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Parasitism of the root-lesion nematode *Pratylenchus thornei* on chickpea

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Pratylenchus thornei–chickpea interactions were investigated under controlled and fluctuating environmental conditions in the growth chamber, greenhouse and shadehouse. Under controlled conditions, *P. thornei* infected chickpea lines 12071/10054 and P 2245 and cultivars Andoum 1, JG 62 and UC 27. Line P 2245 and cv. JG 62 were the most susceptible genotypes on the basis of root damage and nematode reproduction, but nematode infection did not significantly reduce root and shoot weights. Cultivars Andoum 1 and UC 27 and line 12071/10054 showed the least root damage and nematode reproduction. Inoculation of cv. Andoum 1 with 2500, 5000 or 10 000 nematodes per plant in pots did not affect shoot weight, regardless of the conditions of water stress of the plants. However, root weight was significantly reduced by nematode infection in plants grown under water stress and fluctuating temperature conditions in the greenhouse, but was not affected by any other treatment. The nematode reproduction index was not affected by soil water content under shadehouse conditions, but was greater on plants watered to soil water-holding capacity than in water-stressed plants under greenhouse conditions. For both environments, the nematode reproduction index decreased when inoculum density was greater than 5000 nematodes per plant.

INTRODUCTION

Pratylenchus thornei, the cereal and legume root-lesion nematode (Fortuner, 1977), has been reported to be associated with chickpea (*Cicer arietinum*) crops in several localities in southern Spain (Castillo *et al.*, 1994). Although necrotic root lesions were observed in nematode-infected plants, no growth reduction or other symptoms were discernible above ground. The pathogenicity of *P. thornei* to chickpea cv. Ghab 1 was recently determined by Di Vito *et al.* (1992) under field conditions. However, differences in pathogenic capability among *Pratylenchus* spp. and populations have been reported (Motalaote *et al.*, 1987; Griffin, 1991; Pinochet *et al.*, 1993). Furthermore, information about the pathological effects of *P. thornei* on chickpea under controlled or greenhouse conditions is limited (Walia & Seshadri, 1985; Tiyagi & Parveen, 1992), and information about the methods of inoculation was not provided.

The objectives of the present study were (1) to evaluate a method for inoculating *P. thornei* on chickpea, and (2) to investigate the response of chickpea lines and cultivars to *P. thornei* under controlled (growth chamber) and fluctuating (greenhouse and shadehouse) environmental conditions with two water-management regimes (water-holding capacity and water stress).

MATERIALS AND METHODS

Response of chickpea lines and cultivars to *Pratylenchus thornei*

Three experiments (I, II and III) were performed in order to determine the response of chickpea lines and cultivars to *P. thornei*. Inoculum was increased from a single female from chickpea roots collected from Cañete de las Torres, Córdoba (experiment I) or from Jerez de la Frontera, Cádiz (experiments II and III). A single female was placed on a carrot disc, incubated at 24°C for 6 weeks, and then multiplied in cultures on several discs (Huettel, 1985). To obtain inoculum, the infected carrot discs were placed on a Baermann funnel. The extracted nematodes were surface sterilized with

0·02% ethoxyethyl mercury chloride followed by 0·1% streptomycin solutions for 2 and 24 h, respectively, thoroughly rinsed several times in sterilized water and the average nematode population densities determined from ten 1-ml aliquots.

