ROTATIONAL MANAGEMENT OF *MELOIDOGYNE JAVANICA* IN A SMALL SCALE GREENHOUSE TRIAL IN CRETE, GREECE

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ABSTRACT

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The crop yields, root galling indices, and population densities of tomato avirulent and virulent root-knot nematode *Meloidogyne javanica* were compared in a greenhouse experiment with two cropping cycles. In the first crop, the avirulent and virulent populations were better controlled by use of resistant (*Mi* gene) tomato and pepper than by application of fenamiphos with susceptible tomato. Susceptible tomato was grown as the second crop in all plots and was treated with fenamiphos, except where it followed the resistant tomato or pepper. At the end of the second crop, fruit yields, levels of root galling and population densities of nematodes were similar for all treatments. The effect of rotational management on *M. javanica* for one cropping cycle seems to be equal to that of nematicide applications on a sequence of susceptible crops.

Key words: crop rotation, fenamiphos, nematicide, pepper, resistance, root-knot nematodes, tomato.

RESUMEN

Tzortzakakis, E. A., M. S. Phillips y D. L. Trudgill. 2000. Manejo rotacional de Meloidogyne javanica en un pequeño ensayo de invernadero en Creta, Grecia. Nematropica 30:167-175.

El rendimiento, índice de agallamiento de la raíz y las densidades poblacionales del nematodo del tomate *Meloidogyne javanica* virulento y avirulento, fueron comparados en un experimento de invernadero con dos ciclos de cultivo. En el primer cultivo, las poblaciones virulentas y avirulentas fueron mejor controladas por el tomate (gen Mi) y ají resistentes, que por los fenamifos en el tomate susceptible. El tomate susceptible, se usó como segundo cultivo en todas las parcelas y fue tratado con fenamifos, excepto donde este siguió al tomate o ají resistentes. Al final del segundo cultivo, los rendimientos de fruta, niveles de agallamiento de la raíz y densidades poblacionales de nematodo, fueron similares en todos los tratamientos. El efecto del manejo rotacional en *M.javanica* para un ciclo de cultivo, parece ser igual al de las aplicaciones de nematicida en una secuencia de cultivos susceptibles.

Palabras claves: ají, fenamifos, nematicida, nematodos agalladores de la raíz, tomate.

INTRODUCTION

Root-knot nematodes (RKN, *Meloidogyne* spp.) are serious pests in the 2000 ha of plastic tunnels used for vegetable production in Crete (Tzortzakakis, 1997), and 600 t of methyl bromide soil fumigant is used annually for their control. Cultural management based mainly on crop rotations of resistant or non-host crops with susceptible crops are the mainstay of most non-chemical control programs and various crop sequences effective in reducing root-knot nematode populations have been reported (Trivedi and Barker, 1986; Netscher and Sikora, 1990; Madulu *et al.*, 1994). In

Crete, the economics of vegetable production limit the options for cultural management with nematode resistant/non-host solanaceous crops (e.g., tomato cvs with the *Mi* gene) in rotation with susceptible plants of the same family (e.g., tomato, eggplant) or cucurbits (e.g., cucumber, melon). Many cultivars of tomato with resistance to *M. javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White) Chitwood, and *M. arenaria* (Neal) Chitwood (Roberts, 1992) conferred by the *Mi* gene are available in local markets. However, virulent populations of *M. javanica* have been detected in a survey of the main vegetable growing areas of Crete (Tzortzakakis and Gowen, 1996; Tzortzakakis *et al.*, 1999a). Several cultivars of pepper were shown to be non-hosts for both virulent and avirulent lines of *M. javanica* but they were hosts for *M. incognita* (Tzortzakakis, 1997; Tzortzakakis *et al.*, 1999a).

The role of resistant tomato in the management of RKN had been previously investigated in experiments on integrated control in a plastic tunnel using a population of *Meloidogyne* avirulent on *Mi* tomato (Tzortzakakis & Gowen, 1994; Tzortzakakis *et al.*, 1999b). In a study reported herein, resistant tomato and pepper were compared with a nematicide for the control of avirulent or virulent *M. javanica* in a small-plot trial in a plastic tunnel.

