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Penetration of Celery and Alfalfa Roots by *Pratylenchus penetrans* as Affected by Temperature

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Abstract: A greater percentage of females than juveniles or males of *P. penetrans* penetrated celery roots grown in infested soil at 5, 18, or 30 C; the difference was greatest at 5 C. The time of initial penetration of alfalfa seedlings inoculated with single nematodes on water agar varied with temperature. Females penetrated the seedlings earlier and over a wider range of temperatures than did males or juveniles. The rate of penetration was highest for females. After initial penetration, the penetration rate decreased with time. At 13–28 C, approximately 80% of roots were penetrated by females and only 25–30% by males and juveniles by the end of the experiment.

Key words: alfalfa, *Apium graveolens,* celery, *Medicago sativum,* root penetration, *Pratylenchus penetrans,* lesion nematode.

At one time only fourth-stage juveniles and adults of the root-lesion nematode, *Pratylenchus penetrans* (Cobb), were believed to penetrate host roots (2). Later, second-stage (3) and third-stage juveniles (7) were shown to penetrate alfalfa roots. Other research showed that soil moisture and temperature affected penetration of cherry and corn (2,6,7). In these studies, however, the numbers of *P. penetrans* in host roots were determined at the end of a specific time at different temperatures; the rates of penetration were not determined. This paper reports on the effect of a range of temperatures on the initiation and rate of penetration of host roots by three life stages of *P. penetrans.*

Materials and Methods

Penetration of celery roots: A *P. penetrans*-infested Tioga fine sandy loam (69% sand, 26% silt, 5% clay) from Dufferin County in Ontario was mixed in an automatic shaker to obtain a uniform distribution of nematodes. One kilogram of infested soil was placed in each of 48 clay pots (12.7 cm d). Each pot of soil was moistened to approximately field capacity (9) and enclosed in a plastic bag, and 16 pots were placed in each of three growth cabinets set at 5, 18, and 30 C. The pots were allowed to equilibrate to cabinet temperature for 72 hours. A slot (11 × 12 cm) was then cut into the soil to the depth of each pot. Washed roots of a 6-week-old celery (*Apium graveolens* L. cv. Utah 52-70) plant were spread into the slot, and the soil was tapped back into place. Celery plants were watered as needed and removed from eight pots from each of the three growth cabinets at 48 and 96 hours. Each root system was severed from its stem, washed on a screen, blotted dry, and weighed. Nematodes were extracted from each washed root system by the pan method (4) for 7 days. Females, males, and juveniles were counted with a dissecting microscope.

Analysis of variance (ANOVA) was used to examine the effects of time and temperature on root weight and numbers of nematodes in roots. The data for each life stage (female, male, and juvenile) first were analyzed separately; terms in the model were temperature, time (48 vs. 96 hours), and the temperature × time interaction. To correct for heterogeneity of variance, nematode counts were transformed ($\log_e [x + 1]$) before analysis. Partial correlations between root weight and both the untransformed and log-transformed counts (adjusted for time and temperature) were calculated.

The data from all three life stages were combined to examine differences among life stages; these comparisons were on a within-pot basis. ANOVA was performed on both the percentage data (number of females/total number of nematodes × 100)

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and on the log-transformed count data. The nematode counts also were expressed relative to the mean life-stage counts observed in 50 g soil at transplanting (150 for females, 47 for males, and 600 for juveniles).

Penetration of alfalfa roots: Pratylenchus penetrans from the Niagara Peninsula in southern Ontario were reared in a greenhouse on celery grown in a Vineland silt loam (VSL) and then on sweet corn, *Zea mays* L. cv. Earlivee, grown in VSL in a greenhouse groundbed. Nematodes were extracted from washed corn roots in a mistifier (4) and stored in a shallow layer of water at 5 C prior to experimentation.

