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The effect of temperature on hatching and penetration of chickpea roots by *Pratylenchus thornei*

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Egg hatch of *Pratylenchus thornei* was influenced by temperature. It took place at all temperatures within the range 10–25°C and was optimal at 20°C. Root penetration increased steadily with increasing time of incubation up to the end of the experiment 11 days after inoculation. Temperature affected penetration rate in chickpea (*Cicer arietinum*) cultivars UC 27 and JG 62 but not in line P 2245, being significantly higher at 20–25°C than that at 15°C. At the end of the experiment, roots of line P2245 held at 15°C contained more *P. thornei* than cultivars UC 27 and JG 62. No difference in percentage penetration among host genotypes was observed at 20 or 25°C. All migratory stages of *P. thornei* penetrated roots of chickpea from the first to 11th days after inoculation.

INTRODUCTION

Pratylenchus thornei, the cereal and legumes root-lesion nematode (Fortuner, 1977), severely affects chickpea (*Cicer arietinum*) and wheat (*Triticum aestivum*) crops (Van Gundy *et al.*, 1974; Di Vito *et al.*, 1992; Tiwari *et al.*, 1992). Recently, *P. thornei* was associated with chickpea crops in southern Spain causing root growth reduction in plants grown under water stress and fluctuating temperature in the greenhouse (Castillo *et al.*, 1995a, 1996).

However, little information is available on the life cycle and pathogenesis of *P. thornei*. Penetration of the host roots by *Pratylenchus* spp. may vary with nematode species and life stages, and is known to be affected by several other factors including soil moisture, temperature and time of incubation (Sontirat & Chapman, 1970; Townshend, 1972; Van Gundy *et al.*, 1974; Olowe & Corbett, 1976; Marbon-Mendoza & Viglierchio, 1980; Olthof, 1982). Because inocula of *Pratylenchus* spp. for pathogenicity studies are composed of all life stages (eggs, second, third, and fourth-stage juveniles, and adults), it is important to determine the effect of temperature and time of incubation on egg hatching and penetration of migratory life stages of *P. thornei* into chickpea roots. A wide range of chickpea genotypes are good hosts of *P. thornei*, and few differences in their susceptibility to the nematode have been reported (Greco *et al.*, 1988; Castillo *et al.*, 1995). Therefore, a study was conducted to determine: (1) the effects of temperature on hatching of *P. thornei* eggs; and (2) the effect of temperature and chickpea genotype on penetration of roots by migratory life stages of *P. thornei.*

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MATERIAL AND METHODS

Hatching

Eggs of *P. thornei* were extracted from carrot-disk cultures by centrifugation (Dunn, 1973). A carrot disk was homogenized in 250 ml of water in a Waring blender at 12 600 r.p.m. for 30 s, then the mixture was centrifuged at 1500 ***g*** for 4 min. The supernatant was decanted, centrifuge tubes were refilled with 250 ml of a sucrose solution ($d = 1{\cdot}15$), mixed with a stirring-vibrator, and centrifuged for 3 min at 1500 ***g*** (Castillo *et al.*, 1995b). The sucrose solution was sieved repeatedly through a 325-mesh sieve and then through a sieve with 15 μm openings. The remaining migratory stages in an egg suspension were picked up under a stereomicroscope. One ml of nematode egg suspension in deionized water, containing about 200 eggs, was transferred to and incubated in petri dishes (50 mm diameter) containing a thin layer of water-agar (1·5%) at 10, 15, 20 or 25°C, in the dark, for 23 days, when experiments were terminated. The exact number of eggs per dish was determined at the beginning of the experiment. Newly hatched second-stage juveniles were counted at 2-day

intervals and the numbers were expressed as cumulative percentages based on the total number of eggs added to each dish. There were eight replications (dishes) for each temperature. The dishes were randomly positioned in an incubator set at the required temperature. The experiment was done twice.

Penetration

The influence of temperature and chickpea genotype on penetration of roots by all migratory life stages of *P. thornei* was determined at 15, 20 and 25°C, using chickpea line P 2245 and cultivar JG 62, both highly susceptible and cv. UC 27, less susceptible to the nematode (Castillo *et al.*, 1995a). A *P. thornei* population obtained from roots of 'Blanco Lechoso' chickpeas in Cañete de las Torres (Córdoba) was used. Inocula for experiments were produced from a single female in carrot disks (Castillo *et al.*, 1995b). For inoculation, nematodes were extracted from carrot-disk cultures by centrifugation as described above. Eggs were discarded by sieving repeatedly through a 300-mesh sieve. Migratory life stages were surface-disinfected with 0·02% ethoxyethyl mercury and 0·01% streptomycin sulphate solutions for 2 and 24 h, respectively, and nematode population densities were determined from 1-ml aliquots.

