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RESEARCH ARTICLE / *ARTIGO*

Reaction of coffee genotypes to different populations of *Meloidogyne* spp.: detection of a naturally virulent *M. exigua* population

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ABSTRACT

The reaction of seven genotypes of *Coffea arabica* to 10 *Meloidogyne* spp. populations collected mainly from coffee plantations in Brazil and Costa Rica was evaluated under greenhouse conditions. The inoculum consisted of 10,000 eggs per plant. Evaluations were done 8 months after inoculations considering the root fresh weight, gall and egg mass indices, number of eggs per gram of root and reproduction factor (RF). The cultivars Obatã IAC 1669-20, Sarchimor IAC 4361 and Tupi Amarelo IAC 5111 exhibited susceptibility to the four Brazilian *M. exigua* populations tested. However, cv. Tupi Vermelho IAC 1669-33 revealed resistance (RF value of 0.7) to the *M. exigua* population from Lavras, Minas Gerais State, Brazil. A population of *M. exigua* from Bom Jesus de Itabapoana, Rio de Janeiro State, Brazil, was highly virulent on cv. IAPAR 59 (RF= 165.7), bearing resistance gene Mex-1, and was also virulent on genotype Paraíso (H 419-5-4-5-2) (RF=396.2). A *Meloidogyne* sp. population on coffee from Garça, São Paulo State, Brazil, reproduced at low rates (RF ranging from 0.1 to 3.9) on all genotypes. All tested cultivars were susceptible to *M. incognita* and *M. paranaensis*. *M. mayaguensis* of guava from Paraná State, Brazil, reproduced at low rates in all coffee genotypes; however, another population of coffee, from Costa Rica, was more aggressive and showed RF value that ranged from 0.8 to 12.4. Results of this study point for the first time to the ability of a naturally occurring *M. exigua* population to overcome the resistance conferred by the Mex-1 gene.
Keywords: *Coffea arabica*, resistance, susceptibility, root-knot nematodes.

RESUMO

Reação de genótipos de cafeeiro a diferentes populações de *Meloidogyne* spp.: detecção de uma população de *M. exigua* naturalmente virulenta

A reação de sete genótipos de *Coffea arabica a* 10 populações de *Meloidogyne* spp. coletadas principalmente em plantios de café no Brasil e Costa Rica foi avaliada em casa de vegetação. O inóculo consistiu de 10.000 ovos por planta. As avaliações foram realizadas 8 meses após as inoculações considerando-se a massa da matéria fresca das raízes, índices de galhas e massas de ovos, número de ovos por grama de raiz e fator de reprodução (FR). As cultivares Obatã IAC 1669-20, Sarchimor IAC 4361 e Tupi Amarelo IAC 5111 exibiram suscetibilidade às quatro populações brasileiras de *M. exigua*. Entretanto, cv. Tupi Vermelho IAC 1669-33 mostrou-se resistente (FR=0,7) a uma população de *M. exigua* proveniente de Lavras, MG, Brasil. A população de *M. exigua* oriunda de Bom Jesus de Itabapoana, RJ, Brasil foi altamente virulenta à cv. IAPAR 59 (FR= 165,7), portadora do gene de resistência Mex-1 e ao genótipo Paraíso (H 419-5-4-5-2) (FR=396,2). A população de *Meloidogyne* sp. do cafeeiro, Garça, SP, Brasil, reproduziu-se em baixos níveis (FR = 0,1 - 3,9) sobre todos os genótipos. Todas as cultivares testadas foram suscetíveis a *M. incognita* e *M. paranaensis*. A reprodução de *M. mayaguensis* obtida de goiabeira, PR, Brasil, foi baixa (FR = 0,0-1,6), em todos os genótipos. Entretanto, outra população obtida do cafeeiro na Costa Rica apresentou valores de FR que variaram de 0,8 a 12,4. Os resultados deste trabalho mostraram, pela primeira vez, a capacidade de uma população de *M. exigua* obtida em campo de superar a resistência conferida pelo gene Mex-1.
Palavras-chave: *Coffea arabica*, resistência, suscetibilidade, nematóides de galha.

