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Research Papers

Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction, and growth of tomato genotypes

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Summary. A pot experiment was conducted to determine the effects of three inoculum levels (2,500, 5,000 and 10,000 eggs/plant) on the reproduction of *Meloidogyne hispanica* and *M. javanica* isolates and growth of the susceptible tomato genotypes Easypeel and Moneymaker, and genotypes Motelle and VFNT-Cherr, which possess the *Mi*-gene, at 25±2°C. Sixty days after inoculation, roots were assessed for gall index (GI), reproduction factor (Rf=final/initial population density) and reproduction index (RI=Rf in the *Mi*-gene tomato plants/Rf in tomato Easypeel × 100). Shoot and root lengths and fresh and dry root and shoot weights were also recorded. Both species of *Meloidogyne* reproduced at all inoculum levels on all four tomato genotypes (4≤GI≤5 and 3.44≤Rf≤317.30). The *M. javanica* isolate, obtained from an infected potato field, was identified as natural and partially virulent to the *Mi*-gene (3.71≤RI≤20.19). This emphasizes the need for new sources of resistance to root-knot nematodes and for testing *Mi*-tomato plants for their susceptibility to local populations. Reproduction of *M. javanica* and *M. hispanica* on the resistant Motelle and VFNT-Cherr was significantly less than on the susceptible Easypeel and Moneymaker. VFNT-Cherr was more resistant than Motelle, which suggest an influence of the genetic background of the plants on the nematode response. For Easypeel and Moneymaker, there was a trend of decreased plant growth parameters with increasing inoculum level, irrespective of the nematode species, due to damage caused by the increasing number of nematodes that invaded plant roots. However, these values on Motelle and VFNT-Cherr remained relatively stable regarding shoot and total shoot plus root dry weight. The reproductive rate of *M. javanica* was greater than that of *M. hispanica* on all four genotypes tested, and tomato plants inoculated with *M. hispanica* had greater growth parameters. The resistance response of the *Mi*-tomato plants was independent of the *Meloidogyne* species, however, because both species gave similar RIs.

Key words: *Mi*-gene, root-knot nematodes, *Solanum lycopersicum* L., virulence.

Introduction

Nematodes of the genus *Meloidogyne*, commonly known as root-knot nematodes (RKNs), belong to a group of plant-parasitic nematodes that is widely dispersed around the world. Root-knot nematodes can parasitize most crops, affecting production and quality. These nematodes constitute a major threat to agriculture in temperate and tropical regions and an obstacle to agricultural production in developing countries (Sasser, 1977; Hussey and Janssen, 2002; Abad *et al*., 2003). During their development inside host roots these nematodes release secretions, and some cells of the vascular parenchyma tissues become hypertrophied, with intense cellular multiplication and hyperplasia, leading to formation of giant cells and galls. *Meloidogyne* species, as obligate sedentary endoparasites, require healthy plants to support their development and reproduction. Differentiation of females is favoured when food is available, since the reproductive function requires a greater expenditure of energy. Alternatively, nematodes differentiate

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into a larger number of males when food availability is insufficient (Triantaphyllou and Hirschmann, 1959; Eisenback and Triantaphyllou, 1991).

Meloidogyne infection affects host water and nutrient absorption and their translocation by root system, decreases the rate of leaf photosynthesis, which is negatively correlated with inoculum levels, and mobilizes photosynthates from shoots to roots, more specifically to giant cells, in order to support nematode development and reproduction (Hussey, 1985; Carneiro *et al*., 1999). Symptoms exhibited by RKN-infected plants lead to suppression of plant yields and are caused by an altered plant metabolism, usually involving debilitation of the root systems and leaf nutritional deficiencies, such as chlorosis with temporary wilting in periods of water stress and high temperatures. Some of these symptoms differ greatly, however, according to plant species and cultivars, and can be confused with the damage associated with poor nutrition or injury caused by bacteria, pathogenic fungi and/or viruses (Hussey, 1985; Whitehead, 1997). Some examples of the effects of these economically important RKNs include: stunting, yellowing, internal potato tissue necrosis and browning and severe galling in potato tubers caused by *M. chitwoodi* and *M. fallax*; forking and hairiness of carrots due to *M. hapla*; yellow patch disease on grass in golf courses caused by *M. minor*; and stunting, wilting and severe galling of hosts of *M. ethiopica* (EPPO/OEPP, 2004, 2011; Moens *et al*., 2009; Wesemael *et al*., 2011). The extent of the damage caused by nematodes is directly proportional to the number of second-stage juveniles (J2) penetrating and becoming established in the host root tissue, and their reproduction rate in plants (Barker and Olthof, 1976; Karssen and Moens, 2006). Increasing the initial population density of *M. javanica* correlated negatively with growth of tomato and pepper, and similarly for sugarbeet infected with *Heterodera shachtii* (Griffin, 1981; Mekete *et al*., 2003). According to Wong and Mai (1973) and Vrain (1982), the greatest reductions in foliage weights of lettuce and root weights of carrots were in plants inoculated with the highest inoculum levels of *M. hapla*, in growth chamber and field experiments. Fortnum *et al*., (1991) recorded declines in leaf area and root, shoot, leaf and total plant dry weights with increasing inoculum levels of *M. incognita* on tomato. Di Vito *et al*., (1983) reported that high population densities of *M. incognita* resulted in severe growth reduction or death, of tomato Roma VF plants, while resistant tomato plants showed negligible growth reduction even at high population densities.

