Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes over three consecutive growing seasons

Francisco Javier Sorribas[1], César Ornat[1], Soledad Verdejo-Lucas[2], Magda Galeano[2] and Jordi Valero[3]

[1]*Department of Food Engineering and Biotechnology, Technical University of Catalonia. Comte d'Urgell 187. 08036 Barcelona, Spain. Fax: +34 934137501. E-mail: francesc.xavier.sorribas@upc.es;* [2] *Department of Plant Protection, Institute for Food and Agricultural Research and Technology, Crta. de Cabrils s/n , 08348 Cabrils, Barcelona, Spain;* [3] *College of Agricultural Engineering of Barcelona and Department of Mathematics, Autonomous University of Barcelona. Comte d'Urgell 187. 08036 Barcelona, Spain.*

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Abstract

Experiments were conducted to determine the effectiveness and profitability of the *Mi*-resistance gene on tomato in suppressing populations of *Meloidogyne javanica* in a plastic-house with a natural infestation of the nematode. Experiments were also conducted to test for virulence and durability of the resistance. Monika (*Mi*-gene resistant) and Durinta (susceptible) tomato cultivars were cropped for three consecutive seasons in non-fumigated or in soil fumigated with methyl bromide at 75 g m^{-2} and at a cost of 2.44 euros m^{-2}. Nematode densities were determined at the beginning and end of each crop. Yield was assessed in eight plants per plot weekly for six weeks. The Pf/ Pi values were 0.28 and 21.6 after three crops of resistant or susceptible cultivars, respectively. Growth of resistant as opposed to susceptible tomato cultivars in non-fumigated soil increased profits by 30000 euros ha^{-1}. The resistant Monika in non-fumigated soil yielded similarly ($P > 0.05$) to the susceptible Durinta in methyl bromide fumigated soil but the resistant tomato provided a benefit of 8800 euros ha^{-1} over the susceptible one because of the cost of fumigation. Selection for virulence did not occur, although the nematode population subjected to the resistant cultivar for three consecutive seasons produced four times more eggs than the population on the susceptible one. Such a difference was also shown when the resistant cultivar was subjected to high continuous inoculum pressure for 14 weeks. The *Mi*-resistance gene can be an effective and economic alternative to methyl bromide in plastic-houses infested with root-knot nematodes, but should be used in an integrated management

context to preserve its durability and prevent the selection of virulent populations due to variability in isolate reproduction and environmental conditions.

Introduction

Increasing environmental concerns and governmental regulations have promoted the use of non-chemical over chemical pest control methods. Plant resistance is the single most important control measure that is able to suppress or retard invasion by a potential pathogen (Holliday, 1989). In Nematology, resistance is the ability of a plant to suppress development or reproduction of nematodes (Roberts, 2002). Tomatoes carrying the *Mi*-resistance gene suppress development or reproduction of root-knot nematodes and can be cultivated on most nematode-infested soil without significant yield losses (Ornat et al., 1997; Philis and Vakis, 1977; Rich and Olson, 1999). The *Mi*-gene was intogressed from *Lycopersicon peruvianum* to *L. esculentum* (Smith, 1944) and is present in all resistant commercial tomato cultivars. The *Mi*-resistance gene confers resistance, but not immunity, to *Meloidogyne incognita*, *M. javanica* and *M. arenaria* (Roberts and Thomason, 1989). Of these, *M. javanica* is the most common species of root-knot nematodes in the Mediterranean region (Philis, 1983; Sorribas and Verdejo-Lucas, 1994; Eddaoudi et al., 1997; Tzortzakakis and Gowen, 1996; Ornat and Verdejo-Lucas, 1999; Verdejo-Lucas et al., 2002).

As resistant plants can change their relative impact on nematodes in poly-specific communities or select intra-specific variants within the nematode population (Roberts, 2002), the effectiveness of resistance should be considered on a long-term basis in order to determine its durability. Virulence, defined as the ability of nematodes to reproduce on a

host plant that possesses one or more resistance genes, occurs naturally in *Meloidogyne* populations on tomato, apparently without previous exposure to or selection by the *Mi*-resistance gene (Netscher, 1976; Prot, 1984; Ornat et al., 2001). Virulent nematode populations may also be selected after repeated exposure to tomatoes with *Mi*-gene resistance (Castagnone-Sereno et al., 1993; Netscher, 1976; Roberts, 1995). The durability of resistance will depend upon the frequency of individual virulent nematodes that are present in a field population. Therefore, durability may be assessed by long-term cropping of resistant plants, or by submitting resistant plants to high continuous inoculum pressure (Esmenjaud, et al., 1992; 1996). Factors known to affect the expression of the *Mi*-resistance gene include temperature (Dropkin, 1969) and gene dosage, depending on whether the resistance gene is in a homozygous (MiMi) or heterozygous (Mimi) condition (Tzortzakakis et al., 1998).

