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Observations on the variability in reproduction of some populations of root-knot nematodes (*Meloidogyne* spp.) on resistant tomatoes in Crete, Greece

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Summary. Experiments under controlled conditions with a few naturally occurring populations and single egg mass lines of root-knot nematodes (*Meloidogyne* spp.) from Crete demonstrated the nematodes ability to produce low numbers of egg masses on several resistant tomato cultivars. Except for one naturally virulent population of *M. javanica*, reproduction on resistant cultivars was significantly lower than on a susceptible cultivar. A quantitative nature of the *Mi* resistance gene was revealed by the differential reproduction of a partially virulent line of *M. javanica* on heterozygous and homozygous resistant tomatoes. Virulent clones were not produced from a few single egg mass lines of *Meloidogyne* spp. on a resistant cultivar during a two generation period. Only one line produced two egg masses for two generations but the evidence is probably insufficient to prove a genetic inheritance of virulence. At high soil temperature (>28 °C) resistance was probably decreased and a non-virulent population reproduced on a resistant cultivar. The requirements for further research and practical recommendations for successful implementation of resistant tomatoes in management strategies are discussed.

Key words: *Lycopersicon esculentum*, *Mi* gene, *Meloidogyne javanica*, *M. incognita*, plant resistance, root-knot nematodes, virulence.

Meloidogyne spp. (root-knot nematodes) are economically serious pests of vegetable crops in Crete and currently fumigation of soil with methyl bromide is the principal method used to control these nematodes (Tzortzakakis, 1993). This chemical is highly toxic, therefore alternative control strategies are being investigated such as the effectiveness of resistant plant species. Host plant resistance has proved to be an effective means for reducing root-knot nematode damage in several crops (Roberts, 1992; Young, 1992) and several commercial tomato cultivars have been bred which are resistant to *Meloidogyne* spp.

The effectiveness against root-knot nematodes occuring in Crete of several commercially available tomato cultivars resistant to *Meloidogyne* spp. was investigated by examining the reproduction of selected, naturally occuring field populations of *Meloidogyne* spp. from a restricted area at Heraklion Province, Crete, and populations derived from single egg masses. The effect of high soil temperatures on the effectiveness of a resistant tomato cultivar also was studied and the results from these experiments are reported here.

MATERIALS AND METHODS

Meloidogyne populations were collected from heavily galled roots of vegetables grown in five fields near the south coast of Heraklion Province, Crete (the most distant fields were c. 25 km apart). The recent cropping history and identities of the populations are presented in Table 1. Some populations were maintained in pots on a susceptible tomato (*Lycopersicon esculentum* Mill cv. Carouso) before use in the experiments. Egg mass lines were obtained from four populations by inoculating individual egg masses onto seedlings of the susceptible tomato cv. Carouso. Juveniles (J_2s) for inoculation to plants were collected from extraction dishes in which egg masses from galled roots had been incubated at 25-27 °C for 3 days (Southey, 1986).

Seeds of susceptible and nematode resistant tomatoes were germinated in a glasshouse in flat trays with commercial compost soil and seedlings at the two leaf stage were transplanted into 125 cm^3 or 600 cm^3 pots filled with a steam-sterilized sandy loam soil. Cultivars with resistance conferred by the *Mi*

gene, were selected on the basis of information supplied by commercial seed companies (information in Tables 2 & 3). After 5-10 days the plants were inoculated by introducing 200-300 juveniles (J_2s) per replicate with the 125 cm^3 pots and 600-700 with the 600 cm^3. Inoculated plants were transferred to a controlled environment room which provided soil temperature of 24-26 °C, with a 16 h photoperiod. During the growing period the plants were watered as required and fertilized twice per month with a nutrient solution (Complesal 5-8-10 NPK). After c. 6-8 weeks the plants were uprooted, the roots washed free from soil and the number of egg masses counted under a stereoscopic microscope.

Four experiments were done as described in the Results section and the data were subjected to ANOVA with the least significant differences ($P \leq 0.01$ and $P \leq 0.001$) calculated amongst the treatment means. Data were transformed to square roots ($\sqrt{x}$ or $\sqrt{(x+1)}$ when $x=0$) where the value for the standard error of a mean was higher than the mean value (Mead & Curnow, 1990).

RESULTS

Experiment 1. Variability in reproduction of field populations and single egg mass lines of *Meloidogyne* spp. on resistant tomatoes.

The reproduction of four field populations (populations 1-4) was compared on the cv. Carouso (susceptible) and three tomato cultivars heterozygous for the *Mi* resistance gene (Table 2). All populations reproduced to differing extents on the three resistant tomato cultivars; the number of egg masses produced by population 1 did not differ between the susceptible and resistant cultivars whereas with populations 2, 3 and 4 it was significantly lower. Population 4, which came from cucumber following a resistant tomato cultivar was next most virulent after population 1. Populations 2 and 3, which were from fields which had not grown a resistant crop in the previous 2 years were the least virulent.

