PLANT PARASITIC NEMATODES ASSOCIATED WITH CHICKPEA IN SOUTHERN SPAIN AND EFFECT OF SOIL TEMPERATURE ON REPRODUCTION OF *PRATYLENCHUS THORNEI*

BY

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Surveys of 63 fields in the chickpea growing area of the Guadalquivir Valley of Andalucía, southern Spain, in 1990 and 1991, indicated that *Pratylenchus thornei* was the predominant plantparasitic nematode, being recovered from 92% of soil and 84% of root samples, with population densities ranging from 2-48/100 cm^3 and 6-70 per g of fresh root. Substantial populations of the ectoparasitic nematodes *Amplimerlinius magnistylus, Aorolaimus perscitus, Helicotylenchus tunisiensis, Merlinius brevidens, Mesocriconema curvatum* and *Paratrophurus loofi* were also found.

Three populations of *P. thornei* were tested in a glasshouse at 15, 20 and 25°C at an inoculum density of 2500 nematodes per plant to determine the effect of temperature on nematode reproduction on chickpea cv. UG 27. Reproduction increased with increase of soil temperature. A population from Jerez had a reproduction rate significantly higher than the other two populations at each of the temperatures. Shoot wight of chickpea was not affected by nematode population or soil temperature. At 25°C root weight was significantly lower with a *P. thornei* population from Cañete.

Keywords: chickpea, *Cicer arietinum,* plant-parasitic nematodes, *Pratylenchus thornei,* root-lesion nematodes, soil temperature, Spain, surveys.

Approximately 60 000 ha of chickpea (*Cicer arietinum* L.) are grown annually in the Region of Andalucía, southern Spain, accounting for about 65% of the total national acreage, more than half of which is concentrated in the Córdoba and Sevilla provinces. *Pratylenchus* spp. and *Tylenchorhynchus* spp. have been reported in association with chickpea crops in central Spain (Bello, 1979) and *Meloidogyne artiellia* Franklin, *Merlinus microdorus* (Geraert) Siddiqi, *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans-Stekhoven, and *Zygotylenchus guevarai* (Tobar Jiménez) Braun & Loof have been found in southern Spain (Tobar Jiménez, 1973; Varo *et al.*, 1970). On a global basis, nematodes cause an estimated yield loss of 14% in chickpea (Sharma & McDonald, 1990; Sharma *et al.*, 1992), but there is no information about crop losses caused by plant parasitic nematodes in Spain.

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Therefore, a survey was undertaken to determine the distribution and population density of plant-parasitic nematodes associated with chickpea in the Guadalquivir Valley of Andalucía, southern Spain, and glasshouse experiments to determine the effect of soil temperature on the reproduction and pathogenicity of different populations of *Pratylenchus thornei*, which is the most abundant and widespread plant parasitic nematode in chickpea crops in the Mediterranean region (Greco *et al.*, 1992; Di Vito *et al.*, 1994a, b).

MATERIALS AND METHODS

Survey. Systematic surveys for plant-parasitic nematodes were carried out in 12 localities of the main chickpea growing areas of Cádiz, Córdoba and Sevilla provinces (Fig. 1). Soil samples were collected from 63 fields when chickpea was flowering in May, 1990 and 1991. The fields were randomly chosen along a predetermined route in each province. Soil samples, consisting of a composite of *ca* 500 cm^3 aliquots, were randomly collected with a hoe to a depth of 30 cm. Four soil subsamples from each field were bulked, thoroughly mixed in a plastic bag, placed in an insulated container to prevent their exposure to heat or direct sunlight, brought to the laboratory within 24 h and stored at 5°C until they