INTRODUCTION

Root-knot nematodes of the genus *Meloidogyne* are more widely distributed throughout the world in coffee (*Coffea arabica* L.) plantations than any other major group of plant-pathogenic nematodes. In Brazil, the most common, damaging and well-known species are *M. exigua* Göldi, 1887, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996 (Campos & Villain, 2005). Among the most damaging species, *M. exigua* is especially common

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in Latin America, where it constitutes a major agronomic constraint. This species is widespread in coffee-producing states in Brazil. *Meloidogyne incognita* occurs in many coffee-growing areas around the world, but it is in Brazil that its effects on coffee plantations have become most serious (Campos & Villain, 2005). In 1996, a new species of the genus *Meloidogyne* was described in the State of Paraná, Brazil and designated as *M. paranaensis* (Carneiro et al., 1996). According to Campos & Villain (2005), *M. paranaensis* is as destructive to coffee plantations as *M. incognita*, especially in the states of Paraná and São Paulo (Carneiro et al*.,* 2005). This species was also detected in Guatemala and Hawaii (Carneiro et al., 2004).

Meloidogyne mayaguensis Rammah & Hirschmann, 1988 seems to be a polyphagous species widespread in many African and American countries (Carneiro, 2003). In Cuba, it is the most damaging species on coffee (Rodríguez et al., 1995). In Brazil, this species is an economically important plant pathogen on guava (*Psidium guajava*) but not detected in coffee under field conditions (Carneiro et al., 2001, 2006). In addition, it has been found infecting many other crops (Almeida et al., 2008). The host response of coffee cultivars to this species is unknown.

The application of nematicides, crop rotations in areas where old coffee plants have been eradicated, planting in pathogen-free soil, the use of healthy seedlings, resistant rootstocks and resistant cultivars are methods used for controlling coffee root-knot diseases (Campos & Villain, 2005). According to Roberts (2002), plant resistance has been found and developed mainly for the highly specialized parasitic nematodes that have a sedentary endoparasitic relationship with their host. Resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode. It can range from low to high resistance. A completely or highly resistant (HR) plant allows no nematode reproduction, or only trace amounts. Partially or moderately (MR) resistant plants allow some intermediate amounts of reproduction. Susceptibility (S) is used as the opposite of resistance; thus a susceptible plant allows normal nematode development and the expression of any associated disease. However, resistance may lack durability due to variability in the nematode population (Starr et al., 2002).

Several lines derived from the interspecific cross between *C. arabica* and *C. canephora* Pierre ex Froehner (Timor Hybrid) showed resistance to *M. exigua* similar to that observed on *C. canephora* (Silvarolla et al., 1998; Bertrand et al., 2001). Recently, a major gene designated Mex-1 locus introgressed from *C. canephora* was identified. This gene possibly presented an incomplete dominant expression (Noir et al., 2003). There are no studies showing the spectrum of resistance of the gene Mex-1 to different populations of *M. exigua*. Resistance or tolerance to *M. incognita* has been found only in coffee genotypes derived from *C. canephora* (Carneiro, 1995; Gonçalves et al., 1996). For *M. paranaensis,* resistance has been found in both *C. canephora* germplasm and Ethiopian *C. arabica* accessions (Anzueto et al., 2001; Campos & Villain, 2005).

Considering the great diversity in *Meloidogyne* spp. populations on coffee (Randig et al., 2002; Carneiro et al., 2004; Hernandez et al., 2004; Muniz et al., 2008), and the fact that resistance may lack durability due to variability in the nematode population (Starr et al., 2002) it is of prime importance to assess the pathogenicity of *Meloidogyne* species and biotypes (races or enzymatic phenotypes) to different coffee cultivars, so as to implement integrated management strategies. The present study investigated the pathogenicity and virulence of ten different *Meloidogyne* spp. populations on seven coffee genotypes.