MATERIALS AND METHODS

Experimental site and nematode infestation. The experiment was in a plastic covered tunnel at the Plant Protection Institute, Heraklion, Crete. The soil was loamy sand (59% sand, 16% clay, 25% loam, EC = 3.27 S m^{-1}, pH = 7.317, $CaCO_3$ = 44 ppm). The same site had been used previously for an experiment on integrated control of root-knot nematodes with *Pasteuria penetrans*, oxamyl and solarization (Tzortzakakis and Gowen, 1994). At the end of that experiment the site was fumigated with methyl bromide, left for 3 years without cultivation, and then again fumigated with methyl bromide (98% a.i., 2% chloropicrin, applied at a dosage of 680 g per $10m^2$).

After fumigation, the soil was disked and susceptible tomato plants cv. Tiny Tim, heavily infested with avirulent or virulent *M. javanica,* were planted alongside the trickle irrigation system. The virulent population was a mixture of two, single egg mass lines able to reproduce on resistant (*Mi* gene) tomato, and the avirulent population was a field population unable to reproduce on resistant tomato (Tzortzakakis and Gowen, 1996).

Twenty two microplots, each containing two rows of four plants (0.8m × 0.4m apart), were planted with 1m between adjacent microplots. The infested tomato plants were produced by transplanting seedlings at the two-leaf stage into individual 50 ml pot pocket seedling trays filled with a commercial compost. Each seedling was inoculated with c. 300 second stage juveniles (J2s) of either the virulent or the avirulent population and grown at 26-32°C for 40 days. In July 1997, the plants were transplanted into the microplots and grown for a further 7 weeks after which their tops were cut and roots left in the soil.

Before planting, soil samples were collected with a 2.5 cm corer at a 20 cm depth, air dried, hydrated and inoculated with *M. javanica* juveniles. After a 2 day incubation period at 25°C, the juveniles were extracted over modified Baerman trays and examined under an inverted microscope for the presence of attached spores of *Pasteuria penetrans*; no spores were observed on the juveniles re-extracted from the soil.

First rotational crop (Autumn 1997). Replicated plots were planted with seedlings at the two leaf stage of the susceptible tomato

cv. Early Pak, resistant tomato cv. Scala, and pepper cv. Indalo (without genes conferring resistance to RKN) at 1 September. Each seedling was inoculated with two egg masses of the appropriate population of *M. javanica* to ensure a high juvenile density around roots. Five treatments were randomized in four blocks:

A) For plots with virulent *M. javanica*:
   1) Susceptible tomato (Early Pak) + fenamiphos.
   2) Resistant tomato (Scala).
   3) Pepper (Indalo).
B) For plots with avirulent *M. javanica*:
   4) Susceptible tomato (Early Pak) + fenamiphos.
   5) Resistant tomato (Scala).

An additional sixth treatment, randomised in two blocks only was a plot inoculated with the avirulent population which grew untreated cv. Early Pak in both cropping cycles.

The fenamiphos (Nemacur 40EC, 40% a.i) was applied as a soil drench with a watering can 4 days after transplanting at 0.6 ml of the product diluted in 100ml water per plant. The soil was lightly irrigated before and after nematicide application. A small proportion of tomato seedlings (5%) rapidly succumbed with damping off symptoms and were immediately replaced. A mixture of propamocarb hydrochloride and benomyl was then applied as a drench twice (at 2 week intervals) to prevent further spread of the disease.

Fruits were harvested and weighed as they ripened and the first cropping cycle was terminated after 26 weeks growth. The plants were uprooted, roots were carefully washed free of soil, blotted dry in tissue paper, and root galling scored using the 0-10 index (0 = no knot on roots, 10 = all roots severely knotted with no root system and plant usually dead) of Bridge and Page (1980). The roots were then chopped into small pieces and the galled tissue separated and the galled and ungalled parts weighed. A representative sub-sample of 50 g of galled parts was placed in water for 2 days until the tissue softened, after which it was macerated in a kitchen blender and sieved through 150 μm and 38 μm sieves. After thorough washing, the eggs in the lower sieve were collected and their numbers estimated under a stereoscopic microscope (×25). Taking into account the proportion of galls per total root weight, the number of eggs per root g was estimated.

