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Effects of Temperature on *Pratylenchus neglectus* and on Its Pathogenicity to Barley

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Abstract: In a petri-dish study, development of the nematode *Pratylenchus neglectus* was observed every 4 days, and stage-specific development times were estimated, using a parameter estimation algorithm for a distributed-delay population model. The lower threshold temperature for development of a population of *P. neglectus* was 7.75 C. Temperatures above 25 C were unfavorable for this population on barley. Total numbers of *P. neglectus* in barley roots and associated soil in pots were greatest at 25 C and lower at temperatures above and below that level. There was no change in nematode numbers per gram of root as temperature increased between 24 C and 32 C because root weights decreased at higher temperatures. Restricted root mass may contribute to the lower total nematode population levels at higher temperature. Maximum number of nematodes moved through a 2-cm layer of sand on a Baermann funnel at about 20 C; lowest number of nematodes moved at 10 C and 30 C.

Key words: barley, development, lesion nematode, lower temperature threshold, movement, nematode, population, *Pratylenchus neglectus*, temperature.

Pratylenchus neglectus (Rensch) Filipjev & Schuurmans Stekhoven is a migratory endoparasite distributed mainly in temperate regions. It has been reported in Europe (2), Canada (4,21,22), the United States (8), Australia (12), Japan (14), and northwestern India (27). *Pratylenchus neglectus* has been found associated with alfalfa, grain, rangeland grasses, and dryland wheat in the western United States (15,18). In California it has been reported on barley, potato, and a wide range of other crop plants (28).

Of the biotic and abiotic factors influencing nematode biology, temperature is particularly important. Temperature affects nematode movement, rate of development and reproduction, host status of plants, amount of associated damage to plants, distribution of these parasites, and many other functions (33). Mountain (19) reported that reproduction of *P. neglectus* on tobacco was maximum at 38 C, with the life cycle being completed in as few as 28 days. The optimum on alfalfa was 30 C (15). On soybean, a *P. neglectus* population increased most rapidly at 30 C but did not increase at 20 C (1). However, in the Klamath basin in northeastern California and southeastern Oregon, the soil temperature is rarely above 25 C, yet high numbers of this species are often found (7).

Temperature may affect reproduction and death rates of *Pratylenchus* spp. differently on various host plants (11). Moreover, nematode populations from different geographic origins have different thermal adaptations; for example, thermal optima differ for *Meloidogyne incognita* populations from the Netherlands and Venezuela (9). The biology and temperature requirements of the *P. neglectus* population occurring on potato and barley in the Klamath basin have not been studied. Our objectives were to determine the effects of temperature on development, reproduction, and movement of *P. neglectus;* the basal threshold temperature for development; and the impact of temperature on the pathogenicity of *P. neglectus* on barley.

MATERIALS AND METHODS

Soil: Soil was obtained from potato fields near Tulelake in the Klamath basin (48% sand, 37% silt, 15% clay; organic matter 11.5%, pH 7.2, cation exchange capacity 41.5 c · mol · Kg^{-1} and electrical conductivity 2.71 dS/m). Soil was sterilized by autoclaving, then aerated for 1 week and sub-

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Voucher specimens of *Pratylenchus neglectus* (UCDNC numbers 2917, 2918, and 2919) are deposited at the University of California Davis Nematode Collection.

sequently used for pot culture and experiments.

Nematode inoculum: P. neglectus was obtained from soil and root samples from the same location as the soil. Nematode cultures were established in pots (1,500-cm^3) containing barley (*Hordeum vulgare*) cv. Steptoe and maintained in the greenhouse at 25 C. For petri-dish experiments, *P. neglectus* was cultured on excised barley roots growing on Gamborg's B5 medium (13). The method described by Huettel and Rebois (16) was followed for establishing the excised roots and, with some modification, for surface disinfesting the nematodes. Nematodes were extracted from the barley roots in culture pots by the mist chamber technique (29) and concentrated to about 10 ml using a 25-μm-pore sieve. An equal volume of a solution containing 0.004% Aretan + 2% streptomycin sulfate was added to the nematode suspension, thus exposing the nematodes to 0.002% Aretan and 1% streptomycin sulfate. This suspension was aerated for about 30 minutes, washed with sterile water on a 25-μm-pore sieve, and collected in a petri dish. About 40 nematodes were hand-picked and transferred aseptically to each petri dish containing excised roots. Dishes were sealed with parafilm and incubated at 20 C. The nematodes were transferred to dishes with fresh medium and excised roots every 3 months.

