Influence of Soil Temperature and pH on *Pratylenchus penetrans* and *P. crenatus* in Alfalfa and Timothy[1]

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Abstract: Numbers of *Pratylenchus penetrans* in alfalfa and timothy, and to a lesser extent *P. crenatus* in timothy, increased substantially as temperature increased from about 10 C to 30 C. However, *P. crenatus* in alfalfa decreased in number as temperature increased. Mobility of *P. crenatus* in vertical soil columns decreased as temperature increased from 9.5 C to 28.5 C. Raising the soil pH from 5.0 to 6.9 in which alfalfa was grown increased the numbers of *P. penetrans* and greatly reduced the numbers of *P. crenatus*. The numbers of both nematode species in timothy were reduced significantly as soil pH was increased. The optimum soil pH for movement of *P. penetrans* was 6.0. *Pratylenchus crenatus* moved equally well over a range of pH 5.0 to 7.0.
Key words: root-lesion nematodes, population size, movement.

The distribution ranges of *Pratylenchus penetrans* (Cobb) Filip. and Sh. Stek. and *P. crenatus* (Loof) overlap throughout much of eastern North America (19), and it is likely that certain conditions favor one species over the other within localized sites. Recent publications have reviewed the various environmental factors which affect nematodes in general (14) and *P. penetrans* in particular (10). Individual studies have investigated the movement of various stages of *P. penetrans* under different conditions (9,15,20). However, there are only a few observations which have compared the influence of environment on *P. penetrans* and *P. crenatus* (3,4). Therefore, the objective of this study was to investigate the effects of temperature, soil pH, and host plant on population size and mobility of both nematode species under greenhouse and laboratory conditions.

MATERIALS AND METHODS

Populations of *P. penetrans* and *P. crenatus* used for inoculum were maintained in a greenhouse on 'Lakeland' red clover (*Trifolium pratense* L.) and 'Climax' timothy (*Phleum pratense* L.), respectively. Host plants for experimental purposes were 'Iroquois' alfalfa (*Medicago sativa* L.) and 'Climax' timothy. Nematodes for inoculum and experimental purposes were recovered from roots or soil by placing samples in a mister or modified Baermann pan (16), respectively, for 7 d. Data were transformed to log (x + 1) or arcsines prior to statistical analysis. In the pot experiments foliage weights at harvest were recorded.

The first experiment examined the influence of temperature on populations of *P. penetrans* and *P. crenatus*. Alfalfa was seeded in pots containing 4.5 kg of sterilized Charlottetown fine sandy loam (80% sand, 10% silt, 10% clay). Four d after planting, seedlings were thinned to five uniform plants in each pot; 3 d later 25,000 nematodes of either species were added to each pot. Pots were arranged randomly in growth cabinets at 10.0, 18.5, and 27.0 C (±0.5 C), and a light-dark regime of 14 and 10 h, respectively. Recommended cultural procedures, including addition of *Rhizobium* sp., were maintained, and the experiment was terminated 3 months after seeding. Nematodes were recovered from roots and soil using the mister or Baermann pan, and plant weights were recorded. The statistical analysis was based on a split plot with four replicates.

The second experiment examined the influence of three temperatures (10, 20, and 30 C) on the population changes of two nematode species in alfalfa and timothy. The procedures for planting, nematode inoculation, experimental design, and cultural practices were similar to the first experiment, except the plants were grown for 7 months. Nematode populations were determined at 3 and 5 months after planting from samples obtained by inserting a 1-cm-i.d. soil probe at random through the root zone in each pot; four samples were collected each time. Pieces of root were separated from soil and placed in 0.1% methyl blue in lactophenol at 40 C for 24 h and then cleared in lactophenol for at least 24 h. After

Received for publication 9 September 1980.
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the experiment was terminated, nematodes were recovered from roots and soil using the mister or Baermann pan and plant weights were recorded.

A third experiment was designed to determine the rate of movement of nematodes through soil. Plastic tubing 3 cm × 0.7 cm i.d. and covered at one end with nylon mesh, was filled with sandy loam (particle size 150μ–300μ) to a depth of 2.0 cm and placed vertically so that the mesh just touched sterile tap water in watch glasses. A mean of 78 ($\pm$3.4 S$\bar{x}$) *P. penetrans* or *P. crenatus* was introduced in a water droplet onto the saturated soil in each tube. Tubes were placed randomly, five replicates to a treatment, in growth cabinets at 9.5, 18.5, and 28.5 C. The numbers of nematodes that moved through the soil columns were determined at 4, 18, and 66 h after inoculation and expressed as a percentage of the original inoculum in each tube. Nematodes remaining in the columns were recovered by rinsing the soil three times. Nematode mobility was determined by estimating the time necessary for 50% and 90% of the nematodes to move down the soil columns.