Second rotational crop (Spring 1998). Susceptible tomato cv. Early Pak was planted in all plots on 18 March. Fenamiphos was applied at the dosage of 1 ml per plant 2 and 21 days after planting to all plots except those which previously grew pepper and resistant tomato infested with the avirulent population. The reason of increasing the dosage and making a double application was that the nematode densities were expected to be higher than in previous crop; in these cases the high dosage of the product and double application is recommended for commercial vegetable crops in Crete. Plants were harvested after 20 weeks and the same variables recorded as with the first crop. Ten soil samples were collected from each microplot with a 2.5 cm corer at a 20 cm depth, mixed, and a 250 ml sample was processed for juvenile extraction on modified Baerman trays. Juveniles collected in water after 4 days were concentrated on a 20 μm sieve and counted under a stereoscopic microscope.

The two plots of the sixth treatment infested with the avirulent *M. javanica* which had been planted with the susceptible tomato in autumn were planted again in the spring crop and did not receive any nematicide application. These served as check plots to estimate whether nematode levels became suppressive to the spring crop, but results were not included in our statistical analysis.

Growing conditions. To assess the likely rate of development of the *M. javanica* populations, soil temperature was recorded at a 10 cm depth under the canopy at 8:00 am and 2:30 pm daily starting in July 1997. The average monthly mean soil temperatures are given in Figure 1. Using a base temperature (Tb) for development for *M. javanica* of c. 13°C and a minimum thermal time requirement (K) for one generation of 343°C days (Trudgill, 1995; Tzortzakakis and Trudgill, 1996), it was calculated that three generations were possible on the first crop (1 Sept 1997-11 March 1998), and five generations on the second (18 March-10 Aug 1998). Plants were watered and fertilized as required according to local practices. Fungicides to control powdery mildew, late blight, target spot and grey mold, and insecticides against caterpillars were applied as required. Color traps effectively controlled white flies and introduction of other insects was prevented by covering the side openings of the greenhouse with a plastic net.

RESULTS

In the first crop, untreated resistant tomato Scala with both populations of *M. javanica* yielded more than fenamiphos-treated susceptible cv. Early Pak, even though the Scala was heavily infected by the virulent *M. javanica* (Table 1). However Scala has a higher yield potential than Early pak in the absence of nematodes. Soil temperatures were too low to break resistance of tomato cv. Scala in the first crop. Untreated pepper and resistant tomato cv Scala acted as non-hosts for the virulent or avirulent *M. javanica*, respectively as no galls or egg masses were found (Table 1).