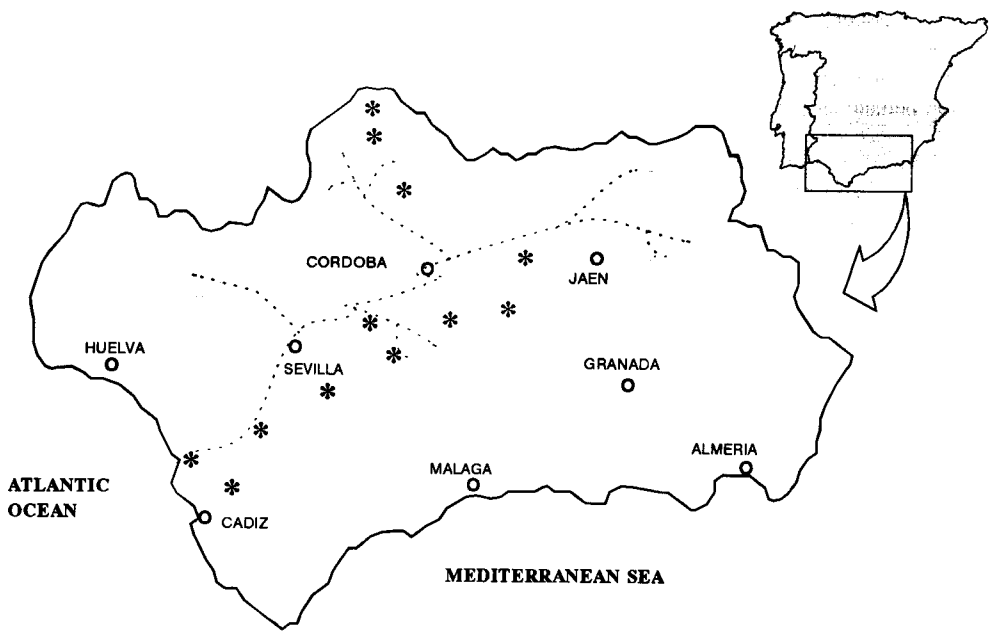

Fig. 1. Map of Andalucía, southern Spain, indicating the location of the twelve sampling localities (*) in the Guadalquivir Valley.

were processed. Root samples, consisting of 12 plants per field (3 plants per subsample) were placed in plastic bags and treated as for soil samples.

Nematodes were extracted from 500 cm^3 subsamples of soil and from 5 g of fresh root by centrifugation (Coolen, 1979). Nematodes were fixed in 4% formaldehyde and processed to glycerin by Seinhorst's (1962) method for species identification.

Influence of soil temperature. Three populations of *P. thornei* were obtained from chickpea root samples at Santaella and Cañete de las Torres, in Córdoba province, and Jerez de la Frontera, in the province of Cádiz. A single female from each population was placed on a carrot disk and incubated at 24°C for six weeks, and the nematode populations increased through several disk cultures (Huettel, 1985). Nematodes were extracted from infested carrot disks in a Baermann funnel to provide inocula for the experiments. Extracted nematodes were surface disinfected with 0.02% ethoxyethyl mercury chloride and 0.1% streptomycin suphate solutions for 2 and 24 h, respectively, thoroughly rinsed several times in sterilized water and nematode population densities determined from a 1 ml aliquot.

Six day-old chickpea seedlings were transplanted into 15 cm diam 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 (15% females, 74% juveniles, 11% eggs for the Santaella population; 10% females, 81% juveniles, 9% eggs for the Cañete population; and 15% females, 68% juveniles, 17% eggs for the Jerez population) in 10 ml of sterile water by adding the nematode suspension around the root ball of each seedling at transplanting. Control plants were treated similarly but with distilled sterile water only. Plants were grown in a glasshouse, in soil tanks at 15, 20 or 25 ± 1°C, and with a 14 h photoperiod of fluorescent light. Plants were watered daily with 100 ml of water and fertilized weekly with 100 ml of a nutrient solution (Hoagland & Arnon, 1950). Average maximum and minimum air temperatures in the glasshouse were 28 ± 5.0°C and 14 ± 2.7°C, respectively. Treatments were replicated ten times in a randomized complete block design and the experiment was repeated once.

Fifty days after nematode inoculation, when plants were at the full bloom to early podding stage, the shoots were cut off at the soil line and the roots washed free of soil. Assessments were made of 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 0-10 scale according to the percentage of necrotic tissue present: 0 = 0% necrotic tissue, and 10 = 100% necrotic tissue. Upon completion of the experiment,

nematodes from 100 cm^3 soil and from 5 g root samples were extracted by centrifugation (Coolen, 1979) to determine final nematode population densities.

Statistical analysis. For data analyses, number of nematodes ($\underline{x}$) were transformed to long ($\underline{x}+1$). Similarity among experimental runs, tested by preliminary analyses of variance using experimental runs as blocks, allowed combining of data for analyses of variance. Treatment means were compared using Fisher's protected least significant difference test (LSD) at $P = 0.05$.

RESULTS

Survey. Twenty-three species of plant-parasitic nematodes were associated with chickpeas in the Guadalquivir Valley (Table I). The root-lesion nematode, *P. thornei*, was found in 92% of the total number of soil and 84% of the root samples. Infested plants had extensive necrotic lesions on the roots but showed no symptoms in the shoots. Population densities of *P. thornei* ranged from 2 to 48 nematodes per 100 cm^3 of soil (Table I) and 6-70 nematodes per g of root. Other endoparasitic nematode species found in relative abundance included *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven, *Pratylenchoides* sp. and *Z. guevarai*, with root populations of 50-124 (one field in Sevilla), 1 (one field in Córdoba) and 3-4 (one field in Cádiz) nematodes per g of root, respectively.