The fourth experiment examined the influence of soil pH on reproduction and survival of *P. penetrans* and *P. crenatus*. Soil pH was adjusted to 4.9, 6.3, and 7.3 using a 60:40 (W:W) mixture of $CaCO_3$ and $MgCO_3$. The procedures were similar to the second experiment. Roots and soil were not disturbed or sampled for nematodes until the final harvest 9 months after seeding. Soil pH at harvest was also determined.

The fifth experiment examined the movement of nematodes through soils of different pH. Tubes, as described in the third experiment, were placed in watch glasses containing tap water adjusted to pH levels of 5, 6, and 7. A mean of 116 ($\pm$4.9 S$\bar{x}$) nematodes of either species was introduced into each tube, and the numbers of nematodes that had moved down through the soil columns into the water were counted after 2, 10, and 26 h. Calculations were the same as in the third experiment.

RESULTS

In experiment one, the numbers of *P. penetrans* increased greatly, while numbers of *P. crenatus* in alfalfa roots decreased significantly, as temperatures increased from 10 C to 27 C (Table 1). In the second experiment, examination of stained roots of timothy and alfalfa 3 months after nematode inoculation indicated that *P. penetrans* and *P. crenatus* numbers increased as temperature increased (Fig. 1). This was still evident in both hosts infested with *P. penetrans* for 5 months but not for *P. crenatus* in alfalfa. The deposition of eggs by *P. penetrans* was much greater at 30 C than at 10 C. Very few eggs were laid in roots of either host by *P. crenatus* at any temperature. The total number of *P. penetrans* at 7 months was greatest at 30 C in timothy and alfalfa pots, as was *P. crenatus* in timothy, though to a lesser extent. However, there were fewer than 1,000 *P. crenatus* per pot of alfalfa at 30 C when the experiment ended.

Table 1. The effect of soil temperature on numbers of *Pratylenchus penetrans* (PP) and *P. crenatus* (PC) 3 months after seeding alfalfa.

No. of nematodes	Species	Soil temperature (C) 10.0	18.5	27.0	Species means
Per g dry root	PP	450*bc	1,210b	23,660a	2,340a
	PC	160c	100c	20d	70b
Temperature means		270a	350a	680a	
Per kg dry soil	PP	3,030	2,430	1,860	2,390a
	PC	1,810	790	850	1,070b
Temperature means		2,340a	1,390a	1,260a	
Per pot	PP	15,340	11,700	11,140	12,600a
	PC	9,640	3,920	4,310	5,460b
Temperature means		12,160a	6,770b	6,930b	

*Geometric mean of four replicates. Letters for Duncan's multiple-range test (P = 0.05) are omitted where species x temperature interaction was not significant.

