

# Effect of Low Temperatures in the Field and Laboratory on Survival of *Pratylenchus penetrans*

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## ABSTRACT

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Effects of winter temperatures on the survival of *Pratylenchus penetrans* in annual ryegrass and orchard grass in upper New York State and in red clover on Prince Edward Island were investigated. The numbers of nematodes in soil depths of 0–15 cm decreased 35–59% from December to April during 4 yr when the average soil temperatures were –1.1 to –0.8 C. Nematode numbers increased 39% during one winter on Prince Edward Island when the soil temperatures ranged from 0.1 to 0.4 C. Nematode mortality in laboratory studies at both –12 and –8 C was about 93% after 4 hr of exposure. The duration of exposure needed to kill 50% of the population at –4, 0, and 4 C was 3.4, 124, and 742 days, respectively. These data may be a useful guide for predicting the size of spring populations of *P. penetrans* from samples collected in the fall or winter.

Throughout most of its range in North America, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven withstands cold, and in some areas, freezing soil for extended periods. Previous studies have indicated that temperatures suitable for movement of *P. penetrans* in soil and penetration into roots are unfavorable for survival in the absence of host roots (11). Survival is enhanced when soil temperature is just above freezing and the low metabolic rate of the nematode enables it to conserve its food reserves (11).

This paper presents data that show the change in numbers of *P. penetrans* from late fall and early winter to spring in upper New York State and Prince Edward Island. Information is included on the effect of low temperatures on nematode populations in the laboratory.

## MATERIALS AND METHODS

A field site (about 100 m<sup>2</sup>) of fine sandy loam (65% sand, 25% silt, 10% clay) at Freeville, NY, which had supported annual ryegrass (*Lolium* sp.) and orchard grass (*Dactylis glomerata* L.) for several years, was sampled for *P. penetrans* on 3 January (shown under December in

Table 1) and 3 April 1972. At each date, four 1.5-L samples were taken randomly with a small spade at depths of 0–15 and 15–30 cm and placed in plastic bags. Each sample was mixed thoroughly and a 50-cm<sup>3</sup> aliquot (estimated at 50 g) of soil with roots was placed on a Baermann pan (10) for 4 days. The criterion for survival was based on the ability of nematodes to move out of soil and roots into the extraction dish. Soil temperature data were obtained at the site during each sample time with an armored thermometer placed at the median height of each depth interval. Daily temperature data were acquired from a weather station 10 km from the site, maintained by the Division of Meteorology, Cornell University.

A field plot (0.45 ha) of red clover (*Trifolium pratense* L.) near Charlottetown, Prince Edward Island, was sampled for *P. penetrans* in November and December and in April and May from 1973 to 1977. Twelve samples were taken from the site at each date and placed in plastic bags. Each sample consisted of 20 soil cores taken randomly at a depth of 15 cm with a 25-mm-diameter soil probe. Soil samples with roots were mixed thoroughly, and 50-g subsamples were placed in a modified Baermann pan (10) for 7 days. The soil type was a fine sandy loam with an approximate particle size distribution of 70% sand, 20% silt, and 10% clay and a pH range of 5.5–5.9. The only cultivation during this period was in May 1976, when the plot was reseeded because of severe winterkill. Soil temperature data were obtained at the site during each sample time with an armored thermometer placed at the median height of each depth interval. Daily temperature data were acquired from a weather station 6 km from the site, maintained by the Agro-

Climatology Section, Prince Edward Island Department of Agriculture.

Mortality and survival of *P. penetrans* at –12, –8, –4, and 4 C were investigated at Cornell University in low-temperature freezers fitted with accessory controls and thermocouples connected to a multi-channel recorder. Nematodes extracted from Ranger alfalfa (*Medicago sativa* L.) callus tissue were mixed into autoclaved soil from the Freeville site. Aliquots of 50 cm<sup>3</sup> were placed in separate plastic bags and incubated in the freezer. Samples were assayed for nematodes by placing the 50-cm<sup>3</sup> aliquots on a Baermann pan for 4 days. The experiments conducted at –4 and 4 C were designed to facilitate probit analysis (5) of the results, treating the exposure temperature as a toxicant and the duration of exposure as the dose. Similar methods were used to study the survival of *P. penetrans* at 0 C at Charlottetown.

At each temperature, four samples were assayed for each time interval. At Cornell University, samples were assayed at –12 C for 0, 2, 4, 8, 11, 14, and 24 hr and at –8 C for 0 and 4 hr. Four tests were conducted at –4 C consisting of readings taken at about 24-hr intervals over 5–7 days. In the experiment to estimate nematode mortality at 4 C, the temperature at which samples usually are stored before processing, nematodes were extracted at 0, 7, 14, 43, 94, 195, and 223 days. Two tests were conducted at 0 C at Charlottetown. Data were acquired at 24-hr intervals over 7 days and subjected to probit analysis.

## RESULTS

Numbers of *P. penetrans* at depths of 0–15 cm decreased from late December to early April by 35–59%, except for a 39% increase in 1974–1975 (Table 1). The same trend was evident for the November-to-May interval. The 18% decrease in numbers at depths of 15–30 cm at Freeville was not statistically significant, though the 59% decline at 0–15 cm was significant and consistent with the observations made at Charlottetown.

