[1] Institute of Agriculture, Goce Delcev bb, 92400 Strumica, Republic of Macedonia
[2] The Graduate School for Experimental Plant Sciences, Laboratory of Nematology, Wageningen University and Research Center, Binnenhaven 10, 6709 PD Wageningen, The Netherlands

MELOIDOGYNE SPP. IN MACEDONIA: DISTRIBUTION AND VIRULENCE FOR *Mi* RESISTANCE IN TOMATO

by
M. DAUTOVA[1,2] and F. J. GOMMERS[2]

Summary. The occurrence of *Meloidogyne* in several areas of Macedonia was surveyed. Seventy-three isolates from nine locations (fields and glasshouses) were identified and their behaviour on two susceptible and two *Mi* resistant tomato cultivars compared. *M. incognita* (47,9%) and *M. javanica* (35,6%) are the predominant species followed by *M. arenaria* (13,7%), and, sporadically, *M. hapla* (2,7%) was found. Mixtures of species were present at nearly all locations. Virulent isolates were found in *M. incognita* (11%) and *M. javanica* (46%) as well as in *M. arenaria* (50%). *M. hapla* isolates were compatible with all tomato genotypes tested. The impact of the occurrence of virulence for *Mi* carrying tomato genotypes is discussed.

The widely distributed polyphagous root knot nematodes (*Meloidogyne* spp.) are among the most damaging plant parasitic nematodes in many economically important crops. Damage by the different species, its populations (intraspecific variation) and subsequent control are important issues. A means of control that is environmentally satisfactory is the growing of resistant cultivars but this is generally hampered because resistance is often not universal i.e. not directed to all *Meloidogyne* species present in an area or by the occurrence of virulent field populations. Resistance against root knot nematodes has been described for various crops, among them tomato (*Lycopersicum esculentum*) (e.g. Fassuliotis, 1987; Robert, 1995).

Resistance in tomato to root knot nematodes was found about 50 years ago in an accession of *L. peruvianum* and introgressed in *L. esculentum* using embryo rescue (Smith, 1944). All currently available root-knot resistant tomato cultivars are derived from this source (Medina-Filho and Tanksley, 1983). A major gene (*Mi*) located on chromosome 6 (Gilbert and McGuire, 1956) controls resistance. The *Mi* gene confers resistance to *M. incognita*, *M. javanica* and *M. arenaria* but not to *M. hapla* (Hadisoeganda and Sasser, 1982; Ammati *et al.*, 1985).

There are two major limiting factors in the use of *Mi*: (i) at high soil temperature (28-30 °C) the resistance does not function (Dropkin, 1969) and (ii) the occurrence of resistance breaking root knot nematode populations. *M. incognita*, *M. arenaria* and *M. javanica* include virulent field populations and isolates selected on *Mi*-bearing cultivars after several generations (e.g. Riggs and Winstead, 1959; Roberts and Thomason, 1986; Ogallo and McClure, 1996; Molinari and Miacola, 1997). Variation in virulence in *M. incognita* populations towards the *Mi* gene has been studied by many authors (Jarquin-Barberena *et al.*, 1991; Castagnone-Sereno

et al., 1992, 1996; Anwar *et al.*, 1994; Mani and Zidgali, 1995). There are also several reports on virulent *M. arenaria* and *M. javanica* populations (Sikora *et al.*, 1973; Prot, 1984; Noe, 1992; Tzortzakakis and Gowen, 1996). Currently seven additional independent dominant *Meloidogyne* resistance genes, designated *Mi-2* to *Mi-8*, have been identified from *L. peruvianum* accessions and one from *L. chilense*. These genes are not yet available in commercial cultivars. They display resistance with different properties from *Mi* and some are resistant to *M. hapla* or confer resistance at 32 °C (see: Williamson, 1998). Because *L. esculentum* and *L. peruvianum* do not normally cross, the behaviour of these resistance genes in a tomato background is as yet hardly known.

In spite of the economic importance of the tomato crop in Macedonia, knowledge on the distribution of *Meloidogyne* species is scarce and even completely absent if intraspecific variations in virulence are considered. These omissions seriously impede implementation of proper management systems for *Meloidogyne*. As a first step towards such a system we report in this paper on the distribution of *Meloidogyne* populations (a)virulent on *Mi*-bearing tomato genotypes in Macedonian vegetable and tobacco fields.

Materials and methods

The susceptible tomato, *L. esculentum* Mill., cvs Moneymaker and Vivia - F 172, and the *Mi* resistant genotypes cv. Carmello GC 204 and cv. Manthos GC 785 were used. S&G Sandoz Seeds, The Netherlands, kindly provided us with the seeds, except for cv. Moneymaker. To exclude nullifying the *Mi* gene at high temperature (Dropkin, 1969) experiments were done at 20-25 °C in a glasshouse. Two week old seedlings were transplanted into 20 cm plastic pots filled with sterilised sand and allowed to establish for two weeks before inoculation with second stage juveniles. Pots were fertilised at regular intervals and watered with tap water as required. To avoid contamination pots were kept separate on saucers.