MATERIALS AND METHODS

Nematode populations, multiplication and inoculation

Ten nematode populations originated from infected coffee and guava roots were used (Table 1). The populations were characterized by perineal pattern, races, esterase phenotypes and/or SCAR (sequence-characterized amplified region) markers using the techniques proposed by Hartman & Sasser (1985), Carneiro & Almeida (2001) and Randig et al. (2002).

The populations were multiplied on coffee cv. Catuaí Vermelho IAC 144 (Mexi 1, Mexi 3, Mexi 4, Minc 6 and Mpar 8) or tomato (*Lycopersicon esculentum* group Santa Cruz cv. Kada, populations Mexi 2, Minc 5, Msp 7, Mma 9 and Mma 10) roots under greenhouse conditions. To recover the inoculum, 3-month-old tomato roots or 6-month-old coffee roots were cut into 1-2 cm segments and blended for 1 minute in a 0.5% sodium hypochlorite solution (Barker, 1985). Eggs were rinsed thoroughly and counted in 1 ml aliquots in Peter's counting slide. Means of three replicates were used to represent the number of eggs per mL. Single 6-month-old coffee plants grown in 3-liter plastic pots containing 1:1:1 mixture (v/v) autoclaved soil, bovine manure and sand were inoculated with approximately 10,000 eggs of each nematode. The inoculum was applied onto the soil surface around the stem base. Plants were arranged in a randomized complete block factorial design (10 *Meloidogyne* spp. populations x 7 coffee genotypes) with eight replicates. Plants were grown with regular watering and fertilization.

Experiment and plant material

The trial was carried out in a greenhouse at temperatures ranging from 22 to 28ºC. All cultivars tested are currently used by farmers in Brazil. Varieties studied are originated from the parental group 'Sarchimor', formed by the cultivars Obatã IAC 1669-20, Tupi Vermelho IAC 1669-33, Tupi Amarelo IAC 5111, Sarchimor IAC 4361 and IAPAR 59, all of them derived from a cross between the cv. 'Villa Sarchi' and the 'Timor Hybrid CIFC 832/2'. The cultivar Paraíso (H 419-5-4-5-2) derived from 'Catuaí Amarelo IAC 30 x Timor Hybrid UFV 445-46 (CIFC 2570)'

Table 1 - *Meloidogyne* spp. populations used for coffee genotype evaluations

Population code	Geographical origin	Species/Race	Esterase phenotype
Mexi 1	Lavras – MG, Brazil	*M. exigua* race 1	E1
Mexi 2	Lavras – MG, Brazil	*M. exigua* race 2	E2
Mexi 3	Bom Jesus de Itabapoana – RJ, Brazil	*M. exigua* race 1	E1
Mexi 4	Campinas – SP, Brazil	*M. exigua* race 1	E2
Minc 5	Avilândia – SP, Brazil	*M. incognita* race 1	I1
Minc 6	Londrina – PR, Brazil	*M. incognita* race 3	I2
Msp 7	Garça – SP, Brazil	*Meloidogyne* sp.	S2
Mpar 8	Londrina – PR, Brazil	*M. paranaensis*	P1
Mma 9	Santa Mariana – PR, Brazil	*M. mayaguensis* race 2[a]	M2
Mma 10	Guanacaste, Costa Rica	*M. mayaguensis* race 2	M2

[a]Population obtained from guava. The remaining populations were obtained from coffee.

and 'Catuaí Vermelho IAC 144' that was used as susceptible standard.

