ROOT-KNOT AND OTHER PLANT-PARASITIC NEMATODES ASSOCIATED WITH FIG TREES IN PORTUGAL

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Summary. A survey was conducted in Portugal to detect root-knot and plant-parasitic nematodes associated with edible fig trees (*Ficus carica*). Fifty-three soil and root samples were collected in seven Portuguese districts. Fruits from edible fig and wild caprifig trees (*F. carica sylvestris*) were also collected. Of the genera and species found, *Helicotylenchus* spp., *Heterodera fici*, *Meloidogyne* spp., *Paratylenchus* sp., *Pratylenchus* sp., and *Xiphinema* spp. were the most widely distributed. Root-knot nematodes are probably the nematodes that are most damaging to fig trees. *Ogma palmatum*, *Mesocriconema xenoplax* and *Schistonchus caprifici* are recorded for the first time from Portugal.

Key words: Esterase phenotypes, *Ficus carica*, geographical distribution, host tests, *Meloidogyne* spp., new records.

The fig tree, *Ficus carica* L., which probably originated from Western Asia, was taken to the Mediterranean region, countries from this area being the largest producers of edible figs (Sadhu, 1990; Tous and Ferguson, 1996). The European Union (EU), in 2004, produced approximately 184 000 t of figs, which corresponds to 17% of world production, Greece being the leading EU producer (Anonymous, 2005). In Portugal, 40% of the fig tree area (3,000 ha) is located in the district of Faro, Algarve (Anonymous, 2003). The first record of a plant-parasitic nematode associated with fig trees was *Meloidogyne* spp. (root-knot nematodes, RKN) in the USA (Neal, 1889). Since then, several plant-parasitic nematode species have been reported parasitizing fig trees in many countries (McSorley, 1981, 1992; Cohn and Duncan, 1990; Campos, 1997; Krnjaić *et al.*, 1997; Li *et al.*, 1999). In Portugal, only two nematodes, *Aphelenchus avenae* Bastian, 1865 and *Heterodera fici* Kirjanova, 1954 were known to occur associated with fig trees (Macara, 1963).

This study was undertaken to provide information on the plant-parasitic nematodes associated with fig trees in Portugal.

MATERIALS AND METHODS

Fifty-three samples were collected from various fig-growing areas of continental Portugal (Tables I and III). Soil and root samples were taken around the fig trees and, when possible, fruits of edible fig trees (28 samples) and two samples of wild caprifig trees, *F. carica sylvestris* L., were also collected. Soil samples, each consisting of five cores, were processed by a Baermann-type method and by $ZnSO_4$ centrifugal-flotation (Jenkins, 1964; Abrantes *et al.*, 1976) of a 300 cm^3 sub-sample. Also, a 500 cm^3 sub-sample of each soil sample was processed using a Fenwick can followed by microscopic examination of screen material for nematode cysts (Shepherd, 1986). Roots were gently rinsed with water and examined under a stereomicroscope for the presence of RKN and cyst nematodes. RKN females and egg masses were extracted from galled roots and 20 perineal patterns of adult females/isolate were prepared for their identification according to Hartman and Sasser (1985). RKN egg masses were used to propagate the isolates on tomato (*Solanum lycopersicum* L. cv. Rutgers), in the glasshouse, to obtain inocula for the North Carolina differential host tests (Taylor and Sasser, 1978) and for biochemical characterization.

Non-specific esterase activity for each RKN isolate was determined from replicate protein extracts of one, three and five females. Extracts of five females of a *M. javanica* isolate were included on each gel as a reference phenotype. Soluble proteins were separated by the automated PhastSystem in polyacrylamide gradient 10-15 PhastGels and the gels were stained for esterase activity. Phenotypes were designated with letter(s) and a number indicating the number of bands according to Esbenshade and Triantaphyllou (1985), Pais *et al.* (1986) and Carneiro *et al.* (2004).

Identification of *Heterodera* isolates was based on observations and measurements of vulval cones prepared from cysts using the glycerine-agar technique (Correia and Abrantes, 1997), and of second-stage juveniles (J2) mounted on slides after releasing them from