In 1996 and 1997 populations of *Meloidogyne* from nine areas (Table I), seven cropped with vegetables (mainly tomatoes) in glasshouses and two tobacco fields were collected from infested roots and rhizosphere soil. Only in the Gevgelija glasshouse was the *Mi* resistant tomato cv. Suzo grown. The seventy-three isolates (lines) were set up from single egg masses and propagated and maintained for about seven months on cv. Moneymaker. To obtain enough second stage juveniles (J_2) for the virulence tests eggs were harvested approximately 12 weeks after inoculation by dissolving egg masses in 0.5% NaOCl-solution (Hussey and Barker, 1973). J_2s were hatched in water and stored at 4 °C until required. The average egg mass contained 200 J_2s. Prior to inoculation nematodes were allowed to pass through a cotton filter and viable juveniles were collected after one day. In virulence tests 400 J_2s (~ 2 egg masses (P_i)) in suspension were pipetted with an automatic syringe onto the soil surface around the stem base of the test plant seedlings followed by light watering. Seven weeks later plants were harvested individually, their roots washed free from sand stained with Phloxine B solution (Daykin and Hussey, 1985) and the egg masses (P_f) and galls counted. Multiplication rates (P_f/P_i) were assessed. Host status was divided into three categories as follows: $P_f/P_i \geq 1.0$, suitable host (SH); $0.1 < P_f/P_i < 1.0$, poor host (PH) and $P_f/P_i \leq 0.1$, non-host (NH). Each nematode population was tested in four replicates on the susceptible and resistant genotypes. For each isolate, after square root transformation of the data (data not shown), pair-wise differences between the means were analysed for significance and Least Significant Difference (LSD) at $P = 0.05$ with ANOVA using Genstat (Payne *et al.*, 1987).

Species composition of the isolates was determined in two ways. (i) Using the morphological criteria of perineal pattern of adult females

TABLE I - *Origin, preceding crop and species designation of populations and isolates used.*

Location	Preceding crop	Number of populations	Number of isolates	Species identified	Code*
Bogdanci	tomato, cv. Priska	1	7	*M. incognita*	MiB
Hamzali	cucumber	3	12	*M. incognita*	MiSH
		1	2	*M. javanica*	MjSH
	tomato, cv. Balka	1	3	*M. arenaria*	MaSH
Bansko	cucumber	1	4	*M. incognita*	MiSB
Ilovica	tobacco	2	5	*M. incognita*	Mil
Prosenikovo	cucumber	2	7	*M. incognita*	MiP
		1	2	*M. javanica*	MjP
		1	1	*M. arenaria*	MaP
Kocani	cucumber	1	3	*M. javanica*	MjK
	tomato, cv. Balka	2	8	*M. javanica*	MjK
		1	1	*M. arenaria*	MaK
		1	1	*M. hapla*	MhK
Gevgelija	tomato, cv. Suzo	1	7	*M. javanica*	MjG
Kumanovo	tomato, cv. Vivia	1	4	*M. javanica*	MjKu
		1	1	*M. hapla*	MhKu
Stuka	tobacco	1	5	*M. arenaria*	MaS

* isolates are coded as indicated followed by numbers for field population and isolate.

and morphometrics of males and J_2s (Jepson, 1987). At least 15 perineal patterns and 25 males and juveniles of each isolate were examined. (ii) By amplifying the intergenic spacer region between the cytochrome oxidase II gene and the 16S rRNA gene in the mitochondrial genome of single juveniles (Powers and Haris, 1993). In this approach ten individual J_2s were handpicked, homogenised in a 15 µl drop of sterile water, frozen for future analysis or immediately processed in a PCR procedure in a final volume of 25 µl as described. Following DNA amplification the products were separated on agarose gel, stained with ethidium bromide and visualised on a UV box. *M. arenaria* is characterised by a unique 1.1 kb fragment. To allow discrimination among species with identically sized amplification products standard restriction digestions (Dra I or Hinf I) of 5 µl of the amplified products were conducted for 2-4 hours at 37 °C and evaluated on 1.5% agarose gels. Digestion with Dra I was carried out in case of the presence of a 0.52 kb amplification product. A four-banded pattern (0.44, 0.29, 0.23 and 0.08 kb fragments) separated *M. hapla* from *M. chitwoodi*, *M. marylandi*, *M. nataliei*, *M. naasi* and *M. fallax*. In case of the presence of a 1.7 kb fragment a Hinf I digestion distinguished *M. javanica* from *M. incognita* on basis of an undigested band and two-banded patterns (0.4 and 1.3 kb fragments) respectively (Williamson *et al.*, 1994). Results were obtained with about 90% of the individual J_2s tested.