Nematode resistance evaluation

Evaluations were carried out 8 months after inoculation. The root systems were carefully washed free of soil, blotted onto paper to damp dry, and weighed (FRW). The whole root system of each plant was soaked in a solution of Phloxine B (0.015g/L of tap water) for 20 minutes to stain egg masses. Galls and number of egg masses produced by the nematode per root system were counted. Gall Index (GI) and Egg Mass Index (EMI) were calculated according to a scale proposed by Hartman & Sasser (1985), where 0 = no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10, 3= 11-30; 4= 31-100, and 5= over 100 galls or egg masses. Host-plant type symptoms were observed.

Considering the large size of roots, eggs were extracted by trituration in a blender for 4 minutes in a 1% NaOCl, according to Hussey & Barker's (1973) methodology modified by Bonetti & Ferraz (1981).The number of eggs per root system (final nematode population = P_f) was counted in triplicate in a Peter's counting slide. This mean value was used to determine the number of eggs per gram of root (eggs/g root) and the reproductive factor (RF), which represents the relation between final and initial nematode population densities for each treatment ($RF=P_f/P_i$), according to Oostenbrink (1966). The genotypes for which FR < 1 were considered as highly resistant (HR), and those for which FR ≥ 1 were considered susceptible (S) or moderately resistant (Sasser et al., 1984). The genotypes were scored as moderately resistant (MR) or resistant (R), according to the terminology proposed by Roberts (2002) and statistical analyses to quantify his concept.

Statistical analyses

Analysis of variance was performed for the experiment after a log (x + 1) transformation of the data for the eggs/g root and RF values, and Scott-Knott test ($P\leq0.05$) was used to evaluate differences among genotypes within the same population and differences between populations for the same coffee genotype. Pearson correlation coefficients between fresh root weight (FRW) and FR were calculated for each genotype. Data were analyzed using the SAS statistical package (SAS Institute Inc., 1988).

RESULTS

Correlations between observed variables

There were no differences among populations and genotypes for the vegetative parameter plant height during the experiments, and plants showed no obvious aerial symptoms. This is probably the result of the constant fertilization of the coffee plants during the 8 months of the bioassay. Statistical analysis revealed significant effects of coffee genotypes and *Meloidogyne* spp. populations and the interactions between FRW, eggs/g root and RF ($P\leq0.05$). Only for the genotypes Catuaí Vermelho IAC 144, Sarchimor IAC 4361, Obatã IAC 1669-20 and Paraíso (H 419-5-4-5-2) was FRW significantly correlated to RF, but with low Pearson correlation coefficients (r = 0.25-0.45), showing a small influence of root weight on final nematode population.

The GI (gall index) and EMI (egg masses index) (Table 2) were subjective parameters because some populations formed typical galls and others did not. In some populations the egg masses did not extend beyond the root tissue and in others the egg-masses emerged outside of the roots and were stained with Phloxine B. Considering that, these two parameters (GI and EMI) were used to describe symptoms but not to evaluate nematode infection. Statistical analysis showed that GI and EMI were correlated with coffee infection (RF) for *M. exigua* (r = 0.59-0.69; 0.36-0.76, respectively), *M. mayaguensis* (r = 0.34-0.51; 0.68-0.73) and *Meloidogyne* sp. (r = 0.42; 0.58), but sometimes with low Pearson correlation coefficient. For *M. incognita* and *M. paranaensis* GI and EMI were not significantly

Table 2 - Mean values of gall index and egg mass index produced by 10 *Meloidogyne* spp. populations on seven coffee genotypes