Research into the relationship between initial population densities and plant damage has been performed for the RKN species which are more widely distributed in agricultural areas, but no studies have been reported with *M. hispanica*. This RKN species was isolated for the first time in Seville, Spain, from a peach rootstock (*Prunus persica silvestris*) (Hirschmann, 1986), and there are records of occurrence in Africa, Asia, Europe, North, Central and South America and Australia (Landa *et al*., 2008). This species has the ability to infect and reproduce in a wide range of plant species and cultivars (Maleita *et al*., 2011a). The nematode is of great concern to tomato producers due to its ability to reproduce on resistant cultivars containing the *Mi*-1.2 gene (Maleita *et al*., 2011b). The purpose of the present study was to determine the effects of increasing inoculum levels of *M. hispanica* and *M. javanica* on nematode reproduction and growth of the tomato genotypes Easypeel and Moneymaker, which are susceptible to *Meloidogyne* spp., and Motelle and VFNT-Cherr, which possess the *Mi-1.2* gene (referred to hereafter as *Mi*-gene).

Materials and methods

Nematode isolates

One isolate each of *M. hispanica* (obtained from infected fig-tree roots collected in Odeceixe, Faro, Portugal) and of *M. javanica* [from a potato field in Celorico da Beira (Espinheiro), Portugal] were maintained on tomato, *Solanum lycopersicum* L., cv. Easypeel, in pots containing sterilized sandy loam soil and sand (v:v;1:1) in the Nematology Laboratory at the University of Coimbra, Portugal. The isolates were characterized according to perineal pattern morphology and isoesterase phenotype (Hi4 and J3, respectively) (Abrantes *et al*., 2008). The isolate of *M. javanica* was included in this experiment for comparison with *M. hispanica.*

Plant material

The susceptible (mimi) tomato genotypes, Easypeel and Moneymaker, and the resistant (MiMi) genotypes Motelle and VFNT-Cherr (obtained from

C. Rick, Tomato Genetics Stock Center, University of California, Davis, USA) were used in this study. Tomato seedlings were grown from seeds in a Petri dish with filter paper soaked in distilled water and placed in a growth chamber at 26–27ºC. They were transplanted to 5-cm diameter plastic pots filled with sterilized sandy loam soil and sand (v:v;1:1) and maintained in a growth chamber at 25±2ºC, with a photoperiod 12 h and at 60% relative humidity. Plants were watered daily and fertilized weekly with Hiponex® (The Hiponex Co., Inc., Copley Ohio, USA), a water soluble fertilizer (7% N, 6% P and 19% K).

Pot experiment

One four-week-old seedling of each tomato genotype was transplanted to each 10 cm-diameter pot (capacity 500 cm^3) filled with sterilized sandy loam soil and sand (v:v; 1:1). The nematode inocula (eggs) were obtained from infected Easypeel tomato roots, using a 0.52% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). Four plants of each genotype were inoculated with 0, 2,500, 5,000 or 10,000 eggs (initial population density, Pi) of *M. hispanica* or *M. javanica* corresponding to 28 treatments with four replicates/treatment (four tomato genotypes × two species of *Meloidogyne* × three inoculum levels, plus four non-inoculated plants of each genotype). Pots were arranged in a completely randomized experimental design in the growth chamber. Some plants of the tomato genotype Motelle died before the end of the experiment, and the number of replicates was reduced to two for the non-inoculated plants and three for those inoculated with *M. javanica*.

Sixty days after inoculation, the plants were removed from pots and the roots were carefully washed free of soil. The number of galls/root system was assessed and assigned a severity scale from 0 to 5 (0=no galls, 1=1–2, 2=3–10, 3=11–30, 4=31–100, 5=>100 galls per root system) (Taylor and Sasser, 1978). Final population densities (Pf) were expressed as the total number of eggs + second-stage juveniles (J2). Eggs were extracted from the entire root system of each plant with a 0.52% NaOCl solution (Hussey and Barker, 1973). Juveniles were extracted from soil by the Whitehead and Hemming tray method (Hooper, 1986), and J2 that migrated to the water were collected 1 week later, concentrated on a 20 μm pore sieve and counted. Suitability of the plant to the nematode was assessed on the basis of root gall index (GI), an indicator of plant damage, and the reproduction factor (Rf=number of eggs and J2 in roots and J2 in soil/Pi), an indicator of nematode reproduction or host efficiency, according to the modified quantitative scheme of Canto-Sáenz (Sasser *et al*., 1984). Because all tomato genotypes were suitable hosts for *M. hispanica* and *M. javanica*, the reproduction index (RI=Rf in tomato genotypes with *Mi*-gene/Rf in tomato Easypeel × 100) was calculated. Plants in which RI was more than 50% were designated as susceptible, 25≤RI≤50%, slightly resistant; 10≤RI≤25%, moderately resistant; 1≤RI≤10%, very resistant; RI≤1%, highly resistant; or immune when nematodes penetrated the roots but did not develop or reproduce (Triantaphyllou, 1975; Hadisoeganda and Sasser, 1982). Plant shoot and root lengths (stems and root systems were carefully stretched and measured with a rule) and fresh and dry root and shoot weights were also recorded.