Results

There were hardly any discrepancies between the time consuming morphological identifications and the rather straightforward and rapid identifications based on amplification of intergenic spacer region of the mtDNA

(Fig. 1). Contradictory results between the morphological and DNA-based identifications were obtained with three isolates (4.1%). The perineal patterns pointed at *M. javanica* and the DNA based identifications at *M. incognita*. The outcomes of the DNA analyses were followed.

Seventy-three isolate originating from 22 *Meloidogyne* populations were collected in 11 areas previously cropped with cucumber, tobacco or tomato. Two glasshouses turned out to be free from *Meloidogyne* infestations. Mostly mixtures of species were found and *M. javanica* (Treub) Chitw. was present in nearly all tomato crops. Thirty-five isolates were identified as *M. incognita* (Kofoid *et* White) Chitw., 26 as *M. javanica*, ten as *M. arenaria* (Neal) Chitw. and two as *M. hapla* Chitw.

Virulence characteristics of the isolates were determined on the susceptible cvs Moneymaker and Vivia and the *Mi* resistant cvs Carmello and Manthos (Table II, Fig. 2). All *M. incognita* isolates reproduced on both susceptible cultivars. It was noticed that often the numbers of egg masses on cv. Moneymaker were higher than the numbers of galls. On cv. Vivia the opposite was true. Four out of the 35 *M. incognita* isolates (11%) (two MiB, MiS and Mil) also produced egg masses on the resistant cultivars. One of the two MiB isolates, being the most virulent, reproduced equally well on cv. Moneymaker and both resistant cultivars.

As also shown in Table II the *M. javanica* isolates generally reproduced well on both susceptible cultivars although numbers of egg

