A Population of *Meloidogyne javanica* in Spain Virulent to the *Mi* Resistance Gene in Tomato

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ABSTRACT

Ornat, C., Verdejo-Lucas, S., and Sorribas, F. J. 2001. A population of *Meloidogyne javanica* in Spain virulent to the *Mi* resistance gene in tomato. Plant Dis. 85:271-276.

A population of *Meloidogyne javanica* virulent to *Mi*-gene in tomato was identified in Spain. It reproduced similarly on resistant and susceptible tomato cultivars in the greenhouse, microplots, and in the field. In monoxenic cultures, reproduction of the virulent *M. javanica* was higher than that of an avirulent population on resistant but not on susceptible tomatoes. The virulent population suppressed tomato yield of both resistant and susceptible tomatoes by 29% in microplots. Initial population density (Pi) was inversely correlated with Pf (final population density)/Pi on both resistant and susceptible tomatoes in the field. A negative correlation was found between Pi and tomato yield for the susceptible but not for the resistant cultivar.

Additional keywords: *Lycopersicon esculentum*, reproductive potential, root-knot nematode, susceptibility, yield loss

The tomato (*Lycopersicon esculentum* L.) is the most important horticultural crop in Spain, and is cultivated on approximately 60,000 ha. Production exceeds 3 million tons a year, half used for fresh consumption and the other half for processing. Spain is the largest producer of fresh tomatoes in the European Union with 30% of total production. This crop is well adapted to most Spanish climatic conditions, and it is cultivated in several crop cycles year round. A wide range of tomato cultivars are grown to meet climatic conditions and market demands.

Meloidogyne spp. are widely distributed and of great economic consequence in the Mediterranean basin. They cause considerable losses in many vegetable, ornamental, and fruit crops (7,12,16,29,30). In order of occurrence, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoed & White) Chitwood are the most widespread species of *Meloidogyne*, whereas *M. arenaria* (Neal) Chitwood is less common (9).

Fumigant and non-fumigant nematicides are used to control *Meloidogyne* on vegetable crops, but increasing environmental concerns and government regulations have contributed to an increased emphasis on other nematode control measures. The use of tomato cultivars resistant to *Meloi-*

dogyne is an ideal management tactic because it is safe, non-polluting, and requires no input of additional resources by growers. Many commercial tomato cultivars possess the *Mi*-gene that confers resistance, but not immunity, to *M. incognita*, *M. arenaria*, and *M. javanica* (27). These cultivars can be grown on most infested land without significant yield loss because they suppress nematode reproduction and are tolerant of initial infection (17,21,23).

Virulent populations of *Meloidogyne* spp. occur naturally, apparently without previous exposure to or selection by tomatoes with *Mi*-gene resistance (15,22). They may also result from selection after repeated exposure to tomatoes with *Mi*-gene resistance under greenhouse or field conditions (3,15,24,27). The occurrence of virulent populations of *Meloidogyne* has been reported in several Mediterranean countries in the last decade (6,10,14,33). In this paper we report the reproduction of 14 populations of *Meloidogyne* from Spain on resistant and susceptible tomato cultivars in a greenhouse test. One population of *M. javanica* was found to produce high numbers of eggs on resistant cultivars. Monoxenic culture, greenhouse, microplot, and field experiments were conducted to confirm the virulence of this population and its impact on tomato yield.

MATERIALS AND METHODS

Preliminary greenhouse test. Isolates of the following 14 nematode populations were used in the study: two *M. incognita* race 1 (MI-35 and MI-39), one *M. incognita* race 2 (MI-28), and two *M. incognita* race unknown (MI-17 and MI-38), five *M. javanica* (MJ-01, MJ-05, MJ-10, MJ-27,