Experiment I

The response of chickpea lines 12071/10054 and P 2245, and cvs Andoum 1, JG 62 and UC 27 to *P. thornei* was evaluated. Andoum 1 is resistant to races 0 and 1 of *Fusarium oxysporum* f.sp. *ciceris*, but susceptible to race 5 [the three dominant races in southern Spain (Jiménez-Díaz *et al.*, 1993a)], while cv. JG 62 is resistant to race 0 and susceptible to races 1 and 5, and line 12071/10054 is susceptible to races 0 and 1 and resistant to race 5 (Jiménez-Díaz *et al.*, 1993a, 1993b). UC 27 and line P 2245 are resistant and susceptible, respectively, to races 0, 1 and 5 (Jiménez-Díaz *et al.*, 1993b). Seeds were sown in sterile sand, and 6 days later the seedlings were removed, washed free from sand and transplanted into 15-cm-diameter clay pots (one per pot) containing 0·5 l of an autoclaved potting mixture (sand: clay loam, 2 : 1, v/v). Plants were inoculated with 2500 nematodes (36% females, 18% juveniles and 46% eggs) in 10 ml of sterile water either by infesting the soil of a pot immediately before transplanting or by adding the nematodes around the root ball of a plant at the time of transplanting. Control plants were treated similarly, but without inoculum. Plants were grown in a growth chamber adjusted to 24 ± 1°C, 60–90% relative humidity and a 14-h photoperiod of fluorescent light at approximately $360{\cdot}5 \pm 24{\cdot}7\ \mu E/m^2/s$. Plants were watered daily with 100 ml of water and fertilized weekly with 100 ml of a nutrient solution (Hoagland & Arnon, 1950). Treatments were replicated ten times in a randomized complete block design, and the experiment was repeated once.

Experiments II and III

The response of chickpea cv. Andoum 1 to *P. thornei* was also tested in two additional experiments, which were performed in a greenhouse (experiment II) and in a shadehouse (experiment III) at the same time. For both experiments, seeds were sown in sterile sand, and 6 days later the seedlings were removed, washed free from sand and transplanted into 15-cm-diameter clay pots (one per pot) containing 0·5 l of the autoclaved potting mixture. Plants were inoculated with 2500, 5000 or 10 000 nematodes (9% females, 67% juveniles, and 24% eggs) in 10 ml of sterile water. The nematode suspension was added around the root ball of a plant at the time of transplanting. Control plants were treated similarly, but with sterile water instead of inoculum.

Plants in the greenhouse were irrigated with either 100 ml (water-holding capacity of soil) or 50 ml (water stress condition) water per pot daily. Plants in the shadehouse were irrigated with the same amount of water as those in the greenhouse, but on alternate days. The water potential for both levels of irrigation, determined at 10-day intervals was $-20{\cdot}8$ and $-10{\cdot}3$ kPa, respectively, for plants in the shadehouse, and $-18{\cdot}3$ and $-9{\cdot}1$ kPa, respectively, for those in the greenhouse. Plants were fertilized weekly with 100 ml of a nutrient solution (Hoagland & Arnon, 1950). The average maximum and minimum air temperatures were $36 \pm 5{\cdot}0$ (25–43)°C and $17 \pm 2{\cdot}8$ (11–23)°C in the greenhouse, and 29 ± 5.6 (16–41)°C and $14 \pm 3{\cdot}3$ (9–21)°C in the shadehouse, respectively. For both experiments, treatments were replicated ten times in a randomized complete block design, and the experiments were repeated once.

Assessment of plant–nematode interaction

All experiments were terminated 50 days after inoculation, when plants were at the full bloom to early podding stage. The shoot of each plant was then cut off at soil level and the roots were washed free from soil. The following parameters were assessed: root necrosis, nematode numbers in roots and soil, nematode reproduction index (Rf = final population/initial population), shoot and root fresh weight. Root necrosis was assessed on a scale of 0–10 according to the percentage of necrotic tissue present, as follows: 0 = 0% necrotic tissue, and 10 = 100% necrotic tissue. Nematodes were extracted from 100 cm^3 soil and 5-g root samples by centrifugation (Coolen, 1979), and their averages were used to estimate the final nematode population densities.

Statistical analysis

For analysis, the number of nematodes (X) and the percentage data were transformed into log $(X + 1)$ and the arcsine-square root, respectively. All experiments were repeated once. Similarity between experimental runs, tested by preliminary analyses of variance using experimental runs as

Table 1. Response of chickpea lines and cultivars to *Pratylenchus thornei*[a]

