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Effect of Temperature on *Pratylenchus penetrans* Development

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Abstract: Reproduction and development of *Pratylenchus penetrans* were studied on genetically transformed ladino clover roots. Solitary females developing on transformed roots in nutrient gellan gum medium (pH 5.5) deposited 1.2, 1.5, 1.6, 1.8, and 2.0 eggs per day at the respective temperatures of 17, 20, 25, 27, and 30 °C. The number of eggs deposited was highly correlated with temperature. A reduction in egg-laying rates at the start of hatching was observed at all temperatures. Juvenile mortality was higher at 17 °C (50.4%), 20 °C (50.3%), and 30 °C (58.4%) than at 25 °C (34.6%) and 27 °C (37.6%). Life-cycle (egg deposition to egg deposition) duration was 46, 38, 28, 26, and 22 days at the respective temperatures. The developmental zero degrees (°C) and the effective accumulative temperatures (degree-days) required for hatching, female emergence, and onset of oviposition (completion of one generation) of *P. penetrans* were estimated to be 2.7 and 200, 4.2 and 548, and 5.1 and 564, respectively. *Pratylenchus penetrans* reproduces over a wide range of temperatures.

Key words: degree-day, developmental zero degree, egg-laying rate, effective accumulative temperature, ladino clover, life cycle, nematode, *Pratylenchus penetrans*, reproduction, transformed hairy root.

Pratylenchus penetrans (Cobb) Filipjev & Schuurmans Stekhoven is recorded on more than 350 hosts and is distributed widely throughout temperate areas of the world (Corbett, 1973). In Japan, this nematode mainly has been reported from areas with an annual mean atmospheric temperature of 15 °C or less (Gotoh, 1974). The rate of development of *P. penetrans* was nearly a linear function of temperature, and higher temperatures (30 °C and 33 °C) affected fecundity (Mamiya, 1971). Although this species completed its life cycle by 35 days at 21 °C on both potato and onion plants (Wong and Ferris, 1968), optimum temperature for reproduction varied depending on the test plants (Acosta and Malek, 1979; Dickerson et al., 1964; Griffin, 1993; Kimpinski and Willis, 1981). Soil pH (Kimpinski and Willis, 1981; Morgan and MacLean, 1968; Willis, 1972) and moisture (Kable and Mai, 1968) also affected reproduction of *P. penetrans*. Because soil temperature affects activities of nematodes and greatly influences or regulates other parameters (Norton, 1978), thermal studies on the functions of reproduction, female maturity rate, egg-laying rate, mortality of each stage, and duration of oviposition have primary importance. High temperatures apparently accelerated *P. penetrans* female development (Mamiya, 1971). The egg-laying rate of *P. penetrans* has been studied at 23 °C (Zunke, 1990b) and at various other temperatures (Mamiya, 1971). However, the effect of temperature on the duration of egg-laying and mortality is unknown. In addition, the developmental zero degree and effective accumulated temperature required for *P. penetrans* to pass through each developmental stage have not been reported.

The objective of this study was to identify how parameters such as egg-laying rate, hatching rate, mortality of juveniles, and duration of life cycle of *P. penetrans* vary with temperature.

MATERIALS AND METHODS

Experiments were conducted using genetically transformed *Triforum repens* L. race *giganteum* (Ladino clover) roots, as application of transformed roots to the study of the life cycle in *Meloidogyne* species has proven successful (Adachi et al., 1992, 1993). Transformed roots produced according to Adachi et al. (1993) were maintained on Woody Plant Medium (WP) (Lloyd and McCown, 1980). Fifteen-mm-long root tips were placed in 4 ml of root culture media (RM)