Population development in petri dishes: Nematodes were extracted aseptically by transferring roots from culture dishes to test tubes containing sterile distilled water and stirring vigorously for about 90 seconds. Two ml of the suspension, containing ca. 55 nematodes of various stages (including eggs), were pipetted onto uniform-sized barley root explants established in petri dishes. Dishes were sealed with parafilm, placed at 20 C for 24 hours to allow the nematodes to become acclimated, and then incubated at 10, 12.5, 15, 20, 25, or 29.5 C. Four replications were maintained at each temperature. After 68 days, the nematodes were extracted from the roots and agar medium by the mist chamber technique (29) and counted with a dissecting microscope. The experiment was not repeated.

Stage-specific development in petri dishes: Roots and agar medium in culture dishes containing high numbers of nematode eggs were transferred to test tubes with sterile distilled water. The tubes were stirred vigorously for 2–3 minutes, and the contents were poured onto petri dishes containing 1.5% water agar. Juvenile and adult stages of the nematode were hand-picked from the dishes, and the eggs were incubated at 25 C. To obtain a single-age cohort of juveniles, the eggs were observed daily, and one freshly hatched second-stage juvenile (J2) was transferred to each of 400 dishes with root explants. The dishes were kept at 20 C for 12 hours for nematode acclimation and were then incubated at 15, 20, 25, and 30 C. From the sixth day onwards, four dishes at each temperature were destructively sampled every 4 days.

The roots were stained with acid fuchsin (6) and the developmental stages of the nematodes were determined (24). Adults prior to egg-laying were classified as "preadults"; those laying eggs were considered "adults." The observations at each temperature were stopped when J2 of the next generation were seen. The data were analyzed in several steps:

1. Assuming 7 C as the minimum threshold temperature for development, the duration in degree-days for each life stage from J2 to preadult at different temperatures was estimated using the parameter estimation algorithm for a distributed-delay population model developed by Schneider and Ferris (25). The goodness-of-fit between observed and predicted numbers at each stage was assessed using weighted least squares (25). The combination of predicted numbers with minimum value for the least squares was selected as the best estimate of the parameters. A chi-square test ($P = 0.01$) for the observed and predicted numbers was done to verify the predicted values.

2. The estimated duration of each stage, in degree days (DD), was converted to julian days. Using different minimum

threshold temperatures from 1 to 10 C, the stage duration in degree days was recalculated. By minimizing the coefficient of variation (CV) for the stage durations across all stages, the minimum threshold temperature was established as 5.25 C. Step 1 was then repeated using 5.25 C as the minimum threshold temperature.

3. The proportion of development per DD, calculated as the inverse of time taken to complete development from J2 to preadult stage, was determined for the different temperatures (15 to 30 C) and fitted to a linear regression model to validate the concept of degree days. The slope of the model did not differ from zero, but it had a very low negative value. By omission of 30 C from the linear model, a positive slope, not significantly different from zero, was obtained. Thus, 30 C was considered to be above the upper threshold temperature, and steps 1 to 3 were repeated, omitting data at 30 C. At step 3, minimum CV was obtained at a threshold temperature of 7.75 C, and this was used in subsequent estimations. Results of both analyses, with and without 30 C, are presented.

The durations of adult and egg stages were not determined because the experiment was terminated before completion of these stages. However, degree days from the first observation of preadult to egg, and from egg to first observance of second-generation J2, were calculated based on the minimum threshold temperature determined for the other stages.