Coffee genotypes		*Meloidogyne* populations									
		Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma 10**
Catuaí Vermel ho IAC 144	GI	5.0	5.0	4.9	3.1	2.8	3.3	0.9	3.6	1.4	3.0
	EMI	5.0	5.0	4.4	3.1	5.0	5.0	1.1	5.0	1.4	4.3
IAPAR 59	GI	0.0	0.0	5.0	0.1	3.6	3.5	0.3	3.3	3.0	2.8
	EMI	0.0	0.0	5.0	0.0	4.9	5.0	0.5	5.0	3.6	2.3
Obatã IAC 1669-20	GI	2.1	2.9	5.0	0.6	3.6	4.3	1.6	3.6	0.0	3.6
	EMI	2.0	2.9	4.8	0.3	5.0	5.0	3.3	5.0	0.0	4.6
Sarchimor IAC 4361	GI	5.0	5.0	4.9	3.4	2.9	3.5	0.8	4.0	0.1	1.8
	EMI	4.4	5.0	4.5	3.3	5.0	4.3	2.9	4.9	0.3	0.9
Paraíso (H419-5-4-5-2)	GI	0.0	0.0	5.0	0.0	2.5	4.0	0.0	4.1	0.0	2.8
	EMI	0.0	0.0	4.4	0.0	5.0	4.9	1.9	5.0	0.0	1.6
Tupi Amarelo IAC 5111	GI	2.6	2.9	4.9	3.1	2.3	3.0	0.0	3.9	0.0	2.9
	EMI	1.6	2.1	3.6	2.1	4.9	4.9	0.3	4.6	1.6	1.1
Tupi Vermelho IA C 1669-33	GI	1.0	3.8	5.0	2.5	3.3	4.4	0.0	3.4	0.1	2.3
	EMI	0.0	2.1	1.4	1.6	5.0	4.9	0.0	4.6	0.4	1.5

Values are means of eight replicate root systems. Population codes are given in Table 1.
Gall Index (GI) and Egg Mass Index (EMI) were based on a 0 – 5 scale, where 0 = no galls or egg masses and 5 = over 100 galls or egg masses for each root system.

correlated with RF. These pathosystems did not allow the evaluation of nematode infection using GI and EMI**.**

Coffee genotypes x *M. exigua*

The root symptoms observed were small to large rounded or elongated galls mostly on new roots, which usually contained external and internal egg masses. The cultivars Obatã IAC 1669-20, Sarchimor IAC 4361 and Tupi Amarelo IAC 1669-33 exhibited susceptibility to the four *M. exigua* populations (Tables 3-4). Considering the RF value of 0.7, cv. Tupi Vermelho IAC 1669-33 can be classified as resistant to the population Mexi 1 from Lavras, MG, Brazil, and susceptible to the others. However, the RFs are relatively small for this cultivar when compared with the others (Table 4).

The reproduction of the populations Mexi1, Mexi 2 and Mexi 4 was very low on IAPAR 59 and Paraíso (H419-5-4-5-2) genotypes (RF= 0.0-0.4) (Table 4). In contrast, for the population Mexi 3, collected in Rio de Janeiro State, Brazil, the RF values on these two cultivars were very high: RF= 165.7 for IAPAR 59 and RF=396.2 for Paraíso (H 419-5-4-5-2). On the IAPAR 59, Obatã IAC 1669-20, Sarchimor IAC 4361 and Paraíso (H419-5-4-5-2) genotypes this population reproduced much more than on Catuaí Vermelho IAC144 (the susceptible control), Tupi Amarelo IAC 5111 and Tupi Vermelho IAC 1669-33 (Tables 3-4).

Coffee genotypes x *M. incognita*

Coffee plants inoculated with this species showed swellings on the root, some galls and necrosis in the tap roots. Egg masses were produced on the root surface. In general, the coffee genotypes responded differently to the attack of the tested populations. For the population Minc5 race 1, the RFs produced in the Obatã IAC 1669-20 and Tupi Amarelo IAC 5111 genotypes were significantly higher than the remaining genotypes. Moreover, population Minc 6 race 3 also reproduced on all the cultivars, but no significant differences were detected in the RF values (Table 4). Based on the nematode reproduction, all cultivars were considered susceptible to both races of *M. incognita*. Considering the RF values, *M. incognita* race 1 seems to be more aggressive to coffee cultivars than race 3 (Table 4).

Coffee genotypes x *Meloidogyne* sp.