Chickpea seeds were germinated in sterile, moist petri dishes at 25°C for 3 days. Seedlings were selected for uniformity of radicle length and planted into 7-cm diameter plastic pots (one per pot) containing 0·21 of an autoclaved potting mixture (sand, clay loam: 2 : 1, v/v). Seedlings were inoculated with 200 nematodes (64% second-stage juveniles, 21% third and fourth-stage juveniles and 15% females) by adding 2·5 ml of nematode suspension around the radicle. Pots with inoculated seedlings were held in growth chambers adjusted to 15, 20 and 25 ± 1°C, 60–90% r.h., and a 14-h photoperiod of fluorescent light of approximately $360 \pm 25\ \mu E/m^2/s$. Roots were harvested at 2-day intervals from 1 to 11 days after inoculation, when observations were terminated. Roots were thoroughly rinsed free from sand in deionized water. Whole root systems were cut into 0·5 cm pieces, homogenized in a blender for 30 s and nematodes extracted by centrifugation as described above (Coolen, 1979). Total numbers of nematodes extracted from the roots of a plant at each sampling date were recorded and converted into percentages based on the number of nematodes added as inoculum. There were six replications (plants) per treatment for each sampling date, with plants completely randomized within a growth chamber. The experiment was done twice.

Statistical analysis

Similarity among experimental runs, tested by preliminary analyses of variance using experimental runs as blocks, allowed data to be combined for analyses of variance and regressions. Cumulative percentages of hatch at each temperature were subjected to linear regression analysis over time of incubation. Data were transformed into $\ln[1/(K - Y)]$ for linear regression analysis, where Y = cumulative hatched eggs and K = the maximum Y reached for each temperature. Cumulative percentages of hatch at the third, fifth, ninth and 11th days of incubation were subjected to non-linear regression analysis over temperature. Regressions were performed using 16 replicates from two experiments. For penetration experiments, percentages of nematodes penetrating the roots (Y) were transformed into $\ln(Y)$. Transformed data were subjected to analyses of variance and regression analyses to express changes over time in numbers of migratory life stages that penetrated into chickpea roots at the tested temperatures. Regressions were calculated using 12 replicates for each treatment combination and sampling date. Data were analysed using Statistix (NH Analytical Software, Roseville, MN). Treatment means were compared using Fisher's protected least significant difference test (LSD) at $P = 0{\cdot}05$. Coefficients of determination (R^2), coefficients of determination adjusted for degree of freedom (R^2_a), and patterns of residuals plotted against expected values were used to indicate appropriateness of the model to describe the data (Campbell & Madden, 1990). For regression analyses, slopes of linear regressions for egg hatch, different chickpea genotypes, temperatures and migratory life stages were compared by *t*-test at $P = 0{\cdot}05$ (Snedecor & Cochran, 1980).

RESULTS

Hatching

Percentage hatch of *P. thornei* eggs in deionized water was significantly ($P < 0{\cdot}05$) influenced by temperature throughout the experiment (Fig. 1). The percentage of egg hatch over time was described by the general equation $\ln[1/(K - Y)] = \beta_0 + \beta_1 t$, in which Y = cumulative hatched eggs, and K = the maximum Y reached for each temperature. Regression lines were:
(10°C) $\ln[1/(K - Y)] = 0{\cdot}257 + 0{\cdot}099t$, $R^2 = 0{\cdot}91$;
(15°C) $\ln[1/(K - Y)] = 0{\cdot}658 + 0{\cdot}087t$, $R^2 = 0{\cdot}97$;
(20°C) $\ln[1/(K - Y)] = 0{\cdot}392 + 0{\cdot}190t$, $R^2 = 0{\cdot}96$;
(25°C) $\ln[1/(K - Y)] = 0{\cdot}994 + 0{\cdot}131t$, $R^2 = 0{\cdot}79$.