and MJ-34), and four *M. arenaria* race 2 (MA-CA, MA-04, MA-12, and MA-26). These isolates came from infested vegetable fields in coastal areas of northeast Spain and had been established as single egg mass lines in monoxenic cultures (16,30). To obtain inocula, nematodes were transferred from monoxenic cultures to potted tomato plants in a greenhouse and allowed to multiply for 8 weeks. Egg inoculum was collected from infected roots with a 0.5% NaOCl solution for 5 min (8). Seedlings of resistant tomato cultivars Carmelo and Luxor and of the susceptible cultivar Precodor were transplanted singly to one-liter pots containing steam-sterilized sand and inoculated with a suspension of 1,000 eggs per plant (one egg per cm³ potted soil) 1 week after transplanting. Each isolate-cultivar combination was replicated seven times, and plants were arranged in a random complete block design on a greenhouse bench. Soil temperature in the pots ranged from 14 to 26°C ($x = 20$) during the test. Plants were watered as needed and fertilized with a slow-release fertilizer (15% N + 10% P₂O₅ + 12% K₂O + 2% MgO₂ + microelements). Number of egg masses and eggs per plant were determined 8 weeks after infestation. Eggs were extracted from the whole root system in 0.5% NaOCl solution for 10 min (8). The Pf (eggs per plant)/Pi (egg inoculum) ratio and the index of reproduction [(Pf on resistant cultivar/Pf on susceptible cultivar) $\times$ 100] were calculated.

***M. javanica* MJ-27 virulence.** This isolate was obtained from susceptible tomato roots in a naturally infested greenhouse located in Pineda de Mar, Barcelona. Nematode inoculum for the greenhouse and microplot experiments was obtained as described for the preliminary greenhouse test. The nematode virulence was measured by assessing number of eggs or juveniles produced on resistant and susceptible plants.

Monoxenic culture experiment. Reproduction of *M. javanica* MJ-27 was compared with that of the avirulent *M. javanica* isolate MJ-05 (17). Excised root cultures of the resistant tomato cv. Luxor and the susceptible cv. Precodor were prepared on 9 cm-diameter petri dishes containing Gamborg's B5 medium (35). Roots were inoculated with 20 ± 4 second-stage juveniles (J2) per root culture. Juveniles were obtained from egg masses collected axenically from monoxenic cultures and 24-h-old J2 were used as inoculum. Each

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Accepted for publication 11 November 2000.

isolate $\times$ cultivar combination was replicated six times. Cultures were maintained in an incubator in the dark at $21 \pm 1^\circ\text{C}$. Eggs masses were counted 30 days after inoculation. The entire root cultures (infected roots + agar) were placed on modified Baermann funnels to determine viability of eggs produced on the resistant and susceptible tomato cultivars. Hatched juveniles were collected twice per week for 8 weeks, and J2 counts were added.

Greenhouse experiment. Seedlings of the resistant tomato cvs. Alpado, Carmelo, Nikita, Ramón, Luxor, and Merlin were transplanted singly to one-liter plastic pots containing steam-sterilised sand. The susceptible tomato cvs. Cobra and Laurisilva were included as controls. Five plants of each cultivar were inoculated with a suspension of 1,000 eggs of *M. javanica* MJ-27 per plant (one egg per cm^3 potted soil) one week after transplanting. Experimental conditions, plant maintenance, and assessment of nematode reproduction were as described for the preliminary greenhouse test. Plants were harvested 9 weeks after soil infestation. Soil temperature in the pots ranged from 13 to 26°C (19°C on average) during the test. The experiment was repeated once.

Microplots experiment. Round bucket-microplots (10-liter capacity) filled with a sandy soil (90% sand, 8% lime, 2% clay) were buried in soil in a shade house (54% area shaded) at the Experimental Station of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Cabrils, Barcelona. Seedlings of the resistant tomato cv. Ramón and susceptible cv. Cobra were transplanted singly to the microplots at the four-true-leaf stage, and five plants of each cultivar were inoculated with a suspension of 10,000 eggs of *M. javanica* MJ-27 per

plant (one egg per cm^3 of soil) 1 week after transplanting. Uninoculated plants served as controls. Plants were watered as required and fertilized with the same slow-release fertilizer used for the greenhouse experiments. Soil temperature during the study ranged from 12 to 27°C (20°C on average) at 15 cm depth. Mature fruit was collected twice a week for 12 weeks, starting 8 weeks after transplanting. Tomatoes were counted and weighed. Root galling and nematode reproduction were assessed 20 weeks after inoculation. Root system were rated for galling on a scale of 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots were dead. Nematodes were extracted from two 500 cm^3 soil subsamples by using Baermann trays. Trays were left for one week and J2 that migrated to the clean water were collected, concentrated on a 25 μm sieve, and counted. This experiment was repeated once.