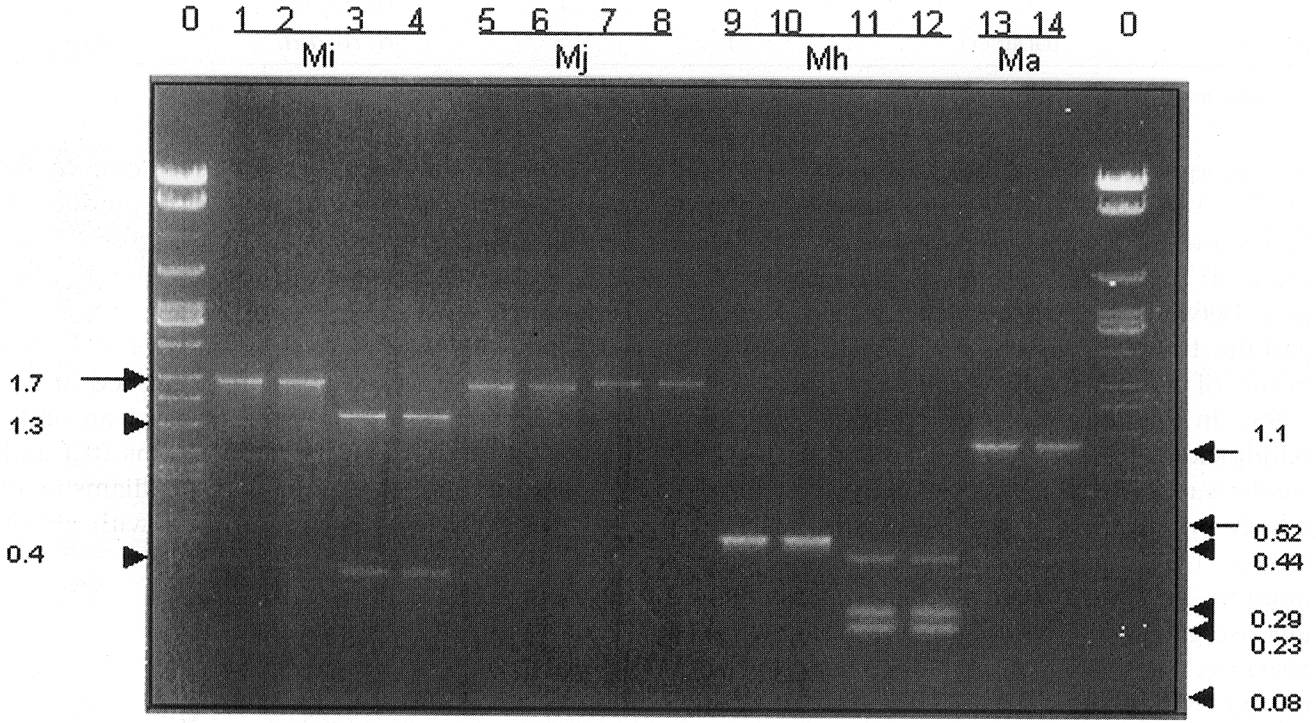

Fig. 1 - PCR amplification of mtDNA of individual J2s. Primers and conditions are as described by Powers and Harris (1993). The 1.7 kb product is characteristic for *Melidogyne incognita* and *M. javanica* (lanes 1, 2 and 5, 6 respectively). After a Hinf I digestion of the 1.7 kb fragment *M. incognita* (lane 3, 4) is identified by a two banded pattern and the *M. javanica* (lane 7, 8) fragments remains undigested. The 0.52 kb product (lanes 9, 10) is characteristic for a number of *Meloidogyne* species. After a Dra I digestion four unique fragments identify *M. hapla* (lanes 11, 12). *M. arenaria* gives a 1.1 kb product (lanes 13, 14). The first and last lanes are standard markers.

TABLE II - *Means of the square root numbers of egg masses of 73* Meloidogyne *isolates on roots of four* Lycopersicum *genotypes differing in presence or absence of the* Mi *gene (means are of four replicates; virulent populations are shaded).*

Isolates	Egg masses / Root system			
	Money-maker	Vivia	Carmello	Manthos
MiB 12.4	8,25 *a	4,47 **b	0,00 c	1,41 c
MiB 12.5	10,72 *a	6,40 **b	1,41 c	0,00 d
MiB 12.6	7,35 *a	11,83 *b	0,00 c	0,00 c
MiB 12.7	3,46 *a	9,64 **b	2,45 **a	2,65 a
MiB 12.8	8,83 *a	9,43 **a	0,00 c	0,00 c
MiB 12.9	9,49 *a	8,43 a	2,00 c	5,10 d
MiB 12.10	5,66 *a	7,28 **b	1,00 c	0,00 d
MiSH 17.4	6,93 *a	0,00	0,00 c	0,00 c
MiSH 17.5	7,87 *a	4,47 **b	0,00 *c	0,00 *c
MiSH 17.7	7,35 a	6,56 a	0,00 c	0,00 c
MiSH 17.9	10,15 a	12,04 *b	0,00 c	1,00 d
MiSH 17.10	16,09 *a	10,10 *b	1,00 c	0,00 d
MiSH 17.12	6,00 a	18,28 *b	0,00 c	1,41 d
MiSH 25.1	6,78 *a	8,77 b	0,00 c	0,00 c
MiSH 25.2	11,96 a	10,91 *a	1,00 c	0,00 d
MiSH 25.3	8,19 **a	13,27 **b	0,00 **c	1,41 *d
MiSH 25.8	13,49 *a	9,80 *b	2,00 c	0,00 d
MiSH 26.2	9,90 a	9,43 a	0,00 c	0,00 c
MiSH 26.3	13,67 a	10,30 **b	0,00 **c	1,00 d
MjSH 15.3	8,72 a	2,65 *b	0,00 c	1,00 d
MjSH 15.10	8,49 a	3,74 b	2,24 c	1,00 d
MaSH 25.4	15,52 a	14,63 *a	1,41 c	1,00 c
MaSH 25.5	7,07 a	7,35 a	1,41 c	1,41 c
MaSH 25.10	14,18 a	11,18 **b	1,41 c	2,00 c
MiSB 19.3	9,38 *a	8,06 a	1,00 c	1,00 c
MiSB 19.6	16,22 a	9,95 b	1,00 *c	0,00 d
MiSB 19.7	7,21 a	4,90 *b	1,00 c	0,00 *d
MiSB 19.9	9,59 a	8,37 *a	0,00 c	0,00 c
Mil 29.3	3,87 a	2,83 a	0,00 c	0,00 c
Mil 29.6	3,16 a	3,16 a	0,00 c	0,00 c
Mil 29.10	11,09 a	8,25 b	1,00 c	2,00 *d
Mil 30.4	7,21 a	7,35 a	0,00 c	0,00 c
Mil 30.7	6,93 a	7,68 a	1,41 c	0,00 d
MiP 32.5	7,62 a	5,10 *b	0,00 c	0,00 c
MiP 32.9	4,00 a	2,00 *b	0,00 c	0,00 c
MiP 32.11	4,58 a	2,45 b	0,00 c	0,00 c
MiP 33.1	6,71 a	5,83 a	0,00 c	0,00 c

