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Effect of Time, Temperature, and Inoculum Density on Reproduction of *Pratylenchus thornei* in Carrot Disk Cultures[1]

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Abstract: Reproduction of *Pratylenchus thornei* on carrot disk cultures at different time periods after inoculation, temperature, and initial inoculum density was studied. At 25 C and with an initial inoculum of 25 females per disk, the final nematode population increased with increasing time after inoculation, although the populations after 25 and 50 days were not different. Nematode numbers increased by 1,255-fold and 3,619-fold at 75 and 100 days, respectively. Over 35 days incubation at 15, 20, 25, and 30 C, the nematode multiplied 1.8, 8.4, 10.5, and 0.4 times, respectively. The optimum temperature for reproduction was between 20 and 25 C, and the nematode life cycle was completed in about 25–35 days. Increasing nematode inoculum (25, 50, 100, 500, 1,000 nematodes per disk) increased the final nematode population but did not increase reproduction rate, the highest being 25.3 at an initial inoculum density of 100 nematodes per disk.

Key words: carrot, inoculum density, lesion nematode, monoxenic culture, nematode, *Pratylenchus thornei*, reproduction, temperature.

Surveys in chickpea (*Cicer arietinum* L.) crops in southern Spain indicated that the cereal and legume root-lesion nematode, *Pratylenchus thornei* Sher & Allen (7), is the most abundant and widespread plant-parasite nematode (3). The nematode was recovered from 90.5% of soil samples and 84.1% of root samples, with population densities of 2–48 nematodes/100 cc of soil and 6–70 nematodes/g of root (3).

Large numbers of *P. thornei* eggs, juveniles, and females are needed for pathogenicity studies on chickpea. Suitable numbers can be produced in monoxenic cultures on carrot (*Daucus carota* L.) disks (9). Availability of standardized inocula for sequenced experiments also would be facilitated if information was available on the influence of initial inoculum, temperature, and time on reproduction of the nematode on carrot disks.

Several reports have been published concerning the monoxenic culture of root lesion nematodes on carrot disks (4,11–17). However, few reports include the influence of time, temperature, and inoculum density on nematode reproduction (1, 15,16). Reproduction of an English population of *P. thornei* on carrot disks has been studied recently (17), but there was no reference to the above-mentioned factors. The objective of this study was to determine the effect of time, temperature, and initial inoculum density on reproduction of a Spanish population of *P. thornei* in monoxenic carrot cultures.

Materials and Methods

Three experiments were conducted with a nematode population obtained from chickpea roots collected in a field at Jerez de la Frontera (Cádiz), southern Spain. Nematodes were increased from a single female in carrot disks (9) at 25 C in the dark. Inoculum for experiments was obtained from established carrot-disk cultures placed on Baermann funnels. Extracted nematodes were surface disinfested with 0.02% ethoxyetil mercury and 0.1% streptomycin solutions for 2 and 24 hours, respectively, and rinsed several times in sterilized water (10). To prepare carrot disks for the experiments, fresh carrots with attached leaves were washed free of soil, surface disinfested in 20% NaOCl solution for 5 minutes, flamed in a laminar flow hood under aseptic conditions, and then peeled and sliced transversely (10

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mm thick, 30–40 mm diameter). A single carrot disk was transferred into a sterile 90 mm petri dish, and the dish was then sealed with parafilm.

Upon completion of experiments, nematodes from carrot disks were extracted by centrifugation (5). A carrot disk was homogenized in 250 ml of water in a Waring blender at 12,600 rpm for 30 seconds, then the mixture was centrifuged at 1,500*g* for 4 minutes. The supernatant was decanted, centrifuge tubes were refilled with 250 ml of a sucrose solution (r = 1.15), mixed with a stirring-vibrator, and centrifuged for 3 minutes at 1,500*g*. The sucrose solution was poured onto a sieve (5-μm pore diameter), and final nematode population densities (eggs, juveniles, and females) were determined from a 1-ml aliquot for experiments 1 and 3, and the complete sample for experiment 2. The experiments were repeated once and pooled data from two experiments were subjected to analysis of variance.

Experiment 1: The effect of length of incubation period on nematode reproduction was determined 25, 50, 75, and 100 days after inoculation by adding 25 surface-disinfested females to each disk. Nematodes were transferred with a sterile handling needle into a drop of sterile distilled water, which was already placed on the carrot disk. Inoculated disks were incubated at 25 ± 1 C in the dark. For each incubation time, disks were placed in the incubator in a completely randomized design with 10 replicates.

Experiment 2: The effect of temperature on nematode reproduction was investigated at 15, 20, 25, and 30 ± 1 C with an initial inoculum density of 25 surface-disinfested females per disk. Nematodes were transferred to carrot disks as for experiment 1. Inoculated disks were incubated at the appropriate temperature in the dark for 35 days. There were 10 replicated disks for each temperature, and disks were completely randomized within each incubator.