Population development and pathogenicity in pots: Nematodes were subjected to a wider range of temperatures in pots than in the petri-dish experiments. Barley seedlings, cv. Steptoe, were grown in 1,500-cm^3 pots filled with autoclaved Tulelake soil. The pots were embedded in 5,000-cm^3 plastic buckets filled with steam-sterilized sand. When the seedlings were 7 days old, the plastic buckets containing the pots were placed in constant-temperature water baths at 8, 15, 20, 24, 28, and 32 ±2 C. After 3 days, 20 ml of nematode suspension containing ca. 2,700 *P. neglectus* were added per pot. The nematode suspension was pipetted into six holes about 5 cm deep between the seedlings and was covered with soil. The control treatments received 20 ml of tap water without nematodes. There were 12 seedlings per pot, and seven replicate pots were maintained at each temperature. After 68 days, nematodes were extracted from roots in the mist chamber for 7 days and from soil by the combination of elutriation and centrifugal flotation (5), and counted with a dissecting microscope. The pathogenicity of *P. neglectus* at different temperatures was assessed by comparing the fresh root and shoot weights of plants with and without nematodes. The experiment was not repeated.

Movement through saturated sand: Twenty-four Baermann funnels with a 2-cm layer of 60-mesh sand were set up in constant temperature rooms at 10 C, 15 C, 20 C, 25 C, and 30 C in the dark. Water level in the funnels was maintained at the top of the sand layer. About 1,350 *P. neglectus* of different growth stages in 2.5 ml of suspension were gently pipetted on top of the sand in each funnel. Six funnels were destructively sampled from each temperature 12, 24, 48, and 72 hours after inoculation. A sample consisted of 25 ml of suspension collected from the bottom of a funnel. One ml of the suspension was placed in a counting slide, and the number of *P. neglectus* was counted by stage with a dissecting microscope. The number of nematodes recovered was regressed on temperature. The cumulative number of nematodes recovered per unit time was fitted to an exponential decay model. The experiment was repeated.

Results

Population development in petri dishes: Pratylenchus neglectus reproduced at all the temperatures tested (Fig. 1). Total numbers of nematodes were linearly related to temperature up to 25 C and then decreased at 29.5 C. Therefore, data at 29.5 C were not included in the linear regression analysis.

Stage-specific development in petri dishes: Without including 30 C in the analysis, the

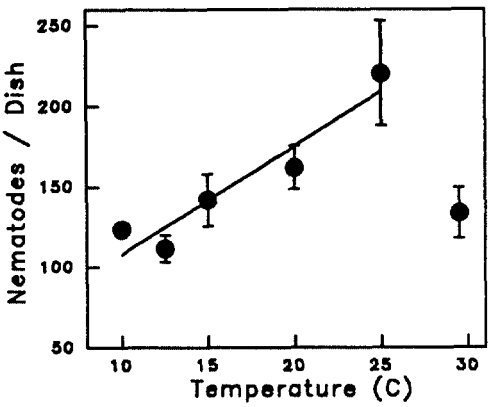

FIG. 1. Effects of temperature on the numbers of *Pratylenchus neglectus* per petri dish, incubated for 68 days, on excised barley roots. The values are the means (±SE) of four replications, and the regression model based on the means is $Y = 40 + 6.8X$, $R^2 = 0.95$.

basal threshold with the minimum CV for the number of degree days to complete development from J2 to preadult stage was 7.75 C (Fig. 2). The duration of each stage was 184 ± 22, 119 ± 7, 145 ± 12, and 120 ± 16 $DD_{7.75}$ for J2, J3, J4 and preadult, respectively (Table 1). The total duration of development for stages J2, J3, J4, and preadult was 568 ± 20 $DD_{7.75}$. The egg was first observed 108 ± 22 $DD_{7.75}$ after the observation of preadult, and the second-generation J2 was first observed 143 ± 4 $DD_{7.75}$ after the first egg (Table 1).