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(Becard and Fortin, 1988) in 35- × 10-mm tissue culture dishes (Falcon 3001, Becton Dickinson, produced by Radie Inc., Takasaki, Gunma, Japan). Each root was incubated at 25 °C for at least 24 hours until inoculation. The *Pratylenchus penetrans* culture used (Chiba-1 isolate) originally was collected from a farmer's carrot field in Funabashi City, Chiba, Japan, and maintained on carrot in the greenhouse. A pure isolate from a kidney bean pot culture, which had been propagated from a single female and seven males obtained from the carrot pot culture, was aseptically inoculated to lucerne callus induced by modified Schenk and Hildebrand medium (Mitsui, 1977), then cultured for a year as a monoxenic callus culture. Inoculum was extracted for 24 hours with Baermann funnels (Mitsui, 1974). Nematodes were washed 3 times with sterile tap water. A female was micropipetted onto RM in a few microliters of water about 10 mm from the root. After inoculation, culture dishes were sealed with a strip of Parafilm "M" (American National Can, Greenwich, CT). Culture dishes were kept in an incubator at 25 °C for 24 hours for the experiments at 17 to 27 °C. Nematodes inside culture dishes were then observed with a dissecting microscope, and dishes with free females or dead females outside root tips were discarded. The remainder were kept in incubators at 17 ± 1, 20 ± 1, 25 ± 0.5, and 27 ± 1 °C. These experiments started 24 hours after the real inoculation. Unlike experiments at other temperatures, the experiment at 30 °C was started soon after inoculation, and kept in an incubator at 30 ± 1 °C. Dishes with free females or dead females outside root tips were discarded after 24 hours. To reduce possible detrimental effects on nematode reproduction due to shortage of nourishment, roots kept at 20, 25, 27, and 30 °C were removed from tissue culture dishes after 14 days; those kept at 17 °C were removed 21 days after inoculation and transferred to dishes filled with fresh medium. Experiments at 17, 20, 25, and 27 °C were composed of two observations, while a single experiment was conducted at 30 °C. Twelve to 16, 8 to 16, or 8 to 12 petri dishes were sampled from 17 and 25 °C, 20 and 27 °C, and 30 °C, respectively, at each interval after inoculation. Removed roots were stored in triethanolamine-formalin (TAF) preservative until they were studied. Nematodes or eggs present in the substrate were counted after roots were removed. Each root preserved in TAF was washed with tap water three times, and placed in 3-ml glass tubes with 1 ml tap water. Roots and nematodes were stained with an NaOCl-acid fuchsin-glycerin method (Byrd et al., 1983). Roots were then pressed between two pieces of glass and observed.

An experimental plan developed by Gadd and Loos (1941) and Mamiya (1971) was used to estimate the onset of events in the life cycle of *P. penetrans* inside roots. Instead of an overall mean, estimation of fecundity was taken from the means of data sets that excluded the lower half of the values and the maximum value from the entire data sets. All regression analyses, except early fertility (eggs laid), were performed using these upper-half means of the data sets. Regression lines for early fertility (eggs laid) were calculated using means of entire replications. The onset (day) of egg deposition and juvenile hatch by the first female were determined by interpolation of the regression equations to the abscissa (day). The first fertility (eggs laid) of the next generation was a mean of groups of eggs that were observed in remote loci from the initial colony. Then, the onset (day) of egg deposition by the next generation was measured by squaring a line having the regression coefficient of the early fertility (eggs laid) in the first generation and passing through the next generation's fertility at the first observed day.

Results

For all temperatures, 88% of initial females were present in root tissue at 2, 4, 7, 10, and 14 days after inoculation, but often disappeared at 17 days and thereafter. Fertility of females under identical temperature and sampling periods was less variable until juveniles started to hatch, but was increas-

ingly variable thereafter. In general, small, stained particles were observed near eggs and juveniles when there were fewer nematodes inside roots. A few females stayed in the culture substrates or were found in the TAF that was used as root preservative. During the first 2 weeks after inoculation, females were observed in roots at their basal trunk, and clusters of eggs and/or juveniles were observed near the females. At the end of the experiment, besides numerous eggs in the first established cluster, more than one group of eggs with or without a female were observed in remote sites. Before maturing, some nematodes migrated from the original sites to colonize remote sites of the root. Tracks of nematode movement on the substrate were observed at all temperatures after 35 days (17 °C), 32 days (20 °C), 14 days (25 °C), 10 days (27 °C), and 17 days (30 °C). The migration of nematodes was frequent on transformed ladino clover roots but rare in preliminary tests with transformed lettuce roots. This characteristic of transformed clover roots proved useful for the study of the life cycle, as the eggs deposited separately by the next generation of females permitted estimation of the first oviposition date by the second-generation females.