Experiment 3: The effect of initial inoculum density on nematode reproduction was determined using 25, 50, 100, 500, and 1,000 surface-disinfested nematodes (all life stages) per disk. Nematodes were transferred to carrot disks as a suspension in sterile water. Nematode inoculum density was estimated in 10 1-ml subsamples of the extracted nematodes. The average percentage of life stages in the inoculum was 14.2% eggs, 56.9% juveniles, and 28.9% females. Inoculated disks were incubated at 25 ± 1 C in the dark for 35 days. For each inoculum density, disks were placed in the incubator in a completely randomized design with 10 replicates.

Statistical analyses: Nematode numbers (X) and their transformation into log (X + 1) were used for data analyses. All experiments were repeated once, and similarity among experimental runs tested by analyses of variance. Then data were combined for analyses of variance and regression. Treatment means were compared using

TABLE 1. Reproduction of *Pratylenchus thornei* on carrot disks after four time periods.

Time (days)†	Number of nematodes			Pf	Rf
	Females	Juveniles	Eggs		
25	22	89	18	129	5.1
50	96	363	175	634	25.4
75	2,060	14,010	15,300	31,370	1,254.8
100	5,850	53,380	31,250	90,480	3,619.3
$LSD_{0.05}$	1,549.1	9,795.9	6,432.6	16,040	641.8

Number of nematodes per carrot disk. Data are the average of two experiments with 10 replicated disks. Actual data are presented for each time period of incubation, but data were transformed to log (X + 1) for analysis. Each disk was inoculated with an initial population (Pi) of 25 females and incubated at 25 ± 1 C.

† Days after inoculation.

Pf = final population; Rf (reproduction factor) = Pf/Pi.

TABLE 2. Regression analysis of the population density and reproduction rate of *Pratylenchus thornei* on carrot disks at 25 ± 1 C over time (t) in days.

Dependent variable	Regression equation	r^2	P
Females	$\log (X + 1) = 0.47 + 0.03t$	0.940	0.0001
Juveniles	$\log (X + 1) = 0.86 + 0.04t$	0.932	0.0001
Eggs	$\log (X + 1) = 0.11 + 0.05t$	0.918	0.0001
Final population	$\log (X + 1) = 1.03 + 0.04t$	0.939	0.0001
Reproduction rate	$\log (X + 1) = -0.28 + 0.04t$	0.937	0.0001

Data are from two experimental runs with 10 replicated disks per time period. Each disk was inoculated with an initial population (Pi) of 25 females and incubated at 25 ± 1 C.

Fisher's protected least significant difference test (LSD) ($P = 0.05$). Transformed and nontransformed data were subjected to linear regression analysis (experiments 1 and 3), or fitted with a quadratic model (experiment 2). Coefficients of determination (r^2), and patterns of residuals were used to determine appropriateness of the model (2,6).

RESULTS AND DISCUSSION

In experiment 1, at 25 C, nematode population density and reproduction rate of *P. thornei* increased with increasing time after inoculation (Table 1). The increase of the nematode population over time was described by the linear equation $\log (X + 1) = \beta_0 + \beta_1 t$, in which X = number of nematodes per carrot disk, or reproduction rate, and t = time of incubation (Table 2). By 25 days after inoculation, there were about four juveniles and eggs per female in the initial inoculum. Apparently the first life cycle was not completed, as the observed number of females at 25 days was close to the initial inoculum. Similarly, by 50 days after inoculation, the nematode has not completed a second life cycle and the nematode population was a mixture of the first and second generation. At this time, the total number of females was close to the number of juveniles 25 days earlier, and there were about 4.8 juveniles and eggs per female in the population. Thus, it appears that at low population density the generation time is between 25 and 50 days, and there is about four-fold increase per generation. At later times, however, crowding and exhaustion of the food supply may have affected nematode life cycle and reproduction. By 75 days after inoculation, the total number of females was more than three times the total number of nematodes 25 days earlier. Experiment 1 showed that reproduction of the Spanish population of *P. thornei* used in our study is higher than that of the English population reported by Verdejo and Pinochet (17), for which reproduction rate was 1,462 after 90 days as compared to 3,619 after 100 days that we recorded. These differences may

TABLE 3. Reproduction of *Pratylenchus thornei* on carrot disks at different temperatures.

	Number of nematodes				
Temperature (C)	Females	Juveniles	Eggs	Pf	Rf
15	12	5	27	44	1.8
20	56	84	70	210	8.4
25	63	122	78	263	10.5
30	6	2	1	9	0.4
$LSD_{0.05}$	11.3	33.8	21.7	54.9	2.2

Number of nematodes per carrot disk. Data are the average of two experiments with 10 replicated disks. Actual data are presented for each temperature, but data were transformed to $\log (X + 1)$ for analysis. Each disk was inoculated with an initial population (Pi) of 25 females, and nematodes were extracted 35 days after inoculation.
Pf = final population; Rf (reproduction factor) = Pf/Pi.

TABLE 4. Regression analysis of the population density and reproduction rate of *Pratylenchus thornei* on carrot disks over incubation temperature (T) (C).

