at the 5% level.

Differences ($P < 0.05$) in dry weight of tops were evident only between crenate-tailed females and smooth-tailed.

Radish: Growth of radish, in terms of fresh weight of both tops and roots, was significantly less for crenate-tailed females of *P. penetrans* than for all other treatments (Table 2, Expt. VI).

Differences in the growth of radish between smooth- and crenate-tailed *P. penetrans* and *P. crenatus* were seen only when analyses were conducted on dry weights. Again, weights of tops and roots and total weight of radish were significantly less with crenate-tailed females of *P. penetrans* than with any other treatment (Table 2, Expt. VII).

Smooth- and crenate-tailed females of

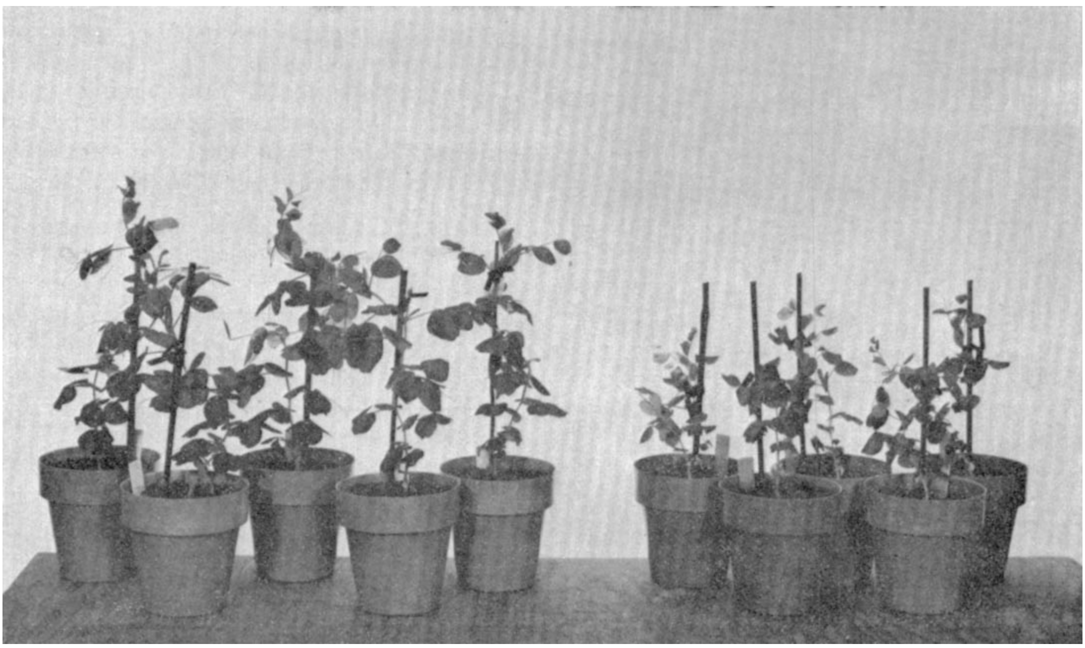

FIG. 1. Effect of morphological variants of *Pratylenchus penetrans* on growth of pea grown at 3,900 lux at 8 wk. *A)* Inoculated with one smooth-tailed female/plant. *B)* Inoculated with one crenate-tailed female/plant.

TABLE 2. Effect of morphological variants of *Pratylenchus penetrans, P. neglectus,* and *P. crenatus* on radish grown at 3900 lux for 7 weeks.

Treatment	Fresh wt. (g) Total	Fresh wt. (g) Top	Fresh wt. (g) Root	Dry wt. (mg) Total	Dry wt. (mg) Top	Dry wt. (mg) Root
Expt. VI						
Control	0.65 a	0.56 a	0.09 a	–	–	–
P. penetrans						
Smooth-tailed[a]	0.47 a	0.49 a	0.08 a	–	–	–
Crenate-tailed	0.33 b	0.29 b	0.04 b	–	–	–
P. neglectus						
Smooth-tailed	0.58 a	0.50 a	0.08 a	–	–	–
Expt. VII						
Control	1.6 a	1.5 a	0.1 a	–	135 a	–
P. penetrans						
Smooth-tailed	1.5 a	1.4 a	0.1 a	126 a	118 a	8 a
Crenate-tailed	1.1 a	1.08 a	0.02 b	62 b	57 b	4 b
P. crenatus						
Smooth-tailed	1.8 a	1.7 a	0.1 a	147 a	139 a	8 a
Crenate-tailed	1.5 a	1.4 a	0.1 a	156 a	115 a	8 a

[a]Each plant inoculated with four females.
[b]Letters indicate Duncan's multiple-range grouping of treatments that do not differ at the 5% level.

