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Species of Root-knot Nematodes and Fungal Egg Parasites Recovered from Vegetables in Almería and Barcelona, Spain

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Abstract: Intensive vegetable production areas were surveyed in the provinces of Almería (35 sites) and Barcelona (22 sites), Spain, to determine the incidence and identity of *Meloidogyne* spp. and of fungal parasites of nematode eggs. Two species of *Meloidogyne* were found in Almería—*M. javanica* (63% of the samples) and *M. incognita* (31%). Three species were found in Barcelona, including *M. incognita* (50%), *M. javanica* (36%), and *M. arenaria* (14%). Solanaceous crops supported larger ($P < 0.05$) nematode numbers than cucurbit crops in Almería but not in Barcelona. Fungal parasites were found in 37% and 45% of the sites in Almería and Barcelona, respectively, but percent parasitism was never greater than 5%. Nine fungal species were isolated from single eggs of the nematode. The fungi included *Verticillium chlamydosporium, V. catenulatum, Fusarium oxysporum, F. solani, Fusarium* spp., *Acremonium strictum, Gliocladium roseum, Cylindrocarpon* spp., *Engiodontium album,* and *Dactylella oviparasitica.* Two sterile fungi and five unidentified fungi also were isolated from *Meloidogyne* spp. eggs.

Key words: antagonist, biological control, *Meloidogyne* spp.

Vegetable crops are grown intensively for fresh market in many countries of the Mediterranean region. The mild winters and warm summers, which are characteristic of this climate, permit two to three crops to be cultivated at the same site from spring through winter. As a result of intensive agriculture and cultivation in plastic houses, root-knot nematodes have become a major pest of vegetable crops. In the Mediterranean region, *Meloidogyne* spp. cause yield losses of 11 to 60%, (Ibrahim, 1985; Lamberti, 1981). Six species of *Meloidogyne* have been described as plant pathogens in Spain (Andrés et al., 1998), but information on their distribution, frequency, and relative importance is limited.

At present, control of root-knot nematodes is based mainly on the use of soil fumigants. Historically, methyl bromide was widely used in some areas of Spain (López Bellido et al., 1994; Verdejo-Lucas et al., 1997), but it is being replaced by other soil fumigants such as dichloropropene and metam sodium (Bello et al., 1997). Increased concern for the environment and human health and the impact of governmental regulations have prompted research to find alternative methods of nematode control. There is a renewed interest in the use of nematode antagonists as management strategies for nematode control. Among these fungal antagonists are the egg parasites. Root-knot nematode eggs are likely the most vulnerable stage of the nematode life cycle to antagonists (Viaene and Abawi, 1998). The objective of this study was to determine the *Meloidogyne* species that were present in two intensive vegetable production areas of north and south Spain, and their egg parasites.

Materials and Methods

Survey for Meloidogyne spp.: Composite soil and root samples were taken from 35 plastic houses in Almería and from 12 plastic houses and 10 fields in Barcelona to assess nematode numbers and fungal parasitism of nematode eggs at the end of the spring crop. Sampling was done in May and July 1998 in Almería and Barcelona, respectively. The sites were selected because they were infested with *Meloidogyne* spp. and were representative of intensive vegetable cropping systems in the Mediterranean region but had different cropping cycles, climatic conditions, and agronomic practices. One to two crops are grown per year in Almería using a sand-mulching system consisting of a layer of manure placed on top of undisturbed soil and covered with a 15 to 20-cm layer of river sand. In Almería, annual temperatures range from 12.0 to 25.8 °C. In Barcelona, two to three crops are grown per year in soils with more than 60% sand, and annual temperatures range from 10.2 to 23.8 °C. In each site, 6 to 10 plants were dug from patches with symptoms of root-knot nematode damage, and galled roots and rhizosphere soil (approximately 2 kg soil per site) were collected. Samples were sieved to separate roots from soil, and one 500-cm^3 soil subsample per site was used for nematode extraction on Baermann trays. Juveniles migrating into the water were collected 1 week later, counted, and expressed per 250 cm^3 of soil. Roots from each site were washed free of soil, chopped, and bulked, and eggs were extracted from a 5-gram root subsample by blender maceration in a 0.5% NaOCl solution for 10 minutes. Females were extracted from an additional 5-gram root subsample by blender maceration, and 10 to 12 females per site were identified according to the morphology of the perineal pattern. The esterase phenotype was determined for 10 to 12 individual females per site. Numbers of nematodes after solanaceous or

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cucurbit crops were compared within each sample area by Student's *t*-test ($P < 0.05$).