Oviposition occurred 4 days after inoculation at 17 °C (Fig. 1A), but was 2 days after inoculation at the other four temperatures (Fig. 1B–E). There were steady increases in egg numbers for the first 17, 14, 10, 7, and 7 days at 17, 20, 25, 27, and 30 °C, respectively (Table 1, Fig. 2). The oviposition rates during this period were 1.2, 1.5, 1.6, 1.8, and 2.0 eggs per day at the respective temperatures of 17, 20, 25, 27, and 30 °C (Table 1, Fig. 2). There was a significant correlation between the egg-laying rate of *P. penetrans* and temperature (Fig. 2). The regression lines at 17, 20, 25, 27, and 30 °C had an estimated onset of oviposition of 2.7, 1.6, 1.1, 0.7, and 1.6 days after inoculation, respectively. The rate of oviposition (eggs per day) at the experimental temperatures diminished to 0.3 (17 °C), 0.3 (20 °C), 0.4 (25 °C), 1.6 (27 °C), and 0.8 (30 °C) at 17, 14, 10, 7, and 7 days after inoculation, respectively.

Juveniles were first seen 17 days after inoculation at 17 and 20 °C (Fig. 1A,B), 14 days at 25 °C (Fig. 1C), 10 days at 27 °C (Fig. 1D), and 14 days at 30 °C (Fig. 1E), then steadily increased thereafter. The estimated onsets of juvenile hatch determinated from the regression equations were 17.5, 13.1, 9.7, 8.8, and 9.2 days after inoculation at the experimental temperatures of 17 to 30 °C, which coincided with the days when rates of oviposition diminished at the five temperatures (Fig. 1A–E). The estimated embryonic periods decreased as temperature increased: 14.7 days (17 °C), 11.4 days (20 °C), 8.6 days (25 °C), 8.1 days (27 °C), and 7.6 days (30 °C) (Table 1).

Adults at 17 °C were not observed until day 49, and the best approximation to adult emergence was 45.5 days after inoculation (Fig. 1A). At 20 °C, males first appeared on day 35, but females were not seen until day 39 (Fig. 1B). Thus, appearance of females at this temperature must have been between days 35 and 39. At 25 °C, males and females first appeared on days 25 and 28, respectively, and female appearance was between days 25 and 28 (Fig. 1C). At 27 °C, males first appeared on day 25, and a possible next-generation female also was first observed on day 25 (Fig. 1D). Adults at 30 °C appeared on day 25, and the appearance of females was between days 21 and 25 (Fig. 1E). Total juvenile development times for females were approximately 28.0 days (17 °C), 23.9 days (20 °C), 16.7 days (25 °C), 16.2 days (27 °C), and 13.8 days (30 °C). The duration from eggs to adult females were 42.7, 35.4, 25.4, 24.3, and 21.4 days for the respective temperatures of 17, 20, 25, 27, and 30 °C (Table 1).

At respective temperatures of 17, 20, 25, 27, and 30 °C, maxima of 30, 34, 30, 46, and 31 offspring (including eggs) were laid by one initial female in 35, 35, 17, 17, and 21 days, although maxima of 107, 69, 115, 127, and 40 offspring were produced during the incubation period (Table 1). The next generation of females laid 4.7 eggs on day 53 (17 °C), 2.9 eggs on day 42 (20 °C), 4.6 eggs on day 32 (25 °C), 1.8 eggs on day 28 (27 °C), and 2.0 eggs on day 25 (30 °C).