Isolates	Egg masses / Root system			
	Money-maker	Vivia	Carmello	Manthos
MiP 33.3	9,33 a	8,25 a	0,00 c	0,00 c
MiP 33.6	5,74 a	4,00 *b	0,00 c	0,00 c
MiP 33.8	9,06 a	8,72 a	1,00 c	1,00 c
MjP 31.1	9,33 a	5,00 **b	0,00 c	1,00 d
MjP 31.2	10,39 *a	8,89 **b	0,00 *c	0,00 c
MaP 33.5	8,12 *a	7,07 **a	0,00 c	0,00 c
MjK 21.1	8,66 a	3,61 b	2,65 bc	2,00 c
MjK 21.4	3,61 *a	5,29 *b	1,00 *c	2,45 d
MjK 21.8	8,89 a	6,00 *b	2,45 c	1,41 d
MjK 2.2	8,25 a	7,00 b	0,00 c	0,00 c
MjK 2.4	9,59 a	5,83 b	0,00 c	0,00 c
MjK 2.6	7,35 a	4,80 b	0,00 c	1,00 d
MjK 2.7	9,49 a	3,61 **b	0,00 c	0,00 c
MjK 2.10	11,05 *a	6,86 *b	0,00 c	0,00 c
MjK 3.3	8,25 *a	8,60 a	1,00 c	3,46 d
MjK 3.12	7,87 a	4,58 b	0,00 c	1,00 d
MjK 3.13	14,35 a	10,10 b	1,00 c	0,00 d
MaK 22.1	11,58 a	12,17 a	2,83 *c	4,58 *d
MhK 2.8	8,66 a	6,71 a	1,73 c	3,61 a
MjG 4.3	9,22 a	6,48 b	1,41 c	3,16 d
MjG 4.4	3,16 a	3,46 a	1,00 c	0,00 d
MjG 4.5	9,27 a	12,53 a	2,24 c	8,89 a
MjG 4.6	11,40 a	5,20 *b	3,00 c	2,65 c
MjG 4.10	7,48 *a	2,83 **b	1,41 *c	0,00 d
MjG 4.11	9,00 a	4,69 b	1,73 c	1,41 c
MjG 4.13	8,49 a	4,58 b	0,00 c	1,41 d
MjKu 7.3	9,59 *a	4,90 *b	1,41 c	2,00 c
MjKu 7.4	8,37 a	7,21 a	0,00 c	0,00 c
MjKu 7.7	10,20 *a	7,35 **b	1,00 c	2,24 d
MjKu 7.9	8,72 *a	5,57 *b	0,00 *c	1,73 d
MhKu 7.6	9,90 *a	5,29 **b	2,00 *c	2,65 **b
MaS 27.2	8,49 a	8,00 a	0,00 *c	0,00 *c
MaS 27.5	13,67 a	12,49 a	0,00 c	1,41 d
MaS 27.6	11,58 a	11,09 a	2,24 c	3,87 d
MaS 27.9	8,83 a	7,94 *a	2,65 *c	2,00 c
MaS 27.11	7,28 a	6,16 a	0,00 c	2,00 d

* numbers of galls less that numbers of egg masses. ** numbers of galls more that numbers of egg masses. Isolate-genotype combinations sharing a common letter do not differ significantly at P = 0.05.