Dependent variable	Regression equation	r^2	P
Females	$\log (X + 1) = -6.32 + 0.75T - 0.02T^2$	0.812	0.0001
Juveniles	$\log (X + 1) = -12.35 + 1.31T - 0.03T^2$	0.895	0.0001
Eggs	$\log (X + 1) = -7.11 + 0.87T - 0.02T^2$	0.839	0.0001
Final population	$\log (X + 1) = -7.94 + 0.97T - 0.02T^2$	0.843	0.0001
Reproduction rate	$\log (X + 1) = -5.90 + 0.64T - 0.01T^2$	0.876	0.0001

Data are from two experimental runs with 10 replicated disks per temperature. Each disk was inoculated with an initial population (Pi) of 25 females, and nematodes were extracted 35 days after inoculation.

relate to differences in carrot-disk culture methods, in nematode extraction methods, or in the optimum temperature for reproduction among northern and Mediterranean populations of *P. thornei.*

In experiment 2, higher numbers of nematodes and reproduction rate occurred at 25 and 20 C than at 15 and 30 C (Table 3). There were no differences between 20 and 25 C or between 15 and 30 C. The influence of temperature on reproduction of *P. thornei* on carrot disks was described by the quadratic equation $\log (X + 1) = \beta_0 + \beta_1 T + \beta_2 T^2$, in which X = number of nematodes per carrot disk, or reproduction rate; and T = temperature (C) (Table 4). The optimum temperature for reproduction of this Spanish population of *P. thornei* was 20–25 C. Acosta and Malek (1) reported similar temperature requirements for reproduction of *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven and *P. vulnus* Allen & Jensen on soybean. In our experiment, the number of females and the reproduction rate at 20 and 25 C at 35 days after inoculation was nearly two-fold that after 25 days at 25 C (Tables 3,4). Therefore, the nematode completes its life cycle in about 25–35 days at 20–25 C. Although optimum temperatures for reproduction on carrot disks may not coincide with that of parasitism on crops, our results can be indicative and useful for studies on the biology of *P. thornei.*

Experiment 3 (Table 5) showed that nematode population density after 35 days at 25 C increased with increasing inoculum density. The influence of inoculum density on reproduction of *P. thornei* on carrot disks was described by the linear equation $\log (X + 1) = \beta_0 + \beta_1 \text{Pi}$, in which X = number of nematodes per carrot disk and Pi = initial number of nematodes per carrot disk (Table 6). The highest reproduction rate occurred with an inoculum of 100 *P. thornei* per disk and declined at higher inoculum levels. However, regression analysis showed that reproduction rate and initial inoculum density were not significantly related by linear or polynomial models.

TABLE 5. Effect of inoculum density on reproduction of *Pratylenchus thornei* on carrot disks.

	Number of nematodes				
Inoculum density	Females	Juveniles	Eggs	Pf	Rf
25	47	180	192	420	16.8
50	56	369	355	780	15.6
100	360	1,204	968	2,532	25.3
500	999	4,684	2,472	8,156	16.4
1,000	1,532	7,930	4,703	14,165	14.2
$LSD_{0.05}$	403	2,213	895	3,192	5.1

Number of nematodes per carrot disk. Data are the average of two experiments with 10 replicated disks. Actual data are presented for each inoculum density, but data were transformed to $\log (X + 1)$ for analysis. Each carrot disk was inoculated with an inoculum density (Pi) of either 25, 50, 100, 500, or 1,000 *P. thornei* mixed life stages, incubated at 25 ± 1 C, and nematodes were extracted 35 days after incubation.

Pf = final population; Rf (reproduction factor) = Pf/Pi.

TABLE 6. Regression analysis of the population density and reproduction rate of *Pratylenchus thornei* on carrot disks at 25 ± 1 C over inoculum density (Pi).

Dependent variable	Regression equation	r^2	P
Females	$\log (X + 1) = 1.93 + 0.001Pi$	0.645	0.0001
Juveniles	$\log (X + 1) = 2.58 + 0.001Pi$	0.663	0.0001
Eggs	$\log (X + 1) = 2.53 + 0.001Pi$	0.714	0.0001
Final population	$\log (X + 1) = 2.92 + 0.001Pi$	0.712	0.0001
Reproduction rate	—	—	ns

Data are from two experimental runs with 10 replicated disks per Pi. Each disk was inoculated with initial populations (Pi) of either 25, 50, 100, 500, or 1,000 *P. thornei* mixed life stages, incubated at 25 ± 1 C, and nematodes were extracted 35 days after incubation at 25 ± 1 C for 35 days.

Reduction of nematode reproduction rate with increasing nematode inoculum density has been recorded for *P. thornei* on chickpea grown under greenhouse and shadehouse conditions (3), as well as for other root lesion nematodes such as *Pratylenchus neglectus* (Rensch) Filipjev and Schuurmans-Stekhoven on wheatgrasses (8). Such a reduction could be interpreted as a result of competition for food resources.

In conclusion, our results indicate that maximum yields of viable *P. thornei* on carrot disk cultures can be obtained after 100 days of incubation at 25 C. The optimum initial inoculum density for reproductive rate was 100 nematodes per disk at 35 days after inoculation and incubation at 25 C. However, if the highest possible numbers of nematodes for inoculum production were desired, higher initial inoculum rates would be indicated.

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