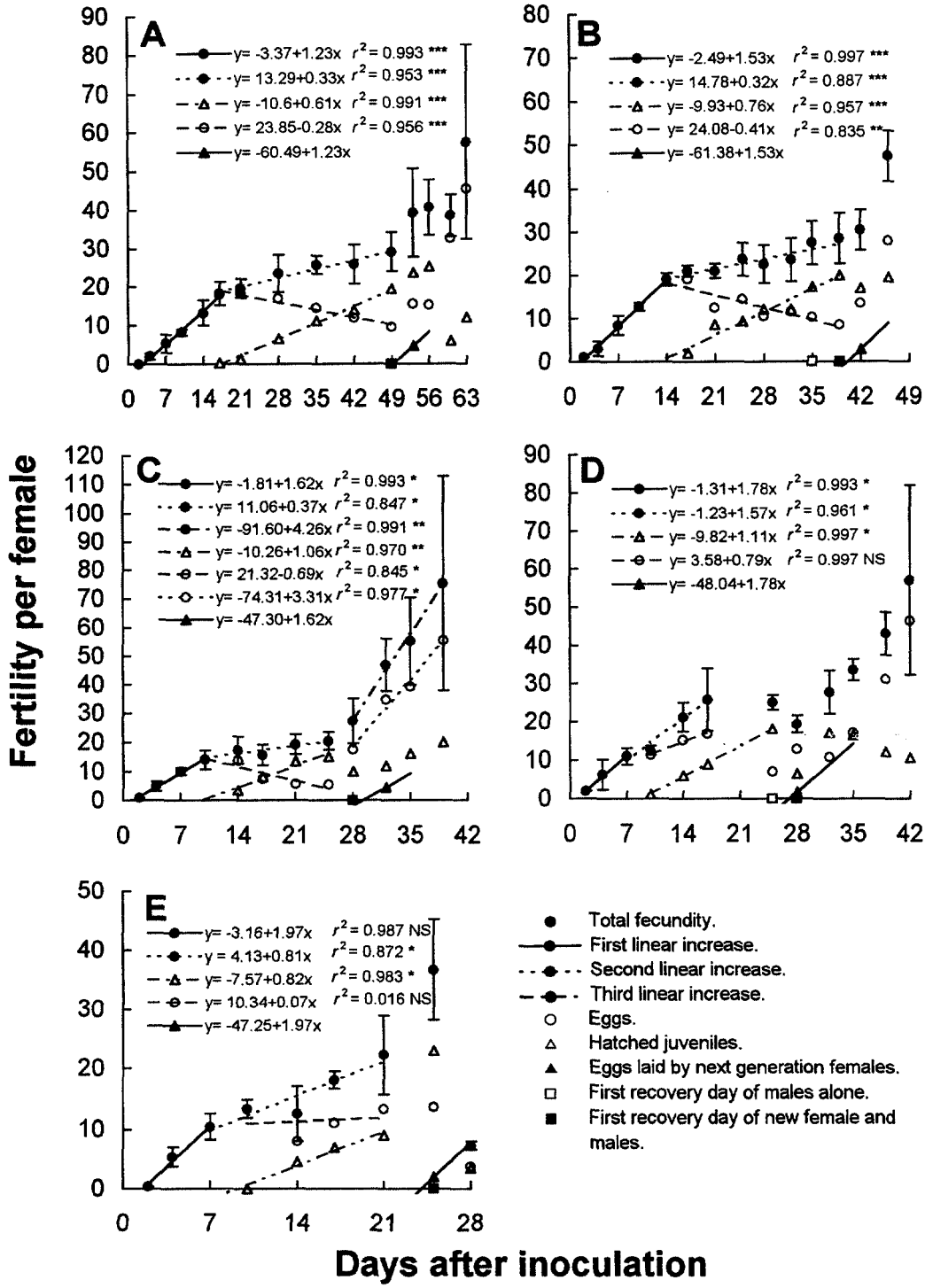

FIG. 1. Time course changes in total fecundity, hatched juveniles, and eggs of a single *Pratylenchus penetrans* female on transformed ladino clover roots. A) 17 °C. B) 20 °C. C) 25 °C. D) 27 °C. E) 30 °C. Bars show standard deviation for total fecundity. NS: not significant.

TABLE 1. Comparative population dynamics and development of *Pratylenchus penetrans* on transformed root explants and whole-seedling experimental systems at various temperatures.

	Transformed ladino clover root system[a] (Present study)					Conifer seedling system (Mamiya, 1971)				
	17 °C	20 °C	25 °C	27 °C	30 °C	15 °C	20 °C	24 °C	30 °C	33 °C
Egg-laying rate per day[b]	1.23	1.53	1.62	1.78	1.97	0.71	1.46	1.25	1.21	0.09
Maximal numbers of offspring during the incubation period (days)	107 (63)	69 (46)	115 (39)	127 (42)	40 (25)	49 (–)[c]	58 (35)	53 (42)	39 (28)	— —
Embryonic development (days)	14.7	11.4	8.6	8.1	7.6	25	14–16	8–10	9–10	—
Postembryonic development (days)	28.0	23.9	16.7	16.2	13.8	53	24	16	17	—
Duration from egg to adult	42.7	35.4	25.4	24.3	21.4	78	38	26	27	—
Duration from egg to egg	46.4	38.5	28.1	26.2	22.4	86	42–44	35	30–31	—

[a] Each temperature with 8 to 16 replications.
[b] Rate determined during a steady egg-laying period 7 to 17 days after inoculation.
[c] Duration in days not given.