This study was conducted to determine the effectiveness and economic benefit of the tomato *Mi*-resistance gene in suppressing populations of *M. javanica* for three consecutive growing seasons in a plastic-house with a natural infestation. Further experiments under controlled conditions were conducted to determine whether three consecutive crops of resistant tomato could select for virulence within the natural nematode plastic-house population. Finally, the Mi-resistance gene was subjected to high continuous inoculum pressure of *M. javanica* to determine if such pressure affects the expression of the resistant response.

Materials and methods

Plastic-house experiment

The study was conducted in an unheated plastic-house naturally infested by *M. javanica* at Cabrils, Barcelona, Spain. The soil was a sandy loam with 85.8% sand, 8.1% silt and 6.1% clay, pH 8.1, 0.9% organic matter (w:w), and 0.40 dS m^{-1} electric conductivity. Individual plots were 3.4 m x 1.5 m and consisted of two rows with six plants of tomato per row spaced 50 cm within the row and 55 cm between rows. Four treatments were investigated. They included: i) non-fumigated soil and the tomato cultivar with the *Mi*-resistance gene; ii) non-fumigated soil and the tomato cultivar without the *Mi*-resistance gene; iii) fumigated soil with methyl bromide (98% methyl bromide + 2% chloropicrin) and the tomato cultivar with the *Mi*-resistance gene, and iv) fumigated soil and the tomato cultivar without the *Mi*-resistance gene. Each treatment was replicated four times according a stratified randomised block design. The fumigant was applied trough a heated serpentine at 70ºC under polyethylene mulch at a rate of 75 g m^{-2} in October 1998. The polyethylene mulch was removed after four days and the soil was prepared for planting. Soil temperature at the time of fumigation at 15 cm deep was 21ºC. No further fumigation was done during the three-year study. One-month-old seedling of the resistant tomato cv. Monika and the susceptible cv. Durinta were transplanted in the same fumigated or non-fumigated plots in March and left to grow until July in 1999, 2000, and 2001. Lettuce, *Lactuca sativa* type Maravilla cv Arena, rotated with tomato from October to February, did not support nematode reproduction (Verdejo-Lucas et al., 2003).

Densities of M. javanica *and evaluation of nematode damage*

Composite soil samples were collected from each plot at the beginning and at the end of each tomato crop to estimate initial (Pi) and final (Pf) nematode population densities, respectively. Individual samples consisted of five soil cores taken to 30 cm deep with a sampling tube (2.5 cm diameter). Samples of approximately 735 cm^3 were mixed thoroughly and nematodes were extracted from a 500 cm^3 soil subsample using Baermann trays (Whitehead and Hemming, 1965). Second-stage juveniles (J2) that migrated to the water were collected one week later, concentrated on a 25-µm-pore sieve, counted and expressed as J2 per 250 cm^3 of soil. The assessment of the nematode damage was based on the root gall index of tomato plants following soil sampling for final J2 densities. Eight plants per plot were dug from the soil, examined, and immediately rated on a scale of 0 to 10, where 0 = a complete and healthy root system (no galls observed) and 10 = plants and roots dead (Zeck, 1971). Roots from each plot were then bulked, chopped in 0.5 cm-long segments and two 10-gram subsamples used to extract eggs by blender maceration in a 0.5% NaOCl solution for 10 minutes (Hussey and Barker, 1973). The number of eggs is expressed per gram of fresh root weight.

Crop yield and value

Tomatoes produced from eight plants in each plot were harvested once per week for six weeks and the cumulative yield was expressed as kilograms per m^2. Individual yield values in euros were calculated for each season according to the average price paid to growers at the central market of Barcelona. The price of 1 kg of tomatoes was 0.47, 0.70 and 0.71 euros in the first, second and third season, respectively. To determine the cost-efficacy of using resistant tomato cultivars *versus* fumigation, an economic estimation was made using

the gain threshold (GT) described by Pedigo (1989), which relates the cost of control to economic damage according to the formula GT = control cost (euros m^{-2}) / marketable crop value (euros kg^{-1}). The cost of controlling the nematode by fumigation with methyl bromide was 2.44 euros m^{-2} which included the product, application and labour. This cost was distributed proportionally for the three tomato crops (0.81 euros m^{-2} $crop^{-1}$) since fumigation maintained nematode densities at undetectable levels for the three consecutive seasons. The cost of controlling the nematode by plant resistance was nil as the price of the seedlings of the resistant and susceptible cultivars was the same. The remaining agronomical practices were similar for all treatments and were not included in the estimation.

Crop management

Soil preparation was carried out by hand cultivation of plots to prevent cross contamination among treatments. Plants received water through a drip irrigation system and were fertilized weekly with a solution consisting of NPK (15-5-30), iron chelate and micronutrients at rates of 31 and 0.9 kg per hectare, respectively. After the final tomato harvest in each year, plants were cut at ground level and removed from the plastic-house to prevent further increase in nematode population. Weeds were removed manually during and between crops. Soil temperatures were recorded daily at 30-minute intervals with temperature probes placed at a depth of 15-cm.