Statistical analyses

Analysis of variance (ANOVA), with no blocking, as appropriate for the completely randomised design, was applied to the data in order to assess the statistical significance of the main effects and interactions between the three experimental factors. For all analyses, a logarithmic (to base *e*) transformation of the data was used to ensure a Normal distribution and constant variance for the data, as checked by plotting the residuals against the fitted values from the ANOVA. Following the extraction of the significant interaction terms from the ANOVA (using the F-test), means of interest in these terms were compared using the appropriate least significant difference (LSD) values, at the 5% level of probability. Statistical analyses were performed using GenStat® (2009, 12th edition, © VSN International Ltd, Hemel Hempstead, UK).

Results

Both species of *Meloidogyne* reproduced on all the tomato genotypes, with high numbers of eggs and J2 recovered from roots and soil, so that all four genotypes were suitable hosts for the nematode and would be considered as susceptible (GI>2 and Rf>1) according Sasser *et al*. (1984) (Table 1). However, tomato genotypes Easypeel and Moneymaker

were better nematode hosts than Motelle and VFNT-Cherr, wherefore the reproduction index was calculated. The RI values for genotype Motelle ranged from 12.72% (*M. javanica* inoculated with 2,500 eggs) to 25.97% (*M. hispanica* inoculated with 10,000 eggs) (Table 1). Genotype Motelle was considered moderately resistant (12.72≤RI≤20.23%) to *M. javanica* and *M. hispanica*, irrespective of the initial inoculum level; except when inoculated with 10,000 *M. hispanica* eggs where it was considered slightly resistant (RI=25.97%). Genotype VFNT-Cherr responded as very resistant to both species at all three initial inoculum levels (3.71≤RI≤8.11%) (Table 1).

Although the interaction between the tomato genotypes and the *Meloidogyne* species was not statistically significant for Pf (P>0.05, F-test), there was a trend for all genotypes to show greater Pf levels when inoculated with *M. javanica* than *M. hispanica*. However, the egg population recovered from roots of all genotypes (Pfr) was significantly greater for *M. javanica* than *M. hispanica* [P<0.05, LSD test; means on log scale from significant (P=0.024, F-test) genotype by isolate interaction: Easypeel - *M. hispanica* 12.770, *M. javanica* 13.553; Moneymaker - *M. hispanica* 12.819, *M. javanica* 13.357; Motelle - *M. hispanica* 11.263, *M. javanica* 11.697; VFNT-Cherr - *M. hispanica* 10.029, *M. javanica* 10.344; SED=0.1090 with 69 df; LSD (5%)=0.2175]. Genotype VFNT-Cherr showed the lowest Pfr values ranging from 15,825 to 37,067 eggs/plant, and this genotype was significantly different (P<0.05, LSD test) from Motelle, as the next most suitable genotype for egg production. The final population recovered from soil (Pfs) was greater for *M. javanica* than *M. hispanica* for all genotypes except Moneymaker, with significant differences between Motelle and VFNT-Cherr [P<0.05, LSD test; means on log scale from significant (P<0.001, F-test) genotype by isolate interaction: Easypeel - *M. hispanica* 10.421, *M. javanica* 10.531; Moneymaker - *M. hispanica* 10.610, *M. javanica* 10.401; Motelle - *M. hispanica* 7.832, *M. javanica* 9.375; VFNT-Cherr - *M. hispanica* 7.266, *M. javanica* 8.395; SED=0.2604 with 69 df; LSD (5%)=0.5194].

Greater Pf and Pfr values were recorded for *M. javanica* than *M. hispanica* at all inoculum levels [P<0.05, LSD test; Pf means on log scale from significant (P=0.013, F-test) inoculum by isolate interaction: 2,500 eggs - *M. hispanica* 11.616, *M. javanica* 12.298; 5,000 eggs - *M. hispanica* 11.754, *M. javanica* 12.338; 10,000 eggs - *M. hispanica* 12.036, *M. javanica* 12.357; SED=0.0869 with 69 df; LSD (5%)=0.1733. Pfr means on log scale from significant (P=0.033, F-test) inoculum by isolate interaction: 2,500 eggs - *M. hispanica* 11.550, *M. javanica* 12.204; 5,000 eggs - *M. hispanica* 11.662, *M. javanica* 12.246; 10,000 eggs - *M. hispanica* 11.949, *M. javanica* 12.264; SED=0.0944 with 69 df; LSD (5%)=0.1884]. The tomato genotypes were affected differently by the increase in inoculum levels as shown by a significant (P<0.001, F-test) genotype by inoculum interaction for both Pf and Pfr. The greatest effect was observed on tomato Motelle, Pf increasing on average by 78% and Pfr by 76%, compared with Pi 10,000 to Pi 2,500. Tomato VFNT-Cherr showed the lowest Pf values of the four genotypes, and GI≥4, for all inoculum levels, and Easypeel and Moneymaker the greatest (GI=5). Genotype Moneymaker was relatively only slightly affected by the increase in the initial inoculum levels (Table 1).