Field experiment. The study was conducted in the greenhouse from which *M. javanica* MJ-27 was originally isolated. No other plant-parasitic nematode was detected in the field soil. The greenhouse had a surface of 3,000 m^2 , and the soil was a loamy sand with a pH of 8.1 and 3.3% organic matter. The crop history of the greenhouse, as far as known, was susceptible tomato in spring and lettuce in autumn-winter for 2 consecutive years, and susceptible tomato and radish the season before the initiation of this study. Thirty-two plots of $5.5 \times 3 \text{ m}$ (16.5 m^2) were used, and soil samples were taken for nematode analysis. Seedlings of the resistant tomato cv. Ramón were transplanted to 16 plots and the susceptible cv. Cobra to another 16 plots in a random complete block design. Plants were spaced 50 cm apart in the row,

and there was 70 cm between rows (2.9 plants per m^2). Soil samples were collected before planting and at the final harvest 20 weeks after transplanting. Individual samples consisted of five soil cores (2.5 cm diameter $\times$ 30 cm deep) taken from each plot. Soil cores were mixed thoroughly, and a 500 cm^3 subsample was used for nematode extraction on Baermann trays. Juveniles were collected one week later and counted and expressed per 250 cm^3 of soil. After final harvest, six root systems from each plot were rated for galling as described earlier. To confirm infestation of the greenhouse by *M. javanica*, infected root samples from the resistant and susceptible cultivars were collected at random. Females were extracted from the roots by blender maceration, collected individually, and the esterase phenotype of 40 individual females from each cultivar was determined.

The effect of *M. javanica* MJ-27 on yield was assessed from six plants in each of eight randomly selected plots planted either with resistant or susceptible tomato. Fruits of 48 plants per cultivar were harvested twice a week for 7 weeks, counted and weighed. Agronomic practices including fertilization and irrigation were carried out by the grower and results from this study represent grower's conditions.

In a second test, seedlings of the resistant tomato cv. Ramón were transplanted to eight *M. javanica* infested plots at the same site following the procedure described previously. Nematode densities were determined at planting and after 12 weeks, when the crop was abandoned due to poor crop performance.

Data analysis. Statistical analyses were performed using the linear model procedure of SAS (6.12 SAS, Cary, NC). As no

Table 1. Egg mass production, multiplication rate (Pf/Pi), and reproductive index of 14 populations of *Meloidogyne* from Spain on *Mi*-gene resistant tomato cvs Carmelo and Luxor, and the susceptible cv Precodor 8 weeks after the inoculation of 1,000 eggs per plant in a greenhouse^x