Penetration of alfalfa roots by females, males, and third-stage juveniles (J3) of *P. penetrans* was studied in separate experiments. Alfalfa (*Medicago sativum* L. cv. Saranac) seeds were surface sterilized for 30 minutes in 37 N sulphuric acid, washed with cold sterile water, and germinated on sterile water agar in inverted petri dishes at 20 C. One nematode was transferred to each of five drops of sterile water placed equidistant on the surface of sterile water agar in a 9-cm-d petri dish. The root of a 72-hour-old alfalfa seedling was placed in each drop of water, covered with sterile silica sand, and moistened further if required. Sufficient plates were prepared to quantify root penetration at temperatures from 3 C to 33 C at 5-C intervals and at different times. Sampling of female-inoculated seedlings incubated at 13–28 C began at 12 hours and concluded at 96 hours after five samplings. Sampling of seedlings incubated at 8, 33, and 3 C began at 24, 48, and 72 hours, respectively, and concluded at 96, 120, and 168 hours, respectively. Sampling of male-inoculated and J3-inoculated seedlings began at 48 hours and concluded as late as 192 hours. There were four plates for each combination of temperature and sampling time. Roots were stained in cotton blue lactophenol (1) and were mounted on slides. Each root system was examined with a dissecting microscope to determine whether penetration had occurred.

ANOVA was used to examine the effect of temperature, time, and the temperature × time interaction on the percentage of penetrated seedlings. Both main effects were partitioned into their linear, quadratic, and higher order components.

Results

Penetration of celery roots: There were no differences ($P > 0.05$) in root weight due to temperature or time (48 vs. 96 hours). Based on the ANOVA of the log-transformed data, the number of nematodes in roots increased ($P < 0.01$) with temperature (Table 1). Although more nematodes appeared to be in roots at 96 hours than at 48 hours, the difference was significant ($P < 0.05$) only for females. The partial correlation between root weight and the numbers of nematodes (total and by life stage) adjusted for temperature and time ranged from 0.50 to 0.65 (0.55 to 0.65 for log-transformed counts), indicating that the amount of penetration was directly related ($P < 0.01$) to root weight.

Fewer ($P < 0.01$) males than females or juveniles were found in roots. In each of the six temperature × time combinations, males represented less than 6% of the nematodes (Table 1). The proportion of females vs. juveniles was affected ($P < 0.01$) by temperature. At 5 C, approximately two-thirds of the *P. penetrans* in the roots were females and one-third were juveniles; these proportions were reversed at the two higher temperatures (Table 1). The percentage of each life stage in roots was not affected ($P > 0.05$) by time. The percentages of life stages found in the roots at the two higher temperatures (32% females, 3% males, 65% juveniles) reflected the relative numbers found in the soil (150:47:600/50 g) at time 0.

Penetration of alfalfa roots: Temperature had a greater effect on time of first penetration of alfalfa roots on agar than on percentage of penetration (Table 2). At comparable temperatures, males and J3 were slower in commencing penetration than were females. At 3 C, female penetration was markedly delayed and males and J3

TABLE 1. Root weight and number of *Pratylenchus penetrans* in celery roots as affected by temperature and time.

Temperature (C)	Time (hours)	Root weight (g)	Number of nematodes				Percentage of total nematodes in roots		
			Females	Males	Juveniles	Total	Females	Males	Juveniles
5	48	2.76	12.8 ± 2.1	0.4 ± 0.2	7.3 ± 1.4	20.4 ± 3.2	65 ± 4	2 ± 1	34 ± 4
	96	2.79	18.6 ± 3.8	0.6 ± 0.5	9.9 ± 3.2	29.1 ± 6.9	68 ± 5	1 ± 1	30 ± 5
18	48	2.29	20.0 ± 7.0	3.3 ± 1.3	48.3 ± 18.7	71.5 ± 26.7	31 ± 3	3 ± 1	66 ± 3
	96	2.22	53.1 ± 28.3	5.6 ± 3.7	96.3 ± 38.0	155.0 ± 68.2	32 ± 4	2 ± 1	66 ± 4
30	48	2.55	38.5 ± 12.5	6.8 ± 3.1	110.1 ± 29.9	155.4 ± 44.5	24 ± 2	3 ± 1	73 ± 2
	96	2.43	110.0 ± 32.8	10.6 ± 2.7	165.0 ± 60.0	285.6 ± 94.3	39 ± 3	5 ± 1	56 ± 2

The soil contained 150 female, 47 male, and 600 juvenile *P. penetrans* per 50 g at time 0.
Pooled standard error of the mean based for root weight, n = 8, was 0.45 g. For number of nematodes and percentage of total nematodes in roots, values are the means ± SE, n = 8.

failed to penetrate at all. Both time and temperature affected ($P < 0.01$) penetration of alfalfa roots by males, females, and J3 (Table 2); the time × temperature interaction was not significant ($P > 0.05$). For both time and temperature, the effect was quadratic ($P < 0.01$). The quadratic effect of time reflected that the daily increase in percentage of penetration decreased with time; the largest increases in percentage of penetration were observed during the early stages of the experiment. In contrast, the quadratic effect of temperature indicated that the highest percentage of penetration occurred at the intermediate temperatures. Discrimination among temperatures was somewhat better for females for which higher penetration was observed than for J3 or males.