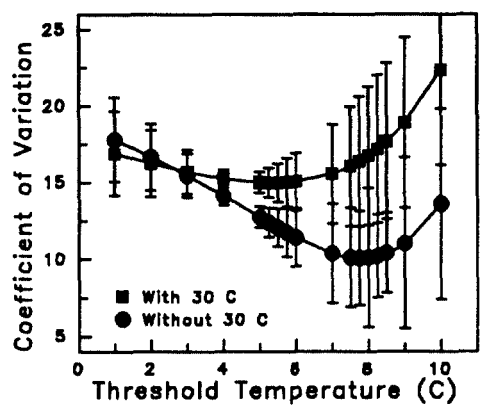

FIG. 2. Coefficient of variation (CV) of number of degree days to develop from second-stage juvenile (J2) to preadult stage. Uniform-aged J2 were incubated at 15, 20, 25, and 30 C until the next generation J2 were seen. The nematodes were observed every 4 days by staining the roots. The stage durations were estimated by the parameter estimation algorithm based on distributed delay population model of Schneider and Ferris, 1986. The minimum CV was determined on data with and without 30 C, as we consider 30 C to be slightly above the upper threshold temperature.

TABLE 1. The stage-specific duration in degree days for development of *P. neglectus* from second-stage juvenile (J2) to preadult (PA) stage, incubated at 15, 20, 25, and 30 C.

Temperature (C)	Developmental stage				PA-Egg†	Egg-J2‡
	J2	J3	J4	PA		
			$DD_{7.75}$			
15	189	119	158	127	87	145
20	208	111	129	97	98	147
25	156	128	147	135	138	138
Mean	184	119	145	120	108	143
SD	22	7	12	16	22	4
			$DD_{5.25}$			
15	254	162	212	171	117	195
20	251	133	157	120	118	177
25	171	156	161	168	158	158
30	220	205	196	212	198	198
Mean	224	164	182	168	148	182
SD	33	26	23	33	33	16

† Degree days from first observation of preadult to first observation of egg.

‡ Degree days from first observation of egg to first observation of second-generation J2. Degree days were calculated using the minimum threshold temperature determined for other stages.

When 30 C was included in the analyses, the lower threshold temperature of 5.25 C yielded the minimum CV (Fig. 2). At this threshold temperature, the development from J2 to preadult was completed in 738 ± 79 $DD_{5.25}$. The egg and second-generation J2 were first seen 148 ± 33 and 182 ± 16 $DD_{5.25}$ after the initial observation of preadult and egg, respectively (Table 1). The observed and predicted values at each stage did not differ ($P = 0.01$) by chi-square tests (data not presented).

Population development and pathogenicity in pots: As in the petri-dish experiment, nematode numbers in pots increased with temperature levels. However, nematode numbers were not linearly related to temperature (Fig. 3A). Hence, a spline curve was fitted to the data. The numbers of nematodes in the roots and in the soil were highest between 24 C and 28 C and signif-

icantly lower at 8 C, 15 C, and 32 C in roots (Fig. 3A). The number of nematodes in the total root system increased with temperature up to 20 C, was similar at 20–28 C, and then declined at 32 C. However, the number of nematodes per gram of root increased up to 32 C (Fig. 3B).

Root weight decreased linearly as soil temperature increased (Fig. 4A). Root weight did not differ among the plants inoculated with *P. neglectus* and the uninoculated controls. Also, there was no interaction between temperature and nematode on the root weight.

The shoot weight of uninoculated plants increased with temperature up to about 20 C and then decreased (Fig. 4B), whereas the shoot weight of plants inoculated with nematodes decreased at temperatures above 15 C. The difference in shoot weight of plants inoculated with *P. neglectus* and the plants without nematodes was greatest at about 24 C and least at higher and lower ends of the temperature range tested.