Fungal egg parasites: Galled roots were chopped and mixed, and 30 to 40 egg masses were handpicked with the aid of a dissecting stereomicroscope from one 5-gram root subsample per survey site. Eggs were dispersed from egg masses with approximately 0.2 ml of sterile water using a pestle in an Eppendorf microcentrifuge tube, spread on a restrictive growth medium (Lopez-Llorca and Duncan, 1986) in three replicate petri dishes, and incubated at 25 °C. Eggs were examined for parasitism after 48 hours at ×50; they were considered parasitized if fungal hyphae grew from them. Parasitized eggs were transferred individually to corn meal agar to establish pure cultures of the fungi. Cultures were stored on 1% (w/v) water-agar slants at 4 ℃. The fungal taxa were identi fied according to their cultural and morphological characteristics. The *Acremonium* Link, *Verticillium* Nees, and *Gliocladium* Corda isolates were grown on 2% malt extract agar (200 mL malt extract [10% sugar], 15 g agar, 800 mL tap water) from a single conidum at 20 ℃. and identi fied according Gams (1971) and Domsch et al. (1980), respectively. *Cylindrocarpon* Wollenweber isolates were inoculated onto potato dextrose agar (PDA; 75 g potatoes [boiled and filtered], 20 g dextrose, 15 g agar, 1 L tap water) and incubated at 20 to 25 ℃ and identi fied to species (Booth, 1966). To identify the *Fusarium* Link strains, the single-spore method (Nelson et al., 1983) was used. When monosporic cultures were established, the isolates were plated onto PDA and carnation-leaf agar (3 to 5 pieces of carnation leaves sterilized by propilene oxide fumigation and placed on solid 2% tap water agar), incubated at 20 to 25 ℃, and identi fied according Nelson et al. (1983). *Engyodontium* de Hoog species were identified according Hoog (1972) after culturing the isolates on oatmeal agar (30 g boiled and filtered oat flakes, 15 g agar, 1 L tap water) at 20 ℃. Finally, a *Dactylella* Grove isolate was grown on half-strength corn meal agar (8.5 g corn meal, 12.5 g agar, 1 L distilled water) at 23 ℃ and identi fied according Rubner (1996). All cultures were incubated under 12 hours of darkness, alternating with 12 hours of cool white fluorescent light. The diameter of the colonies was taken after incubation for 7 and 14 days. The measurements of the microscopic structures were made in tap water mounts, using a Leitz Dialux 20 EB microscope.

Results

Incidence of Meloidogyne spp. In Almería, 63% of the sites sampled were infested with *Meloidogyne javanica* (Treub) Chitwood and 31% of the sites were infested with *M. incognita* (Kofoid & White) Chitwood (Table 1). They were the only plant-parasitic nematodes detected in 94% of the plastic houses except for the migratory ectoparasitic nematode *Tylenchorhynchus cylindricus* Cobb that was also present in sites D-4 and N-57, and *Paratylenchus* spp. in site N-3. *Meloidogyne javanica* was the dominant species (92% of the samples) in the Bajo Andarax County, where tomato is grown in monoculture. Numbers of nematodes were higher ($P < 0.05$) after a solanaceous (tomato or eggplant) ($\bar{x} \pm SE$ = 19,890 ± 29,480 juveniles/250 cm^3 soil, and 100,370 ± 66,100 eggs/g root) than after a cucurbit crop (melon, watermelon, squash, or cucumber) (6,260 ± 5,580 juveniles/250 cm^3 soil, and 21,090 ± 19,000 eggs/g root).