Testing for virulence

Two experiments were conducted to compare the reproduction index ((Pf on resistant cultivar / Pf on susceptible cultivar) x 100) of the *M. javanica* populations coming from plots cultivated with resistant (population RT3) or susceptible (population ST3) tomato cultivars for three consecutive seasons. In experiment 1, Bond (resistant) and Palosanto (susceptible) tomatoes were transplanted singly to one-litre pots containing steam-sterilised sand and inoculated with 3000 *M. javanica* eggs per plant. The egg inoculum was collected from tomato roots of the third resistant (population RT3) or susceptible (population ST3) tomato crop. Inocula of both populations were prepared by macerating the infected roots in a 0.5% NaOCl solution for 5 min (Hussey and Barker, 1973). Aliquots of the egg suspensions were pipetted into two holes made in the soil at 2 cm from the stem of the plants. Eight replicate pots were prepared for each population-cultivar combination and plants were arranged at random on a greenhouse bench. Soil temperatures in the pots were under 27^{o}C throughout the test. Plants were irrigated as needed and fertilized with a slow-release fertilizer (15N + 10P + 12K + 2MgO + microelements). The number of eggs from each root system was determined 8 weeks after nematode inoculation. Eggs were extracted from the roots in a 0.5% NaOCl solution for 10 min (Hussey and Barker, 1973). The reproduction index of each population of *M. javanica* was calculated.

In experiment 2, soil from plots that had been cultivated with the *Mi*-resistance gene (population RT3) or susceptible (population ST3) tomato from 1999 to 2001 was collected after one year of clean fallow (2003) and used for the experiment. The infested soils were mixed separately with steam-sterilized sand (1:1; v:v) and placed into one-litre pots. Population densities in the potting mix were determined using Baermann trays. Initial J2 densities of population RT3 and ST3 were 580 and 830 per 250 cm^3 soil, respectively. Monika (resistant) and Durinta (susceptible) tomatoes were transplanted singly into the

potting mix. Twelve pots were prepared for each nematode population-cultivar combination and plants were arranged at random on a greenhouse bench and maintained and fertilized as described previously. The number of eggs per plant was determined 10 weeks after transplanting, and the reproduction index of each population was calculated as before.

Durability of the resistance response

The experiment was conducted in 2003, in the same plots used for the study in the plastic-house after one year of clean fallow. Monika (resistant) and Durinta (susceptible) tomatoes were transplanted alternatively to plots containing the RT3 or ST3 population. In each plot, there were six plants of each cultivar placed 25 cm apart within the row in the following sequence R S R S R S R S R S R S. Each resistant tomato was transplanted in front of a susceptible one in the opposite row and viceversa (Figure 1). To determine the initial population densities, soil samples were collected as described for the plastic-house experiment. Six plants of each cultivar were alternately harvested per plot eight weeks after transplanting to assess the reproduction of the nematode after the first generation. The plants left behind remained 50 cm apart within the row and were allowed to grow for six additional weeks. During this period, resistant plants were subjected to continuous high inoculum pressure provided by their neighbour's susceptible cultivars (Esmenjaud et al. 1992; 1996) placed in front of them. At each harvest, root galling and the number of eggs per gram of root were determined following the procedures indicated previously.

Data analysis

Statistical analyses were performed using the general linear model of the SAS software version 8 (SAS institute Inc. Cary, NC). The number of J2 in soil and eggs per gram of root were transformed to [log (x+1)] and then along with data on gall ratings and yields of tomato were subjected to analysis of variance. When the overall F test was significant ($P \leq 0.05$), means were separated by the Least Significant Difference (LSD) method. Regression analysis was used to determine the relationship between Pi and Pf/Pi on the susceptible tomato cultivar in plots infested with *M. javanica* in the plastic house. In the tests for virulence and the experiment on durability of the resistance, data on nematode reproduction were transformed to [log (x+1)] before being subjected to analysis of variance, and least square means were separated by Tukey-Kramer adjustment for the multiple comparison method. Data on the reproduction index were transformed to arc sine and the means were separated by the Student t-test.

Results

Soil temperatures were below 28ºC from March to July in 1999, 2000, and 2001. Temperatures ranged from 14.8 to 28ºC (x = 21.6) in the first season, from 16.1 to 26.7 (x = 21.7) in the second one, and from 12.8 to 26.7ºC (x = 21.6) in the third one. In the soil fumigated with methyl bromide the population of *M. javanica* remained at undetectable levels throughout the three cropping seasons, regardless of the resistance or susceptibility of the tomato cultivar planted in each plot. In non-fumigated plots planted with resistant and susceptible tomato cultivars, the J2 populations were 660 and 480 per 250 cm^3 soil before the first planting, and 190 and 10350 J2 per 250 cm^3 soil, respectively, after three consecutive cropping cycles (Table 1). The Pf/Pi relationships were 0.29 and 21.6 after

three consecutive crops of resistant or susceptible tomato, respectively, in plots without fumigation.