Root length was only affected by the *Meloidogye* species (P=0.002, F-test). Longer roots were observed in plants infected by *M. javanica* than *M. hispanica* [P<0.05, LSD test; means on log scale: *M. hispanica* 3.068, *M. javanica* 3.174; SED=0.0332 with 79 df; LSD (5%)=0.0661]. Statistically significant differences in shoot and total shoot plus root lengths were detected between the genotypes and *Meloidogyne* species, and genotypes and inoculum levels (significant interactions, P<0.001, F-tests). Shoot and total shoot plus root lengths were more reduced in Moneymaker and Motelle when inoculated with *M. javanica* than for *M. hispanica*, whereas genotype VFNT-Cherr had longer shoot and total shoot plus root lengths when inoculated with *M. javanica* than with *M. hispanica*. Shoot and total shoot plus root lengths decreased with increasing inoculum levels in all genotypes, but most dramatically in Moneymaker, with an average reduction of 42% and 44%, respectively for the two nematodes, compared with non-inoculated plants (Figure 2a).

Smaller root fresh weight (RFW) and root dry weight (RDW) were recorded for all tomato genotypes infected by *M. javanica* compared to *M. hispanica*, with significant genotype by isolate interactions (P<0.001, F-tests) for both variables (Figure 2b and c). Differences were significant (P<0.05) for all genotypes except Easypeel [means on log scale of RFW: Easypeel *M. hispanica* 1.9037, *M. javanica* 1.8334; Moneymaker *M. hispanica* 1.7167, *M. javanica* 1.5405; Motelle *M. hispanica* 1.6791, *M. javanica* 1.5859; VFNT-Cherr *M. hispanica* 1.7722, *M. javanica* 1.3861;

Table 1. Effects of increasing inoculum levels on the reproduction of *Meloidogyne hispanica* and *M. javanica* on tomato, *Solanum lycopersicum* L., genotypes Easypeel, Moneymaker, Motelle and VFNT-Cherr, 60 days after the inoculation of 2,500, 5,000 or 10,000 eggs per plant in a growth chamber at 25±2°C. See Results section for data means on natural log (to base *e*) scale to consider for statistical comparison.

Inoculum level (eggs)	Parameters evaluated	Easypeel		Moneymaker		Motelle		VFNT-Cherr	
		M. hispanica	*M. javanica*	*M. hispanica*	*M. javanica*	*M. hispanica*	*M. javanica*	*M. hispanica*	*M. javanica*
2,500	GI[a]	5	5	5	5	5	5	4	4
	Soil (J2)[b]	26 832±7 670	52 850±28 887	33 775±6 876	46 642±23 443	1 407±1 757	21 047±587	1 498±997	3 803±1 616
	Root (eggs+J2)[b]	287 400±48 336	740 400±82 890	310 350±16 325	722 100±48 521	57 650±15 269	88 400±17 016	23 975±5 461	34 667±5 809
	Pf[c]	314 232±43 590	793 250±90 108	344 125±21 950	768 742±31 046	59 057±13 658	109 447±16 458	25 473±5 145	38 470±6 034
	Rf[d]	125.69±17.44	317.30±36.04	137.65±8.78	307.50±12.42	23.62±5.46	43.78±6.58	10.19±2.06	15.39±2.41
	RI[e]	—	—	—	—	18.79±4.35 (MR)	12.72±2.07 (MR)	8.11±1.64 (VR)	4.85±0.76 (VR)
5,000	GI	5	5	5	5	5	5	4	4
	Soil (J2)	45 267±17 436	56 242±41 921	50 433±23 412	31 183±32 033	3 178±2 702	11 960±4 180	1 373±388	4 560±1 823
	Root (eggs+J2)	392 533±97 229	827 700±57 334	409 867±121 189	643 800±187 037	85 400±30 750	141 600±41 365	15 825±7 906	28 200±11 200
	Pf	437 800±112 176	883 942±59 860	460 300±106 386	674 983±191 408	88 578±32 917	153 560±37 345	17 198±7 763	32 760±10 990
	Rf	87.56±22.44	176.79±11.97	92.06±21.28	135.00±38.28	17.72±6.58	30.71±7.47	3.44±1.55	6.55±2.20
	RI	—	—	—	—	20.23±7.52 (MR)	17.37±4.22 (MR)	3.93±1.77 (VR)	3.71±1.24 (VR)
10,000	GI	5	5	5	5	5	5	5	4
	Soil (J2)	39 733±22 826	28 983±10 555	46 767±23 387	53 742±55 673	9 933±2 745	11 793±3 117	1 967±325	5 867±1 509
	Root (eggs+J2)	40 5000±93 325	764 100±197 142	412 267±57 220	579 300±168 403	105 550±20 881	148 333±41 546	33 950±3 296	37 067±19 251
	Pf	444 733±103 719	793 083±206 614	459 033±59 961	633 042±161 892	115 483±22 744	160 127±44 634	35 917±3 165	42 933±20 704
	Rf	44.47±10.37	79.31±20.66	45.90±6.00	63.30±16.19	11.55±2.27	16.01±4.46	3.59±0.32	4.29±2.07
	RI	—	—	—	—	25.97±5.14 (SR)	20.19±5.63 (MR)	8.08±0.71 (VR)	5.41±2.61 (VR)