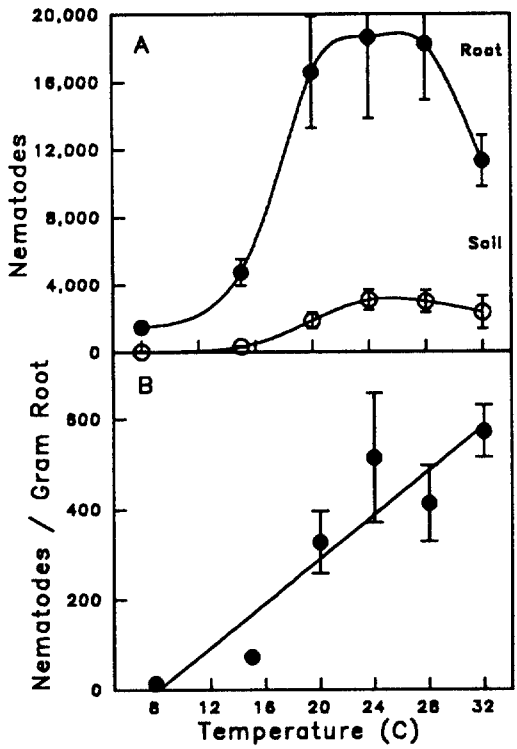

FIG. 3. Effects of temperature on *Pratylenchus neglectus*. A) Total numbers of nematodes in barley roots and associated soil in pots. The nematodes were extracted from the roots in the mist chamber for 7 days and from the soil by the combination of elutriation and centrifugal flotation. The values are the means (±SE) of seven replications; B) Numbers of *P. neglectus* per gram of root. The values are the means (±SE) of seven replications, and the regression model based on the means is $Y = 202 + 25X$, $R^2 = 0.94$.

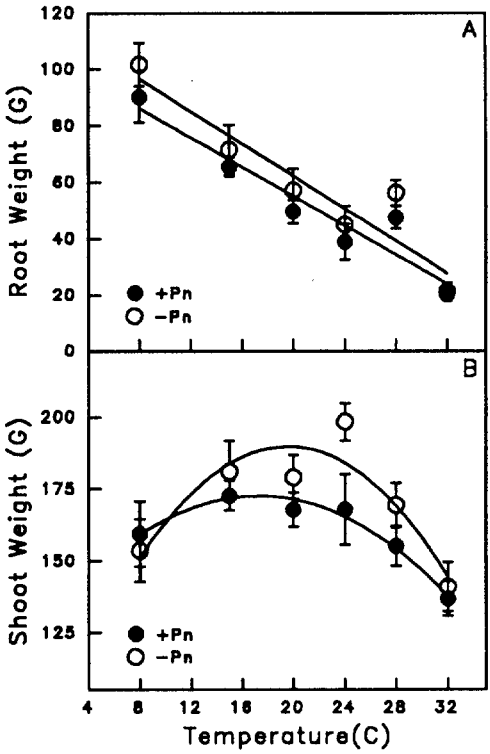

FIG. 4. Influence of *Pratylenchus neglectus* on the growth of barley at different temperatures. A) Root weights (−Pn = plants without nematodes; +Pn = plants inoculated with *P. neglectus*). The values are the means (±SE) of seven replications, and regression models based on the means were −Pn: $Y = 119 - 2.9X$, $R^2 = 0.94$; and +Pn: $Y = 106 - 2.6X$, $R^2 = 0.95$; B) Shoot weights (−Pn = plants without nematodes; +Pn = plants inoculated with *P. neglectus*.) Regression was done on the means, and the values are the means (±SE) of seven replications. Outliers in treatments, −Pn at 20 C and 32 C, and +Pn at 24 C and 32 C, were removed; thus these treatment values are means (±SE) of six replications. The regression models based on the means were −Pn: $Y = 78.9 + 11.4X - 0.29X^2$, $R^2 = 0.92$; and +Pn: $Y = 125.7 + 5.5X - 0.16X^2$, $R^2 = 0.99$.