The life cycle or generation time of *P. penetrans* were estimated to be 46.4, 38.5, 28.1, 26.2, and 22.4 days at 17, 20, 25, 27, and 30 °C, respectively. Generation time of *P. penetrans* (28.1 days at 25 °C) may be an underestimate because the numbers of eggs already had increased rapidly 28 days after inoculation (Fig. 1C).

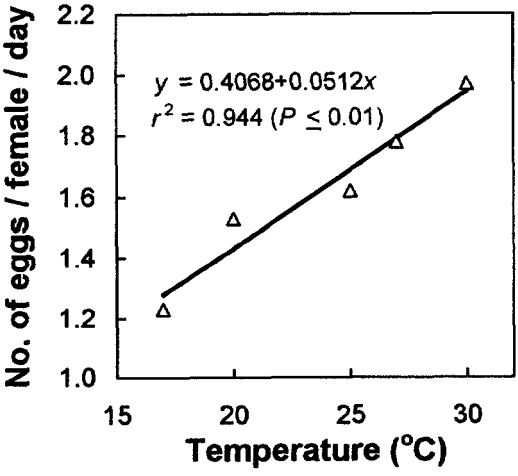

FIG. 2. Changes of number of eggs deposited per female *Pratylenchus penetrans* per day as influenced by temperature. Nematodes were inoculated onto transformed ladino clover roots cultured in nutrient medium.

Nematodes outside roots at 17 °C were first found on day 35 (16% of total nematodes excluding eggs), leveled off at 6–10% during days 35 to 56, then quickly increased to 95% on day 63 (Fig. 3). Males and females were found outside roots on day 49 and thereafter. At 20 °C, nematodes were not found outside roots until 28 days (one male observed), fluctuated between 10 to 31% of the entire population from day 32 to 42, and increased thereafter to a maximum of 67% on day 46 (Fig. 3). Nematodes at 25 °C were not seen outside roots until 14 days, when 29% of all juveniles were observed. Nematodes were not seen outside roots on days 17 and 21 after inoculation; however, they increased on day 25 and thereafter, reaching almost 100% on day 35 (Fig. 3). At 27 °C, many juveniles (33%) were found outside roots on day 14. The percentage of nematodes outside roots fluctuated between 4% and 23% from day 17 to day 39, and then increased to 68% on day 42 (Fig. 3). At 30 °C, nematodes outside roots were not seen until day 21, when 13% of all nematodes were observed on the medium surface. Nematodes outside roots then increased to 26% on day 25 and to 36% on day 28.

Eggs at all temperatures usually were laid

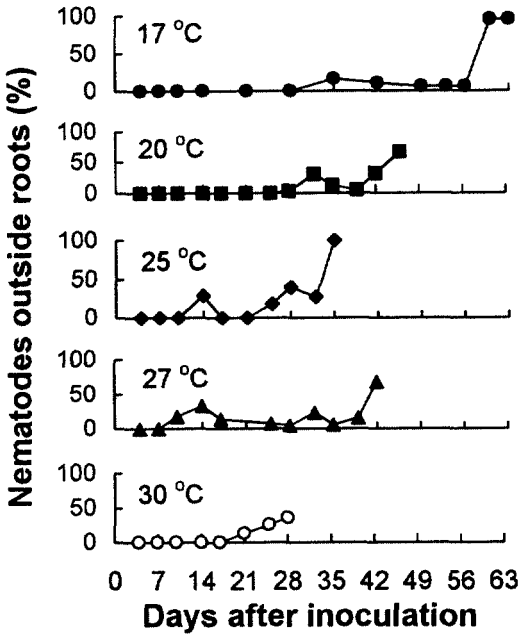

FIG. 3. Time course changes of hatched *Pratylenchus penetrans* outside roots as affected by temperature. The *y*-axis denotes population (juveniles and adults) outside roots as percentage of total population. Nematodes are the descendants of a single female in a transformed ladino clover root cultured in nutrient medium.

inside roots. Less than 3% of all eggs at 17 °C were found outside roots at 21, 35, and 63 days after inoculation. A few eggs (2%, 3%, and 1% of all eggs, respectively) were also found on day 46 at 20 °C, day 35 at 25 °C, and day 25 at 30 °C, respectively. However, at 27 °C, although only 2% and 1% of eggs were outside roots on day 17 and 28, as many as 12% of eggs were outside roots on day 42.