Inoculation method	Cultivars and lines	Root necrosis severity[b]	Root weight (%)[c]	Shoot weight (%)	Nematodes Soil (100 cm^3)	Nematodes Roots (g)	Rf[d]
Root ball inoculation	Andoum 1	1·5b	107·0	104·1	97·5c	443·1b	3·3c
	UC 27	1·3b	95·5	95·2	201·5b	633·2b	4·6c
	JG 62	2·0a	182·3*	117·3*	394·5a	2381·3a	15·7a
	12071/10054	1·5b	109·1	97·2	199·0b	1564·0a	7·1b
	P2245	2·2a	92·8	89·8	300·0a	1634·2a	9·3b
	Mean	1·7A			238·5A	1331·2A	8·0A
Soil infestation	Andoum 1	0·7a	102·5	101·2	48·0a	160·6bc	1·3a
	UC 27	0·6a	97·9	99·0	43·5a	125·8c	1·0a
	JG 62	0·7a	100·5	101·4	39·0a	351·4a	1·4a
	12071/10054	0·6a	106·5	94·9	42·5a	309·7a	1·5a
	P2245	0·6a	113·1	97·0	31·5a	225·5b	1·4a
	Mean	0·6B			40·9B	234·6B	1·3B

[a] Data are the average of two experiemnts with 10 replicated plants per treatment combination in each experiment. For each inoculation method, means followed by the same letter do not differ significantly ($P = 0{\cdot}05$) according to Fisher's protected LSD test. Upper-case letters refer to mean comparisons between treatments for inoculation method. Actual data are presented for each inoculation method, but numbers of nematodes and percentages were transformed to log $(X + 1)$, and to arcsine-square root for analysis, respectively.
[b] Assessed on a scale of 0 (0% necrotic tissue) to 10 (100% necrotic tissue), 50 days after inoculation.
[c] Average percentage weight of inoculated plants relative to controls; * significantly different at $P < 0{\cdot}05$.
[d] Nematode reproduction index= final nematode population density per plant/initial nematode population density per plant.

blocks, allowed the data to be combined for analyses of variance. Treatment means were compared using Fisher's protected least significant difference test (LSD) at $P = 0{\cdot}05$.

RESULTS

Response of chickpea to *Pratylenchus thornei*

Growth chamber experiment

All five chickpea lines and cultivars were infected by *P. thornei* (Table 1). Both the method of inoculation and the host genotype, as well as their interaction, significantly ($P < 0{\cdot}05$) influenced the severity of root necrosis, nematode population density in soil and roots, and the nematode reproduction index. All these parameters were significantly greater ($P < 0{\cdot}05$) when the nematode suspension was poured around the root ball of a plant at the time of transplanting than when nematodes were added to the soil immediately before transplanting (Table 1). Chickpea lines and cultivars differed significantly in their reaction to nematode infection. In root ball-inoculated plants, the severity of root necrosis was in the range 1·3–2·2 within a scale of 0–10. Although all five chickpea lines and cultivars were effective hosts of *P. thornei*, cv. JG 62 and line P 2245 showed the greatest root necrosis, and cv. JG 62 had the greatest nematode reproduction index (Table 1). However, root and shoot weight were not significantly ($P = 0{\cdot}05$) reduced as a result of nematode infection, except for a significant plant growth stimulation in cv. JG 62 (Table 1).

Greenhouse and shadehouse experiments

The results varied between the two environments. Under greenhouse conditions, the nematode inoculum density significantly influenced the severity of root necrosis in cv. Andoum 1, but no differences were observed with regard to the water content in soil (Table 2). Similarly, the nematode population density in roots increased significantly ($P < 0{\cdot}05$) with increasing nematode inoculum density from 2500 to 10 000 nematodes per plant, irrespective of the water content of the soil (Table 2). However, both the

Table 2. Response of chickpea cv. Andoum 1 to *Pratylenchus thornei* under conditions of water stress in greenhouse and shadehouse experiments[a]