Nematode mortality in the laboratory at –12 C was 92% after 4 hr of exposure and 97% after 24 hr. Similar results were obtained in the test at –8 C, where 94% of the nematodes had died after 4 hr. At –4 C, the median lethal exposure time averaged about 3.4 days, and the estimated mortality after 4 wk of

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**Table 1.** Overwintering of *Pratylenchus penetrans* at Freeville, NY, and Charlottetown, Prince Edward Island

Location Season	Nematodes per kilogram of dry soil ( $\times 100$ ) <sup>a</sup>					Percent change		Mean soil temperature (C) <sup>c</sup>	
	Nov.	Dec.	Apr.	May	SEM <sup>b</sup>	Nov.-May	Dec.-Apr.	Min.	Max.
Freeville									
1971-1972 <sup>d</sup>	...	17	7	...	0.103*	...	-59	-1.0	...
	...	11	9	...	0.065	...	-18	0.0	...
Charlottetown									
1973-1974	98	86	35	56	0.078*	-43	-59	-0.9	-0.6
1974-1975	149	155	215	195	0.044*	31	39	0.1	0.4
1975-1976	189	139	90	60	0.063*	-68	-35	-1.0	-0.6
1976-1977	106	97	57	57	0.061*	-46	-41	-1.5	-1.1

<sup>a</sup> Geometric means of four replicates for the Freeville data and 12 replicates for the Charlottetown data.

<sup>b</sup> Standard errors of the means in log<sub>10</sub> units since the analyses of variance were conducted on this scale. An asterisk indicates that the variance ratio was significant ( $P = 0.05$ ).

<sup>c</sup> Based on daily recordings at depths of 5 and 15 cm for January to March, inclusive.

<sup>d</sup> First and second rows correspond to depths of 0-15 and 15-30 cm, respectively, at Freeville. Charlottetown samples taken at 0-15 cm.

exposure was about 90% (Table 2). The time needed to kill 50% of the population at 0 C was about 124 days, and the estimated mortality after 4 wk was 42%. The median lethal exposure time of *P. penetrans* at 4 C was calculated at 742 days, and the estimated mortality after 4 wk was 13%.

## DISCUSSION

The overwinter survival rate at both field locations during 4 yr (1974-1975 excluded) averaged about 50% when mean minimum and maximum soil temperatures were about -1.1 and -0.8 C, respectively, from December to April. This is similar to the 33-65% reduction in numbers of *P. penetrans* during two winters in frozen loamy sand tobacco soils in southern Ontario (6,7). In contrast, Dickerson et al (3) in Wisconsin recorded an increase of 40% in the size of a population of *Pratylenchus* spp. during winter in frozen soil. The study, however, was carried out in a muck soil in which moisture relations were very different from those in the mineral soils used in our studies. Also, the nematode populations were very small and no statistical analyses were presented, so an estimate of the variability in the Wisconsin data is not possible.

The increase in numbers of *P. penetrans* during the winter of 1974-1975 at Charlottetown may have been due to the higher soil temperatures, which averaged between 0.1 and 0.4 C. Snowfall in January (123 cm) was twice the long-term average, and deep snow cover kept the soil from freezing. Townshend (11) stated that survival of *P. penetrans* is optimal when the temperature is just above 0 C. The lack of significant change in populations at Freeville at depths of 15-30 cm, where the minimum temperature was 0 C, is also consistent with the thesis that populations of this species are stable near 0 C. There also is some evidence that embryonic development can take place at temperatures slightly above freezing (4); Corbett et al (2) reported that a

**Table 2.** Mortality of *Pratylenchus penetrans* in soil in the laboratory at -4, 0, and 4 C calculated from probit analysis

Temperature (C)	Regression equation of probit mortality on log time	Median lethal exposure time (days) <sup>a</sup>	Estimated mortality <sup>b</sup> after 4 wk of exposure
-4	$Y = 3.98 + 1.61X$	3.4	90
0	$Y = 4.33 + 0.32X$	124	42
4	$Y = 2.73 + 0.79X$	742	13

<sup>a</sup> Duration of exposure necessary to kill 50% of the population.

<sup>b</sup> Based on percentage of initial population.

population of *Pratylenchus* spp. in a wheat field increased in size during the winter in England. Another plant-parasitic nematode, *Rotylenchus robustus* (de Man) Filipjev, has been observed feeding on perennial ryegrass (*Lolium perenne* L.) on agar plates at 0.5 C (1).

Average soil temperatures in the field never declined to levels where nematode numbers were reduced drastically as was observed in the laboratory studies at -12, -8, and -4 C. The acute mortality when temperatures dropped to -4 C or lower suggested that some major injury was suffered by *P. penetrans*. Sayre (9) reported that the body fluids of several nematode species passed from the super-cooled liquid state to the frozen state when temperatures declined to about -4 C. Mortality at 0 C was much less, with many of the deaths occurring during the first 24-hr period. This initial mortality, occurring when the drop in temperature was rapid compared with field conditions, may also have been responsible for the modest decline in nematode numbers at 4 C.

It is generally not feasible in the northern United States and Canada to collect large numbers of samples in the spring and return information to the grower before planting. The data presented here should be of use when attempting to predict the size of spring populations of *P. penetrans* from samples collected in the fall or winter. The sampling scheme in this study, however, provided estimates only to within about 30-60% of the true population mean (8).

Therefore, advice on nematicide treatments would have to be given with a cautionary note.

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