On the resistant Monika, initial and final nematode densities, as well as gall rating decreased significantly ($P < 0.05$) after two or three consecutive crops (Table 1). Final densities at the end of the study were 71% lower than those at the beginning. The percentage of resistant plants with galls was 75%, 9% and 22% after one, two and three consecutive crops, respectively, with most plants showing gall ratings of 1 (very few small galls only detected upon close examination). Hence, significant differences in the gall rating were due to an increased number of plants with galls after one crop. Egg production after three crops of resistant Monika was 53 times higher than after two crops but the observed differences were not significant (Table 1).

On the susceptible Durinta, the Pf/Pi values were 62, 43 and 20 after one, two or three consecutive crops, respectively, and there was a highly significant negative correlation ($y = -0.76x + 3.59$; $R^2 = 0.7324$; $P = 0.0004$) between Pi and the Pf/Pi. All plants of Durinta exhibited high gall ratings.

The susceptible Durinta yielded more ($P < 0.05$) in fumigated than non-fumigated soil every season whereas the resistant Monika produced lower yield ($P < 0.05$) in non-fumigated than fumigated soil but only in the first crop. Across seasons, the average tomato yield in methyl bromide fumigated soil was similar in plots planted with the resistant or susceptible cultivar (Table 2). The resistant cultivar yielded 56% more ($P < 0.05$) than the susceptible one in non-fumigated soil (Table 2), which in turn provided a profit increase of 30000 euros ha^{-1}. The resistant Monika in non-fumigated soil yielded similarly ($P > 0.05$) to the susceptible Durinta in methyl bromide fumigated soil, but growing the resistant cultivar in non-fumigated soil provided a benefit of 8800 euros ha^{-1} over the susceptible one

in methyl bromide fumigated soil because of the cost of fumigation. In non-fumigated soil, the resistant Monika gave a benefit of 10600 euros ha^{-1} compared with methyl bromide fumigated soil. In methyl bromide fumigated soil, the susceptible Durinta provided a benefit of 21200 euros ha^{-1} compared to non-fumigated soil.

Testing for virulence.

The reproduction index of the *M. javanica* populations RT3 and ST3 was similar ($P > 0.05$) in both experiments (Table 3). The numbers of eggs produced by the *M. javanica* RT3 and ST3 were lower ($P < 0.05$) on the resistant compared to the susceptible cultivar (Table 3). In experiment 1, population RT3 produced 4.3 times more ($P < 0.05$) eggs than population ST3 on Palosanto (susceptible). In experiment 2, population RT3 produces 4 times more ($P < 0.05$) eggs than population ST3 on Monika (resistant).

Durability of the resistant response

Gall rating and egg production by RT3 and ST3 populations of *M. javanica* were lower ($P < 0.05$) on the resistant Monika compared to susceptible Durinta in the plastic-house 8 and 14 weeks after transplanting (Table 4). Differences between populations occurred after 14 weeks exposure to high nematode densities. The RT3 population showed higher ($P < 0.05$) gall rating and egg production than population ST3 on the resistant Monika. The percentage of Monika with galls induced by the RT3 and ST3 populations was 87% and 46%, respectively, whereas 100% of the plants of Durinta showed galled roots irrespective of the origin of the population.

Discussion

The results of this study demonstrate that the *Mi*-resistance gene in tomato can be a technical and economic alternative to methyl bromide fumigation in plastic-houses infested with damaging levels of the root-knot nematodes because it provided a high level of nematode suppression and increased the yield value. However, caution should be taken in the use of resistant tomato as a management tactic because of different responses of local root-knot nematode populations and the frequency of virulent populations (Roberts and Thomason, 1989). Our previous studies showed that resistant tomatoes have a high level of resistance to populations of *M. incognita* and *M. arenaria,* but are less resistant to *M. javanica* (Busquet et al., 1994; Sorribas and Verdejo-Lucas, 1999; Ornat et al., 2001). We examined over 30 root-knot nematode populations from Spain, and found only one population of *M. javanica* virulent to the *Mi*-resistance gene occurring naturally without previous exposure to the resistance gene (Ornat et al., 2001). In the present study, the percentage of plants with galls increased from 9% after two crops to 22% after three crops of resistant tomato, and there was an increase in the number of eggs per gram of root, which suggested that a virulent population might have developed within the field population. However, the greenhouse tests showed that the *M. javanica* RT3 population exposed to the *Mi*-resistance gene for three cropping cycles remained avirulent since low egg production and reproduction indexes were consistently obtained on resistant cultivars. Previous studies using excised tomato root cultures showed the avirulent status of this population of *M. javanica* (Ornat et al., 2001). Repeated cultivation of a resistant plant in the same site may lead to increased egg production by the nematode as the results from the

pot and plastic-house experiments pointed out. Thus, high inoculum pressure exerted on the resistant Monika when interplanted with the susceptible plant resulted in an increase in eggs and reproductive index in plots with a history of resistant tomatoes but not with susceptible ones. Increased egg production maybe the first step in the process of selecting a virulent population, although it appears that it can be reversed since the increase in egg numbers changed from 4.2 to 1 times after one year of clean fallow. In nature, the frequency of virulent nematode populations to the *Mi*-resistance gene is still relatively rare, and much less common than is virulence to specific resistance genes as in potato to *Globodera rostochiensis* and *G. pallida* or in soybean to *Heterodera glycines* (Starr et al., 2002). Whereas the potential for virulence in a *Meloidogyne* population should not be overlooked, neither it is certain or even probable that virulence will develop in any one field or plastic-house after a given period of use of a single resistance gene.