Nematode population		Egg masses per plant			Pf/Pi			Reproductive index ^y	
		Carmelo	Luxor	Precodor	Carmelo	Luxor	Precodor	Carmelo	Luxor
<i>M. javanica</i>	MJ-27	117 a	133 a	216 abc	37 a	40 a	73 abc	51	55
	MJ-34	2 bc	6 bcd	206 abc	1.9 bc	4.5 bcd	69 abcd	2.7	6.5
	MJ-05	3 bc	10 bc	236 abc	1.5 bc	4 bcd	79 abc	1.8	5
	MJ-01	7 bc	9 bc	257 ab	4 bc	5 b	115 ab	3.7	4
	MJ-10	7 bc	na ^z	298 a	2.5 bc	na	153 a	1.6	na
	Average	27 A	32 A	243 A	9.4 A	11 A	98 A	10	14
<i>M. incognita</i>	MI-28	47 ab	36 b	100 abc	9 b	9.4 b	32 bcd	28	29
	MI-17	0 c	44 bcd	185 abc	0.2 bc	11 bcde	71 abc	0.3	15
	MI-35	0 c	0 e	118 abc	0.02 c	0.1 e	18 d	0.1	0.6
	MI-38	0 c	0 e	93 c	0.04 c	0.2 e	24 cd	0.2	0.6
	MI-39	11 c	1 de	180 abc	5.5 bc	0.2 de	58 abc	9	0.4
	Average	12 B	16 B	136 B	2.9 B	4 B	41 B	7	10
<i>M. arenaria</i>	MA-04	13 c	2 cde	111 abc	2.8 bc	1.9 bcde	49 abcd	5.6	3.8
	MA-CA	2 c	2 cde	102 bc	0.3 bc	1.3 bcde	48 abcd	0.7	2.8
	MA-12	18 bc	5 bcde	137 abc	3.5 bc	0.5 cde	48 abcd	7.2	1.1
	MA-26	0 c	1 de	164 abc	0.1 c	0.2 de	72 abc	0.2	0.3
	Average	8 B	2 B	128 B	1.6 B	1 B	54 B	3	2

^x Each value is mean of seven replications. Statistical analyses of data were based on $\log_{10}(x + 1)$ transformed data. Means within a column followed by the same letter in lower case indicate no difference among populations, and in upper case indicate no difference among species according to Tukey test ($P = 0.05$).

^y Eggs per plant on resistant cultivar divided by susceptible cultivar $\times$ 100.

^z Not available.

significant differences existed between the repeated experiments, results were pooled for statistical analysis. Data on nematode reproduction in monoxenic culture, microplots, and field experiments were transformed to $[\log(x + 1)]$ and compared by the Student *t* test. Data from the greenhouse studies were subjected to analysis of variance followed by mean separation with the Tukey test. In the microplot experiment, yield data from inoculated and uninoculated plants of each cultivar were compared with Student *t* test. The relationship between Pi and Pf/Pi, and between Pi and yield in the field experiment were determined by regression analysis.

RESULTS

Preliminary greenhouse test. Across species, the Pf/Pi of the three *Meloidogyne* species (14 populations) was higher ($P \leq 0.05$) on the susceptible than on the resistant tomato cultivars (data not shown). Reproduction of *M. javanica* and *M. arenaria* (490 and 480 eggs per female, respectively) were higher ($P \leq 0.05$) than *M. incognita* females (330 eggs per female) regardless of the tomato cultivar. *M. javanica* showed a Pf/Pi relationship higher ($P \leq 0.05$) than *M. incognita* or *M. arenaria* on the susceptible as well as on the resistant cultivars (Table 1). Females of *M. javanica* produced similar numbers of eggs on resistant (576 and 545 eggs per female on Carmelo and Luxor, respectively) and susceptible tomatoes (384 eggs per female). Across populations, the Pf/Pi on the susceptible cultivar was variable and ranged from 18 to 153 times the Pi. On resistant cultivars, the majority of *M. incognita* and *M. arenaria* populations showed reproductive indices lower than 10%, with the exception of *M. incognita* MI-28 that showed a moderate reproduction level. Two isolates of *M. incognita*, MI-35 and MI-38, showed a low Pf/Pi on both susceptible and resistant cultivars. *M. javanica* MJ-27 was the only population that showed high reproductive rates on resistant cultivars.

Monoxenic culture experiment. The virulent *M. javanica* MJ-27 produced more ($P \leq 0.05$) egg masses and had a higher Pf/Pi ratio than the avirulent MJ-05 on the resistant tomato but there was no difference on the susceptible cultivar (Table 2). The susceptible cultivar supported higher ($P \leq 0.05$) reproduction of *M. javanica* MJ-27 than did the resistant one; only 35% of the J2 inoculum became fecund females on the resistant tomato compared with 60% on the susceptible cultivar. Nevertheless, fecund females produced similar numbers of offspring on both tomato cultivars; 314 and 380 J2 per female on the resistant and susceptible cultivars, respectively. Most eggs (90%) produced on either resistant or susceptible cultivar hatched during the first 3 weeks after being placed on Baermann funnels, but eggs of *M. javanica* MJ-27

from susceptible tomato roots continued hatching for an additional 5 weeks. This additional hatching was responsible for the significant difference found ($P \leq 0.05$) between the accumulated number of J2 recovered from each tomato cultivar.