The inoculated plants had slight symptoms of root destruction and some egg masses were observed in some cultivars. On Catuaí Vermelho IAC 144, Obatã IAC 1669-20, Sarchimor IAC 4361 and Paraíso (H419-5-4-5-2) genotypes this population reproduced more than on the other genotypes (Table 4), with RF values ranging from 1.1 to 3.9. Although isolated from coffee, the population Msp 7 can be considered as a weak parasite of this host.

Coffee genotypes x *M. paranaensis*

The symptoms observed in the inoculated plants were swellings on the roots, without producing typical root knot nematode galls**.** Several developed egg masses were observed outside and inside the roots. This population was able to reproduce on all cultivars (Table 4) with RF values ranging from 8.8 to 31.5. The cultivars IAPAR 59 and Paraíso (H419-5-4-5-2) were as susceptible as the control Catuaí Vermelho IAC 144. The lower RF values observed in Obatã IAC 1669-20, Sarchimor IAC 4361, Tupi Amarelo IAC 5111 and Tupi Vermelho IAC 1669-33 genotypes were

Table 3 - Mean values of the number of eggs per gram of root system for ten *Meloidogyne* spp. Populations on seven coffee genotypes

Coffee genotypes	*Meloidogyne* populations									
	Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma10**
Catuaí Vermelho IAC 144	11314.3 Dc	6077.5 Dc	10354.4 Ac	3373.0 Bb	6626.3 Bc	1476.4Ab	89.9 Ba	2358.2Bb	48.4Ba	2565.0 Bb
IAPAR 59	3.4 Aa	20.6 Ab	17770.5 Be	42.3 Ab	2983.6 Ad	4550.0 Bd	14.7Ab	2336.6Bd	195.2Cc	3014.8Bd
Obatã IAC 1669-20	5116.5 Cb	4085.9 Cc	16148.6 Bd	638.0 Aa	9650.4 Bd	1509.6 Ac	695.4 Bb	1741.9 Ac	10.5 Aa	1666.1Bc
Sarchimor IAC 4361	8110.5 Dc	4404.5 Dc	12016.8 Bc	4167.6 Bb	3150.0 Ac	1469.7 Ac	372.6 Bb	2145.9 Bc	20.2 Aa	339.8 Ab
Paraíso (H 419-5-4-5-2)	26.3 Bb	25.3 Ab	37466.2 Bf	35.4 Ab	2191.8 Ae	2806.8 Be	98.5 Bc	3851.2 Be	5.8 Aa	751.4 Bd
Tupi Amarelo IAC 5111	2034.9 Cc	913.2 Bc	13742.8 Ad	1737.9 Ab	6968.8 Bd	1565.1 Ad	17.6 Aa	1614.1Ad	183.6 Bb	339.7 Ac
Tupi Vermelho IAC 1669 -33	70.9 Bb	1154.1 Bc	2065.3 Ad	1040.1 Ab	4925.6 Bd	1125.4 Ad	21.7 Aa	1013.0 Ad	27.8 Ab	389.7 Ac

Values within columns followed by same capital letter or row followed by same lower letter represent data not significantly different from each other at 5% probability using the Scott-Knott test. Values are means of eight replicate root systems. Population codes are given in Table 1.

Table 4 - Reproduction factor (RF) and type of reaction on seven coffee genotypes assessed eight months after inoculation with 10,000 eggs per plant of 10 *Meloidogyne* populations in greenhouse conditions