Irrigation management[b]	Nematode inoculum[c] (per plant)	Root necrosis severity[d]	Root weight (g)	Shoot weight (g)	Nematodes: Soil (100 cm³)	Nematodes: Roots (g)	Rf[e]
Greenhouse experiment							
Water-holding capacity	0	0	15·0a	19·8a	0	0	0
	2500	2·1b	17·2a	20·6a	250c	132·7b	1·0a
	5000	3·5a	16·0a	19·4a	820b	385·0a	1·3a
	10 000	3·0a	14·7a	19·0a	1140a	527·2a	0·8a
	Mean	2·9A	15·7A	19·7A	553A	261·2A	0·8A
Water stress	0	0	8·2a	9·1a	0	0	0
	2500	2·6a	5·5b	7·6a	940c	158·6c	0·8a
	5000	2·9a	6·0b	8·0a	1800b	292·6b	0·8a
	10 000	2·7a	5·9b	8·2a	3300a	513·7a	0·7a
	Mean	2·7A	6·4B	8·2B	1510B	241·2A	0·6B
Shadehouse experiment							
Water-holding capacity	0	0	12·9a	18·2a	0	0	0
	2500	1·6b	12·1a	17·3a	340c	728·3b	3·6a
	5000	2·5a	10·3a	16·5a	670b	1695·4a	3·5a
	10 000	2·9a	11·9a	17·0a	1060a	1535·8a	1·9b
	Mean	2·3A	11·8A	17·2A	519A	989·9A	2·3A
Water stress	0	0	4·8a	7·2a	0	0	0
	2500	1·4b	4·6a	6·7a	1160c	1587·1b	3·5a
	5000	2·3a	4·8a	7·1a	2020b	1958·2a	2·5ab
	10 000	3·0a	4·2a	6·0a	2910a	2898·8a	1·7b
	Mean	2·2A	4·6B	6·7B	1523B	1611·0B	1·9A

[a] Data are the average of two experiments with 10 replicated plants per treatment combination in each experiment. Actual data are presented for each treatment, but data were transformed to log $(X+1)$ for analysis. For each irrigation management, numbers in a column followed by the same letter do not differ significantly ($P = 0{\cdot}05$) according to Fisher's protected LSD test. Upper-case letters refer to mean comparisons between treatments for irrigation management.
[b] Plants in the greenhouse were irrigated with 100 ml (water-holding capacity) or 50 ml (water stress conditions) water per pot daily. Plants in the shadehouse were watered with the same amount of water as those in the greenhouse, but on alternate days.
[c] Given numbers of nematodes in 10 ml of water were added around the root ball of a plant at the time of transplanting.
[d] Assessed on a scale of 0 (0% necrotic tissue) to 10 (100% necrotic tissue), 50 days after inoculation.
[e] Nematode reproduction index = final nematode population density per plant/initial nematode population density per plant.

nematode inoculum density and the water content of the soil significantly influenced the nematode population density in soil, as well as the nematode reproduction index (Table 2). The nematode population in soil was significantly larger ($P < 0{\cdot}05$) when plants were water stressed than when water-holding capacity was maintained in the soil, and under both conditions the nematode population increased steadily with increasing nematode inoculum density (Table 2). The nematode reproduction index was significantly ($P < 0{\cdot}05$) greater in plants watered to soil water-holding capacity than in those growing under conditions of water stress, but it was not affected by inoculum density in either watering regime.

When plants were grown in a shadehouse, where the air temperature was slightly lower than in the greenhouse, both severity of root necrosis and nematode reproduction were significantly ($P < 0{\cdot}05$) influenced by nematode inoculum

density, but not by the water content of the soil (Table 2). The severity of root necrosis was in the range 1·4–3·0, but it increased with nematode inoculum density greater than 2500 nematodes per plant. The nematode reproduction index decreased when the inoculum density was greater than 5000 nematodes per plant, but it was not influenced by the water content of the soil. The nematode population density in both soil and roots was significantly influenced by nematode inoculum density and water content in the soil. Nematode population density in roots and soil was significantly ($P < 0·05$) higher when plants were grown under water stress than when the water-holding capacity of the soil was maintained. The nematode population density in the soil increased steadily with increasing nematode inoculum density from 2500 to 10 000 nematodes per plant (Table 2). However, the nematode population density in the roots did not increase significantly above an inoculum density greater than 2500 nematodes per plant.

In the two experiments, the root and shoot weights of cv. Andoum 1 chickpeas were significantly ($P < 0·05$) reduced by water stress (Table 2). However, nematode parasitism affected root growth but not shoot growth only when the plants were grown under water stress under greenhouse conditions (Table 2).