Greenhouse experiment. The virulent MJ-27 population reproduced on each of the five resistant cultivars tested, though some variation occurred between cultivars (Table 3). Female fecundity varied among cultivars, from 453 to 895 eggs per female on cvs. Cobra and Carmelo, respectively. When data were pooled and compared nematode reproduction was higher ($P \leq 0.05$) on susceptible than on resistant cultivars though both showed a similar Pf/Pi. Because nematode increase on the susceptible cv. Laurisilva was 1.5 times higher than on cv. Cobra, values of the reproductive index were lower when cv. Laurisilva was used as the reference cultivar.

Microplot experiment. Reproduction of the virulent MJ-27 did not differ ($P \leq 0.05$) on resistant or susceptible cultivars (Table 4). The nematode increased the fresh root weight, but decreased accumulated yield of both cultivars. The yield reduction was due to the lower weight of the fruit produced by the nematode-infected resistant cultivar, and to both the lower number and weight of fruit from the nematode-infected susceptible cultivar. The percentage yield

reduction (29%) caused by the virulent MJ-27 was similar on both resistant and susceptible cultivars.

Field experiment. Densities of the MJ-27 population before transplanting tomatoes to plots with the resistant or susceptible cultivars were 582 ± 706 (range 4 to 2,840) and 312 ± 444 (range 18 to 1,672) J2 per 250 cm³ soil $\pm$ SD, and final densities were $1,824 \pm 1,524$ (range 400 to 5,494) and $2,710 \pm 1,294$ (range 820 to 5,340) J2 per 250 cm³ of soil, respectively. The population densities did not differ ($P \leq 0.05$) between the resistant and susceptible cultivars either before planting or at final harvest. Resistant and susceptible tomato plants showed similar gall indices: 5.0 ± 2.0 and 5.6 ± 1.3 , respectively. The typical esterase phenotype of *M. javanica* was observed in every female examined from both resistant and susceptible cultivars.

A highly significant ($P \leq 0.0001$) negative correlation was observed between the Pi and the Pf/Pi on the resistant and on the susceptible cultivar (Fig. 1). Both regression lines had a similar slope and intercept value. The nematode population density increased (Pf/Pi > 1) in each of the 16 plots with the susceptible cultivar, and in 13 of 16 plots with the resistant cultivar. Population densities at or below the equilibrium density (Pf/Pi = 1) in the remaining three plots occurred at Pi higher than 800 J2 per

Table 2. Reproduction in monoxenic culture maintained at 21°C of a virulent and an avirulent population of *Meloidogyne javanica* on *Mi*-resistant and susceptible tomato cultivars inoculated with 20 $\pm$ 4 second-stage juveniles per culture^x

<i>M. javanica</i> population	Resistant cv. Luxor		Susceptible cv. Precodor		Reproduction index (%) ^y
	Egg masses	Pf / Pi	Egg masses	Pf / Pi	
Virulent MJ-27	7 $\pm$ 3 a ^z	101 $\pm$ 69 a ^z	12 $\pm$ 2 a	234 $\pm$ 74 a	43.0
Avirulent MJ-05	0.5 $\pm$ 0.8 b	2.9 $\pm$ 0.5 b	12 $\pm$ 4 a	196 $\pm$ 42 a	1.5

^x Each value is mean of six replications $\pm$ standard deviation.

^y Pf on resistant cultivar divided by Pf on susceptible cultivar $\times$ 100.

^z Indicates differences between nematode populations according to Student *t* test ($P \leq 0.05$).