P. penetrans had identical infectivity, but greater ($P < 0.05$) than that of smooth-tailed *P. neglectus* (Table 3, Expt. VI). Recovery of nematodes from roots of radish was greater for crenate-tailed *P. penetrans* than for smooth-tailed (Table 3, Expt. VI). Similarly, smooth- and crenate-tailed *P. penetrans* had identical infectivity, but greater ($P < 0.05$) than that of *P. crenatus* (Table 3, Expt. VII). In turn, crenate-tailed *P. crenatus* had greater infectivity than smooth-tailed ($P < 0.05$). Again, recovery of nematodes from roots of radish was greater for crenate-tailed *P. penetrans* than for smooth-tailed (Table 3, Expt. VII). Neither *P. neglectus* nor *P. crenatus* was recovered from radish.

TABLE 3. Infectivity and reproductivity of morphological variants of *Pratylenchus penetrans, P. neglectus,* and *P. crenatus* on radish.

Treatment	Number of nematodes: Entered roots	Number of nematodes: Recovered from roots[b]
Expt. VI		
Control	0	0
P. penetrans		
Smooth-tailed	3.57 a[a]	1.00 b
Crenate-tailed	3.64 a	7.18 a
P. neglectus		
Smooth-tailed	2.43 b	0
Expt. VII		
Control		0
P. penetrans		
Smooth-tailed	3.86 a	3.17 b
Crenate-tailed	3.86 a	9.50 a
P. crenatus		
Smooth-tailed	2.29 c	0
Crenate-tailed	3.07 b	0

[a]Letters indicates Duncan's multiple-range grouping of treatments that do not differ at the 5% level.
[b]Radish was grown for 7 weeks.

Onion: At 14 C only crenate-tailed females of *P. penetrans* affected growth of onion (Table 4, Expt. VIII) as indicated by the lower fresh weights ($P < 0.05$). Several

TABLE 4. Effect of morphological variants of *Pratylenchus penetrans* and *P. crenatus* on onion grown at 14 C for 9 weeks.

Treatment	Fresh wt. (g) Total	Fresh wt. (g) Top	Fresh wt. (g) Root
Expt. VIII			
Control	2.37 a[b]	1.48 a	0.90 a
P. penetrans			
Smooth-tailed[a]	2.60 a	1.50 a	1.10 a
Crenate-tailed	1.13 b	0.70 b	0.43 b
P. crenatus			
Smooth-tailed	3.20 a	1.83 a	1.37 a
Crenate-tailed	3.31 a	1.97 a	1.34 a

[a]Each plant inoculated with four females.
[b]Letters indicate Duncan's multiple-range grouping of treatments that do not differ at the 5% level.

replicates inoculated with crenate-tailed females died, and data for them were not included in the analysis.

Smooth- and crenate-tailed females of *P. penetrans* were similar in infectivity to the same types of *P. crenatus*. However, more *P. penetrans* than *P. crenatus* entered the roots (3 vs 2; $P < 0.05$). Nematodes were recovered from the roots of onion inoculated with the two types of *P. penetrans* but there was no reproduction. *P. crenatus* was not recovered from onion.

DISCUSSION

Morphological variations as markers are a convenient means of studying the biological capacities within a species. Tarté and Mai (6) studied the sex expression of smooth- and crenate-tailed females of *P. penetrans*. These tail types became the markers in our study on pathotypes in species of *Pratylenchus*. Differences in reproductivity and in pathogenicity proved to be related to the smooth- and crenate-tailed markers, although only in *P. penetrans*. Crenate-tailed females of *P. penetrans* were pathogenic to pea, radish, and onion and stunted their growth, while in some instances the smooth-tailed females stimulated growth. It is especially noteworthy that only one to three crenate-tailed females were required to cause stunting, yet no lesions developed. Perhaps the nematodes induced a hormonal response in the plant that inhibited growth. Further biological separation of the two morphological variants occurred on the basis of reproductivity.

On radish, crenate-tailed females of *P. penetrans* produced more larvae than did smooth-tailed. Infectivity did not separate the two variants, for each type penetrated roots of the three test plants equally well, although *P. penetrans* had greater infectivity than either *P. crenatus* or *P. neglectus*.

Physical conditions that stress the host plant appear to be necessary to detect the pathogenic capacity of crenate-tailed females of *P. penetrans*. The pathogenic response was obtained with low light intensity, 3,900 lux. Low temperature also provided conditions to detect differences in pathogenicity (1). Use of a heavy clay soil might further stress an infected plant at low light and temperature, accentuating differences in growth.

Races or types of *P. penetrans* were first noted by Slootweg (3), in 1956, who found that certain populations attacked only lilies and not narcissi. In 1968, Olthof (2) found that a population isolated in a greenhouse for 10 years neither stunted nor multiplied in selected varieties of tobacco whereas a population directly from the field did. Neither worker noted morphological differences in the tail of females in their populations.

The occurrence of pathotypes in *P. penetrans* provides new challenges in research with this species. There is a need to know whether pathotypes occur in the field on the basis of tail type. Rotation crops should be reevaluated because certain crops favor the build-up of crenate-tailed females (5). Crop loss predictions of *P. penetrans* may lack accuracy as long as there is need to know what numbers of each tail type will damage a crop and under what conditions. In all, this study and that by Tarté and Mai (5) emphasize the need to describe the whole range of morphological variation in a species and the danger of arbitrarily using a character such as tail type to separate species.

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