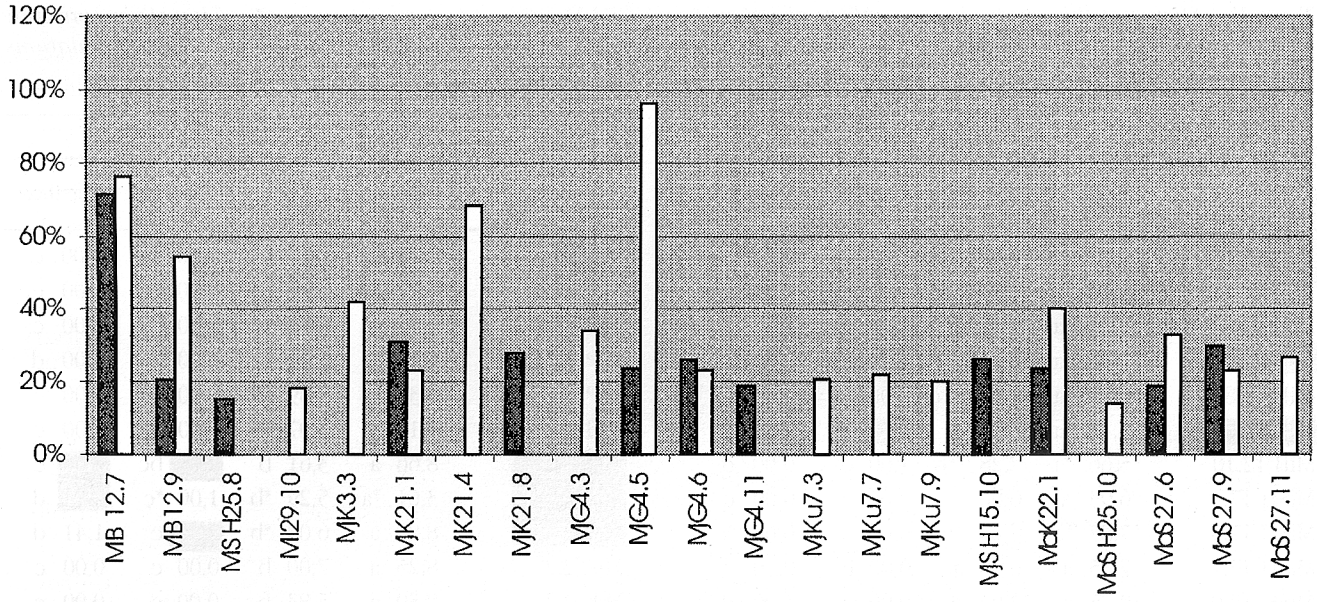

Fig. 2 - Multiplication rates of four *M. incognita*, twelve *M. javanica* and five *M. arenaria* isolates virulent to two *Mi*-bearing *Lycopersicum* genotypes relative to the susceptible cv. Moneymaker. ■ cv. Carmello, □ cv. Manthos.

masses on cv. Vivia tended to be less than on cv. Moneymaker. Twelve out of the 26 isolates (46%) were virulent at least on one of the *Mi*-bearing cultivars. Some of the isolates (MjG, MjK and MjSH) produced slightly more egg masses on cv. Carmello than on cv. Manthos, while for some other isolates from the same locations (MjG, MjK and MjKu) the opposite was true.

The highest number of virulent isolates (50%) was found with *M. arenaria*. All isolates reproduced on the two susceptible cultivars. Isolate MaK and two MaS isolates were also fully compatible with the both resistant cultivars and MaSH and one of the MaS lines reproduced better on cv. Manthos than on cv. Carmello.

Both *M. hapla* isolates were fully compatible with all four tomato genotypes.

Discussion

To distinguish *M. hapla* from a set of other *Meloidogyne* spp. a four-banded instead of a two-banded pattern (Powers and Harris, 1993) was found upon Dra I digestion of the 0.52 kb amplification products and electrophoresis on an agarose gel. The 0.29 kb and 0.23 kb bands were as published. Apparently a second 0.52 kb product was amplified which was digested into a 0.44 kb and a weak 0.08 kb band. Similarly Williamson *et al.* (1994) found deviating results after a Hinf I digestion of the PCR amplification products and electrophoresis on agarose of *M. incognita* and *M. javanica* with a two-banded (1.3 an 0.4 kb) and a single undigested 1.7 kb band respectively. Powers and Harris (1993) published the occurrence of three (1.0, 0.4 and 0.3 kb) and two (1.0 and 0.7 kb) bands (Fig. 1). The reason for such discrepancies is not known but is probably due to differences in the origin of the PCR enzymes used.

In this survey on the occurrence of *Meloidogyne* in Macedonia 73 isolates originating from nine locations in vegetable and tobacco growing areas were identified and their behaviour on two susceptible and two *Mi* resistant tomato cultivars compared. *M. incognita* (47.9%) and

M. javanica (35.6%) predominated followed by *M. arenaria* (13.7%), and, sporadically, *M. hapla* (2.7%) was found. Often species mixtures were found. These figures are consistent with other surveys on *Meloidogyne* in the Mediterranean area (Sorribas and Verdejo-Lucas, 1994; Eddaoudi *et al.*, 1997; Tzortzakakis, 1997).

All isolates reproduced well on both susceptible cultivars. Cv. Moneymaker was in general a better host than cv. Vivia. The virulent *M. incognita*, *M. javanica* and *M. arenaria* isolates reproduced, although to different degrees, on both resistant cultivars (Fig. 2). Most of the isolates reproduced better on cv. Manthos than on cv. Carmello. As expected the two *M. hapla* isolates were fully compatible with the four tomato genotypes (Medina-Filho and Stevens, 1980; Hadisoeganda and Sasser, 1982; Santo, 1982; Ammati *et al.*, 1985; Brown *et al.*, 1997).

Many authors reported on variability in virulence in field populations of *M. incognita*, *M. javanica* and *M. arenaria* from different parts of the world (Roberts *et al.*, 1990; Castagnone-Sereno *et al.*, 1993; Guskova and Al-Bakkur, 1996; Anwar *et al.*, 1996; Kaloshian *et al.*, 1996). Virulent populations may occur naturally or arise from avirulent populations after repeated selection pressure by Mi-carrying cultivars (e.g. Castagnone Sereno *et al.*, 1993). All tomato glasshouses surveyed were, as far as known, until now cropped with susceptible cultivars with the exception of Gevgelija where the *Mi* resistant cv. Suzo was grown. Tzortzakakis and Gowen (1996) found fully virulent *M. javanica* lines from populations never exposed to the *Mi* gene. Therefore it remains unclear if the fairly high incidence of virulent *M. javanica* isolates from the Gevgelija area resulted from selection or that they are already present naturally.