[a] GI=Gall Index (scale 0-5): 0=no galls, 1=1–2, 2=3–10, 3=11–30, 4=31–100, 5= >100 galls per root system.
[b] Number of second-stage juveniles (J2) and eggs + J2 extracted from soil and roots, respectively.
[c] Pf=final population density on soil + roots. Data are means of three (Motelle inoculated with *M. javanica*) or four (all other plants) replicates±standard deviation.
[d] Rf (reproduction factor)=Pf/initial population density.
[e] RI (reproduction index)=(Rf in the *Mi*-gene tomato plants/Rf in the susceptible genotype Easypeel) × 100.
Resistance designations (in parenthesis): SR=slightly resistant (25≤RI≤50%); MR=moderately resistant (10≤RI≤25%); VR=very resistant (1≤RI≤10%) (Hadisoeganda and Sasser, 1982).

SED=0.04087 with 79 df; LSD (5%)=0.08136; means on log scale of RDW: Easypeel *M. hispanica* -0.441, *M. javanica* -0.627; Moneymaker *M. hispanica* -0.739, *M. javanica* -0.943; Motelle *M. hispanica* -0.742, *M. javanica* -1.003; VFNT-Cherr *M. hispanica* -0.596, *M. javanica* -1.241; SED=0.0593 with 79 df; LSD (5%)=0.1181]. Easypeel had the greatest RFW in both inoculated and non-inoculated conditions. There were also significant genotype by inoculum interactions for RFW (P=0.026, F-test) and RDW (P=0.036, F-test). When the nematode inoculum increased, RFW and RDW values decreased for all genotypes, except Easypeel [means on log scale of RFW by inoculum levels: 2,500 eggs - Easypeel 1.8412, Moneymaker 1.6284, Motelle 1.7320, VFNT-Cherr 1.5991; 5,000 eggs - Easypeel 1.8702, Moneymaker 1.6693, Motelle 1.6377, VFNT-Cherr 1.5728; 10,000 eggs - Easypeel 1.8943, Moneymaker 1.5881, Motelle 1.5279, VFNT-Cherr 1.5657; SED=0.05006 with 79 df; LSD (5%)=0.09964; means on log scale of RDW by inoculum levels: 2,500 eggs - Easypeel -0.556, Moneymaker -0.778, Motelle -0.781, VFNT-Cherr -0.769; 5,000 eggs - Easypeel -0.501, Moneymaker -0.797, Motelle -0.909, VFNT-Cherr -1.040; 10,000 eggs - Easypeel -0.546, Moneymaker -0.947, Motelle -0.927, VFNT-Cherr -0.946; SED=0.0727 with 79 df; LSD (5%)=0.14460. Genotype Motelle had the greatest decrease (9%)] in RFW comparing non-inoculated plants to those with the greatest amount of inoculum, although both genotypes Motelle and VFNT-Cherr appeared to show a slight increase compared to non-inoculated plants for the lowest amount of inoculum [means on log scale of RFW by inoculum levels: non-inoculated - Motelle: 1.6195, VFNT-Cherr 1.5707; 2,500 eggs - Motelle 1.7320, VFNT-Cherr 1.5991; 5,000 eggs - Motelle 1.6377, VFNT-Cherr 1.5728; 10,000 eggs - Motelle 1.5279, VFNT-Cherr 1.5657; SED=0.06131 with 79 df; LSD (5%)=0.12204] (Figure 2b).