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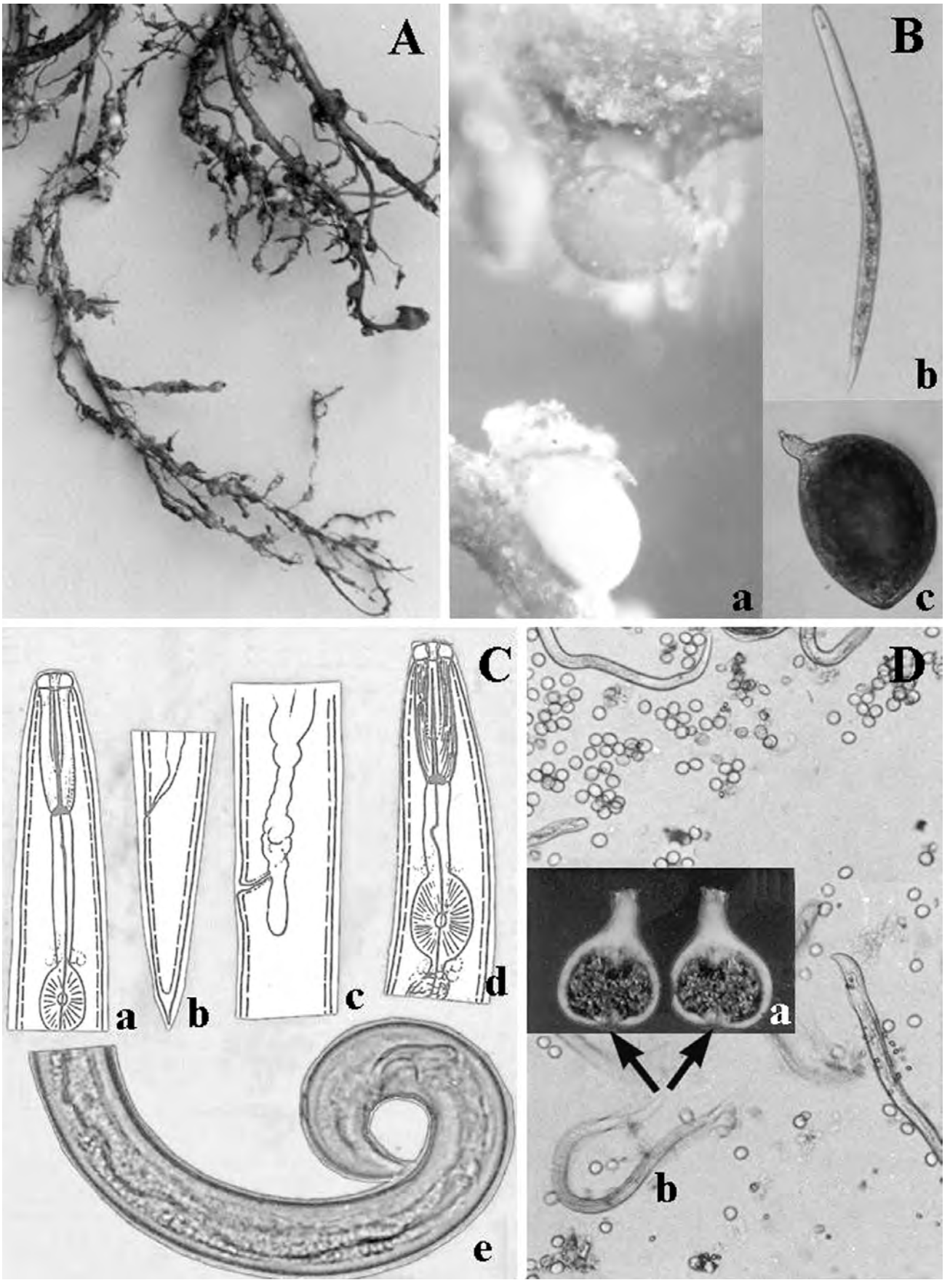

Fig. 1. Plant-parasitic nematodes parasitizing fig trees. A: Fig roots severely infected with *Meloidogyne incognita*; B: *Heterodera fici* - fig roots with a cyst and a white female (a), second-stage juvenile (b), cyst (c); C: *Schistonchus caprifici* female - anterior, posterior and vulva regions (a,b,c), and male - anterior and posterior regions (d,e); D: Caprifig fruits (a) and *Schistonchus caprifici* (b) - specimens obtained from staminate fig florets.

cysts by crushing the cysts.

Roots were stained with acid-fuchsin to detect nematode-infected plant tissues (Byrd *et al*., 1983). Fruits were cut into several pieces and incubated in water. After 24 h, the suspensions were examined under a stereomicroscope (Vovlas *et al*., 1992).

The genera and/or species of other plant-parasitic nematodes found in the various samples were determined through microscopic examination of semi-permanent mounts, but only when sufficient adults were present in samples to provide an accurate identification.