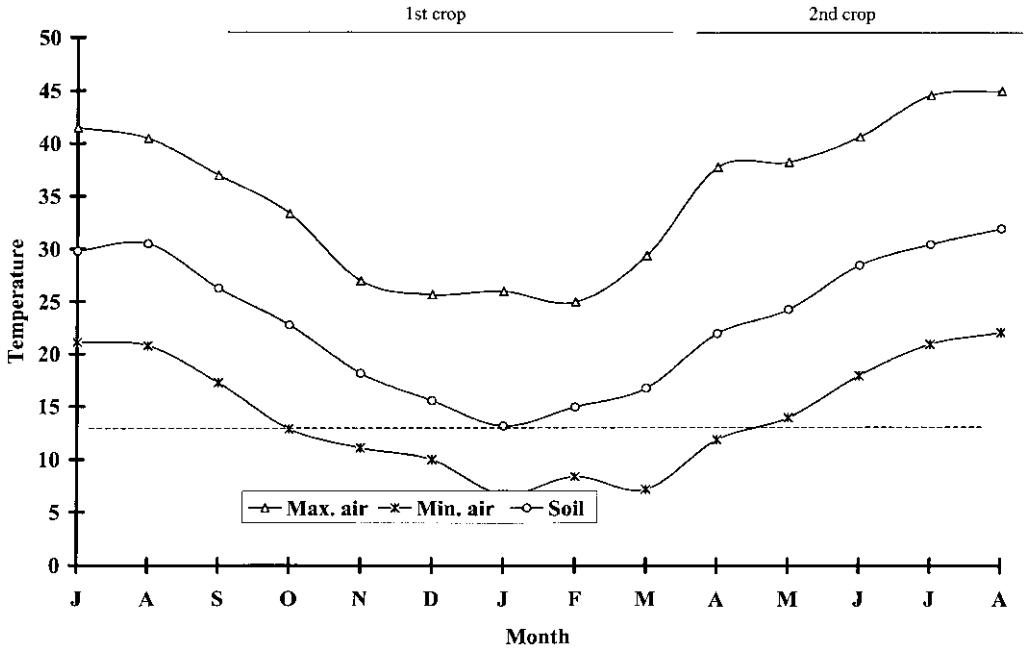

Fig. 1. Average minimum and maximum air temperature and average soil temperature at 10 cm depth recorded at 8:00 am and 2:30 pm under the plant canopy from July 1997 until August 1998. Broken line indicates base temperature for development of *M. javanica* (13°C).

In the second crop, unfavorable high temperatures in June-August 1998 (Fig. 1) damaged the tomato flowers and yield was lower than normal (Table 2). Therefore, the residual effects of the first crop treatments on fruit production of the second crop could not be accurately determined due to insufficient yield data. Untreated Early Pak in check plots infested with avirulent *M. javanica* were severely stunted, and chlorotic and produced the lowest yield. Their roots were also the most severely galled (index = 8.1) and at harvest had begun to decay. In contrast, the roots of plants from the remaining treatments were comparatively healthier, and total root weight did not differ between them (data not shown). Susceptible tomato plants following either non-host (pepper) for virulent or resistant tomato for avirulent responded similarly to these following susceptible tomato and treated with fenamiphos in both crops; their roots contained similar numbers of eggs, gall ratings did not differ, nor did juvenile population density in soil (Table 2). However, none of the treatments were highly effective in reducing egg production and residual numbers of juveniles in soil. Yield, eggs/g root, and gall index did not differ on susceptible tomato inoculated with virulent vs. avirulent *M. javanica* on both crop cycles while at the end of the second crop the juvenile density in soil was significantly higher for the virulent population.

DISCUSSION

In this study, results obtained from previous pot tests (Tzortzakakis *et al.*, 1999a) were further investigated for their practical application in a commercial-style greenhouse experiment comparing a rotational and a chemical strategy for managing *M. javanica* that is virulent or avirulent on resistant tomato. A previous experiment in Crete (Tzortzakakis and Gowen, 1994) indicated that growing a resistant tomato from March to August significantly reduced RKN populations and associated damage to a subsequent crop of cucumber. Resistant tomato grown as a spring crop (February to July) in Spain proved a successful management strategy for reducing population densities of RKN before planting a susceptible crop (Ornat *et al.*, 1997). Similarly, in Tanzania, non-host rotational crops planted in summer-autumn reduced RKN population densities in subsequent tomato or tobacco crops (Madulu *et al.*, 1994). However, comparisons with chemical treatments were not included in these experiments.

Our results confirm that growing a resistant tomato for one cropping cycle in a site infested with avirulent *M. javanica* and followed by a susceptible tomato without nematicide application resulted in final nematode population densities similar to those produced by fenamiphos applications to a sequence of two susceptible crops. In the case of the virulent *M. javanica,* the resistant tomato became infected but pepper acted as a non-host and population densities on a following susceptible tomato were similar to these on a sequence of two susceptible crops treated with fenamiphos. Therefore, the effect of rotational management for a crop cycle was equal to that of nematicide application on susceptible crops. However the system of rotation with a resistant/non host was not sufficiently effective to protect the subsequent crop as population densities were at high levels. This is in accordance with the results of a previous experiment at the same site with an avirulent *M. javanica* population and oxamyl treatments (Tzortzakakis *et al.*, 1999b). As observed by Ehwaeti *et al.* (1998), a small residual population is left after the end of a crop which can rapidly increase to damage a successive crop.