For shoot fresh weight (SFW), there were significant genotype by isolate and genotype by inoculum interactions (P<0.001, F-tests). SFW was greater for tomato genotypes Easypeel, Moneymaker and Motelle inoculated with *M. hispanica* than *M. javanica*, the difference being significant (P<0.05, LSD test) for Motelle [means on log scale by genotype: Easypeel - *M. hispanica* 1.722, *M. javanica* 1.713; Moneymaker - *M. hispanica* 2.223, *M. javanica* 2.091; Motelle - *M. hispanica* 2.555, *M. javanica* 2.155; VFNT-Cherr - *M. hispanica* 2.152, *M. javanica* 2.241; SED=0.0832 with 79 df; LSD (5%)=0.1656]. SFW decreased with increasing inoculum levels for all genotypes, except Motelle [means on log scale of SFW by inoculum levels: 2,500 eggs - Easypeel 1.961, Moneymaker 2.411, Motelle 2.133, VFNT-Cherr 2.227; 5,000 eggs - Easypeel 1.685, Moneymaker 2.234, Motelle 2.445, VFNT-Cherr 2.226; 10,000 eggs - Easypeel 1.506, Moneymaker 1.825, Motelle 2.488, VFNT-Cherr 2.136; SED=0.1019 with 79 df; LSD (5%)=0.2028]. For shoot dry weight (SDW), there was a genotype by inoculum interaction (P<0.001, F-test) and a species by inoculum interaction (P<0.001, F-test). SDW decreased more with increasing amounts of inoculum of *M. hispanica* than *M. javanica*, but SDW was greater for tomato plants inoculated with *M. hispanica* [means on log scale of SDW by inoculum levels: 2,500 eggs - *M. hispanica* 0.505, *M. javanica* 0.358; 5,000 eggs - *M. hispanica* 0.337, *M. javanica* 0.142; 10,000 eggs - *M. hispanica* -0.033, *M. javanica* 0.044; SED=0.0496 with 79 df; LSD (5%)=0.0986]. SDW decreased markedly for genotypes Easypeel and Moneymaker with increasing amounts of inoculum, but were similar for Motelle and VFNT-Cherr [means on log scale of SDW by inoculum levels: 2,500 eggs - Easypeel 0.170, Moneymaker 0.410, Motelle 0.646, VFNT-Cherr 0.501; 5,000 eggs - Easypeel -0.195, Moneymaker 0.090, Motelle 0.593, VFNT-Cherr 0.471; 10,000 eggs - Easypeel -0.595, Moneymaker -0.461, Motelle 0.654, VFNT-Cherr 0.425; SED=0.0701 with 79 df; LSD (5%)=0.1395] (Figure 2b and c).

For total shoot plus root fresh weight (TFW) there were significant genotype by isolate (P=0.008, F-test) and genotype by inoculum (P<0.001, F-test) interactions. TFW was less for plants infected by *M. javanica* than *M. hispanica* [means on log scale of TFW by genotype: Easypeel - *M. hispanica* 2.522, *M. javanica* 2.475; Moneymaker - *M. hispanica* 2.709, *M. javanica* 2.551; Motelle - *M. hispanica* 2.907, *M. javanica* 2.655; VFNT-Cherr - *M. hispanica* 2.674, *M. javanica* 2.597; SED=0.0449 with 79 df; LSD (5%)=0.0894]. Also, TFW decreased with increasing amounts of inoculum for all genotypes except for Motelle [means on log scale of TFW by inoculum levels: 2,500 eggs - Easypeel 2.601, Moneymaker 2.790, Motelle 2.712, VFNT-Cherr 2.661; 5,000 eggs - Easypeel 2.477, Moneymaker 2.689, Motelle 2.817, VFNT-Cherr 2.656; 10,000 eggs - Easypeel 2.417, Moneymaker 2.411, Motelle 2.813, VFNT-Cherr 2.589; SED=0.0550 with 79 df; LSD (5%)=0.10940]. There were significant genotype by inoculum (P<0.001, F-test) and species by inoculum (P=0.039, F-test) interactions for total shoot plus