Table 3. Reproduction of a virulent population of *Meloidogyne javanica* MJ-27 on five *Mi*-resistant and two susceptible tomato cultivars 60 days after the inoculation with 1,000 eggs per plant in a greenhouse^x

Tomato cultivar	Root weight (g)	Egg masses/plant	Pf/Pi	Reproduction index (%) ^y	
				Cobra	Laurisilva
Resistant					
Alpado	33 $\pm$ 9 ab	488 $\pm$ 175 a	209 $\pm$ 62 ab	95	64
Carmelo	23 $\pm$ 4 bc	210 $\pm$ 124 b	201 $\pm$ 102 ab	91	62
Merlin	17 $\pm$ 3 c	340 $\pm$ 153 ab	156 $\pm$ 75 b	71	48
Nikita	26 $\pm$ 9 bc	340 $\pm$ 150 ab	285 $\pm$ 146 ab	129	88
Ramón	28 $\pm$ 9 ab	430 $\pm$ 105 a	328 $\pm$ 92 a	149	101
Susceptible					
Cobra	36 $\pm$ 8 a	493 $\pm$ 117 a	221 $\pm$ 94 ab		
Laurisilva	33 $\pm$ 10 ab	420 $\pm$ 192 ab	325 $\pm$ 158 ab		
Average					
Resistant	25 $\pm$ 9	361 $\pm$ 173	236 $\pm$ 114	107	84
Susceptible	34 $\pm$ 10 ^z	456 $\pm$ 167 ^z	273 $\pm$ 137		

^x Data are the combined results of two experiments. Each value is mean of 10 replications $\pm$ standard deviation. Means in columns followed by the same letter are not significantly different according to Tukey Test ($P \leq 0.05$).

^y Pf on resistant cultivar divided by Pf on susceptible cultivar $\times$ 100.

^z Indicates difference ($P \leq 0.05$) between resistant and susceptible cultivars according to Student *t* test.

250 cm³ soil. Tomato yield did not differ between resistant or susceptible tomatoes; 2.0 and 2.3 kg per plant, respectively, nor did the average fruit weight (data not shown). Tomato yield decreased as the initial population increased in plots with the susceptible cultivar ($y = -0.4608x + 3.2937$; $R^2 = 0.9055$; $P \leq 0.003$), but no correlation was found in plots with the resistant cultivar. In the second field trial, the Pi of the *M. javanica* MJ-27 population on the resistant cultivar was 49 ± 50 , and changed to 215 ± 237 J2 per 250 cm³ soil after 12 weeks of growth.

DISCUSSION

Variability in virulence in species and populations of *Meloidogyne* was confirmed by the results of the preliminary greenhouse test (26,27). This variability was clearer on resistant than on susceptible cultivars. Populations of *M. javanica* multiplied ($Pf/Pi > 1$) on nearly all individual plants of the resistant cultivars, whereas populations of *M. incognita* MI-39, *M. arenaria* MA-04, and MA-12 only did on one of the seven replicated plants of the resistant Carmelo, and *M. incognita* MI-17 did so on one plant of the resistant Luxor. In this experiment and others designed to compare the reproductive potential of populations of *M. incognita*, *M. javanica*, and *M. arenaria* from Spain (2,31), populations of *M. javanica* showed a consistently higher reproductive potential than those of *M. incognita* or *M. arenaria* on resistant and susceptible tomato cultivars. The increased virulence of *M. javanica* has been reported by others (26). This species of *Meloidogyne* is the most common in many production areas of the Mediterranean basin (6,9,12,19,33) including Spain (16). The moderate to high reproduction of *M. javanica* populations on resistant tomatoes emphasizes the need for testing resistant cultivars against local populations of the nematode before use for management of *Meloidogyne* in infested soils.

