VARIABILITY IN REPRODUCTION OF *MELOIDOGYNE JAVANICA* AND *M. INCOGNITA* ON TOMATO AND PEPPER

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RESUMEN

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Doze variedades de ají, *Capsicum annuum*, mostraron resistencia a dos líneas individuales de huevos de *M. javanica*, las que pueden ser capaces o no de reproducirse en tomates resistentes que portan el gen Mi. Sin embargo todas estas varidades fueron susceptibles a una línea de huevos de *M. incognita* que no se reproduce en tomates resistentes. Estas diferencias en la reproducción, se observaron en poblaciones de *Meloidogyne* originadas en un área restringida de Creta, lo que indica la importancia de la identificación de especies y la estimación de la reproducción, antes de decidir una estrategia de manejo para variedades resistentes. La importancia del ají, como sistema biológico de ensayo para la predicción de daños y en las decisiones sobre la rotación de cultivo, son enfatizadas en este trabajo. Durante un periodo de 56 días y con el uso de placas con agar, se estimó el numero de huevos en la masa y la velocidad de producción de estos en 24 horas, por cada hembra de *Meloidogyne* presente en pequeños segmentos de raíces. Este ensayo incluyó a las tres líneas de *Meloidogyne* sobre especies de plantas que resultaron ser buenos hospedantes. Se registraron diferencias en la reproducción de las líneas de *Meloidogyne* para una misma planta hospedante y dentro de la misma línea de *Meloidogyne* en hospedantes diferentes. Este método de ensayo de la fecundidad, puede ser usado para proveer información sobre la dinámica de la población en nematodos.

Palabras claves: ají, Mi, *Meloidogyne incognita*, *Meloidogyne javanica*, nematodos agalladores, tomate.

In Crete, root-knot nematode species (*Meloidogyne* spp.) are serious pests of vegetables grown in plastic tunnels (Tzortzakakis, 1997). The use of resistant and non-host plants in crop rotations could be an economical and environmentally safe management strategy for use by vegetable growers if such plants are economically attractive. Pepper cultivars commonly grown in Crete are rarely infected by local populations of root-knot nematodes and therefore may be useful in crop rotations.

The economic importance of damage caused by *M. incognita* (Kofoid & White) Chitwood on pepper in the tropics and subtropics has been characterized as moderately important for sweet pepper and chilli (*Capsicum annuum*) but there is less information for *M. javanica* (Treub) Chitwood. Damage by *M. incognita* on Cayenne pepper (*C. frutescens*) is moderately important, but of limited or local importance for *M. javanica* (Netscher and Sikora, 1990). A test of the reaction to root knot nematodes of nine pepper cultivars commonly grown in Nigeria indicated that *M. incognita* reproduced on more cultivars than *M. javanica* (Ogbuji and Okafor, 1984). The roots of the pepper cv. Yolo Wonder formed normal giant cells when invaded by *M. incognita*, but reacted with slowly developing giant cells and slow necrosis in response to *M. javanica* (Hendy *et al.*, 1985). The number of developing juveniles and mature females in roots of the nematode susceptible pepper cv. Suryamukhi Green was significantly less for *M. javanica* than for *M. incognita*. Some pepper cultivars susceptible to *M. incognita* were resistant or immune to *M. javanica*

(Khan Ahmad and Khan Wajid, 1991a, 1991b).

The purpose of this investigation was to compare reproduction of two single egg mass lines of *M. javanica* and one of *M. incognita* on some pepper cultivars commonly grown in Crete. These lines are representative of a wide range of genotypes of local *Meloidogyne* populations (Tzortzakakis, 1997; unpublished data). We also compared the fecundity of individual females on tomato and pepper cultivars which were found to be good hosts for the specific nematode lines.

Single egg mass lines of root-knot nematodes were established previously from populations collected from galled vegetable roots in the south of Heraklion Province, Crete. All lines were identified by distinct morphological characteristics (perineal pattern of mature females, tail length and hyaline portion of J_2) and by the North Carolina differential host test (Hartman and Sasser, 1985; Jepson, 1987). The lines were characterized as *M. javanica* (virulent), able to reproduce on susceptible and heterozygous- and homozygous-resistant (with Mi gene) tomato cultivars at moderate soil temperature (<26 C); *M. javanica* (avirulent) indicating minimal or zero reproduction on those cultivars (Tzortzakakis, 1997; Tzortzakakis and Gowen, 1996; Tzortzakakis, Trudgill and Phillips, unpublished); and *M. incognita*, which did not reproduce on the resistant tomato cultivars (Tzortzakakis, 1997). *Meloidogyne incognita* was maintained on pepper cv. California Wonder, and lines of *M. javanica* were maintained on susceptible and resistant tomatoes as appropriate for several generations before experimental use.

