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Survey of *Meloidogyne* spp. in Tomato Production Fields of Baix Llobregat County, Spain

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Abstract: A survey was conducted to determine the frequency and abundance of *Meloidogyne* spp. in tomato production sites located in Baix Llobregat County, Barcelona, Spain. Forty-five sites were sampled before planting and at harvest from February to October, 1991. *Meloidogyne* spp. occurred in 49% of the sites sampled. Preplant population densities ranged from 10 to 220 ($\bar{x}$ = 110) juveniles/250 cm^3 soil, and final population densities ranged from 20 to 1,530 ($\bar{x}$ = 410) juveniles/250 cm^3 soil. Final population densities were higher in open fields than in field greenhouses, but initial population densities were higher in greenhouses than in fields. *Meloidogyne incognita, M. javanica,* and *M. arenaria* were found in this survey. *Meloidogyne* populations that reproduced on *M. incognita*-resistant tomato cultivars in the field sites did not circumvent the *Mi* gene resistance in greenhouse tests.

Key words: greenhouse, *Lycopersicon esculentum, Meloidogyne,* nematode, resistance, root-knot nematode, Spain, survey, susceptibility, tomato.

Meloidogyne is the most important genus of plant-parasitic nematodes that affect vegetable crops in Spain. Recent reports have shown an increased concern about the rapid spread and wide distribution of *Meloidogyne* spp. throughout the country (1–3,8,17,19,20,26,28). In the 1960s, *Meloidogyne* was limited to a few localities and infestation levels were low (9,10,15). More recently, however, *Meloidogyne* has become a major nematode problem in some areas of the country, and it has displaced other economically important nematodes (17,29). Information on the distribution and abundance of *Meloidogyne* spp. in vegetable crops in Catalonia (northeast Spain) was lacking, although the nematode had been observed on tomato and other crops in this region (15,18).

Baix Llobregat County is the third-largest regional producer of fresh market vegetables in Catalonia (27). A multiple-cropping system is practiced, with up to 15 different vegetable crops grown in rotation. Tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), and lettuce (*Lactuca sativa*) are the vegetables produced most frequently in field greenhouses. The types of vegetables grown in open fields are more numerous and diverse, but tomato and lettuce are the most important annual crops. Tomato is cultivated as an early crop from February to June in field greenhouses, and from April to October as the main crop in open fields. Farms are family-operated, and the average size is 2,500 m^2.

The objective of this study was to survey tomato production sites for the presence, distribution, and abundance of *Meloidogyne* spp.

MATERIALS AND METHODS

During the 1991 growing season, 45 fields representing 11 ha were sampled for *Meloidogyne*. Sites were located in three municipalities, Gavà, Sant Boi, and Viladecans in Baix Llobregat County, Barcelona, Spain. Sites to be planted to tomato were identified through direct grower contacts. Composite soil and root samples were collected from 16 field greenhouses, and 29 open fields from February to October. Samples were taken from the top 30 cm soil layer with a soil auger (2.5-cm-d × 50 cm deep) preplant (initial population) and at harvest (final population). Samples were sieved to separate roots from soil, and nematodes in 250-cm^3 soil subsamples were extracted by the centrifugal-flotation method (14). At harvest, *Meloidogyne* eggs were collected from roots blended in a 0.5% NaOCl solution for 10 minutes (13).

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Soil texture (Bouyoucos densimeter and USDA classification) and pH (1:2.5, w/v in water) were determined for each site. Data on previous crops, chemical treatments, and agricultural production practices were obtained from interviews with growers (Table 1).

For identification of species, soil samples that contained *Meloidogyne* second-stage juveniles (J2) were potted and a susceptible tomato seedling (cv. Precodor) was transplanted into each pot. *Meloidogyne* species were identified based on the perineal patterns of the females (7) and polymerase chain reaction (PCR) analysis (5). The DNA was isolated from J2 cultured monoxenically from single egg masses (30) according to the procedure described by Cenis (5).