Meloidogyne incognita, M. javanica, and *M. arenaria* (Neal) Chitwood were found in Barcelona with an incidence of 50, 36, and 14%, respectively (Table 1). *Meloidogyne* spp. were the most abundant plant-parasitic nematodes in all sites except site E-66, where *Helicotylenchus* spp. occurred at densities of 1,200 per 250 cm^3 soil. Other plant-parasitic nematodes occurring at low population densities included *Tylenchorhynchus* spp. (55% of the samples) and root-lesion nematodes, *Pratylenchus* spp. (9%). *Meloidogyne javanica* was found in 46% of the samples in El Maresme County, whereas *M. incognita* dominated in Baix Llobregat County (67% of the samples). Number of nematodes after a solanaceous (tomato or green pepper) (2,400 ± 2,370 juveniles/250 cm^3 soil, and 44,050 ± 69,030 eggs/g root) or cucurbit crop (watermelon, cucumber, or pumpkin) (3,430 ± 1,940 juveniles/250 cm^3 soil, and 23,040 ± 16,090 eggs/g root) did not differ in this province.

Fungal egg parasites. Fungal parasites were found in 37% and 45% in Almería and Barcelona, respectively, but percentage parasitism was never greater than 5%. A diversity of fungal species was identified, including *Verticillium chlamydosporium, V. catenulatum* (Kamyschko ex Barron & Onions) Gams, *Fusarium oxysporum* Schlechtendahl: Fries, *F. solani* (Martius) Saccardo, *Acremonium strictum* Gams, *Gliocladium roseum* Bainier, *Cylindrocarpon* spp, *Engiodontium album* (Limber) de Hoog, and *Dactylella oviparasitica* Stirling & Mankau. We also isolated two unidentified *Fusarium* spp., and seven unidentified genera including two with sterile mycelium. A single fungal species was identified in most sites, although *V. chlamydosporium* and *Fusarium* co-existed in sites B-75 and B-81, and *E. album* and *Fusarium* in site E-61. Eggs of *Meloidogyne* that were parasitized by fungi were found in fields infested with *M. incognita* (12 isolates), *M. javanica* (16 isolates), and *M. arenaria* (2 isolates).

Discussion

The most common species of root-knot nematodes in the Mediterranean region, *Meloidogyne javanica, M. incognita,* and *M. arenaria* (Ibrahim, 1985; Lamberti, 1981), were identified in Barcelona, which confirms previous reports on the distribution of these species in northeast Spain (Ornat and Verdejo-Lucas, 1999; Sorribas and Verdejo-Lucas, 1994). In Almería, the presence of *M. javanica* and *M. incognita* was known (Escuer

TABLE 1. Species of *Meloidogyne* spp. recovered from vegetables in plastic houses in Almería and Barcelona, Spain.

Almería			Barcelona		
Site code	Plant host	*Meloidogyne* spp.	Site code	Plant host	*Meloidogyne* spp.
	Campo Dalías			El Maresme	
D-1	Melon	*M. javanica*	E-60	Cucumber	*M. javanica*
D-6	Melon	*Meloidogyne*	E-62	Cucumber	*M. javanica*
D-7	Melon	*M. javanica*	E-64	Cucumber	*M. incognita*
D-51	Melon	*M. javanica*	E-65	Cucumber	*M. javanica*
D-4	Watermelon	*M. javanica*	E-66	Cucumber	*M. incognita*
D-25	Watermelon	*M. incognita*	E-76	Cucumber	*M. arenaria*
D-27	Watermelon	*M. incognita*	E-63	Green pepper	*M. incognita*
D-26	Cucumber	*M. javanica*	E-69	Green pepper	*M. arenaria*
D-2	Eggplant	*M. incognita*	E-61	Tomato	*M. javanica*
D-3	Eggplant	*M. incognita*	E-67	Tomato	*M. incognita*
D-52	Eggplant	*M. javanica*	E-68	Tomato	*M. javanica*
D-54	Eggplant	*M. javanica*	E-77	Tomato	*J. incognita*
D-5	Tomato	*M. incognita*	E-78	Tomato	*M. javanica*
D-53	Tomato	*M. javanica*			
	Campo Níjar			Baix Llobregat	
N-6	Melon	*M. incognita*	B-72	Parsley	*M. incognita*
N-8	Watermelon	*M. javanica*	B-71	Watermelon	*M. incognita*
N-10	Watermelon	*M. javanica*	B-70	Pumpkin	*M. incognita*
N-11	Watermelon	*M. incognita*	B-73	Pumpkin	*M. incognita*
N-4	Squash	*Meloidogyne*	B-74	Pumpkin	*M. incognita*
N-5	Squash	*M. incognita*	B-75	Pumpkin	*M. incognita*
N-7	Tomato	*M. incognita*	B-79	Tomato	*M. javanica*
N-9	Tomato	*M. javanica*	B-80	Tomato	*M. arenaria*
N-12	Tomato	*M. incognita*	B-81	Tomato	*M. javanica*
	Bajo Andarax				
N-29	Tomato	*M. incognita*			
N-1	Tomato	*M. javanica*			
N-2	Tomato	*M. javanica*			
N-3	Tomato	*M. javanica*			
N-28	Tomato	*M. javanica*			
N-30	Tomato	*M. javanica*			
N-31	Tomato	*M. javanica*			
N-55	Tomato	*M. javanica*			
N-56	Tomato	*M. javanica*			
N-57	Tomato	*M. javanica*			
N-58	Tomato	*M. javanica*			
N-59	Tomato	*M. javanica*			