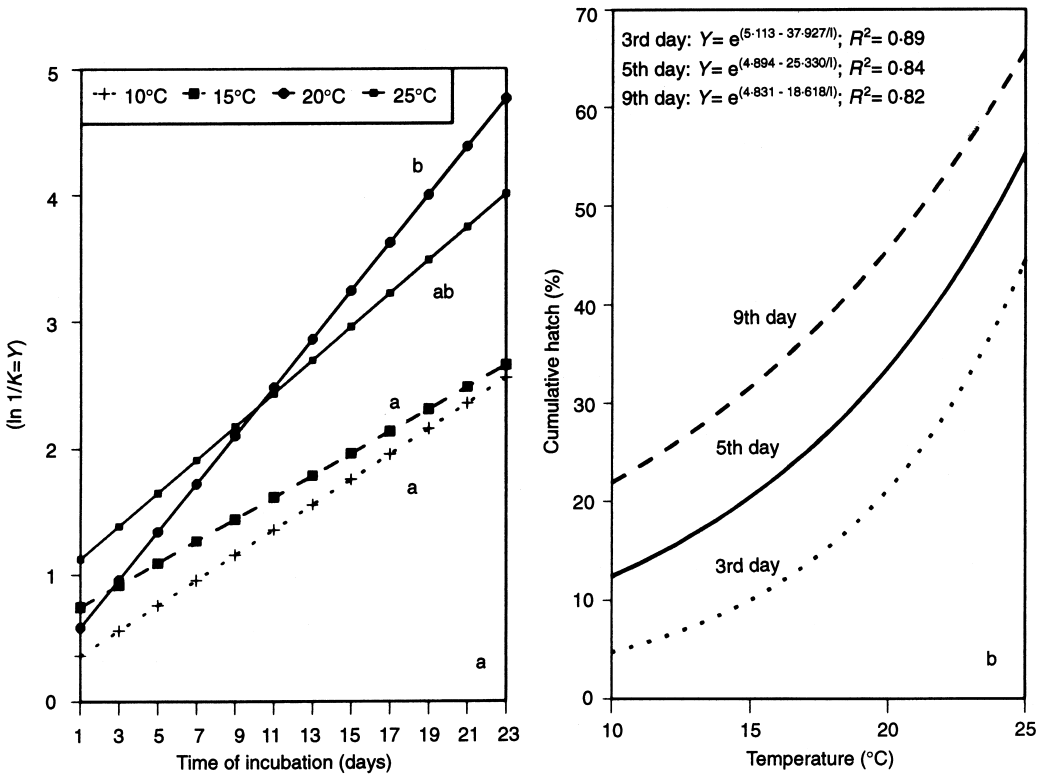

Fig. 1 (a) Linear regression of cumulative percentage hatch (Y) of *P. thornei* eggs over 23 days of incubation at different temperatures. Data were transformed into $\ln[1/(K - Y)]$ for regression analysis. Slopes of linear regressions with a letter in common do not differ significantly ($P = 0{\cdot}05$), according to *t*-test. (b) Influence of temperature on egg hatch after 3, 5, and 9 days of incubation. Percentage hatch was fitted to exponential models by the general equation $Y = e^{(a+b/T)}$. T, temperature of incubation; Y, cumulative percentage hatch.

All coefficients of determination were significant at $P = 0{\cdot}01$, except for 25°C when it was at $P = 0{\cdot}05$ (Fig. 1a). The intrinsic egg hatch rate (β_1) at 20°C was significantly higher ($P < 0{\cdot}05$) than that at 10 and 15°C, but did not differ from that at 25°C (Fig. 1a). Percentage hatch increased with temperature of incubation over the period (3–9 days), and was described (Fig. 1b) by the general equation $Y = e^{(a+b/T)}$, in which T = temperature, and (a, b) are constants for each day of incubation. Thereafter, cumulative percentage hatch increased to 20°C but decreased at 25°C (Fig. 2), and data were described by the general equation $Y = [a + bF(T)]^{1/2}$, in which Y = cumulative percentage hatch, T = temperature, the function $F(T) = \exp[-[\ln(T/20)]^2/(2c^2)]$, and a, b, c = constants (Fig. 2).