Rate of development per day for each temperature was given as a reciprocal of the days required to complete the life cycle. There was a significant correlation between the rates of development (y) and temperature (x) (Table 2). The regression equation was $y = -0.0090 + 0.001772x$. The temperature of 5.08 °C was the point where the line intersected the abscissa and thus was the estimated developmental zero point of *P. penetrans* for completion of its life cycle. The total effective temperature (K) required to complete a given developmental stage was expressed as follows:

$$K = (V - A) \times T;$$

where V = incubation temperature,
A = developmental zero degree,
T = days required to pass through a stage.

Pratylenchus penetrans required 564 degree-days to complete its life cycle (egg to egg). The developmental zero point (°C) and the total effective temperature (degree-days) required to pass through the respective developmental stages, including completion of the life cycle, are presented in Table 2. The developmental zero degrees differed depending on stages: 2.74 for passing through embryonic period; 4.18 for adult female emergence. Hatching and female maturation required 200 and 548 degree-days, respectively.

DISCUSSION

We observed that variability of nematode populations at all temperatures became increasingly large during the experiments. Because all the nematodes outside roots were counted, the lower fertility in some replications might be attributed to lower nematode populations inside roots due to death of

TABLE 2. Developmental zero degrees (°C) and total effective degree-days required to pass through three different developmental stages of *Pratylenchus penetrans*.

	Equation[a]	Developmental zero (°C)	Degree-days
Incubation period (Egg-deposition to hatch)	$y = -0.0137 + 0.005004x$ $r^2 = 0.9773$ ($P \leq 0.01$)	2.74	200
Female emergence period (Egg deposition to adult female)	$y = -0.0076 + 0.001824x$ $r^2 = 0.9928$ ($P \leq 0.001$)	4.18	548
Life-cycle duration (Egg deposition to egg deposition)	$y = -0.0090 + 0.001772x$ $r^2 = 0.9966$ ($P \leq 0.001$)	5.08	564

[a] y = velocity of development, x = temperatures (°C).

nematodes. Nusbaum and Barker (1971) considered that the mortality rate in phytoparasitic nematode populations might be related more to physiological responses of the host than to nematode density. In this study, we observed little necrosis (hypersensitive response) in ladino clover roots. However, Sawhney and Webster (1979) suggested the presence of other mechanisms of resistance besides the hypersensitive response on nematode-resistant tomato. Similarly, Veech (1981) noticed the presence of hypersensitivity without browning. Fate of the dead nematodes inside roots has never been illustrated, but we could observe the collapsed or shrunken eggs and juveniles of *Pratylenchus coffeae* inside sterile transformed soybean roots (unpubl.). Thus, it seems reasonable to hypothesize that plant roots have the ability to decompose dead nematodes. This hypothesis helps to explain why low nematode populations inside ladino clover roots often were accompanied by numerous stained particles. Given a decomposition ability of host roots, it seems reasonable to interpret the increasing variability of nematodes as correlated with increasing fluctuation of a root defense response. Thus, in the present regression analysis, we chose to use the means of upper-half data of the replications, expecting them to be adequately representative of potential fecundity.

The present study confirms the limited mobility of *P. penetrans* females inside roots (Mamiya, 1971). Females stayed near the sites where they entered after inoculation for at least 2 weeks. The presence of nematodes outside roots (Fig. 3) suggests that the movement of nematodes from the initial infestation took place through the medium, which presumably would be through soil under natural conditions. Zunke (1990b) observed that when *P. penetrans* penetrated roots, it subsequently exited the same hole and moved to another area of the root. Observation of a 1- to 2-week lag from juvenile hatching to the first peak of nematodes outside the root at lower and higher temperatures (17, 20, and 30 °C) is similar to results from *P. scribneri* (MacGuidwin, 1989), that the older stages (J4, adult) are more likely to leave roots than J2 and J3. Conversely, the first peak of juveniles outside roots at 25 and 27 °C occurred immediately after their hatching, indicating that migration activity of second-stage *P. penetrans* was greatest at 23–27 °C. However, an extremely high second peak of *P. penetrans* outside roots at the end of every experiment can be attributed to the nematode's response to root aging in the exhausted medium. MacGuidwin (1989) estimated 20–50% of field plot populations of *P. scribneri* to be in the soil. The present observation of eggs outside roots, along with ectoparasitic feeding behavior (Kurppa and Vrain, 1985; Zunke, 1990a), suggests that, as in *Pratylenchus agilis* (Rebois and Huettel, 1986), some *P. penetrans* can complete their life cycle outside roots as ectoparasites.