The reactions of four *M. incognita*-resistant cultivars and one susceptible tomato cultivar to two isolates of *M. incognita* and one of *M. javanica* were tested under greenhouse conditions. The populations were cultured from single egg masses (30) on potted Precodor tomato plants. The *M. incognita*-resistant tomato cultivars tested were Carmelo, Carpy, Mina, and Rambo. The susceptible cultivar Precodor was also tested for comparison. Seedlings of each cultivar were transplanted 4 weeks after germination to pots (500 cm^3 soil capacity) containing steam-sterilized sand. A suspension of 500 eggs of each nematode isolate was pipetted in two holes made in the soil near the plant. Each isolate–cultivar combination was replicated nine times, and plants were maintained in a greenhouse. Plants were watered when needed and fertilized with a slow-release fertilizer (15N + 10P + 12K + 2MgO + microelements). Dry top weight, fresh root weight, and numbers of eggs per plant were determined 7 weeks after infestation. Eggs were extracted from roots in a 0.5% NaOCl solution for 10 minutes (13). Numbers of eggs per plant included egg shells and unhatched eggs. Numbers of eggs per plant were transformed (log $[x + 1]$) and subjected to analysis of variance. Means were compared by the Tukey test ($P = 0.05$).

Results and Discussion

Root-knot nematodes were detected in 22 out of 45 sites sampled (12 field greenhouses and 10 open fields). The nematodes were found in preplant soil samples from 11 sites (24%), and in soil or root samples from 11 additional sites at harvest (Table 2). Preplant population densities ranged from 10 to 220 ($\bar{x} = 110 \pm 85$) J2/250 cm^3 soil (Table 2). Final population densities in soil ranged from 20 to 1,530 ($\bar{x} = 410 \pm 60$) J2/250 cm^3 soil. The number of eggs per g root ranged from 110 to 26,810 ($\bar{x} = 7{,}570 \pm 460$) (Table 2). In general, final nematode population densities were greater in open fields than in field greenhouses. In contrast, *Meloidogyne* was detected more frequently at the beginning of the season in field greenhouses than in open fields. *Meloidogyne* was found at the end of the season in 67% of the greenhouse sites that had been fumigated just before the initial sampling (Table 2). The use of soil fumigants probably reduced the increase of nematode population densities in these sites.

Numbers of *Meloidogyne* J2 were below detectable level before planting the crop in 76% of the sites, but increased after growing a single crop of tomato. This increase could be explained in some cases by the vertical migration of the nematodes from deeper to upper soil layers in the presence of the host plant (22). Also, the nematodes may have escaped detection in preplant samples as eggs within egg masses (11,12). The efficiency of our extraction method (14) is not satisfactory for separating nematode eggs from the soil.

There was no correlation between the numbers of J2 in the soil and soil texture or pH (range 7.3 to 8.4). Silty loam soils were dominant in this survey, and *Meloidogyne* was present in 45% of the sites with this soil texture. Other soil textures where *Meloidogyne* were present included sandy loam (27%), loamy sand (23%), sand (9%), and loam (5%).

The *Meloidogyne* species identified were *M. incognita* in sites 6, 30, 32, 35; *M. ja-*

TABLE 1. Soil characteristics and history of tomato production sites where *Meloidogyne* spp. were detected in Baix Llobregat County, Barcelona, Spain.