The reproduction of 20 single egg mass lines (ten each from populations 3 and 5) were compared on susceptible (cv. Dombito) and resistant (cv. Menglo, heterozygous for the *Mi* gene) tomatoes. Six of the ten single egg mass lines from *M. javanica* population 3 produced one, or a maximum of two egg masses, on the resistant cultivar. When the egg masses were reinoculated on the same cultivar one line produced two egg masses and all the others produced none. Although both populations produced many egg masses on cv. Dombito the other ten single egg mass lines from population 5 did not produce any egg masses on resistant cv. Menglo.

Population 2 reproduced only slightly on pepper cv. California Wonder and a line was established, from a single *M. incognita* egg mass. This line was increased on pepper and batches of 300 J_2s were used to infect seedlings of the susceptible tomato cv. Carouso and eight resistant cultivars (Scala, 7352 Silco, Myrto, Menglo, Rakata, GC 785, GC 788 Alpado and Bermuda). After 7 weeks no reproduction was observed on any of the resistant cultivars whereas on susceptible tomato an average of 35 egg masses per plant were produced.

Experiment 2. Variability in reproduction of three single egg mass lines of *M. javanica* and a line of *Meloidogyne* spp. on resistant tomatoes.

Three single egg mass lines of *M. javanica* were raised on susceptible tomato from populations 1 and 3 (two from population 1 and one from population 3). The lines had been characterized by their morphology and by the North Carolina differential host test (Hartman & Sasser, 1985; Jepson, 1987). The two lines from population 1 (1HVa and 1HVb) had been previously characterized as highly virulent due to their high reproductive rates (which did not differ significantly from that obtained with the susceptible cultivar) on 14 resistant tomato cultivars [Scala, 7352 Silco, 7353, 7358 (Rijk Zwaan), Menglo, Myrto, Rakata, W 1964, W 1967, W 2913 (De Ruiter Seeds), GC 785, GC 788 Alpado, 399 (Sluis & Groot Sandoz Seeds) and Bermuda (Enza Zaden BV)] at a soil temperature of 22-24 °C. The line from population 3 (3AV) had been characterized as avirulent due to the absence of galls and egg masses on any of the above resistant cultivars (Tzortzakakis & Gowen, 1996). A fourth line, an undetermined *Meloidogyne* spp., was derived from population 5. This line was also avirulent as it did not reproduce on resistant tomato cv. Menglo in the previous test. The reproduction of these four lines was tested to determine their virulence on six resistant tomato cultivars which are commonly grown in Crete (Table 3).

Lines 1HVa and 1HVb were consistently virulent on the six resistant cultivars tested and produced as many egg masses on the resistant as on the susceptible cultivars. Line 3AV and the avirulent line of *Meloidogyne* spp. from population 5 produced none, or only a few egg masses on the resistant tomato cultivars.

Experiment 3. Reproduction of a high and low virulent line of *M. javanica* on a heterozygous resistant hybrid and two homozygous resistant inbred lines of tomato.

With the tomato hybrid F_1 Scala, resistance to root-knot nematodes is conferred by the *Mi* gene in a heterozygous state and two F_8 inbred lines deriving

Table 1. Origins and identities of root-knot nematode populations (identification based on the morphology of perineal patterns of 15-20 females).

Population	Host origin	Crops in previous two years	Identity
1	Resistant tomato	Resistant tomato	*M. javanica*
2	Cucumber	Susceptible tomato and cucumber	*M. javanica*, with a few *M. incognita* and an intermediate form
3	Susceptible tomato	Susceptible tomato	*M. javanica*
4	Cucumber	Resistant tomato and cucumber	*M. javanica*, with a few *M. incognita* and an intermediate form
5	Susceptible tomato	Susceptible tomato and cucumber	*M. javanica*, with a few *M. incognita* and an intermediate form

Table 2. Number of egg masses on a susceptible (Carouso) and three heterozygous resistant tomato cultivars infected by 300 J_2s from four populations of *Meloidogyne* spp. (mean of four replicates).

Tomato	Seed Company	Population 1	Population 2	Population 3	Population 4
Carouso (S)		31.75 (5.56)'	27.00 (5.11)'	48.50 (6.84)'	40.00 (6.22)'
Menglo (R)	De Ruiter Seeds	37.75 (5.94)	1.25 (1.13)***	1.25 (1.13)***	10.25 (3.18)**
Rakata (R)	De Ruiter Seeds	44.25 (6.63)	7.00 (2.52)**	2.50 (1.54)***	4.75 (2.09)***
Bermuda (R)	Enza Zaden BV	37.00 (6.03)	8.00 (2.73)**	7.50 (2.62)**	10.00 (3.14)**
SED		(0.97)	(0.69)	(0.84)	(0.64)

Differences from the susceptible tomato based on LSD values; ( )' ANOVA on square root tranformed data; ** and *** significantly lower at $P<0.01$ and $P<0.001$ respectively.