M. javanica MJ-27 was able to overcome the resistance conferred by the *Mi*-gene in F1 tomato hybrids in five experiments under different experimental conditions, and this population showed an index of reproduction of 53% in the preliminary greenhouse test, 43% in monoxenic cultures, 86% in greenhouse potted plants, and

93% in the microplot and field experiments. This virulence was demonstrated in experiments involving one or several generations of the nematode, and also on a range of resistant tomatoes. In Spain, populations of *Meloidogyne* able to increase initial densities on resistant tomatoes have been reported in the Canary Islands (28) and Catalonia. The latter populations showed multiplication rates on resistant cultivars were lower than on susceptible ones in temperature-controlled experiments (30). We have examined over 30 populations of *Meloidogyne* from Spain for their intraspecific variability and virulence characteristics using differential host tests and *Mi*-gene resistant tomatoes, respectively (31), and have found only *M. javanica* MJ-27 to be virulent for the *Mi*-gene. Hence, the frequency of occurrence of virulent populations of the nematode is low, and this is the first report of a virulent root-knot population in tomato in Spain.

The numbers of egg masses produced on roots by the MJ-27 population in the monoxenic and greenhouse experiments was

lower on the resistant than on the susceptible cultivars, but successful J2 that developed into fecund females produced similar numbers of eggs per egg mass on both resistant and susceptible cultivars, suggesting that female fecundity is not affected by the *Mi*-gene. The virulent population showed no preference for any of the resistant cultivars tested. It is assumed this virulence occurred naturally because there was no prior exposure of the nematode to the *Mi*-gene in the recent cropping history of the greenhouse from which the population was originally isolated. A single female line of the field population was obtained in 1994, and was maintained in monoxenic cultures for eight generations before being used for the monoxenic culture, greenhouse, and microplot studies. The consistency of the results under different experimental conditions indicates that the pathogenicity of the population had not been altered, and that this population has a compatible mechanism overcoming the resistance conferred by *Mi*-gene. Castagnone et al. (3) demon-

Fig. 1. Correlation between initial population densities of *Meloidogyne javanica* MJ-27 and multiplication rate (Pf/Pi) on the resistant tomato cv. Ramón ($y = 0.8992x + 3.0597$; $R^2 = 0.84$) and susceptible cv. Cobra ($y = 0.8755x + 3.159$; $R^2 = 0.81$) grown in a greenhouse naturally infested with the nematode.

Table 4. Reproduction of *Meloidogyne javanica* MJ-27 population, gall index, root weight, and yield of the resistant tomato cv. Ramón and susceptible cv. Cobra inoculated with 10,000 eggs of the nematode per plant in microplots^x

Tomato cultivar	Pf/Pi	Gall index ^y	Root weight (g)	Yield (kg/plant)	No. fruits/plant	Average fruit weight (g)
Ramón						
Inoculated	303 ± 104	6.5 ± 0.5	207 ± 82 ^z	1.64 ± 0.4	17 ± 5	95 ± 51
Uninoculated			103 ± 23	2.29 ± 0.6 ^z	19 ± 5	118 ± 56 ^z
Cobra						
Inoculated	326 ± 177	6.5 ± 0.5	148 ± 33 ^z	1.44 ± 0.5	15 ± 2	92 ± 46
Uninoculated			87 ± 15	2.04 ± 0.1 ^z	19 ± 4 ^z	105 ± 48 ^z

^x Data are the combined results of two experiments. Each value is mean of 10 replications ± standard deviation.

^y Based on scale from 0 (none) to 10 (severe).

^z Indicates differences between inoculated and uninoculated plants of each tomato cultivar according to Student *t* test ($P \leq 0.05$).

strated that the induced virulence from avirulent populations in the laboratory and the natural virulence shown by some populations of *M. incognita* was stable and was maintained after reproduction of the nematode for 18 generations on susceptible tomato cultivars.

Females from either resistant or susceptible cultivars collected in the field site provided diagnostic *M. javanica* isozyme patterns, indicating this was probably the dominant *Meloidogyne* species at the site. Nevertheless, the virulent population could have displaced other species that may have co-existed before in the site although there is no evidence to support this hypothesis. Mixed species *Meloidogyne* have not been found in this production area.