Site Number	Site Cultivation†	Locality	Area (m^2)	Soil texture	pH	Preplant soil treatment and year	Previous crops‡	Tomato cultivar 1991
1	Greenhouse	Sant Boi	2,700	Silty loam	7.73	Metham-Na + DD, 1991	T, Cu, F	Mereto
4	Greenhouse	Viladecans	1,750	Loam	7.86	Methyl bromide, 1991	P, Cf	Precodor
6	Greenhouse	Viladecans	3,000	Silty loam	7.55	Methyl bromide, 1990	T, L	Precodor
13	Greenhouse	Sant Boi	630	Silty loam	7.32	Methyl bromide, 1991	T, L	Mereto
12	Greenhouse	Sant Boi	630	Silty loam	7.79	Methyl bromide, 1991	T, L	Mereto
15	Greenhouse	Viladecans	1,500	Silty loam	7.59	Methyl bromide, 1991	T, L	Precodor
2	Greenhouse	Sant Boi	850	Silty loam	7.56	Metham-Na, 1991	T, L	Mereto
10	Greenhouse	Viladecans	1,000	Sandy loam	7.65	Methyl bromide, 1991	P, T	Precodor
14	Greenhouse	Viladecans	4,000	Silty loam	7.77	Methyl bromide, 1991	T, P, L	Luxor
5	Greenhouse	Viladecans	2,500	Silty loam	7.54	Methyl bromide, 1990	T, P, L	Carmelo
16	Greenhouse	Gavà	1,600	Sandy loam	8.14	DD + methyl isothiocyanate, 1991	T, L	Carmelo
3	Greenhouse	Gavà	1,300	Loamy sand	8.04	Untreated	P, R	Carmelo
30	Field	Viladecans	7,000	Sandy loam	8.40	Methyl bromide, 1989	L, S	Royesta
36	Field	Viladecans	2,500	Sand	8.11	Untreated	T, R	Monix
32	Field	Viladecans	2,500	Loamy sand	8.20	Untreated	R	Dario
34	Field	Gavà	2,000	Loamy sand	8.28	Untreated	T, R	Royesta
35	Field	Viladecans	2,000	Sandy loam	8.19	Untreated	T, R, Ce	Monix
39	Field	Gavà	2,500	Sandy loam	8.20	Untreated	L	Riogrande
27	Field	Gavà	4,000	Sand	8.06	Untreated	Po, Cr	Riogrande/ Iberia
28	Field	Gavà	2,500	Loamy sand	7.87	Untreated	Fallow	Iberia
26	Field	Viladecans	5,000	Sandy loam	7.99	Untreated	L, Ce	Cobra
29	Field	Viladecans	2,500	Sandy loam	7.60	Untreated	S, Ca, Cf	Cobra

† "Greenhouse" refers to producion in a field site with a temporary covering used to extend the growing season. "Field" refers to production in uncovered sites.
‡ C: cabbage, Ce: celery, Cf: cauliflower, Cr: curly leaf lettuce, Cu: cucumber, F: french bean, E: endive, L: lettuce, P: pepper, Po: potato, R: radish, S: spinach, T: tomato.

TABLE 2. *Meloidogyne* spp. population densities in tomato production sites in Baix Llobregat County, Barcelona, Spain.

Site: Cultivation	Site: Number	Cultivar	Preplant soil treatment	Nematodes/250 cm^3 soil: Preplant	Nematodes/250 cm^3 soil: Harvest	Number eggs/g root
Greenhouse	1	Mereto (S)†	+‡	209	920	276
	4	Precodor (S)	+	140	29	519
	6	Precodor (S)	+	196	284	427
	13	Mereto (S)	+	42	180	0
	12	Mereto (S)	+	0	54	0
	15	Precodor (S)	+	0	1,235	0
	2	Mereto (S)	+	209	0	0
	10	Precodor (S)	+	144	0	0
	14	Luxor (R)	+	16	0	0
	5	Carmelo (R)	+	0	0	108
	16	Carmelo (R)	+	0	19	0
	3	Carmelo (R)	−	218	0	0
Field	30	Royesta (R)	+	0	44	0
	36	Monix (R)	−	10	0	0
	32	Dario (R)	−	0	504	13,551
	34	Royesta (R)	−	0	42	9,756
	35	Monix (R)	−	0	0	1,578
	39	Riogrande (S)	−	0	260	0
	27	Riogrande (S)	−	0	70	15,220
	28	Iberia (S)	−	0	494	7,483
	26	Cobra (S)	−	15	1,530	26,814
	29	Cobra (S)	−	26	510	0

† S = susceptible; R = resistant cultivar to *M. incognita*.
‡ + = Methyl bromide applied prior to initial sampling in 1991 except for sites 5 and 6, and 30 that were fumigated in 1990 and 1989, respectively. Sites 1, 2, and 16 were treated with DD + Na methyldithiocarbamate, Na-methyldithiocarbamate and DD + methylisothiocyanate, respectively; − = untreated.

vanica in sites 2, 6, 28, 34; and *M. arenaria* in sites 1, 10, 27, 29. Isolation and identification of *Meloidogyne* species from sites where population densities were low was not achieved.