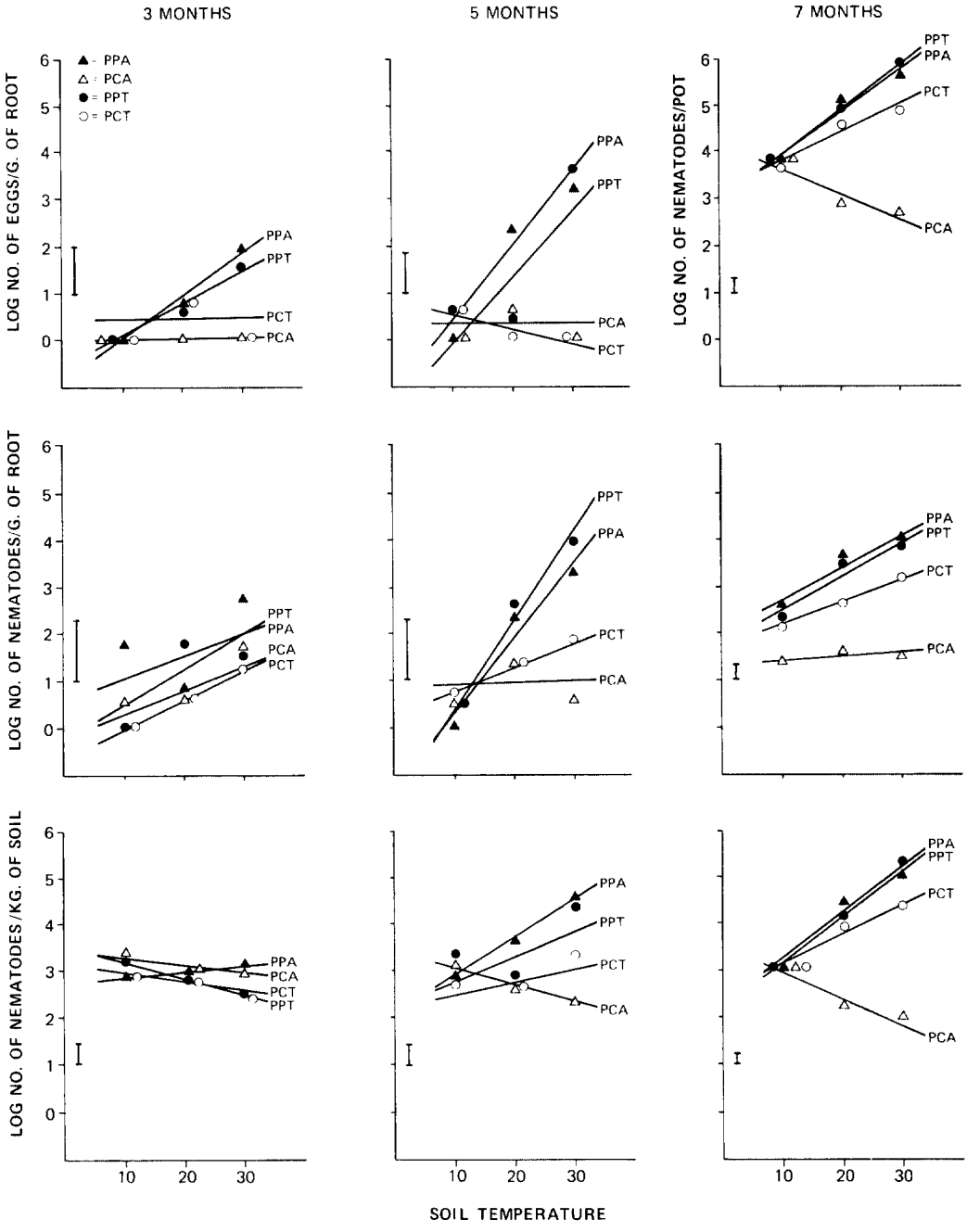

Fig. 1. Effect of temperature on *Pratylenchus penetrans* (PP) and *P. crenatus* (PC) in alfalfa (A) and timothy (T) at 3, 5, and 7 months after nematode inoculation. Vertical bars represent the standard error.

The third experiment examined nematode movement at different temperatures in the absence of a host. *Pratylenchus penetrans* was more active than *P. crenatus* as the times required for 50% or 90% of the populations to move down the soil columns were always less for *P. penetrans* (Table 2). Movement was optimum for *P. penetrans* at 18.5 C. *Pratylenchus crenatus* was very sluggish at 28.5 C, and the 50% and 90% categories could not be calculated as only 13% of the population had migrated down the

Table 2. Number of hours required for 50% (t_{50}) and 90% (t_{90}) of nematodes to pass through 2-cm vertical soil columns at different temperature or pH.

Treatment		$t_{50} \pm S\bar{x}$		$t_{90} \pm S\bar{x}$	
		P. crenatus	*P. penetrans*	*P. crenatus*	*P. penetrans*
Temperature	9.5 C	13.8 ± 1.4	4.8 ± 1.4	43.9 ± 8.2	32.3 ± 17.9
	18.5 C	10.2 ± 3.8	0.3 ± 0.4	47.0 ± 19.1	9.8 ± 3.4
	28.5 C	>66	1.9 ± 0.6	>66	36.2 ± 11.0
Soil pH	5	12.7 ± 0.8	6.2 ± 0.4	37.3 ± 4.1	16.5 ± 2.0
	6	12.3 ± 0.7	5.8 ± 0.2	31.8 ± 4.9	13.6 ± 1.9
	7	14.3 ± 2.4	7.4 ± 1.0	35.9 ± 5.9	21.7 ± 4.1

columns; the majority of specimens left in the soil at the end of the experiment were still alive.

The main response to higher soil pH levels in alfalfa was the sharp drop in the numbers of *P. crenatus* and the significant increase in the numbers of *P. penetrans* (Fig. 2). The numbers of both nematode species in timothy at pH 5 were approximately 50 times greater than the initial inoculum of 25,000 nematodes per pot, and this decreased significantly as the soil pH increased to 6.9. *Pratylenchus penetrans* was more active than *P. crenatus*, and the times required for 50% and 90% of the populations to move down the columns were always less for *P. penetrans* (Table 2). *Pratylenchus penetrans* moved most efficiently at pH 6, but though statistically significant, the difference in relation to pH 7 was not large. Movement of *P. crenatus* did not differ significantly among the various pH levels.

Yields were significantly lower in alfalfa infected with *P. penetrans* than in plants harboring *P. crenatus* in both the temperature and pH experiments. Timothy yields did not differ in their response to either nematode species, and yields of both plant hosts increased as temperature and soil pH were increased (numerical yield data were omitted).