Movement through saturated sand: All the growth stages of *P. neglectus* were active at all the temperatures tested. The total number of all motile stages of nematodes recovered increased with temperature up to

about 20 C and decreased slightly with further increase in temperature at all time intervals tested (Fig. 5A). A similar pattern of recovery was observed for all life stages except for J2 at the 12-hour interval, which had a linear relationship with temperature up to 30 C (data for different stages not presented). The number of nematodes recovered per unit time was fitted to an exponential decay model, $Y = K(1 - e^{-at})$, where Y = the number of nematodes recovered, K = the maximum number of nematodes that can possibly be recovered, a = the decay rate, and t = the time in days. Between 88–95% of the variation is explained by this model (Fig. 5B). The decay rate was higher at temperatures at which the nematodes were more active.

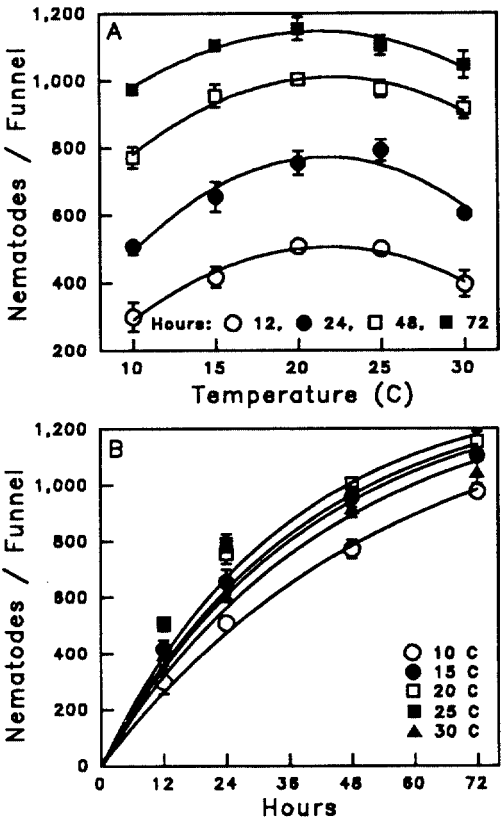

FIG. 5. Influence of temperature on the movement of *Pratylenchus neglectus*. A) Nematode movement through a 2-cm layer of saturated sand at different time intervals. The models for regression of nematode movement on temperature were for 12 hours: $Y = -228 + 67X - 1.6X^2$, $R^2 = 0.99$; for 24 hours: $Y = -198 + 90X - 2.1X^2$, $R^2 = 0.97$; for 48 hours: $Y = 237 + 71X - 1.6X^2$, $R^2 = 0.98$; and for 72 hours: $Y = 545 + 57X - 1.4X^2$, $R^2 = 0.98$. The values are the means (±SE) of six replications, and regressions were based on the means. The results of two trials were similar, and data from the second trial are presented here; B) Movement of *P. neglectus* over time fitted to a decay-rate model, $Y = K(1 - e^{-at})$, where Y = the number of nematodes recovered, K = the maximum number of nematodes that can possibly be recovered, a = decay rate, and t = time in days. (Symbols = means [±SE] of six observed values; line = values predicted by the model.) The value of K = 1,356 for all temperatures. The value of a at different temperatures is as follows—10 C: 0.0179, $R^2 = 0.95$; 15 C: 0.0247, $R^2 = 0.93$; 20 C: 0.0283, $R^2 = 0.92$; 25 C: 0.0259, $R^2 = 0.88$; and 30 C: 0.0224, $R^2 = 0.88$.

DISCUSSION

Numbers of *P. neglectus* on excised barley roots in petri dishes were correlated with temperature up to 25 C. Temperature above 25 C appears to be detrimental to nematode development, as the population at 29.5 C was significantly lower than at 25 C on barley.