The rate of development of *P. penetrans* was found to be a linear function of temperature. This relationship also exists in *Ditylenchus dipsaci* (Griffith et al., 1996) and in species of the genera *Meloidogyne, Heterodera, Globodera, Longidorus, Goodeyus,* and *Caenorhabditis* (Trudgill, 1995). Egg-laying rate and development of *P. penetrans* from 17 to 30 °C were compared with results from a conifer (*Cryptomeria japonica*) seedling system (Mamiya, 1971) (Table 1). Mamiya (1971) found that higher temperatures (30 °C and 33 °C) suppressed the fecundity of *P. penetrans;* in the present study, 30 °C did not suppress fecundity. Temperature and number of eggs deposited ($r^2 = 0.944$; $P \leq 0.01$) were highly correlated, with a maximum of 2.0 eggs deposited per day at 30 °C (Fig. 2, Table 1). High fecundity of *P. penetrans* at 30 °C observed in this study helps to explain why this nematode often has been found in southern Japan (unpubl.) and in subtropical and tropical regions such as India and the Philippines (Corbett, 1973), Algeria (Lamberti et al., 1975), Egypt (Oteifa, 1962), and Brazil (Swarup and Sosa-Moss, 1990). Other thermotypes of this species may occur (Gotoh, 1974).

A sudden decline in egg-laying rate at each temperature was a common characteristic of this and a previous study (Mamiya, 1971). This phenomenon cannot be attributed to a reduction in the egg-laying rate

after production of a given number of eggs because the accumulated numbers of eggs (egg-laying rate × days) at these turning points did not result in constant values: 21, 21, 16, 18, and 14 eggs at 17, 20, 25, 27, and 30 °C, respectively. We interpret the phenomenon as the loss of juveniles after hatching because the turning point coincides with the onset of juvenile hatching at each temperature, and a juvenile regression line had lower regression coefficients than an early egg-laying line (Fig. 1A–E). Thus, the juvenile population was lost at a certain rate after hatching. Assuming the lost juveniles inside the roots decomposed after their death, as already described, the early juvenile mortality (death rate) can be computed by the following formula:

$$M_j = 1 - (R_j/R_e);$$

where, M_j = mortality of juvenile,
R_j = regression coefficient in the juvenile regression equation,
R_e = regression coefficient in the early fertility (eggs laid) regression equation.

Mortality of the juveniles thus computed was 50.4, 50.3, 34.6, 37.6, and 58.4% at 17, 20, 25, 27, and 30 °C, respectively. We assumed that this mortality is due to temperature effects because host response effects were removed when we chose to use the mean of the upper half of the replications (excluding the maximum value) as the potential fertility. Juvenile mortality was lower (one-third of fertility) at 25 and 27 °C than at 17, 20, and 30 °C (half of fertility), providing a possible explanation for why temperatures of about 25 °C are optimal for the multiplication of *P. penetrans* on corn (Dickerson et al., 1964) and soybean (Acosta and Malek, 1979). Conversely, Dunn (1973), Acosta and Malek (1979), and Griffin (1993) reported highest reproduction on alfalfa at 30 °C, and Dickerson et al. (1964) reported 16 °C to be the optimum on potato. Reproduction of *Pratylenchus* appears to depend on the nematode-host interaction, not only on the nematode (Dickerson, 1979). Temperature also affects plant resistance to nematodes, and resistance is often ineffective at high temperatures (Brueske and Dropkin, 1973; Okamoto and Mitsui, 1977).

Our results confirm the importance of temperature to the development of *P. penetrans*. Temperature affects egg-laying rate and juvenile mortality, probably through interaction with other factors. The information also is valuable in forecasting the occurrence of *P. penetrans*. The effects of temperature on longevity or the egg-laying period of female *P. penetrans* remain to be investigated.

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