The ability of the nematode to reproduce on plants with the *Mi*-resistance gene can develop either gradually or suddenly (Williamson, 1998) and it seems that development of virulent populations in the field could occur, although only after long exposure to the *Mi*-resistance gene. In Morocco, for instance, populations of *M. javanica* from fields with a history of resistant tomato for 3 to 8 years broke resistance on genotypes in the homozygous (Mi Mi) and heterozygous state whereas populations exposed for one in every two or three years only broke resistance in the heterozygous resistant tomato (Eddaoudi et al., 1997). In north Florida (USA), three continuous plantings of resistant tomato Sanibel did not decrease the effectiveness of the *Mi*-resistance gene against *M. javanica* (Rich and Olson, 1999) but in central Florida, a resistance breaking biotype of *M. incognita* developed after five continuous plantings of Sanibel (Noling, 2000). Moreover, there is variability in the reproduction of different populations of *Meloidogyne* on resistant tomatoes and

differences in genotype response to the nematode (Roberts and Thomason, 1989; Sorribas and Verdejo-Lucas, 1994; Tzortzakakis and Gowen, 1996; Eddaoudi et al., 1997; Tzortzakakis et al., 1998). In addition, the durability of the resistance is affected by the frequency of virulent individuals within the nematode population. Some populations have shown genetic potential for breaking resistance in controlled selection experiments whereas other populations lack such potential (Jarquin-Barberena et al., 1991). Another important consideration when using resistant tomatoes is that soil temperatures higher than 28ºC may reduce the effectiveness of the resistance (Dropkin, 1969). Hence, planting during the hot season should be avoided, and moist soil conditions must be maintained during the first weeks after transplanting until plant canopy cover can help in maintaining soil temperature below the threshold that breaks resistance (Rich and Olson, 1999).

Although F1 tomato hybrids with the *Mi*-resistance gene have been available for more than 20 years, their use as a management tactic against root-knot nematodes is not widespread despite their highly suppressive effect on nematode reproduction. However, the effectiveness of the *Mi*-resistance gene has been shown when cucumbers were double-cropped with resistant tomatoes indoor (Ornat et al., 1997) and outdoors (Hanna et al., 1993) during the same season but whether there is a carry over effect in consecutive seasons is unknown. In this study, Pf values and individual yields were similar after two or three crops of resistant tomato, which suggests that protection of a successive susceptible crop maybe attained with at least two crops. Therefore, from a practical standpoint, it will be important to determine how frequently a resistant tomato must be cultivated in a rotation scheme to achieve a high level of nematode suppression. Alternatively, fumigants such as 1,3 dichloropropene or metam sodium accepted as alternatives to methyl bromide by the Methyl Bromide Technical Options Committee (2002) can be used in heavily infested soils

to substantially reduce nematode densities before planting resistant cultivars and diminish yield losses to the first crop, which in turn will likely delay any potential development of virulent populations. The resistant Monika produced a 13% less in the first crop in non-fumigated soil.

Methyl bromide gave an excellent and lasting control of *M. javanica* over three growing seasons probably due to thoroughly soil preparation, fumigant application, and sanitation practices during cultivation. Observation of these premises resulted in undetectable root-knot nematode levels in plastic-houses for at least two years (Verdejo-Lucas et al., 2003), although the nematode can be found in methyl bromide fumigated soils after cultivation of a single crop (Sorribas et al., 1994). Since agriculture is an economic activity, any control method can only be justified if the increased value of the crop is equal or greater than the cost of the control method. The cost-efficacy of plant resistance according to gain threshold (GT) values indicated that the use of tomatoes with the *Mi*-resistance gene was economically justified because the resistant Monika yielded 5.6, 4.4, and 4.7 Kg m^{-2} more than the susceptible Durinta in nematode-infested soil after one, two or three consecutive crops, respectively. In addition, the *Mi*-resistance gene in Monika provided yield stability with regard to the susceptible cultivar as does NemX, a cotton cultivar with resistance to *M. incognita* (Ogallo et al., 1999). The use of methyl bromide instead of resistant tomato was economically unjustified in this study, because the susceptible tomato in fumigated soil might yield 1.7, 1.2 and 1.1 Kg m^{-2} more than the resistant tomato in non-fumigated soil after one, two or three crops, respectively. However, the susceptible Durinta in methyl bromide fumigated soil compared to the resistant Monika in non-fumigated soil yielded 3.1, 0.1, and –2.4 Kg m^{-2} after one, two or three crops, respectively. Nevertheless, the relative

benefit of resistant tomatoes with respect to fumigation will vary depending on the seasonal fruit market value.