Although resistance to *M. incognita* is available in commercial tomato cultivars, the results of this survey indicated that different species of *Meloidogyne* can increase to high numbers on *M. incognita*-resistant tomatoes in the field. Thus, *M. incognita*-resistant tomato cultivars, such as Royesta and Monix, supported large population densities of *Meloidogyne* in sites 34 and 35, but not in sites 30 and 36, respectively (Tables 1 and 2). Large numbers of eggs were also found on roots of the *M. incognita*-resistant tomato cv. Dario in site 32. Isolates that reproduced well on *M. incognita*-resistant tomatoes (*M. incognita* in sites 32 and 35, and *M. javanica* in site 34) were selected for the greenhouse test because they were suspected to be resistance-breaking populations; however, the results of the greenhouse test showed otherwise. The *M. incognita* isolates found in sites 32 and 35 did not reproduce on the *M. Meloidogyne*-resistant cultivars tested, whereas the *M. javanica* isolate from site 34 did reproduce; nonetheless, *M. javanica* reproduction was lower on the resistant cultivars than on the susceptible one (Table 3). Numbers of eggs did not differ ($P = 0.05$) among the three isolates tested on the *M. incognita*-susceptible cv. Precodor, although the *M. javanica* isolate produced 3.4 and 2.6 times more eggs than the *M. incognita* isolates from sites 32 and 35, respectively. The use of *M. incognita*-resistant cultivars may cause a shift in root-knot populations to other species as it has been shown in tobacco fields naturally infested by *M. incognita* and *M. javanica* (6,16). However, the *M. incognita* isolates from

TABLE 3. Reproduction of *Meloidogyne incognita* isolates from two sites and *M. javanica* from one site on four *M. incognita*-resistant cultivars and one susceptible cultivar of tomato 49 days after inoculation with 500 nematode eggs.

	Final population/initial population		
	M. incognita		*M. javanica*
Cultivar	Site 32	Site 35	Site 34
Resistant			
Carmelo	0.1† b	1.9 b	3.8 b
Carpy	0.1 b	0.1 b	4.8 b
Mina	0.2 b	0.8 b	5.6 b
Rambo	0.6 b	0.8 b	17.8 b
Susceptible			
Precodor	24.7 a	33.0 a	85.2 a
Mean	4.9	7.5	23.4
CV	79.6	72.8	42.1

† Values are means of nine replications. Means followed by the same letter within the same column are not different ($P = 0.05$) according to the Tukey test.

sites 32 and 35 were the predominant species because they were identified from soil and root samples collected at harvest (Table 2). The greenhouse test showed genetic variation in the tomato cultivars (F_1 hybrids) tested. Thus, most plants (6–7 plants) of cvs. Carmelo, Carpy, and Mina were highly resistant to *M. incognita* from site 32, and four of nine plants also reacted as highly resistant to *M. incognita* from site 35. *Meloidogyne javanica* from site 34, however, completed a life cycle and produced eggs on all plants of all cultivars tested. Many *M. incognita*-resistant tomato cultivars support nematode production, and some cultivars have been reported as susceptible to *M. incognita* (31), *M. javanica* (24), and *M. arenaria* (23).

The *M. javanica* from site 34 had a high Pf/Pi on the *M. incognita*-susceptible cv. Precodor, and also parasitized *M. incognita*-resistant tomato cultivars both under natural and experimental conditions.

The results of this survey confirm other reports on the widespread distribution of *Meloidogyne*, its high frequency of occurrence, and its potential as a problem for vegetable production in Spain (1,2,4,28). Species identification is important and should be considered when selecting the cultivar or the crop to be planted.

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