Penetration

The percentage of *P. thornei* penetrating roots of the three chickpea genotypes studied at 15, 20, or 25°C (including all migratory stages) increased steadily with increasing time of incubation up to the last sampling date 11 days after inoculation (Fig. 3). The increase of percentage nematodes penetrating the roots (including all migratory stages) over time was described by the general equation $\ln(Y) = \beta_0 + \beta_1 t$, in which Y = percentage nematodes penetrating a plant, and t = time after inoculation (days) (Fig. 3). Coefficients of determination were significant at $P = 0{\cdot}01$. Percentage nematode penetration of UC 27 roots at 20 and 25°C increased over time at a rate significantly higher ($P < 0{\cdot}05$) than that at 15°C; while for cultivar JG 62 differences in the rate of increase were significantly different ($P < 0{\cdot}05$) for 25 and 15°C, only; there were no differences among rates of increase of nematode penetration of P 2245 roots over time at 15, 20 and 25°C.

In the first three days of the experiment, temperature slightly affected penetration of *P. thornei* into roots (Table 1). At the end of the

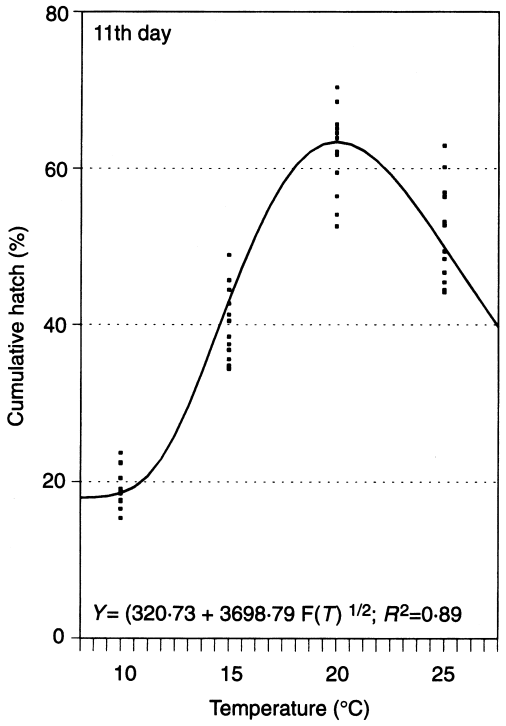

Fig. 2 Non-linear regression analysis of cumulative percentage hatch (Y) of *P. thornei* eggs over temperature of incubation at 11 days after incubation began. Symbols are the observed values (16 replicates from two experiments) used for regression analysis. $F(T) = \exp[-[\ln(T/20)]^2/(2^2)]$, T, temperature of incubation; c, a constant.

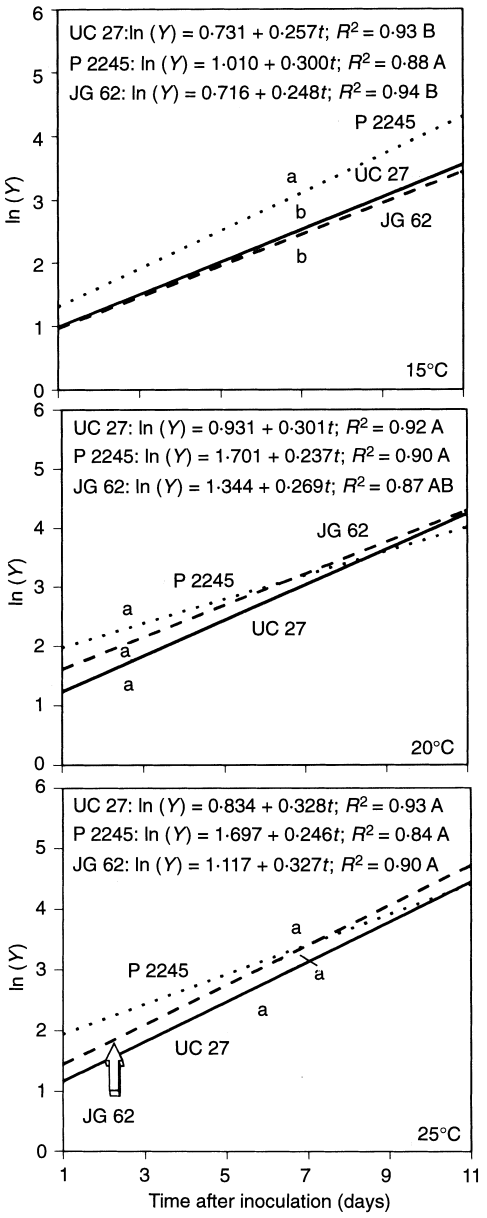

Fig. 3 Influence of temperature on penetration of *P. thornei* into roots of chickpea line P 2245 and cultivars JG 52 and UC 27. Percentage of nematodes penetrating the root from 1 to 11 days after inoculation were transformed into $\ln(Y)$ for regression analysis over time. Slopes of lines within each temperature with a lower-case letter in common do not differ significantly ($P = 0{\cdot}05$), according to t-test. Slopes of lines within each chickpea geno-type with an upper-case letter in common do not differ significantly ($P = 0{\cdot}05$), according to t-test. t, time after inoculation (days); Y, percentage penetration.