Table 3. Number of egg masses on susceptible (Earley pak) and six resistant tomato cultivars heterozygous for the *Mi* gene by 200 J_2s from four single egg mass line of *M. javanica* and *Meloidogyne* spp. (mean of five replicates).

Tomato	Seed Company	*M. javanica*			*Meloidogyne* spp.
		Line 1HVa	Line 1HVb	Line3AV	Lines
Earley pak (S)		31.60	23.00	31.20 (5.63)'	24.20 (4.97)'
Veronica (R)	Rijk Zwaan	21.80	24.20	0.00 (1.00)***	not tested
Adelina (R)	Rijk Zwaan	27.20	31.00	0.20 (1.08)***	1.00 (1.34)***
2001 (R)	Agrosystem	30.20	26.80	0.00 (1.00)***	00.0 (1.00)***
Kastalia (R)	Bruisma	22.60	28.20	00.0 (1.00)***	00.0 (1.00)***
DRW 3544 (R)	De Ruiter Seeds	35.80	33.80	00.0 (1.00)***	0.80 (1.24)***
DRW 3386 (R)	De Ruiter Seeds	35.60	25.60	00.0 (1.00)***	2.60 (1.84)***
SED		4.53	5.73	(0.18)	(0.30)

Data analysis as for Table 2; ( )' = $\sqrt{(x+1)}$ transformations.

Table 4. Number of egg masses on a susceptible (Rutgers), a heterozygous resistant (F_1 Scala) and two homozygous resistant (F_{8A} and F_{8B}) tomato cultivars inoculated with 600 J_2s from two single egg mass lines of *M. javanica* (mean of six replicates).

Tomato	Line 1HVb	Line 3LV
Rutgers (S)	56.50	54.50 (7.41)'
F_1 Scala (R)	79.00	23.83 (4.95)***
F_{8A} (R)	71.20	0.33 (1.13)***$^+$
F_{8B} (R)	65.30	0.00 (1.00)***$^+$
SED	13.75	(0.24)

Data analysis as for Table 2; *** significantly lower than susceptible at $P<0.001$; $^+$ significantly lower than the heterozygous at $P<0.001$; ( )' = $\sqrt{(x+1)}$ transformations.

Table 5. Numbers of invading, emigrating juveniles and egg producing females on roots of a susceptible (Carouso) and heterozygous resistant (Bermuda) tomato cultivar inoculated with 700 J_2s from a *M. javanica/M. incognita* population (mean of four replicates at soil temperature 28 to 31 °C).

	Day after inoculation	Tomato		SED
		susceptible	resistant	
Numbers of invading juveniles	4	67.50 (8.20)	10.50 (3.18)***	(0.40)
	18	79.00 (8.76)	25.25 (5.01)**	(0.87)
Numbers of emerging juveniles	4-10	10.00 (2.79)	36.50 (5.91)**	(0.90)
Numbers of egg producing females	30	79.00 (8.76)	28.00 (5.25)**	(0.92)

Data analysis as for Table 2; ** and *** significantly lower at $P<0.01$ and $P<0.001$ respectively.

from this hybrid (F_{8A} and F_{8B}) had been developed with homozygous resistance by the Rijk Zwaan seed company (P.L.J. Egelmeers, Plant Breeder, Rijk Zwaan, personal communication). The reproduction of two single egg mass lines of *M. javanica* previously characterized as lines of high (1HVb) and low (3LV) virulence (Experiment 2, Tzortzakakis & Gowen, 1996) were compared on the susceptible tomato Rutgers, the heterozygous resistant Scala and the homozygous resistant F_{8A} and F_{8B}. The lines were derived from populations 1 and 3 with high and low virulence respectively.

On the heterozygous resistant tomato, line 3LV produced significantly fewer egg masses than on the susceptible (Table 4). However, on the homozygously resistant F_{8B} it produced no egg masses and on F_{8A} the number of egg masses produced was very small and significantly less than on the heterozygously resistant cv. Scala. In contrast, reproduction of the virulent line 1HVb did not differ between any of the cultivars tested (Table 4).

Experiment 4. Invasion and development of juveniles of an avirulent population of *Meloidogyne* spp. on susceptible and resistant tomato cultivars at a soil temperature 28 °C.