In Macedonia, glasshouses for tomato cropping are normally treated with systemic nematicides or disinfected by steam sterilisation in December. Tomatoes are transplanted in January. After harvest early in the summer glasshouses are left fallow and often in the autumn a second different crop will be grown. The results presented here show that growing *Mi*-resistant tomato cultivars in a cropping system as outlined will be beneficial in case virulent populations are present. The set backs are mainly found in damage to a second crop in case high *Meloidogyne* densities have built up in the spring. This problem is even more pronounced in tomatoes grown as a field crop because of the absence of soil disinfection and the relatively high numbers of (a)virulent *Meloidogyne* populations found.

Acknowledgements. This survey was supported by a combined grant (contract number 08-2720/4-95) of the Ministry of Science, Skopje, Macedonia and the Institute of Agriculture, Strumica, Macedonia, and a sandwich fellowship (contract number P1178) of the Wageningen University, Wageningen, The Netherlands. Mrs Prof. Dr. B. Choleva, Faculty of Biology, University of Sofia, Bulgaria, assisted in the morphological identifications.

Literature cited

AMMATI M., THOMASON I. J. and ROBERTS P. A., 1985. Screening *Lycopersicon spp.* for few new genes importing resistance to root-knot nematodes (*Meloidogyne spp.*). Plant *Disease*, *69*: 112-115.

ANWAR S. A., ABBASI W. S. and FATIMA G., 1996. Penetration, development and reproduction of *Meloidogyne incognita* on seven tomato cultivars. *Afro-Asian J. Nematol.*, *6*: 74-77.

ANWAR S. A., TRUDGILL D. L. and PHILIPS M. S., 1994. The contribution of variation in invasion and development rates of *Meloidogyne incognita* to host status differences. *Nematologica*, *40*: 579-586.

BROWN C. R., MOJTAHEDI H., SANTO G. S. and WILLIAMSON V. M., 1997. Effect of the *Mi* gene in tomato on reproductive factors of *Meloidogyne chitwoodi* and *Meloidogyne hapla*. *J. Nematol.*, *29*: 416-419.

CASTAGNONE-SERENO P., BONGIOVANNI M. and DALMASSO A., 1992. Differential expression of root-knot nematode resistance genes in tomato and pepper: evidence with *Meloidogyne incognita* virulent and avirulent near-isogenic lineages. *Ann. appl. Biol.*, *120*: 487-492.

CASTAGNONE-SERENO P., BONGIOVANNI M. and DALMASSO A., 1993. Stable virulence against the tomato resistance *Mi* gene in the parthenogenetic root-knot nematode *Meloidogyne incognita*. *Phytopathology*, *83*: 803-805.

CASTAGNONE-SERENO P., BONGIOVANNI M., PALLOIX A. and DALMASSO A., 1996. Selection for *Meloidogyne incognita*

virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. *European J. Plant Pathol., 102*: 585-590.

DAYKIN M. E. and HUSSEY R. S., 1985. Staining and histopathological techniques in nematology. pp. 39-48. In *An advanced treatise on Meloidogyne* (K. R. Barker, C. C. Carter and J. N. Sasser eds), Raleigh, North Carolina State University graphics, USA.

DROPKIN V. H., 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 the resistance breaking populations of *Meloidogyne* on tomatoes in Marocco and their effect on new sources of resistance. *Fundam appl. Nematol., 20*: 285-289.

FASSULIOTIS G., 1987. Genetic Basis of Plant Resistance to Nematodes. pp. 364-371. In *Vistas on Nematology* (J. A. Veech and D. W. Dickson eds). Society of Nematologists, Inc. Hyattsville, Maryland.

GILBERT J. C. and MCGUIRE D. C., 1956. Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. *Proc. Amer. Soc. Horticult. Science, 68*: 437-442.

GUSKOVA L. A. and AL-BAKKUR H., 1996. An assessment of resistance in glasshouse-grown tomato cultivars to *Meloidogyne incognita*, race 2, from Latvia and *M. javanica* from Russia. *Russian J. Nematol., 4*: 181-183.

HADISOEGANDA W. W. and SASSER J. N., 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. *Plant Disease, 66*: 145-150.

HUSSEY R. S. and BARKER K. R., 1973. A comparison of methods of collecting inocula of *Meloidogyne spp.*, including a new technique. *Plant Dis. Rept., 57*: 1025-1028.