TABLE 2. Percentage of alfalfa roots penetrated by different life stages of single *Pratylenchus penetrans* as affected by temperature after 12 to 192 hours.

Temperature C	12	24	48	72	96	120	144	168	192
				Females					
3			0	10	20	30	35	40	
8	0	25	35	45	60	65			
13	20	30	45	60	70	75			
18	20	35	55	65	75	80			
23	30	35	55	70	75	80			
28	20	35	50	70	80	75			
33			25	35	55	65	60		
				Males					
8			0	10	15	15	20	20	25
13		0	10	15	20	25	25	30	
18		0	20	25	25	25	30	30	
23		0	15	25	20	20	25	30	
28		0	15	15	20	20	20	25	
33			0	15	15	20	20	20	
				Juveniles					
8				0	10	15	20	20	25
13		0	10	15	20	20	30	25	
18		0	10	15	20	20	25	25	
23			0	15	20	25	25	25	
28			0	15	25	25	25	25	
33			0	5	10	15	20	20	

Each value is a percentage based on 20 roots, one nematode added per root.
Some trials were not run at 12, 24, and 48 hours because penetration was not anticipated until later; some were not run at 144, 168, and 192 hours because penetration was deemed to have reached a maximum earlier.

Estimated coefficients for the equations relating percentage of penetration to temperature and time were similar for males and juveniles (Table 3). Because the effect of time was quadratic, it was inappropriate to use these equations to predict penetration percentage beyond the times used in this study.

DISCUSSION

This study and others (3,7) have shown that all mobile life stages of *P. penetrans* are able to penetrate host roots. However, more females than males and juveniles penetrated the celery roots, relative to the number of each life stage in the infested soil. The Tioga fine sandy loam used in this experiment is similar to Fox sandy loam which has been shown to be very suitable for the movement of *P. penetrans* (5) and

TABLE 3. Coefficients of quadratic equation relating percentage of alfalfa seedlings penetrated by *Pratylenchus penetrans* to time and temperature.

Life stage	B_0	B_1	B_2	B_3	B_4
Female	−54.160 ± 4.708	0.906 ± 0.087	−0.003 ± 0.001	7.188 ± 0.460	−0.176 ± 0.013
Male	−25.884 ± 5.506	0.387 ± 0.067	−0.001 ± 0.0003	2.171 ± 0.476	−0.053 ± 0.012
Juvenile	−30.703 ± 5.782	0.412 ± 0.075	0.001 ± 0.0003	2.135 ± 0.473	−0.053 ± 0.011

Equation: penetration percentage = $B_0 + B_1H + B_2H^2 + B_3T + B_4T^2$ where H is hours since nematodes placed on agar and T is maintenance temperature.
Values are the regression coefficient ± SE.

for the penetration of host roots (6). Soil moisture also was maintained near field capacity to provide a moisture tension most suitable for the penetration of host roots (6).

Temperature affected penetration of roots by J3, females, and males. At 3 C, penetration was delayed for females and totally inhibited for J3 and males. Extreme conditions may be more deleterious to these latter stages. Temperature previously has been shown to affect the mobility of *P. penetrans* (5) and probably affects the search for preferred sites of penetration (7). Because most physiologic processes and activity are temperature dependent in invertebrates, temperature probably affects the probing of the epidermis and the activity of enzymes secreted by *P. penetrans*.

The capacity to penetrate roots rapidly, in greater numbers, and at lower temperatures indicates that females may survive adverse conditions better than do juveniles and males. *Pratylenchus penetrans* prefers sandy soils which have a low moisture holding capacity and which tend to dry rapidly during July and August. The female is able to penetrate roots in 12 hours and thus may not be forced into anhydrobiosis (8). Once in the roots, females can commence laying eggs in a protected environment. Also, females are able to penetrate roots in late autumn and early winter when soil temperatures are just above freezing.

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