In all experiments, a susceptible (Early pak) and a heterozygous resistant (Menglo or Scala) tomato cultivar were included as controls to confirm the previous characterization of *Meloidogyne* lines. Seeds of all treatments were germinated in trays filled with commercial compost soil in a glasshouse and transplanted at the first leaf stage into 125 ml pots (Experiment 1) or 30ml plastic tubes (Experiment 2) filled with steam sterilized sandy loam soil. A total of 12 pepper cultivars[†] were tested in both experiments; 4 replicates of 6 cultivars in Experiment 1, and 5 replicates of 10 cultivars in Experiment 2. Four pepper cultivars from Experiment 1 were also tested in Experiment 2. Second stage juveniles (J_2) were obtained by incubating egg masses in extraction dishes (Southey, 1986). Juveniles recovered during the first 24 h were discarded, and those collected between two and five days were inoculated two days after transplanting at a rate of 450 per plastic pot or 80 per tube. Plants were grown in a controlled environment with 16 h photoperiod at 22-26 C air temperature and were watered as required. Soil temperature ranged from 23 to 26°C. Liquid fertilizer (5-8-10, N-P-K) diluted 200 times in water was applied weekly at rates of 10 ml per pot or 3 ml per tube. The plants were gently uprooted after 8 weeks, the roots were carefully washed and the numbers of egg masses were counted with the aid of a stereobinocular microscope (×25). Data were subjected to ANOVA and the least significant differences ($P \leq 0.05$ and $P \leq 0.01$) between treatment means calculated. Data were transformed to square roots before analysis to standardize variances (Mead and Curnow, 1990).

Neither line of *M. javanica* reproduced on any of the pepper cultivars tested,

[†]Lamuyo 355, DRS 360, DRS 522, DRS 1138, DRS 6344 (De Ruitter Seeds): Gracia, Sammy, 3560 (Rijk Awaan); Rupia, Indalo, Latino (Bruismah) and Agio (Leende Mos). All seeds originated from The Netherlands.

Table 1. Number of egg masses[x] on a susceptible tomato (cv. Early Pak), a heterozygous resistant tomato (cv. Menglo) and six pepper cultivars inoculated[y] with single egg mass lines of *M. javanica* or *M. incognita*.

Plant	*M. javanica* virulent		*M. javanica* avirulent		*M. incognita*	
Tomato						
Early pak (s)	54	(7.31)[z]	46	(6.73)	47	(6.78)
Menglo (r)	36	(5.94)	1	(0.50)	1	(0.60)
Peppers						
Gracia	0	(0.00)	0	(0.00)	56	(7.43)
Sammy	0	(0.00)	0	(0.00)	46	(6.58)
Indalo	0	(0.00)	0	(0.00)	43	(6.52)
Latino	3	(1.69)	5	(1.49)	46	(6.75)
Lamuyo 355	0	(0.00)	0	(0.00)	26	(4.99)
DRS 360	0	(0.00)	0	(0.00)	54	(7.23)
LSD 5%		(0.70)		(0.94)		(1.66)
LSD 1%		(0.95)		(1.28)		(3.72)

[x]Average of four replicates per treatment
[y]Plants inoculated with 450 J_2 and grown in 125 ml pots
[z]Mean of square root transformed data in parentheses.

except for minimal reproduction on cvs. Latino and DRS 6344 (Tables 1 and 2). The *M. incognita* line reproduced on all pepper cultivars at rates which did not differ from or were significantly lower ($P \leq 0.05$) than that on the susceptible tomato (Early pak). The variable reproduction of *M. javanica* lines on the resistant tomato cvs. Menglo and Scala confirmed their previous characterizations as able or unable to reproduce on resistant tomato.

A method described by Tzortzakakis and Trudgill (1996) was used to assess whether there were differences in the egg laying rates and numbers of eggs laid by individual females of the three lines when they infected suitable hosts. Essentially, the method involves cutting small root segments that contain a single egg mass, removing and counting the eggs, dissecting root tissue to expose the posterior end of the female, and placing the root segment with its female on water agar. Eggs produced during the following 24 hrs are counted. Seedlings of tomato cvs. Early pak and Scala and pepper cv. Rupia, grown in 125 ml plastic pots filled with steam sterilized soil, were infested with ca. 200 juveniles, collected over 4 days from extraction filters. Each cultivar was infested with the line(s) which reproduced successfully in the previous tests. The plants were maintained in a controlled environment with a 16 h photoperiod and a temperature of 27 ± 3°C inside the cups. They received water and nutrient solution as previously described. Single plants were removed, their root systems soaked and gently washed free of soil at 17, 21, 26, 31, 40, 46 and 56 days after inoculation and the agar assays prepared. Six replicate assays were prepared for each *Meloidogyne* line and host combination at each observation time. Assay dishes were maintained in the