et al., 1996), but not their relative abundance. This study contributes to the knowledge of the distribution of *Meloidogyne* in the area of highest concentration of plastic houses in Europe.

The larger final population densities on solanaceous than cucurbit crops can be partially explained by the host response to the nematode. Solanaceous crops usually are more tolerant to nematode damage than cucurbit crops; therefore, they may support higher nematode densities. In the survey areas, climatic and physical soil conditions are suitable for nematode multiplication; and host plants for nematode reproduction are available year round. Temperatures above the invasion threshold of 16 °C (Roberts et al., 1981) prevail for more than 6 months of the year, and population densities decline only by 50% on average during the short fallow periods between successive crops (Ornat et al., 1997).

A diversity of fungal species were associated with root-knot nematode eggs. The three isolates of *Verticillium* came from outdoor fields in the northern cooler province of Barcelona, and none were detected in plastic houses in either province. *Fusarium* was the genus most frequently isolated (14 sites) and showed a similar frequency of occurrence in both areas. Isolates of *F. solani* came from Almería, whereas isolates of *F. oxysporum* came from Barcelona. The *A. strictum* isolates came from the southern, warmer province of Almería. Both *F. oxysporum* and *A. strictum* are considered to be facultative saprophytes as defined by Garret (1956). Nigh et al. (1980) showed that *F. oxysporum* and *A. strictum* can grow saprophitically in eggs of *H. schachtii* Schmidt killed by heat. *Dactylella oviparasitica* was isolated only once in Almería. All fungi identified in this study have been previously reported as parasites of root-knot nematodes (Morgan-Jones et al., 1981; Roccuzo et al.,

1993), with the exception of *E. album*. However, low percentage of egg parasitism occurred in the surveyed areas. The abundance and frequency of fungal species may have been greater had we used a bioassay of field samples in the greenhouse (Nigh et al., 1980; Viaene and Abawi, 1998).

More than 90% of the vegetable production in Almería is done on a sand-mulching system into plastic houses (López Bellido et al., 1994). The sand mulching remains undisturbed for 3 to 5 years to prevent the manure layer from mixing with the sand. Due to the presence of this manure layer, we expected but did not find a rich mycota associated with nematode eggs. As manure ages, the microbial community may change, and possibly the antagonists introduced with the manure may not survive for long periods of time. Moreover, the frequent use of soil fumigants and other biocides (one or two times per season) may have prevented the establishment of fungal antagonists of the nematode. In Almería, soil disinfection is recommended in plastic houses with a history of root-knot nematodes before planting a new crop (Belda et al., 1994).

Although fungal parasites were isolated from several sites in Almería and Barcelona, they were not parasitizing a large proportion of *Meloidogyne* eggs and were probably not having a large impact on the nematode population. The activity of the fungi seems to be poor in fields with high (Barcelona) or extremely high (Almería) densities of *Meloidogyne*. For example, 5,790 juveniles/250 cm^3 soil and 16,130 eggs/g root were found after resistant tomato cv Royesta in site 81 (Barcelona), where three nematode antagonists, *V. chlamydosporium, Fusarium* spp., and *Pasteuria penetrans* (Sorribas, pers. comm.), were present. Sampling for antagonists in areas where the nematode is present but is not causing crop damage may provide a higher diversity of antagonists and better candidates for biological control of *Meloidogyne* than surveying areas with nematode problems.

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