Ectoparasitic and semi-endoparasitic nematode species that were common in the chickpea rhizophere included *Amplimerlinius magnistylus* Castillo, Gómez-Barcina, Vovlas and Navas, *Helicotylenchus tunisiensis* Siddiqi, *Mesocriconema curvatum* (Raski) Loof & De Grisse, *M. sphaerocephalum* (Taylor) Loof & De Grisse, *Xiphinema brevicolle* Lordello & Da Costa, *X. ingens* Luc & Dalmasso, *Longidorus* sp., *Merlinius brevidens* (Allen) Siddiqi, *Paratrophorus loofi* Arias, *Paratylenchus sheri* (Raski) Raski & Luc, and *Trophurus imperialis* Loof.

Influence of soil temperature. Nematode population densities in soil and roots, and the nematode reproduction index, increased with increasing soil temperature; nematode populations differed significantly ($P < 0.05$) at each soil temperature (Table II). Nematode density in the roots was higher the Jerez population than with Santaella and Cañete populations at 15 or 20°C but Jerez and Cañete populations were similar at 25°C (Table II). Reproduction of the Jerez population was significantly ($P < 0.05$) higher than the Santaella and Cañete populations at each of the soil temperatures (Table II).

Severity of root necrosis in plants was not affected by soil temperature or *P. thornei* population (Table II). Root weight significantly ($P < 0.05$) decreased

TABLE I

Number of plant-parasitic nematodes per 100 cm^3 of soil from 63 chickpea fields in Cádiz, Córdoba, and Sevilla provinces of Andalucía, southern Spain, recovered May 1990, 1991

	Cádiz			Córdoba			Sevilla		
	No. of fields	No. nematodes		No. of fields	No. nematodes		No. of fields	No. nematodes	
		Mean	Range		Mean	Range		Mean	Range
Amplimerlinius magnistylus	9	5	2-10	13	3	1-4	11	16	6-30
Aorolaimus perscitus	8	3	1-6	5	1	1-1	13	4	3-6
Bitylenchus huesingi	1	1	–	5	1	1-2	5	1	–
Ditylenchus sp.	–	–	–	3	1	1-1	–	–	–
Helicotylenchus tunisiensis	–	–	–	1	3	2-7	8	5	1-11
Longidorus sp.	2	1	–	–	–	–	–	–	–
Meloidogyne artiellia	–	–	–	–	–	–	1	1	–
Merlinius brevidens	12	11	8-16	13	2	1-3	14	5	2-7
Merlinius microdorus	–	–	–	7	16	10-23	–	–	–
Mesocriconema curvatum	–	–	–	3	10	1-20	–	–	–
Mesocriconema sphaerocephalum	–	–	–	3	1	–	1	1	–
Paratrophurus loofi	12	20	8-36	16	14	2-26	9	16	4-28
Paratylenchus microdorus	–	–	–	2	1	–	–	–	–
Paratylenchus sheri	7	3	2-4	5	1	–	8	3	1-7
Pratylenchoides sp.	–	–	–	1	1	–	–	–	–
Pratylenchus penetrans	–	–	–	–	–	–	2	42	39-60
Pratylenchus thornei	14	25	8-48	25	9	2-22	19	19	6-26
Trophurus imperialis	4	1	–	3	2	1-4	3	2	1-2
Tylenchorhynchus clarus	–	–	–	2	1	–	3	4	1-7
Xiphinema brevicolle	–	–	–	–	–	–	6	1	–
Xiphinema ingens	–	–	–	1	1	–	–	–	–
Zygotylenchus guevarai	2	1	–	–	–	–	–	–	–

TABLE II

Effect of temperature on reproduction of three populations of Pratylenchus thornei *from Cañete, Jerez and Santaella, Spain and on growth of chickpea cv. UC 27*[1)]

Temperature (°C)	*P. thornei* populations	Number of nematodes in soil (100 cm^{-3})	Number of nematodes in root (g^{-1})	Rf[2)]	Root weight (g)	Shoot weight (g^{-1})	Root necrosis[3)]
15	Control	0	0	0	24.99a	15.99a	0
	Santaella	48.44a	75.88a	0.70a	22.92a	14.90a	1.6a
	Cañete	32.50b	74.40a	0.70a	23.18a	14.71a	2.4a
	Jerez	70.31c	125.34b	1.12b	23.51a	15.19a	1.9a
20	Control	0	0	0	20.01a	15.72a	0
	Santaella	95.00a	173.98a	1.36a	18.78a	14.81a	2.6a
	Cañete	67.81a	234.46b	1.86b	19.54a	14.56a	2.2a
	Jerez	69.38c	417.05c	3.09c	19.59a	14.85a	1.7a
25	Control	0	0	0	16.85a	15.04a	0
	Santaella	105.94a	346.80a	2.20a	15.36ab	14.84a	2.5a
	Cañete	121.56a	624.87b	3.38b	12.49b	13.49a	1.7a
	Jerez	137.81a	695.44b	4.41c	16.91a	14.43a	1.7a

[1)]Data are the average of two experiments with 10 replicated plants per treatment combination in each experiment. Plants were inoculated with 2 500 nematodes. Actual data are presented for each treatment, but data were transformed to $\log(x+1)$ for analysis of variance. For each temperature, numbers in a column followed by the same letter do not differ significantly ($P = 0.05$), according to Fisher's protected LSD test.