DISCUSSION

The observation that numbers of *P. penetrans* increased as temperature increased agreed with Acosta and Malek (1), who noted that population increase was greatest in soybean (*Glycine max* L. Merr.)

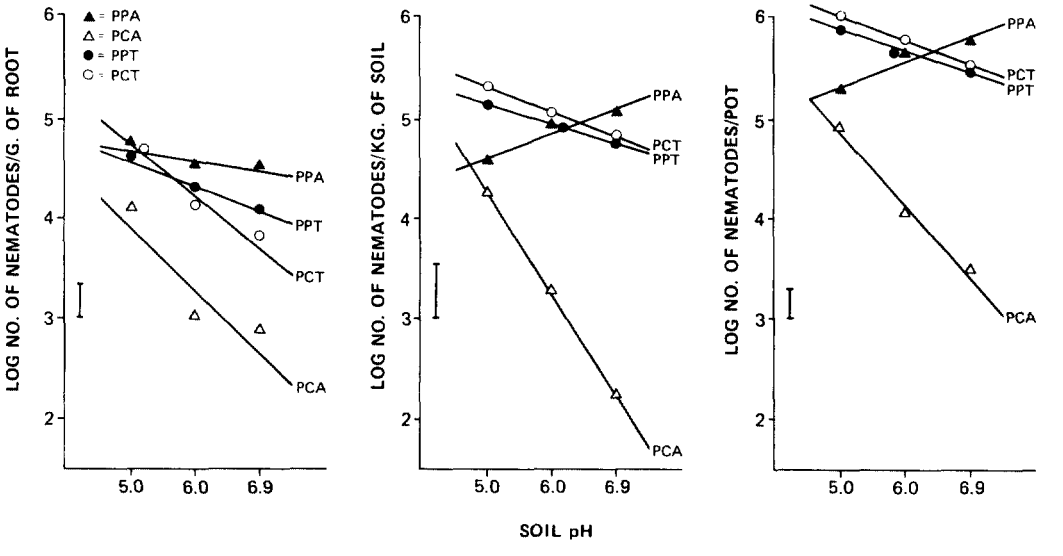

Fig. 2. Effect of soil pH on *Pratylenchus penetrans* (PP) and *P. crenatus* (PC) in alfalfa (A) and timothy (T) 9 months after nematode inoculation. Vertical bars represent the standard error.

at 25 C to 30 C. Dunn (8) observed that population growth in alfalfa callus culture increased as temperature increased from 9 C to 27 C. Mamiya (11) and Dunn (8) working with conifers (*Cryptomeria japonica* D. Don) and alfalfa, respectively, reported that the length of the life cycle of *P. penetrans* was shortest at 30 C. Bhatt and Rohde (2) found that respiration of *P. penetrans* from alfalfa callus tissue increased as temperature increased from 10 C to 35 C.

The reduction of *P. crenatus* populations in alfalfa at higher temperatures agreed with Dao (5) who showed that numbers in corn were greatest at 10 C to 15 C but were less numerous as temperature increased. However, the *P. crenatus* population in this study increased on timothy as temperature rose. Dickerson et al. (7) previously recorded a temperature times host-plant interaction where the most rapid population increase for *P. penetrans* was 24 C on corn and 16 C on potatoes. Dickerson (6) has stressed that temperature limitation depends on the nematode host interaction and not on the nematode itself.

Movement, which is a prerequisite for invasion (21), was optimum for *P. penetrans* at 18.5 C, and this agreed closely with Townshend (17,18) who observed that penetration of corn and alfalfa roots was greatest at 20 C. The greater activity of *P. penetrans* in relation to *P. crenatus* at all temperatures and pH levels appeared to be an inherent difference between the two species. It was noted previously (9,15,20) that adults and 4th stage juveniles of *P. penetrans* were more active than younger stages. Such information is not available for *P. crenatus,* so it is difficult to compare the species on this basis. However, the proportion of different vermiform stages at inoculation was similar, and the only obvious difference between the two species was the absence of males in *P. crenatus.*

Previous studies by Morgan and MacLean (12) and Willis (22) in vetch (*Vicia sativa* L.) and alfalfa, respectively, indicated that *P. penetrans* does best in the range of pH 5.2 to 6.4, with reproduction dropping as the pH approaches 7. However, Myers (13) working with tomatoes recovered more nematodes at pH 6 than at pH 5. This probably indicates that the optimum pH range for root lesion nematodes varies with the species of host plant, as was the case with temperature. What is evident here in alfalfa, and what has been observed previously on cabbage and carrot (3,4), is that *P. penetrans* appears to prefer a higher optimum pH range than does *P. crenatus.*

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