DISCUSSION

The results obtained from three experiments in this study indicate that the chickpea lines and cultivars tested are effective hosts for *P. thornei*. However, it appears that in inoculated plants grown for 50 days, parasitism by the nematode has a pathogenic effect on chickpea root growth only when the plants are cultivated under conditions of water stress and highly fluctuating temperatures in the greenhouse. The average maximum temperatures and temperature range in the greenhouse were greater than those in the shadehouse and the growth chamber, and this may have imposed more stresses on plant growth. Our data for the effects of nematode parasitism on plant growth under artificial conditions are not in agreement with the results of other researchers, who found that *P. thornei* damaged chickpea root and shoot growth significantly when inoculated plants were grown under artificial conditions for 115 days (Walia & Seshadri, 1985; Tiyagi & Parveen, 1992). Differences in the pathogenic capability of *Pratylenchus* species and populations have been reported by several authors (Motalaote *et al.*, 1987; Griffin, 1991; Pinochet *et al.*, 1993). Thus the conflicting data could be caused by differences in the environment and experimental conditions, the susceptibility of chickpea lines and cultivars, or the virulence of the *P. thornei* populations used. Furthermore, our results with regard to the influence of plant growth stresses on the plant response to nematode parasitism relate to crop damage by *P. thornei* under field conditions (Di Vito *et al.*, 1992).

In our experiments, inoculation by infesting the root ball of a plant at the time of transplanting increased *P. thornei* invasion more than infesting the soil with nematodes just before transplanting, and it resulted in increased root necrosis and nematode reproduction. The fact that the nematode reproduction index was higher under shadehouse than under greenhouse conditions, irrespective of the water content of the soil, could be due to the detrimental effects of high temperature on nematode development, as the maximum air temperature in the greenhouse (> 30°C) was higher than that in the shadehouse. Increased nematode inoculum caused a rise in nematode infection, and there was a significant effect on root growth in plants grown under water stress in greenhouse conditions, but shoot growth was not affected in either environment or watering regime. However, the reproductive rate was decreased with high nematode inoculum density in the shadehouse. Similar reductions have been reported for parasitism of a population of *P. thornei* on mint in India (Hasseb & Shukla, 1994) and a population of *P. neglectus* on wheatgrasses (Griffin, 1992). These findings could be the result of intraspecific competition for nutrients or root tissue availability, as a result of which a smaller proportion of the inoculum will develop successfully. The higher nematode population density observed within roots compared to that in soil at the water-holding capacity in the shadehouse may be related to environmental conditions. Townshend (1972) reported that penetration of corn roots by *P. penetrans* was favoured by soil moisture and moderate temperatures (20°C). In our experiments, high water content in the soil and moderate temperature in the shadehouse favoured infection and development of nematodes within roots.

In chickpea, several plant-parasitic nematodes, including *P. thornei*, *Meloidogyne incognita*, *M. javanica* and *Rotylenchulus reniformis* are suspected of increasing fungal disease severity (Sharma & McDonald, 1990; Sharma

et al., 1992). The interaction between *P. thornei* and *Fusarium oxysporum* f. sp. *ciceris* is of much concern. *Fusarium oxysporum* f.sp. *ciceris* causes Fusarium wilt, which is a major disease of chickpea in Spain (Trapero-Casas & Jiménez-Díaz, 1985). This disease can be controlled by means of resistant cultivars (Jiménez-Díaz *et al.*, 1992), but nematodes might modify the host resistance to *Fusarium oxysporum* f.sp. *ciceris*, as indicated by Sharma & Cerkauskas (1985) for root-knot nematodes. Although *P. thornei* does not appear to damage chickpeas significantly under our experimental conditions, the high frequency of this nematode in chickpea fields at the Guadalquivir Valley indicates its potential importance, especially if it interacts with root-infecting fungi. Studies are in progress to determine whether or not *P. thornei* influences the reaction of chickpeas to *Fusarium oxysporum* f.sp. *ciceris*, in both compatible and incompatible plant–fungus interactions.

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