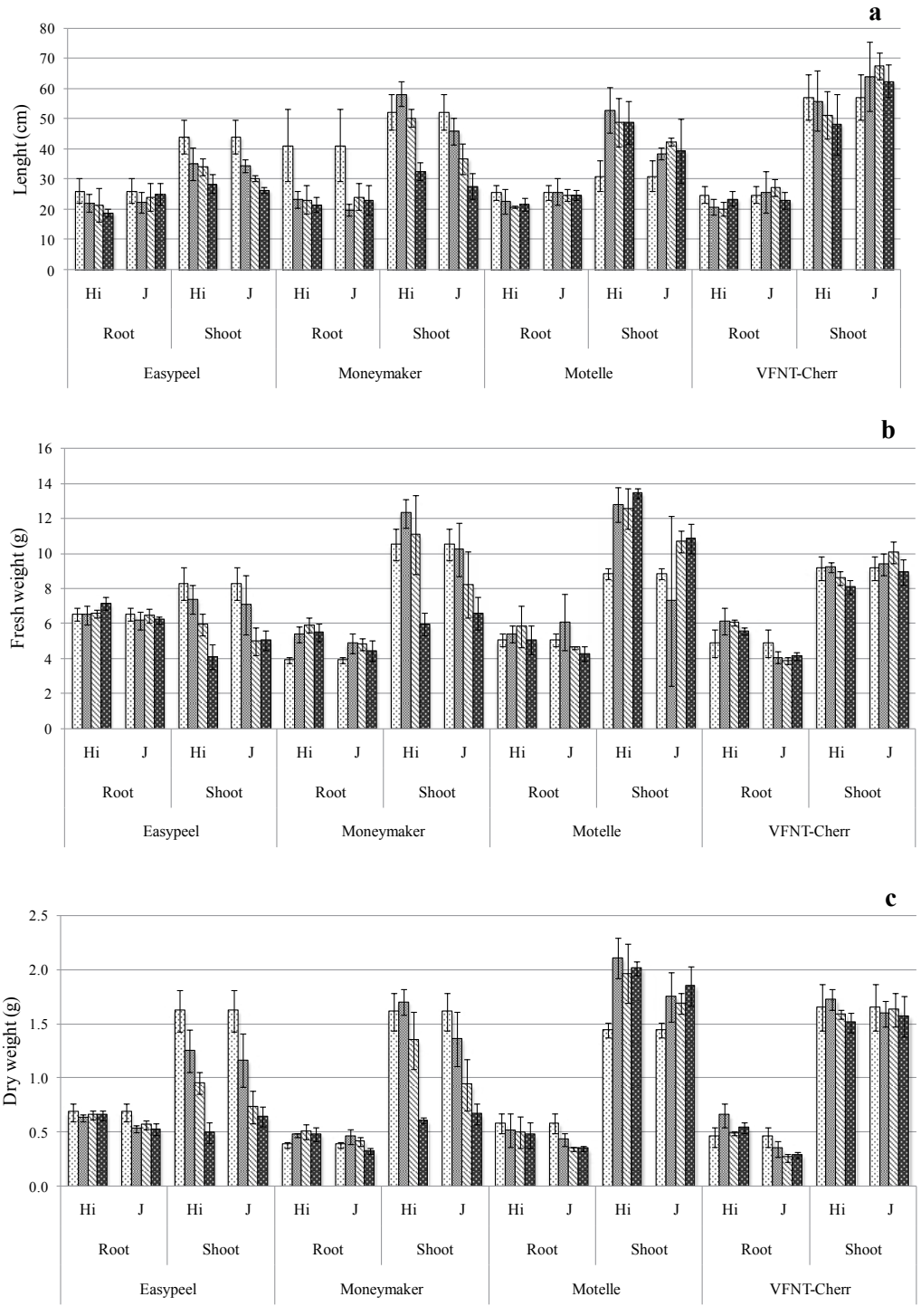

Figure 1. Effects of different initial inoculum levels (0, 2,500, 5,000 or 10,000 eggs / plant) of *Meloidogyne hispanica* (Hi) and *M. javanica* (J) on mean plant length (a) and fresh (b) and dry (c) weights, of tomato genotypes Easypeel, Moneymaker, Motelle and VFNT-Cherr. Bars represent standard deviations. See Results section for data means on natural log (to base *e*) scale for statistical comparisons.

root dry weight (TDW). Genotypes inoculated with *M. hispanica* showed greater TDW than those inoculated with *M. javanica* at all inoculum levels ($P<0.05$, LSD test). However, with increasing inoculum levels, the greatest reduction was observed in plants inoculated with *M. hispanica* [means on log scale for TDW by inoculum levels: 2,500 eggs - *M. hispanica* 0.8041, *M. javanica* 0.6317; 5,000 eggs - *M. hispanica* 0.6746, *M. javanica* 0.4654; 10,000 eggs - *M. hispanica* 0.4543, *M. javanica* 0.3777; SED=0.03728 with 79 df; LSD (5%)=0.07421]. TDW was significantly less in genotypes Easypeel and Moneymaker with the greatest amount of inoculum, with reductions of up to 70% compared to non-inoculated plants. There was only a slight reduction in TDW for genotype VFNT-Cherr but no change for Motelle [means on log scale for TDW by inoculum levels: 2,500 eggs - Easypeel 0.5676, Moneymaker 0.6798, Motelle 0.8652, VFNT-Cherr 0.7590; 5,000 eggs - Easypeel 0.3590, Moneymaker 0.4419, Motelle 0.8006, VFNT-Cherr 0.6786; 10,000 eggs - Easypeel 0.1329, Moneymaker 0.0260, Motelle 0.8437, VFNT-Cherr 0.6614; SED=0.05273 with 79 df; LSD (5%)=0.10495] (Figure 2b and c).

Discussion

Meloidogye hispanica and *M. javanica* were able to reproduce (Rf>1) at all inoculum levels on Motelle and VFNT-Cherr, both of which are homozygous at the *Mi* locus. The *Mi*-gene in tomato confers resistance to the three most common warm climate RKN, *M. arenaria*, *M. incognita* and *M. javanica* (Williamson, 1999) but not immunity. However, some isolates of these species, and other *Meloidogyne* species such as *M. enterolobii*, *M. exigua*, *M. floridensis*, *M. hapla* and *M. hispanica*, can reproduce on tomato genotypes possessing the *Mi*-gene (Brown *et al*., 1997; Brito *et al*., 2007; Abd-Elgawad and Molinari, 2008; Silva *et al*., 2008, Maleita *et al*., 2011b).