RESULTS AND DISCUSSION

Plant-parasitic nematodes were identified in nineteen of the 53 samples. *Meloidogyne* spp. were the most common plant-parasitic nematodes in fig roots. Galls were found in eight of the nineteen fig-infected root samples (Fig. 1A and Table I). According to the perineal pattern morphology, *Meloidogyne hapla* Chitwood occurred in only one sample, *M. hispanica* Hirschman in four, and *M. incognita* (Kofoid *et* White) Chitwood in three (Table I). Results of the differential host tests showed that isolate F13 gave a host response similar to *M. hapla* whereas F25, F26 and F36 gave host responses similar to *M. incognita* race 4 (Table II). *Meloidogyne hispanica* species and races cannot be identified on the basis of their responses in the differential host test. Isolates F15 and F16 gave host responses similar to *M. arenaria* (Neal) Chitwood race 2 or *M. javanica* (Treub) Chitwood whereas isolate F24 gave a response similar to *M. incognita* race 3 and isolate F32 gave a response similar to *M. incognita* race 2 or *M. arenaria* race 1 and to the original population ("Seville") of *M. hispanica* (Taylor and Sasser, 1978; Hirschmann, 1986). These results indicated that *M. hispanica* isolates F15, F16 and F24 were distinct from those already described for this species, for which only cotton and peanut had been considered to be non-hosts (Hirschmann, 1986). Analyses of the esterase phenotypes revealed inter- and intra-specific variability among the RKN isolates (Fig. 2). Phenotype H_1 occurred in isolate F13 and I_1 in isolates F25, F26 and F36. *Meloidogyne hispanica* isolates exhibited some variability in minor and fainter bands. Three phenotypes (Hi_2, Hi_3 and Hi_4) were detected and all the isolates shared two common major bands that have been used to characterize isolates of that species (Fig. 2). Similar phenotypes were reported for *M. hispanica* by Janati *et al*. (1982), Esbenshade and Triantaphyllou (1985), Fargette (1987), Pais and

Table I. Species and races of root-knot nematodes, *Meloidogyne* spp., found in fig tree, *Ficus carica,* roots in Portugal.

Meloidogyne species and race	District Locality (isolate)
M. hapla	Coimbra
	Coimbra (F13)
M. hispanica	Faro
	Armação de Pêra (F15), Portimão (F16), Odeceixe (F32)
	Setúbal
	Grândola (F24)
M. incognita race 4	Setúbal
	Melides (F25), Santiago do Cacém (F26)
	Faro
	Sagres (F36)

Table II. Differential host plant reactions of *Meloidogyne* isolates obtained from fig tree samples.

Meloidogyne species (isolate)	Differential host [a]					
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Tomato
M. hapla						
(F13)	+[b]	-[b]	+	-	+	+
M. hispanica						
(F15)	+	-	-	+	-	+
(F16)	+	-	-	+	-	+
(F24)	-	+	+	+	-	+
(F32)	+	-	+	+	-	+
M. incognita						
(F25, F26, F36)	+	+	+	+	-	+

[a] Tobacco (*Nicotiana tabacum*) 'NC95'; cotton (*Gossypium hirsutum*) 'Deltapine 61'; pepper (*Capsicum annuum*) 'California Wonder'; watermelon (*Citrullus vulgaris*) 'Charleston Grey'; peanut (*Arachis hypogaea*) 'Florunner'; and tomato (Solanum *lycopersicum*) 'Rutgers'.

[b] + = host; - = non-host, see Taylor and Sasser (1978).

Abrantes (1989) and Carneiro *et al.* (2004). The esterase electrophoretic patterns, in conjunction with the perineal pattern morphology, were useful characters for the identification of the RKN isolates.

The morphological and morphometrical characters of the vulval cones and J2 of the five isolates of *Heterodera* (Fig. 2), found in root and soil samples (Table III), agree with those reported for *H. fici* (Mulvey and Golden, 1983). The virus vector nematode *Xiphinema index* Thorne *et* Allen and *Paratylenchus* sp. were also found in the roots (Table III). In the soil samples, nematodes encountered were: *Helicotylenchus* sp., *Helicotylenchus dihystera* (Cobb) Sher, *Mesocriconema xenoplax* (Raski) Loof *et* De Grisse, *Ogma palmatum* (Siddiqi *et* Southey) Siddiqi, *Paratylenchus* sp., *Pratylenchus* sp., *X. index*, *X. pachtaicum* (Tulaganov) Kirjanova, and *Xiphinema* sp. (Table III). *Schistonchus caprifici* (Gasparrini) Cobb was found in all the inflorescences of the 28 edible fig samples and in pistillate florets of one caprifig sample (Fig. 1C, D).