Juveniles from a mixed population of *M. javanica/M. incognita* (population 5) were inoculated into pots filled with steam-sterilized soil and planted with seedlings of susceptible (Carouso) or resistant (Bermuda) tomato. Inoculation rates were c. 700 J_2s per pot. Plants were maintained on a glasshouse bench for 2.5 months; the soil temperature in the pots ranged from 20 to 25 °C. Roots were examined after this period and the susceptible cultivar was heavily infected but on the resistant cultivar no galls or egg masses were found. Similarly, seedlings inoculated with the same population were also transferred to a growth chamber with soil temperatures ranging from 28 to 31 °C and with a 16 h photoperiod. Four replicate plants from each treatment were uprooted 4 and 18 days after inoculation, the roots washed free from soil, stained with acid fuchsin and the number of invading juveniles counted under a stereoscopic microscope (Southey, 1986). To determine emergence rates of juveniles from roots, four seedlings were thoroughly washed free from soil (to remove any juveniles adhering to the root surface) and incubated in beakers containing 50 cm^3 of water for 6 days. The emerging juveniles were collected daily on a 20 μm aperture mesh sieve and counted. The number of egg masses per root after 30 days was determined in a further four replicates.

Juveniles invaded the resistant tomato at the high soil temperature. However, after one month their number and that of females producing eggs were significantly lower than on the susceptible cultivar. A significantly greater number of juveniles emerged from the roots of the resistant compared to that of the susceptible cultivar (Table 5).

DISCUSSION

The experiments in this study were designed to examine virulence differences between populations and single egg mass lines of *Meloidogyne* spp. originating from five fields in a relatively small area in Heraklion Province, Crete. One population of *M. javanica* and two single egg mass lines derived from it were completely virulent on tomato heterozygous for the *Mi* resistance gene. Three of the populations, also mainly *M. javanica*, produced a few egg masses on the resistant tomatoes, but always significantly fewer ($P<0.05$) than on the susceptible cultivar; single egg mass lines gave either the same response or were completely avirulent.

The fully virulent population of *M. javanica* (population 1) and the respective single egg mass lines, came from a field where resistant tomato had been previously grown for 2 years. It is uncertain whether their virulence is natural or, as observed by (Castagnone-Sereno, 1994; Castagnone-Sereno *et al.*, 1994) it is the outcome of selection over several

generations on a resistant cultivar. However, although tested for only two generations, none of the six lines from the low virulent population 3 showed any capacity for increasing virulence. To determine whether this population has the capacity for further adaptation will probably require more generations of selection.

Although *M. javanica* was the dominant species in all populations, two contained some *M. incognita* which reproduced on pepper cv. California Wonder which is an alternative crop to tomato. When an *M. incognita* line was inoculated onto resistant tomatoes, there was no reproduction indicating that rotations including resistant tomatoes and pepper might be used to reduce nematode damage, as the *M. javanica* lines tested have only a limited ability to reproduce on pepper (Tzortzakakis & Trudgill, unpublished).

Perhaps the most interesting result was the observation that tomatoes homozygous for the *Mi* gene were more resistant than the heterozygous cultivars to a partially virulent line of *M. javanica*. This is the first time a quantitative or dose effect of the *Mi* gene has been reported. However, both the heterozygous and homozygous resistant tomato cultivars were equally susceptible to the fully virulent line. Even so, the reduced loss of resistance of *Mi* heterozygous tomato at temperatures 28 °C was confirmed, and this complicates their use in polythene tunnels in Crete during the summer.

An experiment conducted in a polythene tunnel three years previously, with inoculum derived from the field site of population 5, revealed that growing a resistant tomato in nematode infested soil for one cropping season (March-July) significantly decreased damage to the following susceptible crop (Tzortzakakis & Gowen, 1994).

Resistant tomato cultivars could therefore, be of increasing value for managing root-knot nematode infestations in vegetable production areas of Crete, especially if the use of methyl bromide is restricted and nematicide applications are discouraged. However, virulence differences and high soil temperatures will probably complicate their use.

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Резюме. Эксперименты, проведенные при контролируемых условиях с галловыми нематодами из природных популяций и селекционных линий, полученных из одного яйцевого мешка на Крите, продемонстрировали способность нематод к откладке пониженного числа яйцевых мешков при развитии на некоторых устойчивых сортах томатов. За исключением одной природной вирулентной популяции *M. javanica*, размножение галловых нематод на устойчивых сортах томатов было значительно ниже, чем на восприимчивых. Доказана количественная природа гена устойчивости *Mi* на основании различий в показателелях размножения частично вирулентной линии *M. javanica* на гетерозиготных и гомозиготных устойчивых томатах. Селекционные линии нематод, полученные из одного яйцевого мешка, не были способны продуцировать вирулентные клоны при развитии на устойчивых сортах на протяжении двух поколений. При высокой температуре почвы (>28 °C) устойчивость, по-видимому, снижалась, и невирулентная популяция была способна размножаться на устойчивом сорте томатов. Обсуждаются возможные направления дальнейшего теоретического поиска и разработки практических рекомендаций для успешного использования устойчивых сортов томатов в интегрированной системе борьбы с нематодами.