experiments, penetration was affected by temperature, host genotype, and their interaction (Table 1). Penetration was maximum (72·8%) at 25°C in cultivar JG 62; however this percentage was not significantly different with those of line P 2245 (60·4%) or cultivar UC 27 (61·9%) at the same temperature (Table 1). Similar results were obtained for 20°C; however, at 15°C percentage penetration was higher ($P < 0{\cdot}05$) in line P 2245 than those in cultivars UC 27 and JG 62 (Table 1, Fig. 3). The percentage of nematodes penetrating chickpea roots 11 days after inoculation was significantly increased ($P < 0{\cdot}05$) with increasing temperature in cultivars UC 27 and JG 62, but it was not affected in line P 2245 (Table 1, Fig. 3).

DISCUSSION

Results for hatching of *P. thornei* at the beginning of the experiment agree with data of Mai *et al.*

Table 1 Percentage of penetration of *Pratylenchus thornei* into roots of chickpea line P 2245 and cultivars JG 62 and UC 27 at 15, 20 or 25°C[a]

Time after inoculation	UC 27			P 2245			JG 82		
	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
1 day	2·1a[b]	3·0b	2·9b	3·4a	5·3a	5·1a	2·8a	3·0a	2·6a
3 days	5·9a	5·1a	5·3a	6·6a	15·6b	14·5b	4·0a	15·3b	10·3c
5 days	7·5a	15·5b	12·8b	12·5a	16·8a	18·3a	7·4a	15·6b	20·0b
7 days	13·1a	27·6b	39·5c	36·3a	34·9a	52·1b	12·8a	27·9b	46·1c
9 days	25·4a	45·9b	45·0b	49·8a	52·6a	49·1a	22·5a	47·4b	61·0c
11 days[c]	28·8aB	47·5bA	61·9cA	49·1aA	59·0aA	60·4aA	27·0aB	57·6bA	72·8cA

Data are means of 12 replicates (from two trials). Actual data are presented for each treatment, but data were transformed to $\ln(Y)$ for analysis. Means within each cultivar followed by the same lower-case letter do not differ significantly ($P = 0{\cdot}05$), according to Fisher's protected LSD test.

[a] Percentage = Number of nematodes in root system/number of nematodes used as inoculum ×100.

[b] Total number of all migratory life stages penetrating roots/number of nematodes used as inoculum ×100.

[c] Means within each temperature followed by the same upper-case letter do not differ significantly ($P < 0{\cdot}05$), according to Fisher's protected LSD test.

(1977) for *P. penetrans* at different temperatures; however, at the conclusion of a 23-day trial we found a higher cumulative percentage egg hatch at 20°C than at other temperatures. These findings indicate that optimum temperature for hatching in *P. thornei* lies between 18 and 22°C (Fig. 2). Temperatures between 10 and 15°C were also favourable for egg hatch, although amounts of egg hatch similar to those at 20 or 25°C after the fifth day of incubation (about 44%) were only obtained at the 19th and 15th days of incubation, respectively. The differences in egg hatch from *P. penetrans* (Mai *et al.*, 1977), which had hatched in similar numbers at different temperatures at the end of a 26-day trial, may indicate physiological differences among species of *Pratylenchus* with respect to temperature requirements (Acosta & Malek, 1979).

All migratory life stages of *P. thornei* penetrated chickpea roots of all cultivars at all temperatures throughout the experiment. Temperature significantly affected percentage penetration of *P. thornei* into roots of chickpea, with temperatures of 20–25°C optimal for penetration in cultivars UC 27 and JG 62; however, temperature did not significantly affect nematode penetration in line P 2245 (Fig. 3).

Host genotype affected percentage penetration at 15°C only, at which penetration was significantly greater in P 2245 than in cultivars UC 27 or JG 62. These findings would indicate that line P 2245 was more susceptible than cultivars UC 27 and JG 62 to infection by *P. thornei* at this temperature, which agrees with our previous results (Castillo *et al.*, 1995a).

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