Table III. Other plant-parasitic nematodes found in roots and/or soil around roots of fig trees, *Ficus carica*, in Portugal.

Nematode genus or species	Root	Soil	District Locality (isolate reference)
Helicotylenchus			Santarém
	-[a]	+[b]	Ferreira do Zêzere (F3), Golegã (F11)
			Coimbra
	-	+	Porto de Bordalo (F14), Enxofães (F20)
			Faro
	-	+	Castro Marim (F22)
H. dihystera			Faro
	-	+	Guia (F17)
Heterodera fici			Santarém
	+	+	Ferreira do Zêzere (F3)
			Portalegre
	+	+	Gavião (F5), Gáfete (F7)
			Coimbra
	+	+	Silvã (F19), Enxofães (F21)
Mesocriconema xenoplax			Faro
	-	+	Armação de Pêra (F15)
Ogma palmatum			Coimbra
	-	+	Santa Clara (F13)
Paratylenchus			Coimbra
	-	+	Santa Clara (F13), Porto de Bordalo (F14), Silvã (F19), Enxofães (F20)
			Faro
	+	+	Pêra (F16), Armação de Pêra (F15)
Pratylenchus			Portalegre
	-	+	Gáfete (F7)
			Coimbra
	-	+	Porto de Bordalo (F14), Silvã (F19), Enxofães (F20)
			Faro
	-	+	Guia (F18), Castro Marim (F22)
Xiphinema index			Portalegre
	+	-	Gavião (F5)
			Santarém
	+	+	Golegã (F11)
			Faro
	-	+	Pêra (F16), Guia (F18)
X. pachtaicum			Santarém
	-	+	Ferreira do Zêzere (F3)
			Coimbra
	-	+	Santa Clara (F13)
			Faro
	-	+	Armação de Pêra (F15), Pêra (F16)
Xiphinema			Coimbra
	-	+	Enxofães (F20)
			Faro
	-	+	Castro Marim (F22)

[a] absent;
[b] present.

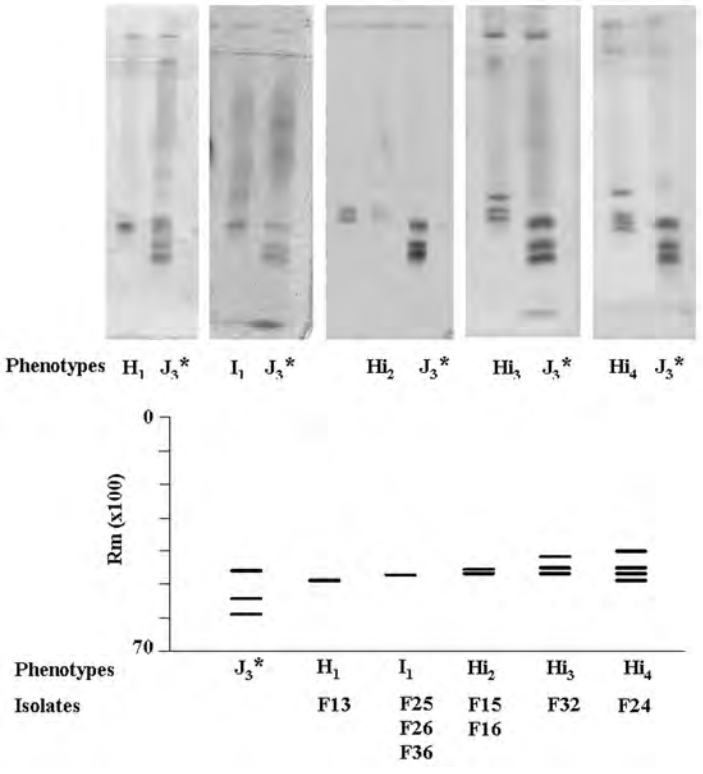

Fig. 2. Esterase phenotypes detected in eight *Meloidogyne* isolates obtained from infected fig tree roots. Phenotypes were designated with a letter(s) and a number(s) indicating the number of bands. J_3 = *M. javanica*, reference isolate; H_1 = *M. hapla*; I_1 = *M. incognita*; Hi_2, Hi_3, and Hi_4 = *M. hispanica*.

Mesocriconema xenoplax, *O. palmatum*, and *S. caprificci* are reported from Portugal for the first time.

These results emphasize the importance in fig cultivation of RKN, which have been considered the most severe nematode problem (Cohn and Duncan, 1990; Li *et al.*, 1999). Further studies are needed to evaluate the pathogenicity and damage thresholds of RKN and other plant-parasitic nematodes infecting fig trees in Portugal as these may also be associated with the decline of fig trees.

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