In conclusion, the *Mi*-resistance gene should be used in an integrated management context to preserve its durability and prevent the selection of virulent populations of *Meloidogyne* due to variability in isolate reproduction, resistant genotypes, and environmental conditions. Resistant tomatoes will be particularly useful for organic farming or integrated production since these systems do not allow the use of chemical control. In addition, the *Mi*-resistance gene also provides resistance against *Macrosiphum euphorbiae* (Rossi et al., 1998) and to *Bemisia tabaci* biotypes Q (Nombela et al., 2001) and B (Jiang et al., 2001).

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References

Busquets O, Sorribas FJ and Verdejo-Lucas S (1994) Potencial reproductor del nematodo *Meloidogyne* en cultivos hortícolas. Investigación Agraria: Producción y Protección Vegetal 9: 1-7.

Castagnone-Sereno P, Bongiovanni, M and Dalmasso A (1993) Stable virulence against tomato resistance *Mi* gene in the parthenogenetic root-knot nematode *Meloidogyne incognita.* Phytopathology 83: 803-805.

Dropkin VH (1969) The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. Phytopathology 59: 1632-1637.

Eddaoudi M, Ammati M and Rammah A (1997) Identification of resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. Fundamental and Applied Nematology 20: 285-289.

Esmenjaud D, La Massèse CS, Salesses G, Minot JC and Voisin R (1992) Method and criteria to evaluate resistance to *Meloidogyne arenaria* in *Prunus cerasifera* Her. Fundamental and Applied Nematology 15: 385-389.

Esmenjaud D, Minot JC and Voisin R (1996) Effects of durable inoculum pressure and high temperature on root galling, nematode numbers and survival of Myrobalan plum genotypes (*Prunus cerasifera* Her) highly resistant to *Meloidogyne* spp. Fundamental and Applied Nematology 19: 85-90.

Hanna HY, Colyer PD, Kirkpatrick TL, Romaine DJ and Vernon PR (1993) Improving yield of cucumbers in nematode infested soil by double-cropping with a resistant tomato cultivar, using transplants and nematicides. Proceedings of the Florida State Horticultural Society 106: 163-165.

Holliday P (1989) A Dictionary of Plant Pathology. Cambridge University Press, Cambridge, UK.

Hussey RS and Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease 57: 1025-1028.

Jarquin-Barberena H, Dalmasso A, de Guiran G and Cardin M (1991) Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. 1. Biological analysis of the phenomenon. Revue de Nématologie 14: 261-275.

Methyl Bromide Technical Options Committee (2002) 2002 Report of the methyl bromide technical options committee. United Nations Environment Program. Ozone Secretariat. Nairobi, Kenya.

Jiang YX, Nombela G and Muñíz M (2001) Analysis by DC-EPG of the resistance to *Bemisia tabaci* on an Mi-tomato line. Entomologia Experimentalis et Applicata 99: 259-302.

Netscher C (1976) Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. Cahier ORSTOM Série Biologie 11: 173-178.

Noling JW (2000) Effects of continuous culture of a resistant tomato cultivar on *Meloidogyne incognita* soil population density and pathogenicity. Journal of Nematology 32:452.

Nombela G, Beitia F and Muñíz M (2001) A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host responses with the B-biotype. Entomologia Experimentalis et Applicata 98: 339-344.

1 Ogallo JL, Goodell PB, Eckert JW and Roberts PA (1999) Management of root-knot
2 nematodes with resistant cotton cv. NemX. Crop Science 39: 418-421.
3 Ornat C and Verdejo-Lucas S (1999) Distribución y densidades de población de
4 *Meloidogyne* spp. en cultivos hortícolas de la comarca de El Maresme (Barcelona).
5 Investigación Agraria: Producción y Protección Vegetal 14: 191-201.
6 Ornat C, Verdejo-Lucas S and Sorribas FJ (1997) Effect of the previous crop on population
7 densities of *Meloidogyne javanica* and yield of cucumber. Nematropica 27: 85-90.
8 Ornat C, Verdejo-Lucas S and Sorribas FJ (2001) A population of *Meloidogyne javanica* in
9 Spain virulent to the *Mi* resistance gene in tomato. Plant Disease 85: 271-276.
10 Pedigo (1989) Entomology and pest management. Macmillan, New York, USA.
11 Philis J (1983) Occurrence of *Meloidogyne* spp. and races on the island of Cyprus.
12 Nematologia Mediterranea 11: 13-19.
13 Philis J and Vakis N (1977) Resistance of tomato varieties to the root-knot nematode
14 *Meloidogyne javanica* in Cyprus. Nematologia Mediterranea 5: 39-44.
15 Prot JC (1984) A naturally occurring resistance breaking biotype of *Meloidogyne arenaria*
16 on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol. Revue
17 de Nématologie 7: 23-28.
18 Rich JR and Olson SM (1999) Utility of *Mi* gene resistance in tomato to manage
19 *Meloidogyne javanica* in North Florida. Journal of Nematology 31: 715-718.
20 Roberts PA (1995) Conceptual and practical aspects of variability in root-knot nematodes
21 related to host plant resistance. Annual Review of Phytopathology 33: 199-221.
22 Roberts PA (2002) Concepts and consequences of resistance. In Starr JL, Cook R and
23 Bridge J (eds.) Plant resistance to parasitic nematodes (pp 23-41) CABI Publishing,
24 Wallingford, UK.