Coffee genotypes	*Meloidogyne* populations									
	Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma 10**
Catuaí Vermelho IAC 144	119.5 C S	58.6 C S	112.8 B S	31.7 C S	36.2 A S	14.1 A S	1.1 B MR	19.3 B S	0.3 A HR	4.7 B S
IAPAR 59	0.1 A HR	0.3 A HR	165.7 B S	0.4 A HR	41.7 A S	10.4 A S	0.1 A HR	20.7 B S	1.6 B MR	7.0 C S
Obatã IAC 1669-20	30.7 B S	73.4 C S	265.1 C S	9.8 B S	61.9 B S	16.5 A S	3.2 B MR	13.9 A S	0.1 A HR	12.4 C S
Sarchimor IAC 4361	84.0 C S	45.3 C S	167.0 B S	42.8 C S	35.3 A S	14.0 A S	3.9 B MR	11.3 A S	0.1 A HR	1.1 B MR
Paraíso (H419-5-4-5-2)	0.2 A HR	0.3 A HR	396.2 C S	0.3 A HR	25.5 A S	13.3 A S	1.4 B MR	31.5 B S	0.0 A HR	2.0 B MR
Tupi Amarelo IAC 5111	26.2 B S	9.3 B S	97.5 B S	16.4 B S	76.5 B S	13.5 A S	0.2 A HR	13.0 A S	1.3 B MR	1.2 B MR
Tupi Vermelho IAC 1669-33	0.7 A HR	13.2 B S	18.9 A S	11.8 B S	29.0 A S	8.6 A S	0.4 A HR	8.8 A S	0.2 A HR	0.8 A HR

HR: highly resistant (RF<1.0), MR: moderately resistant (RF>1.0); S: susceptible (RF>1.0) (Roberts, 2002)
Values within columns followed by same capital letter represent data not significantly different from each other at 5% probability using the Scott-Knott test.
Values are means of eight replicate root systems. Population codes are given in Table 1.

not significantly different from each other. According to the RF values, all the coffee genotypes were susceptible.

Coffee genotypes x *M. mayaguensis*

The root symptoms included swellings on the root tips and necrosis. There was no formation of typical galls. Egg masses were observed outside the root tissues. The population Mma 9 from guava reproduced on IAPAR 59 and Tupi Amarelo IAC 5111 cvs with RF values of 1.6 and 1.3, respectively. On the remaining genotypes this population reproduced very weakly (RF < 1) or did not reproduce at all (Tables 3, 4). Therefore, populations of *M. mayaguensis* from guava can be considered as a weak parasite of coffee.

For the population Mma10 from coffee (Costa Rica) the RF produced on Sarchimor IAC 4361, Paraíso (H 419-5-4-5-2), Tupi Amarelo IAC 5111 and Tupi Vermelho IAC 1669-33 were significantly lower than that observed for Catuaí Vermelho IAC 144, IAPAR 59 and Obatã IAC 1669-20. It should be noted that Tupi Vermelho IAC 1669-33 showed RF < 1. This genotype can be considered as resistant to this population of *M. mayaguensis* from coffee.

DISCUSSION

The galling and egg mass index were not reliable indicators of nematode multiplication rates because the symptoms of damage caused by different species of *Meloidogyne* on coffee are variable and very difficult to quantify. Based on these findings, the most pertinent variable for assessing reproduction of *Meloidogyne* spp. on coffee germplasm and also the host status of the coffee genotype for these nematodes is the number of eggs per gram of roots or the reproduction factor (RF). This result disagrees with the observations made by Hernandez et al. (2004), who considered galling index as a relatively good indicator of nematode multiplication rate for different *Meloidogyne* species on coffee. Most information on virulence in *Meloidogyne* spp. is known with regard to the Mi resistant gene in tomato. Selection experiments under laboratory conditions have shown that the proportion of virulent nematodes gradually increases after each successive generation on resistant tomato plants (Netscher, 1977). The same was observed for *M. exigua* on resistant IAPAR 59 plants (data not shown).