Table 2. Number of egg masses[x] on a susceptible tomato (cv. Early Pak) and a heterozygous resistant tomato (cv. Scala) and ten pepper cultivars inoculated[y] with single egg mass lines of *M. javanica* or *M. incognita.*

Plant	*M. javanica* virulent		*M. javanica* avirulent		*M. incognita*	
Tomatoes						
Early pak (s)	23	(4.80)[z]	26	(5.03)	19	(4.30)
Scala (r)	21	(4.49)	6	(2.44)	1	(0.68)**
Peppers						
Rupia	0	(0.00)	0	(0.00)	13	(3.47)*
Indalo	0	(0.00)	0	(0.00)	13	(3.56)
Latino	0	(0.00)	0	(0.00)	10	(3.06)**
Agio	0	(0.00)	0	(0.00)	13	(3.55)
Gracia	0	(0.00)	0	(0.00)	12	(3.45)*
3560	0	(0.00)	0	(0.00)	12	(3.36)*
DRS 360	0	(0.00)	0	(0.00)	12	(3.50)
DRS 522	0	(0.00)	0	(0.00)	12	(3.44)*
DRS 1138	0	(0.00)	0	(0.00)	19	(4.24)
DRS 6344	1	(0.6)	0	(0.00)	9	(2.91)**
LSD 5%		(0.46)		(0.36)		(0.80)
LSD 1%		(0.61)		(0.48)		(1.07)

[x]Average of five replicates per treatment.
[y]Plants inoculated with 80 J_2, and grown in 30 ml tubes.
[z]Mean of square root transformed data in parentheses.
* and ** significantly lower than susceptible tomato at $P \leq 0.05$ and $P \leq 0.01$, respectively.

dark in a closed plastic box in the same controlled environment with plants.

Females were found in all roots and egg masses were first associated with a few females of *M. incognita* and *M. javanica* (avirulent) at 21 days after inoculation (Table 3). In the subsequent 24 h, eggs were mainly produced by females which had eggs in their egg mass when dissected from the roots (Table 4). Females from *M. javanica* (virulent) on either tomato host laid no eggs before or after being placed on agar. At 26 days after inoculation, egg masses were present in females from all treatments except *M. javanica* (virulent) on resistant tomato. Regardless of host, most females layed eggs after being placed on the agar surface, including some which did not have egg masses when dissected from the roots. At 31 days there were still some females of the two *M. javanica* lines without egg masses (data not shown); however all layed eggs after being placed on the agar surface. Embryonated eggs were observed on day 31 and empty egg shells and hatched juveniles on day 40 in all treatments, indicating that the minimum period required for one generation under these conditions was between 32 and 40 days.

The pepper cultivars tested were not known to have any genes conferring resistance to root-knot nematodes (personal

Table 3. Number of eggs/egg mass[x] laid by individual females at 21, 26, 31 40, 46 and 56 days after inoculation on a susceptible tomato (cv. Early pak) and a heterozygous resistant tomato (c.v. Scala) and pepper (Rupia) cultivars inoculated with 200 juveniles per plant from single egg mass lines of *M. javanica* and *M. incognita.*

Day	*M. incognita* (Early pak) Tomato(s)	*M. incognita* (Rupia) Pepper	*M. javanica (virulent) (Early pak) Tomato(s)*	*M. javanica (virulent) (Scala) Tomato(r)*	*M. javanica (avirulent) (Early pak) Tomato(s)*	F test level of significance	LSD at level of significance
21	2 (0.69)[y]	4 (0.76)	0 (0.00)	0 (0.00)	1 (0.28)	NS[z]	—
26	20 (2.89)	41 (6.02)	16 (2.72)	0 (0.00)	1 (0.52)	1%	3.05
31	108 (9.32)	87 (8.49)	60 (5.00)	14 (2.04)	36 (3.76)	NS	—
40	296 (17.04)	384 (19.12)	172 (12.15)	367 (18.63)	352 (18.55)	5%	2.44
46	345 (18.36)	461 (21.38)	291 (16.93)	283 (16.64)	388 (19.67)	1%	3.64
56	319 (17.76)	408 (19.74)	441 (20.90)	395 (19.63)	539 (23.18)	5%	3.54

[x]Means of 6 replicates.
[y]Square root transformed data for ANOVA in parentheses.
[z]NS = not significant at 5% level.

communication with respective Seed Companies). Our results suggest that these pepper cultivars are likely to be resistant to *M. javanica* and susceptible to *M. incognita* populations from Crete. Thus, the cultivars may be useful as indicator plants in bioassay systems for damage prediction and crop rotation decisions. A survey of *Meloidogyne* populations in vegetable growing areas of Crete indicated that *M. javanica* is the prevalent species, whereas *M. incognita* has a limited distribution on the