The virulent MJ-27 caused similar root gallings and percentage yield loss to the resistant and susceptible tomatoes in the microplot experiment. Reproduction of this virulent population in the field tended to be lower on resistant than susceptible cultivars. This tendency was also observed in monoxenic cultures, greenhouse, and microplots. Resistant cultivars apparently retain some of their resistance capability or the virulence of the nematode is incomplete (24). Because residual population densities (Pf values) were similar after cropping resistant or susceptible tomato cultivars at the field site, the beneficial effects of planting a resistant cultivar on a subsequent crop were negated due to the virulence of *M. javanica* MJ-27. Other control measures will be needed such as use of nematocides, poor host plants, clean fallow periods, or probably a combination of these to reduce nematode densities.

Management of *Meloidogyne* with resistant plants has two major advantages: crop self-protection due to the level of tolerance of resistant plants to injury caused by initial infection, and the rotation value of resistance in cropping systems by suppressing nematode population densities (24). Resistant tomatoes for fresh market consumption were first introduced in the Mediterranean basin in the 1980s, and were reported to suppress nematode densities (9,20,21,28), but later reports indicate that they were not always effective for nematode control perhaps due to a progressive adaptation of *Meloidogyne* to reproduce on resistant cultivars or a selection for parasitism by its repeated exposure to *Mi*-resistant tomatoes (6,28). There is, however, no direct evidence of such a process occurring in the field though it has been demonstrated to occur under greenhouse conditions (4,15). Because the *Mi*-gene confers resistance but not immunity, nematode reproduction on resistant cultivars in the field can be explained by the inter- and intra-specific variability existing in the genus *Meloidogyne*, the variable phenotypic expression of the resistance depending on the genotype background (24,26,27), or the allelic composition of the

resistance character. Thus, the preliminary greenhouse test and previous studies (31) showed that the Pf/Pi tended to be higher on the resistant Luxor than on Carmelo. The *Mi*-gene is in an heterozygous state in the majority of the commercial F1 tomato cultivars. Avirulent root-knot populations produce less galling and egg masses on homozygous (MiMi) than heterozygous plants (1,13), but both types of plants respond as susceptible when inoculated with virulent populations regardless of the state of the *Mi*-gene (32,34).

Naturally virulent populations of *M. incognita*, *M. javanica*, and *M. arenaria* have been identified (3,5,15,22,25) though those from Mediterranean countries (6,14,33), including Spain were coincidentally all species of *M. javanica*. These populations were identified in greenhouses with two to three crops per year per decade after the introduction of resistant tomatoes. The presence of plant hosts year round, temperatures prevailing inside the greenhouses, and the higher survival rate of the nematode in greenhouses than outdoors (18) may enhance the appearance of resistant breaking populations in intensive agriculture under protected covers. In California processing tomatoes with the *Mi*-gene have been grown outdoors as a single crop per year for many years (24), and resistant breaking populations were not detected until 1996 (11). However, no widespread occurrence of these populations in tomato fields has been reported. From a practical standpoint it is important to determine the frequency of virulent populations in a given production area so rotational choice of resistant cultivars and rotational sequences can be properly made. Long-term studies in nematode-infested fields are needed to ascertain if avirulent populations develop to virulent ones after continuous cropping with resistant tomatoes since some populations have shown genetic potential for resistant breaking in controlled selection experiments whereas other populations lack such potential (10). No evidence of resistant breaking biotypes of *M. javanica* was observed after three successive resistant tomato crops in Florida (23). The virulent *M. javanica* MJ-27, identified by differential host test, perineal-pattern morphology, and esterase phenotype, did not differ from other populations of the same species, therefore, rapid diagnosis of virulent populations without the need to bioassay will be desirable since available diagnostic techniques do not distinguish virulent and avirulent forms.

ACKNOWLEDGMENTS

The first author was recipient of a grant from Instituto Nacional de Investigaciones Agrarias (INIA). This research was supported by INIA grant no. SC95-049. Thanks are given to P. A. Roberts and J. Aramburu for critical reading of the manuscript, and to the Dorlan family for allowing the authors to carry out the field study on their property.

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