The variation in the number of degree days for development of each stage at different temperatures could be due to the observation of a different subset of the cohort at each sample time. The variation probably would have been less if the same nematode was observed throughout the course of its development. The differences in the estimates may also have been reduced by monitoring more frequently. The stage duration of the first-generation J2, 184 $DD_{7.75}$, seems disproportionately long. This situation may reflect a time lag by the nematode to overcome the shock of handling and finding a suitable feeding site. The duration of the second-generation J2, or those that emerge from eggs within the root, may be lower. The stage durations for adult and egg could not be estimated using the algorithm; hence, the durations are presented from preadult to first observation of egg and from egg to first observation of second-generation J2.

The effects of temperature on nematode development may be direct or indirect by altering the physiology of the host root. It is difficult to differentiate between

the two effects. Conceivably, if the thermal relationships of the host are different from those of the nematode, the apparent effect of temperature on the nematode is likely to vary with host. Barley-root mass decreased with increasing soil temperature. Because of this effect on root mass, the population of *P. neglectus* per gram of root increased with temperature and was maximum at 32 C, although the total population decreased at temperatures above 24 C.

Population levels in both the excised root and whole-plant experiment followed the same trends with relation to temperature. Relatively cool to moderate soil temperatures (20 C to 25 C) favored the development and reproduction of this population of *P. neglectus*. Small increases in soil temperature above 15 C resulted in large increases in the *P. neglectus* population size. Maximum population levels of *P. neglectus* at moderate temperatures may reflect its adaptation to cooler regions of the Pacific Northwest. The population levels in the pot experiment appear to be similar between 24 and 28 C. This may represent a carrying capacity of the root system, or the number of generations that were completed in the duration studied.

From our experiments, we infer that the optimum temperature for development of this population of *P. neglectus* is about 25 C. In studies on soybean and alfalfa, the optimum temperature for reproduction of *P. neglectus* was 30 C (1,15). The difference in optimum temperature for reproduction between these and the present studies may be due to adaptation of the nematode to its local environment (9) or to indirect effect of the host plant. The *P. neglectus* populations in the earlier studies were from Nebraska and Utah, where summer soil temperatures are higher than in the Klamath basin. Dickerson (11) observed that maximum reproduction of *Pratylenchus scribneri* and *P. alleni* occurred at 35 C; however, they did not reproduce at temperatures below 27.5 C on soybean but reproduced at 20 C on tomatoes. Such studies indicate that temperature limitation depends on the nematode–host interaction and not on the nematode alone.

Although *P. neglectus* is primarily a parasite of grasses, including cereals (32), it has a wide host range. This nematode species is commonly found on wheat, oats, and barley (17). In our greenhouse experiment, *P. neglectus* appeared to be a weak pathogen. It did not affect barley root weight, but it suppressed shoot growth at about 24 C. Likewise, *P. neglectus* was inferred to be a weak pathogen on Stephens wheat under experimental conditions (18). According to earlier studies, pathogenicity of this nematode varies with host plants and soil types. Growth (dry weight) of barley was restricted (3), whereas seedling emergence and seedling stand of alfalfa cv. Saranac were not affected (31). In marine clay soil, *P. neglectus* did not restrict the tuber yield of potato (26), but it limited the total number and weight of tubers in loamy sand (20).

Temperature is a significant factor in nematode activity and behavior. For instance, Prot and Van Gundy (23) demonstrated that *M. incognita* and *M. hapla* differ in their ability to migrate and to infect tomato roots at 14 C. Similarly, maximum penetration of corn roots was at 20 C for *P. penetrans* and at 30 C for *P. minyus* (= *P. neglectus*) (30). In our study, maximum activity of *P. neglectus,* as indicated by its movement through sand, occurred at about 20 C. The difference in activity optima may correspond to the cooler spring soil temperatures in the Klamath basin when nematodes are locating and penetrating host roots.

The decay rate of nematode movement with time was higher at temperatures at which they are more active. Thus, the proportion of nematodes moving is reduced at a faster rate at these temperatures. We speculate that the nematodes deplete energy resources earlier at temperatures at which they are more active.

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