Table 1. Crop yield and population densities of *Meloidogyne javanica* in roots of resistant and susceptible tomato and pepper at the end of the first crop (1 September 1997-11 March 1998).

M. javanica	Treatment	Kg/plant	No of fruits/plant	Gall index[z]	Eggs/g of root
Virulent	Susceptible tomato + fenamiphos[x]	4.85	23	2.4	198
	Resistant tomato	6.03	23	5.9	889
	Pepper[y]	1.52	8	0	0
	SED	0.73	2.09	0.43	91
	P value	>0.05	>0.05	<0.01	<0.01
Avirulent	Susceptible tomato + fenamiphos [x]	4.3	22	3.8	378
	Resistant tomato	5.3	21	0	0
	Check plots [y]	3	17	6.7	1095
	SED	0.23	1.10	—	—
	P value	<0.05	= 0.05	—	—

Data are means of four microplots per treatment with eight plants in each.
[x]One application 4 days after planting at the dosage of 0.6 ml per plant.
[y]Not included in analysis.
[z]Scale 0-10.

Table 2. Yield and population densities of *Meloidogyne javanica* in roots of resistant and susceptible tomato at the end of the second crop (18 March-10 August 1998).

M. javanica	Treatment in previous crop	Treatment in current crop	Kg/plant	No of fruits/plant	Gall index[z]	Eggs/g of root (X1000)	J2s/g of soil
Virulent	Susceptible tomato + fenamiphos	Susceptible tomato + fenamiphos[x]	1.58	10	6.6	4.351	12
	Resistant tomato	Susceptible tomato + fenamiphos[x]	1.44	9	6.6	5.034	7
	Pepper	Susceptible tomato	1.49	9	6.1	3.289	6
		SED	0.22	1.22	0.42	0.96	2.07
		P value	>0.05	>0.05	>0.05	>0.05	>0.05
Avirulent	Susceptible tomato + fenamiphos	Susceptible tomato + fenamiphos[x]	1.20	7	6	4.904	3
	Resistant tomato	Susceptible tomato	1.56	10	6.1	3.646	4
	Check plots	Check plots[y]	0.59	4	8.1	4.610	2
		SED	0.47	1.88	0.27	0.96	1.41
		P value	>0.05	>0.05	>0.05	>0.05	>0.05

Data are means of four microplots per treatment with six plants in each.
[x]Two applications, 2 and 21 days after planting at the dosage of 1 ml per plant.
[y]Not included in analysis.
[z]Scale 0-10.

Consequently, more than one crop cycle with a resistant/non host crop may be required to fully control RKN, although this may be unacceptable to farmers in Crete. Considering the need for a high financial return per unit production, a resistant tomato or pepper for more than one cropping cycle may not be acceptable. Repeated use of resistant cultivars has the added risk of accelerating selection of virulent RKN. Although selection of *M. incognita* with increased virulence against the *Mi* resistant gene using a single egg mass line/clone has been reported (Castagnone-Sereno *et al.*, 1994), not all populations have the capacity to adapt to resistant tomatoes (Trudgill *et al.*, 1996). Selection over three generations in a pot experiment with single egg mass lines of *M. javanica* populations and of the avirulent population tested in this work failed to achieve increased virulence (Tzortzakakis *et al.*, 1999a).

To improve the proposed management strategy, it may be necessary to integrate resistant/non host plants for one cropping cycle with nematicide applications in subsequent susceptible crops. Furthermore, growing the resistant/non-host crop in the spring period may be more effective, as spring temperatures are likely to generate more nematode generations. A study comparing these rotational strategies with methyl bromide treatments is ongoing to determine the cost and efficacy of the system.

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