Table 4. Number of eggs[x] laid by individual females within 24h at 21, 26, 31, 40, 46 and 56 days after inoculation on a susceptible tomato (cv. Early pak) and a heterozygous resistant tomato (c.v. Scala) and pepper (Rupia) cultivars inoculated with 200 juveniles per plant from single egg mass lines of *M. javanica* and *M. incognita.*

Day	*M. incognita* (Early pak) Tomato(s)	*M. incognita* (Rupia) Pepper	*M. javanica (virulent) (Early pak) Tomato(s)*	*M. javanica (virulent) (Scala) Tomato(r)*	*M. javanica (avirulent) (Early pak) Tomato(s)*	F test level of significance	LSD at level of significance
21	4 (1.33)[y]	10 (2.41)	0 (0.00)	0 (0.00)	2 (0.57)	5%	1.65
26	8 (1.70)	23 (4.46)	23 (3.83)	16 (3.23)	10 (1.84)	NS[z]	—
31	41 (6.30)	49 (6.86)	26 (4.80)	16 (3.54)	18 (3.96)	1%	2.58
40	44 (6.50)	57 (7.48)	57 (7.58)	65 (8.09)	68 (8.24)	5%	1.07
46	40 (6.21)	47 (6.79)	61 (7.74)	55 (7.32)	54 (7.20)	NS	—
56	47 (6.81)	39 (5.96)	68 (8.19)	25 (4.74)	28 (4.91)	1%	2.47

[x]Means of 6 replicates.
[y]Square root transformed data for ANOVA in parentheses.
[z]NS = not significant at 5% level.

island, being associated mainly with pepper cultivation (Tzortzakakis, unpublished data). Our work demonstrates that most pepper cultivars grown in Crete are potentially valuable to suppress *M. javanica* populations, even those able to reproduce on tomato with the Mi gene. However, all pepper cultivars were susceptible to *M. incognita* emphasizing the importance of identifying the species that are present before making the decision to rotate susceptible plants with resistant tomatoes or peppers. The decision is obviously more complex when both species coexist.

The results in Tables 3 and 4 indicate that the number of eggs laid within a 24 h period and the total number of eggs per egg mass vary with time depending on the nematode species and the host plant. Khan Wajid and Haider (1991) reported that *M. javanica* and *M. incognita* differed in their reproductive ability on a common susceptible host when soil population (J_2 and males), root population (juveniles and females) and number of eggs per root were compared fifty days after inoculation. Our findings indicate differences in egg output rate and numbers of eggs/egg mass between three *Meloidogyne* lines on susceptible tomato and also from the same line on different hosts. However, there was no clear trend in either of these comparisons, and the variation can probably be attributed to differences in the period of egg laying. If the daily egg laying rate for a time period (eg. between day 21 and 26) is estimated as the average of the number of eggs laid on the first and the last day, then the total number of eggs laid in this interval can be estimated by multiplying the daily rate by the number of days in the time interval. By summing these values we can estimate the total number of eggs laid per female between day 21 and 56 (Table 5). For *M. javanica* (avirulent) that was estimated to be 1 263. A previous study with the same line on another susceptible tomato cultivar indicated that the total number of eggs laid from 21 to 65 days after inoculation at ca. 26°C was 1973

Table 5. Estimated number of eggs/female and eggs per plant for a period between 21 and 56 days after inoculation on a susceptible (Early Pak) and a heterozygous resistant (Scala) tomato and pepper (Rupia) cultivars inoculated with 200 juveniles per plant from single egg mass lines of *M. javanica* and *M. incognita*.

Nematode Host	*M. incognita* (Early pak) Tomato(s)	*M. incognita* (Rupia) Pepper	*M. javanica* (virulent) (Early pak) Tomato(s)	*M. javanica* (virulent) (Scala) Tomato(r)	*M. javanica* (avirulent) (Early pak) Tomato(s)
Day 21-26	30	83	58	40	30
Day 26-31	123	180	123	80	70
Day 31-40	383	477	374	365	387
Day 40-46	252	312	354	360	366
Day 46-56	435	430	645	400	410
Total eggs/female	1223	1482	1554	1245	1263
Egg masses/plant'	19	13	23	21	26
Total egg/plant	23 237	19 266	35 742	26 145	32 838

'Data from Table 2.

(Tzortzakakis and Trudgill, 1996). Using the number of egg-producing females per plant from Table 2 we can estimate a relative total number of eggs per plant. These estimates indicate that *M. javanica* may produce more offspring than *M. incognita* on the susceptible tomato and a line of *M. javanica* reproducing on both tomato cvs. may produce more offspring on susceptible than on resistant tomato. The results also illustrate the enormous reproductive potential of *M. javanica* and *M. incognita* on good hosts (Trudgill *et al.*, 1992) and demonstrate a possible method to obtain life stage data for modelling the population dynamics of these nematodes.

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