[2)]Nematode reproduction index = final nematode population density per plant/initial nematode population density per plant.

[3)]Assessed on a scale of 0 (0% necrotic tissue) to 10 (100% necrotic tissue) 50 days after inoculation.

with increasing soil temperature, but was not influenced by nematode population at 15 or 20°C (Table II). However, at 25°C soil temperature, root weight of plants inoculated with the Cañete population was lower than those of the controls and the Jerez population. Shoot weight was not significantly affected by soil temperature or nematode population.

DISCUSSION

Pratylenchus thornei has been reported from several hosts and at various localities in Spain (Arias & Romero, 1971; Bello, 1969; Castillo *et al.*, 1985).

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DISCUSSION

Pratylenchus thornei has been reported from several hosts and at various localities in Spain (Arias & Romero, 1971; Bello, 1969; Castillo *et al.*, 1985).

Included in the hosts is wheat (Fortuner, 1977) which is extensively used in crop rotations with chickpea in the Guadalquivir Valley. In our survey nematode population densities of *P. thornei* were not large, but nevertheless the species must be considered as potentially capable of damaging chickpea in Spain. Di Vito *et al.* (1992) established a threshold density of 3.1 nematodes per 100 cm^3 soil under field conditions in Syria.

The nematode fauna of chickpea in Spain is quite similar to that found in other countries of the Mediterranean region, except for the absence of important cyst nematodes such as *H. ciceris* Vovlas *et al.* in Syria (Greco *et al.*, 1992) and Turkey (Di Vito *et al.*, 1994b) and a larger number of root-lesion nematode species in North Africa and Turkey (Greco *et al.*, 1992; Di Vito *et al.*, 1994a, b).

The three Spanish populations of *P. thornei* infested chickpea cv. UC 27, but only the Cañete population caused a significant ($P < 0.05$) reduction of root growth of about 25% at 25°C. Nematode reproduction increased with temperature, although it was not high at 20 and 25°C. Moreover, the decline of the Santaella and Cañete at 15°C supports the hypothesis that a proportion of nematodes might have died soon after inoculation. Nevertheless, the three populations differed in their ability to reproduce on chickpea at 20 or 25°C, the reproduction index of the Jerez population being significantly higher at any of the soil temperatures. The influence of temperature on nematode reproduction may, in turn, affect plant growth and productivity. These results support the existence of strains of *Pratylenchus* species with different parasitic fitness reported by several authors (Griffin, 1991; Motalaote *et al.*, 1987; Pinochet *et al.*, 1993) and are consistent with data from other studies with root-lesion nematodes (Griffin, 1991; Griffin & Gray, 1990; Kimpinski & Willis, 1981).

Since *P. thornei* is the most frequently found endoparasitic nematode in chickpea crops in southern Spain, studies have been initiated to determine whether it influences the reaction of chickpea to *Fusarium oxysporum* f.sp. *ciceris*, both in compatible and incompatible plant-fungus interactions.

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RÉSUMÉ

Nématodes phytoparasites associés au pois chiche dans le sud de l'Espagne et effet de la température du sol sur la reproduction de Pratylenchus thornei

Une enquête ayant porté sur 63 champs de pois chiche de la vallée du Guadalquivir (Andalousie, sud de l'Espagne) a démontré que *Pratylenchus thornei* constitue l'espèce de nématode phytopara-

site prépondérante; elle a en effet été rencontrée dans 92% des échantillons de sol et 84% de ceux de racines avec des densités de population s'échelonnant de 2 à 48 individus pour 100 cm^3 de sol et 6 à 70 individus par gramme de racine fraîche. Des populations notables de nématodes ectoparasites *Amplimerlinius magnistylus, Aorolaimus perscitus, Helicotylenchus tunisiensis, Merlinius brevidens, Mesocriconema curvatum* et *Paratrophurus loofi* ont également été trouvées.

Trois populations de *P. thornei* ont été testées en serre, à 15, 20 et 25°C avec un inoculum de 2500 individus par plante, pour déterminer l'effet de la température sur la reproduction du nématode sur le pois chiche cv. UC 27. La reproduction augmente en même temps que la température. Une population originaire de Jerez montre un taux de reproduction significativement plus élevé que celui des deux autres populations, et ce quelle que soit la température. Le poids des pieds de pois chiche n'est affecté ni par la population du nématode, ni par la température. A 25°C, le poids des racines est significativement plus faible dans le cas d'une population de *P. thornei* provenant de Cañete.

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