Our results reveal that the Portuguese isolate of *M. javanica* can be considered as natural and partially virulent (Tzortzakakis and Gowen, 1996) as it reproduces (Rf>1) on *Mi*-tomato plants, but at lower levels than on susceptible control plants (3.71<RI<20.19%) This isolate was obtained in 1980, from an infested potato field with high yield losses, and has been maintained in the laboratory on susceptible tomato genotypes. The crop rotation scheme used in this field was potato and bean rotated with ryegrass (*Lolium multiflorum* Lam.) and rye (*Secale cereale* L.). Thus, this *M. javanica* population seems to have an inherent ability to reproduce on *Mi*-tomato plants as there was no prior exposure of the nematode to the *Mi*-gene, at least in recent cropping history of the field, although the possibility of previous exposure in evolutionary time cannot be discarded (Roberts, 1995; Ornat *et al*., 2001). According to Castagnone-Sereno *et al*. (1993), the acquired nematode virulence to plants with the *Mi*-gene is stable and inherited, even after extended growth of the virulent nematode on susceptible plants. Natural and selected resistance-breaking populations of *M. javanica* have already been reported in Cyprus, Egypt, Greece, Jordan, Morocco, Spain, Tunisia and Turkey (Philis and Vakis, 1977; Eddaoudi *et al*., 1997; Tzortzakakis *et al*., 1999, 2005; Ornat *et al*., 2001; Molinari and Caradonna, 2003; Verdejo-Lucas *et al*., 2009; Devran and Söğüt, 2010). Selection of virulent populations can occur due to pressure exerted on the nematodes by their frequent cultivation in the field or repeated inoculation in laboratory conditions, on *Mi*-resistant tomato plants. The occurrence of nematode populations able to overcome the *Mi*-resistance gene in tomato genotypes emphasizes the need for developing new sources of resistance to RKN, and also for screening genotypes against local populations of *Meloidogyne* for their ability to overcome the *Mi*-resistance gene. Otherwise, the successful use of tomato plants with the *Mi*-gene in integrated control strategies such as crop rotation could be compromised.

The reproduction of both *Meloidogyne* species on tomato genotypes Motelle and VFNT-Cherr, with the *Mi*-gene, was less (3.44≤Rf≤43.78) than on Easypeel and Moneymaker (44.47≤Rf≤317.30) that lacked this gene. Genotype VFNT-Cherr, considered very resistant to both nematode species irrespective the initial amount of inoculum (3.71≤RI≤8.11%), was more resistant than Motelle (12.72≤RI≤25.97%). These results suggest that the *Mi*-gene provides partial protection against the development of *M. hispanica* and *M. javanica*, and nematode reproduction is influenced by the genetic background of the plants (Tzortzakakis *et al*., 1998; Jacquet *et al*., 2005; Maleita *et al*., 2011b).

For all tomato genotypes inoculated with either isolate, and increasing inoculum levels, there was a decrease in root length and shoot/total (shoot plus root) length. This was most likely due to damage caused by the increasing number of nematodes that invaded plant roots, probably causing reduced nutrient and water uptake (Karssen and Moens, 2006).

According to Carneiro *et al*. (1999), in nematode-infected soybean plants, reduction in shoot length is strongly influenced by increased partitioning of carbohydrates to the roots and may vary with nematode species. Mobilization and accumulation of photosynthetic products from shoots to roots reaches maximum levels when adult females start laying eggs (Karssen and Moens, 2006). The growth responses of tomato genotypes can be related to the presence of the *Mi*-gene and consequently the inability of the nematodes to reproduce at the same levels as on the susceptible genotypes, which suggests incomplete or partial nematode virulence (Roberts, 1995). The results for root and shoot dry weights confirmed the differences between species and genotypes and are considered a more realistic measure of the effects of the nematode on plant growth than the other growth parameters evaluated (Fortmun *et al*., 1991).

Both genotypes Motelle and VFNT-Cherr showed slight increases in root fresh weight at the lowest inoculum level compared to non-inoculated control plants. This increase has been attributed to gall formation and secondary root proliferation (Carneiro *et al*., 1999; Abrão and Mazzafera, 2001).

The resistance response of the *Mi*-tomato plants Motelle and VFNT-Cherr was independent of the RKN species, because both species provided similar RI for each genotype. However, nematode reproduction and growth parameters differed between *M. javanica* and *M. hispanica.* Although the increase in the amount of inoculum led to reductions in growth parameters, tomato plants inoculated with *M. hispanica* showed greater growth parameters than those with *M. javanica*, and lower values of Rf. The *M. javanica* isolate used in this study presented greater reproductive and destructive potential than the isolate of *M. hispanica*, and both nematode species seriously affected the growth of the tomato genotypes tested.

This study has demonstrated that *M. hispanica* can be a threat to tomato production and suggests that it will be important to monitor the performance of tomato plants with the *Mi*-gene in fields infested with *M. javanica*.

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