JARQUIN-BARBERENA H., DALMASSO A., DE GUIRAN G. and CARDIN M-C., 1991. Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. Biological analysis of the phenomenon. *Rev. Nématol., 14*: 261-275.

JEPSON S. B., 1987. *Identification of root-knot nematodes* (Meloidogyne *species*). Wallingford, Cab International, UK, 252 pp.

KALOSHIAN I., WILLIAMSON V. M., MIYAO G., LAWN D. A. and WESTERDAHL B. B., 1996. "Resistance-breaking" nematodes in California tomatoes. *California Agriculture, 50*: 18-19.

MANI A. and ZIDGALI A., 1995. Screening of tomato cultivars for resistance against *Meloidogyne incognita*. *Nematol. medit., 23*: 269.

MEDINA-FILHO H. P. and STEVENS M. A., 1980. Tomato breeding for nematode resistance: survey of resistant varieties for horticultural characteristics and genotype of acid phosphatase. *Acta Horticulturae, 100*: 383-391.

MEDINA-FILHO H. P. and TANKSLEY S. D., 1983. Breeding for nematode resistance. pp. 904-923. In *Handbook of Plant Cell Culture*. (D. A. Evans, W. R. Sharp, P. V. Ammirato and Y. Yamada eds), New York, USA.

MOLINARI S. and MIACOLA C., 1997. Interactions between resistant tomato cvs and *Meloidogyne spp.* in vitro. *Nematol. medit., 25*: 63-71.

NOE J. P., 1992. Variability among populations of *Meloidogyne arenaria*. *J. Nematol., 24*: 404-414.

OGALLO J. L. and MCCLURE M. A., 1996. Systemic acquired resistance and susceptibility to root-knot nematodes in tomato. *Phytopathology, 86*: 498-501.

PAYNE R. W., LANE P. W., AINSLEY A. E., BICKNELL K. E., DIGBY P. G. N., LEECH P. K., SIMPSON H. R., TODD A. D., VERRIER P. J., WHITE R. P., GOWER J. C., TUNNICLIFFE WILSON G. and PATERSON L. J., 1987. *Genstad 5 reference manual.*, Clarendon press. Oxford, UK. 749 pp.

POWERS T. O. and HARRIS T. S., 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *J. Nematol., 25*: 1-6.

PROT J-C., 1984. A naturally occurring resistance breaking biotype of *Meloidogyne arenaria* on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol. *Rev. Nématol.*, 7: 23-28.

RIGGS R. D. and WINSTEAD N. N., 1959. Studies on resistance in tomato to root-knot nematodes and on occurrence of pathogenic biotypes. *Phytopathology, 49*: 716-724.

ROBERTS P. A., 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Ann. Rev. Phytopathol., 33*: 199-221.

ROBERTS P. A., DALMASSO A., CAP G. B. and CASTAGNONE-SERENO P., 1990. Resistance in *Lycopersicon peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. *J. Nematol., 22*: 585-589.

ROBERTS P. A. and THOMASON I. J., 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *Meloidogyne javanica* on resistant tomato genotypes. *Plant Disease, 70*: 547-551.

SANTO G. S., 1982. Reaction of tomato cultivars to *Meloidogyne chitwoodi* and *M. hapla*. *Plant Disease, 66*: 406-407.

SIKORA R. A., SITARAMAIAH K. and SINGH R. S., 1973. Reaction of root-knot nematode-resistant tomato cultivars to *Meloidogyne javanica* in India. *Plant Dis. Rept., 57*: 141-143.

SMITH P. G., 1944. Embryo culture of a tomato species hybrid. *Proc.Amer. Soc. Of Horticult. Science, 44*: 413-416.

SORRIBAS F. J. and VERDEJO-LUCAS S., 1994. Survey of *Meloidogyne spp.* in tomato production fields of Baix Liobregat County, Spain. *J. Nematol., 26* (supplement): 731-736.

TZORTZAKAKIS E. A., 1997. Observations on the variability in reproduction of some populations of root-knot nematodes (*Meloidogyne spp.*) on resistant tomatoes in Crete, Greece. *Russian J. Nematol., 5*: 1-6.

TZORTZAKAKIS E. A. and GOWEN S. R., 1996. Occurrence of a resistance breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. *Fund. appl. Nematol., 19*: 283-288.

WILLIAMSON V. M., 1998. Root-knot nematode resistance genes in tomato and their potential for future use. *Ann. Rev. Phytopathol., 36*: 277-293.

WILLIAMSON V. M., CASWELL-CHEN E. P., WU F. F. and HANSON D., 1994. PCR for nematode identification. pp. 119-127. In *Advances in Molecular Plant Nematology* (F. Lamberti F., C. De Giorgi and D. Mck. Bird eds), Plenum Press, New York and London.

Accepted for publication on 22 March 2000.