Roberts PA and Thomason IJ (1989) A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon.* Agricultural Zoology Reviews 3: 225-252.

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE and Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proceedings of the National Academy of Sciences, USA. 95: 9750-9754.

Smith PG (1944) Embryo culture of a tomato species hybrid. Proceedings of the American Society of Horticultural Science 44: 413-416.

Sorribas FJ and Verdejo-Lucas S (1994) Survey of *Meloidogyne* spp. in tomato production fields of Baix Llobregat county, Spain. Journal of Nematology 26: 731-736.

Sorribas FJ and Verdejo-Lucas S (1999) Capacidad parasitaria de *Meloidogyne* spp. en cultivares de tomate resistentes. Investigación Agraria: Producción y Protección Vegetal 14: 237-247.

Starr JL, Bridge J, and Cook R (2002) Resistance to plant-parasitic nematodes: History, current use and future potential. In Starr JL, Cook R and Bridge J (eds.) Plant resistance to parasitic nematodes (pp 1-22) CABI Publishing, Wallingford, UK.

Tzortzakakis EA and Gowen SR (1996) Occurrence of a resistance-breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. Fundamental and Applied Nematology 19: 283-288.

Tzortzakakis EA, Trudgill DL and Phillips MS (1998) Evidence for a dosage effect of the *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. Journal of Nematology 30: 76-80.

1 Verdejo-Lucas S, Ornat C, Sorribas FJ and Stchigel A (2002) Species of root-knot
2 nematodes and fungal egg parasites recovered from vegetables in Almería and Barcelona,
3 Spain. Journal of Nematology 34: 405-408.
4 Verdejo-Lucas S, Sorribas FJ, Ornat C and Galeano, M (2003) Evaluating *Pochonia*
5 *chlamydosporia* in a double-cropping system of lettuce and tomato in plastic houses
6 infested with *Meloidogyne javanica.* Plant Pathology 52: 521-528.
7 Whitehead AG and Hemming JR (1965) A comparison of some quantitative methods of
8 extracting small vermiform nematodes from soil. Annals of Applied Biology 55: 25-38.
9 Williamson VM (1988) Root-knot nematode resistance genes in tomato and their potential
10 for future use. Annual Review Phytopathology 36: 277-293.
11 Zeck WM (1971) A rating scheme for field evaluation of root-knot nematode infestations.
12 Pflanzenschtz-Nachrichten Bayer 24: 141-144.
13

Table 1. Initial (Pi) and final (Pf) population densities of *Meloidogyne javanica* in soil, number of eggs per gram of root, and gall rating on *Mi*-resistance gene and susceptible tomato cultivars for three consecutive growing seasons in a plastic-house with natural infestation of the nematode

Tomato cultivar	Year	Nematodes 250 cm^{-3} soil		Gall rating [a]	Eggs g^{-1} root
		Pi	Pf		
Monika (R)	1999	660 ± 413 a	860 ± 338 a	0.8 ± 0.3 a	ne[b]
	2000	10 ± 8 b	190 ± 235 b	0.1 ± 0.1 b	88 ± 95 a
	2001	28 ± 30 b	190 ± 236 b	0.3 ± 0.4 b	4700 ± 9300 a
Durinta (S)	1999	480 ± 240 a	29710 ± 4770 a	7.0 ± 0.2 a	ne
	2000	310 ± 186 b	13400 ± 5560 ab	6.5 ± 0.8 a	50300 ± 18000 a
	2001	530 ± 103 a	10356 ± 4475 b	7.0 ± 0.3 a	42700 ± 14400 a

(R) = resistant; (S) = susceptible. Values are mean ± standard deviation of four replicated plots. For each tomato cultivar, values within the same column followed by a different letter are significantly different according to the LSD test ($P \leq 0.05$).

[a] Based on a scale from 0 (none) to 10 (severe) (Zeck, 1971). 32 plants of each cultivar were examined.

[b] Data not evaluated.