The results of this study were partially consistent with earlier findings by Bertrand et al. (2000) and Salgado et al. (2005). These authors detected the resistance of the cultivar IAPAR-59 to different populations of *M. exigua.* Their resistance was also observed in genotype Paraíso (H419-5-4-5-2) for the three populations of *M. exigua*. However, our results showed the first naturally resistance–breaking field populations of *M. exigua* on the cultivar IAPAR 59 derived from Hybrid carrying the gene Mex-1. In addition, this virulent population was observed even when they were not previously exposed to resistant cultivars. This resistance-breaking was also observed in the Paraíso (H 419-5-4-5-2) genotype. Since *M. exigua* is a meiotic parthenogenetic species (Triantaphyllou, 1985), mechanisms of genetic recombination or other mechanisms must be responsible for the increasing virulence. According to Cook & Evans (1987), meiotic parthenogenesis maintains the opportunity for sexual reproduction and this permits recombination between homologous chromosomes. These authors suggested that parthenogenesis does not reduce mutation rates and this may generate atypical populations.

This ability to multiply on such cultivars and their uniformly higher rate of reproduction compared with other *M. exigua* populations used in this study have to be viewed as very important and potentially dangerous characteristics of this population. Other reports of differential behavior of populations of *M. exigua* in Brazil have been suggested by Barbosa et al. (2007); however, the RF value for 'IAPAR 59' and 'Paraíso (H 419-5-4-5-2)' were very small (0.75 and 1.18, respectively) and it was difficult to characterize the virulence.

The nematode resistance originated from *C. canephora* and introgressed by crossing with Timor Hybrid has been considered as monogenic with an incomplete dominant expression (Noir et al., 2003), and also shown to induce postinfection reaction (Salgado et al., 2005). According to Anthony et al. (2005) resistance conferred by the Mex-1 gene is strongly associated with a hypersensitive reaction (HR). Recently, Alzipar et al. (2007) concluded that Mex-1 could have incomplete dominant expression because most of the F2 populations showed a gall index higher than the mean value of the resistant parent.

In the present work, all coffee cultivars were susceptible to two *M. incognita* populations, but race 1 (EST I1) from São Paulo was more aggressive than race 3 (EST I2) from Paraná State. Several studies have been done on the reaction of *C. arabica* or *C. canephora* genotypes to *M. incognita* under greenhouse or field conditions in Brazil and other countries (Carneiro, 1995; Anzueto et al., 2001; Hernandez et al., 2004; Tomazini et al., 2005). In these studies, some progenies of *C. canephora* or Ethiopian *C. arabica* accessions were proved to be effective against those nematodes. However, in these studies, the *Meloidogyne* species were sometimes incorrectly identified (Carneiro et al., 2004).

Meloidogyne sp. collected from coffee in Garça, São Paulo State, presented low aggressiveness to coffee genotypes in greenhouse and field conditions (Gonçalves W, pers. comm.). This population displaying the esterase phenotype S1 (= S2), presented a perineal pattern resembling *M. incognita* (Oliveira et al., 2006). Other morphological characters using scanning electron microscopy and SCAR markers should be used to characterize this species.

Meloidogyne mayaguensis is considered the most dangerous species in coffee fields in Cuba (Rodríguez et al., 1995; 2001). The two populations of *M. mayaguensis* (from guava, Brazil and from coffee, Costa Rica) revealed differences in aggressiveness suggesting a physiological

specialization of this species on coffee. Moreover, the low values of RF indicate that coffee is a poor host for *M. mayaguensis***.**

In Brazil, *M. mayaguensis* was reported for the first time in the semi-arid zone of the northeastern region in the States of Pernambuco and Bahia causing severe damage in guava plantations (Carneiro et al., 2001). However, despite its pathogenicity and distribution, there is no report of this species as being a potential coffee parasite in Brazil. Considering the diversity of *Meloidogyne* species able to parasitize coffee in Brazil and Central America (Carneiro et al., 2004; Hernandez et al., 2004; Muniz et al., 2008) and in terms of virulence, experiments must be conducted with more than one population of each *Meloidogyne* species when evaluating resistance of new coffee genotypes. Such information will be of considerable interest for the development of integrated management programs and, especially, for the development of durable resistant cultivars adapted to the different situations in coffee-growing areas.

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