Table 2. Tomato yield and yield value of *Mi*-resistance gene and susceptible tomato cultivars cultivated in methyl bromide fumigated and non-fumigated plots infested with *Meloidogyne javanica* for three consecutive growing seasons in a plastic-house

		Tomato yield (kg m^{-2})		Yield value [b] (euros m^{-2})	
Tomato	Year	Fumigated [a]	Non-fumigated	Fumigated	Non-fumigated
Monika (R)	1999	13.9 ± 1.0 a *	12.1 ± 0.9 a	6.53	5.69
	2000	13.4 ± 0.8 a	14.1 ± 1.7 a	9.40	9.85
	2001	13 ± 1.6 a	14.6 ± 2.2 a	9.22	10.37
Durinta (S)	1999	15.2 ± 1.0 a *	6.5 ± 1.2 b	7.14	3.05
	2000	14.2 ± 1.1 a *	9.7 ± 1.6 a	9.93	6.79
	2001	12.2 ± 1.3 b *	9.9 ± 1.2 a	8.62	7.05
Mean					
Resistant		13.4 ± 1.2 a	13.6 ± 1.9 a	8.38	8.63
Susceptible		13.9 ± 1.7 a *	8.7 ± 2.1 b	8.56	5.63

(R) = resistant; (S) = susceptible. Data are mean ± standard deviation of 32 plants. For each tomato cultivar, data within the same column followed by different letter are significantly different according to the LSD test ($P \leq 0.05$). Data within the same row with * are significantly different according to the Student t-test ($P \leq 0.05$).

[a] Methyl bromide at a rate of 75 g m^{-2} in October 1998.

[b] Average price of tomato was 0.47, 0.70 and 0.71 euros kg^{-1} in 1999, 2000 and 2001, respectively.

Table 3. Number of eggs per gram of root, and reproduction index of *Meloidogyne javanica* populations RT3 and ST3 on Mi-resistance gene and susceptible tomato cultivars in pot experiments to test for virulence

	Tomato cultivar	Eggs per g^{-1} root		Reproduction index [b]	
		RT3 [a]	ST3	RT3	ST3
Exp. 1	Bond (R)	1300 ± 1300 b	1100 ± 1100 b	6 ± 9	14 ± 14
	Palosanto (S)	58300 ± 42600 a *	13700 ± 8400 a		
Exp. 2	Monika (R)	800 ± 400 b *	200 ± 300 b	26 ± 13	11 ± 24
	Durinta (S)	2600 ± 600 a	2600 ± 1000 a		

(R) = resistant; (S) = susceptible. In experiment 1, values are mean ± standard deviation of eight plants assessed 8 weeks after inoculation of 3 eggs cm^{-3} soil. In experiment 2, values are mean ± standard deviation of 12 plants assessed 10 weeks after planting in soil infested with 2.3 and 3.3 juveniles cm^{-3} soil of populations RT3 or ST3, respectively.

For each experiment, values within the same column followed by a different letter, and values within the same row with *, are significantly different according to the Tukey-Kramer adjustment for a multiple comparison method ($P \leq 0.05$).

[a] Populations RT3 and ST3 came from plots cultivated with the *Mi*-resistance gene or susceptible tomato, respectively, for three consecutive seasons.

[b] Reproduction index: ((final population on resistant cultivar / final population on susceptible cultivar) x 100)

Table 4. Number of eggs per gram of root, gall rating, and reproduction index of *Meloidogyne javanica* populations RT3 and ST3 on Mi-resistance gene and susceptible tomato cultivars after eight and 14 weeks of growth in a plastic house to determine the durability of the resistant response

		Gall rating [a]		Eggs per g root		Reproduction index [c]	
Harvest (weeks)	Tomato cultivar	RT3 [b]	ST3	RT3	ST3	RT3	ST3
8	Monika (R)	1.8 ± 0.6 a	0.7 ± 0.4 a	4000 ± 340 a	600 ± 600 a	13 ± 14	6 ± 3
	Durinta (S)	4.3 ± 0.3 b	3.4 ± 0.7 b	42400 ± 46200 b	11800 ± 10200 b		
14	Monika (R)	2.2 ± 1.0 a *	0.5 ± 0.4 a	14300 ± 14800 a *	1100 ± 600 a	31 ± 33	4 ± 1
	Durinta (S)	6.1 ± 0.5 b	5.1 ± 0.8 b	49600 ± 7100 b	27600 ± 12700 b		
8 vs 14	Resistant	NS	NS	NS	NS	NS	NS
	Susceptible	S (P = 0.007)	S (P = 0.014)	NS	NS		

(R) = resistant; (S) = susceptible. Values are mean ± standard deviation of 24 plants. For each harvest, values within the same column followed by a different letter, and values within the same row with * are significantly different according to the Tukey-Kramer adjustment for a multiple comparison method ($P \leq 0.05$). NS: not significant. S: significant.

[a] Based on a scale from 0 (none) to 10 (severe galling) (Zeck, 1971).

[b] Populations RT3 and ST3 came from plots cultivated with *Mi*-resistance gene or susceptible tomato, respectively, for three consecutive seasons.

[c] Reproduction index: ((final population on resistant cultivar / final population on susceptible cultivar) x 100)

Figure 1. Planting arrangement of the resistant tomato cultivar Monika (R) and the susceptible cultivar Durinta (S) in plots containing the population RT3 or ST3 of *M. javanica* to determine the durability of the resistance response in plastic-house. Plants inside the rectangle were harvested eight weeks after transplanting and the remaining ones after 14 weeks.

1

RT3

R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R

ST3

R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R
R	S
S	R

2
3