Parasitism of Meloidogyne exigua races 1 and 2 in coffee plants derived from Timor Hybrid

INTRODUCTION

Most of the Brazilian coffee crop is comprised by susceptible cultivars to Meloidogyne exigua Goeldi, 1887. The parasitism of this species of root knot nematode (RKN) has been causing decreases of 20 to 68% in the production of susceptible coffee trees infested by nematodes (BARBOSA et al., 2004). Several management strategies have been recommended to minimize the damage caused by RKN in coffee (CAMPOS & SILVA, 2008); however, genetic resistance is the most effective method to cultivate coffee in infested soils. Coffea arabica L. cultivars with genetic resistance to nematodes has been selected from the introgression of resistance genes present in C. canephora, like MGS Catiguá 3, IAPAR 59, IAC 125 RN, Acauã, Catucaiam 78515 (CARVALHO et al., 2008) and IPR 100 (REZENDE et al., 2017). The first three are derived from the Timor Hybrid, the penultimate of Icatu x Catuaí and the

ABSTRACT: To investigate the degree of parasitism of two populations of Meloidogyne exigua, the gall index (GI) and the reproduction factor (RF) of M. exigua races 1 (Est E2) and 2 (Est E1) were analyzed in 47 progenies on F 3:4 or F4:5 generation derived from the crossing between Coffea arabica cv. Catuaí Amarelo and Timor Hybrid. C. canephora cv. Apoatã IAC 2258 and C. arabica cv. Catuai Vermelho IAC 144 were used as resistance and susceptibility checks, respectively. The genotypes that were classified as resistant or susceptible by RF were similarly classified by GI, showing a close relationship between both methodologies. The data also indicated no differences in virulence between the nematode populations, since the progenies showed similar resistance reactions to the M. exigua races 1 and 2. According to GI from the 47 mother plants evaluated, 27 progenies (57.4%) were classified as resistant to M. exigua races 1 and 2, with GI ranging from 0.0 to 1.4 and 20 progenies (42.6%) were susceptible with GI from 2.6 to 4.4. These results showed that most of the evaluated germplasm was very promising in relation to the development of new Arabica coffee cultivars with resistance to M. exigua.

Key words: root knot nematode, genetic resistance, Coffea arabica.

RESUMO: Com o objetivo de investigar o grau de parasitismo de duas populações de Meloidogyne exigua, o índice de galhas (IG) e o fator de reprodução (FR) de M. exigua raças 1 (Est E2) e 2 (Est E1) foram analisados em 47 progêniases na geração F 3:4 ou F4:5, derivadas do cruzamento entre Coffea arabica cv. Catuaí Amarelo e Híbrido de Timor. Plantas de C. canephora cv. Apoatã IAC 2258 e C. arabica cv. Catuai Vermelho IAC 144 foram usadas como padrão de resistência e de suscetibilidade, respectivamente. Os genótipos que foram classificados como resistentes ou suscetíveis pelo FR foram similarmente classificados pelo IG, mostrando uma estreita relação entre as duas metodologias para a avaliação da resistência. Os dados também indicaram que não houve diferenças quanto à virulência entre as duas populações do nematoide, uma vez que as progêniases mostraram similar reação de resistência a M. exigua raça 1 e 2. De acordo com o IG, das 47 plantas-mãe avaliadas, 27 progêniases (57,4%) foram classificadas como resistentes a M. exigua raças 1 e 2, com IG variando de 0,0 a 1,4 e 20 progêniases (42,6%) foram suscetíveis, com IG variando de 2,6 a 4,4. Esses resultados mostraram que a maioria dos germoplasmas avaliados foi muito promissora em relação ao desenvolvimento de novas cultivas de café Arábica com resistência a M. exigua.

Palavras-chave: nematoide das galhas, resistência genética, Coffea arabica.

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latter is derived from BA-10 (PEREIRA & BAIÃO, 2015; SERA et al., 2017). The grafting cultivar of *C. canephora* Apoatã IAC 2258 is also highly resistant (FAZUOLI et al., 2002).

After the report of coffee rust (*Hemileia vastatrix* Berk. & Br.) in Brazil, in 1970, germplasms carrying genes of resistance to *H. vastatrix,* notably coffee plants derived from the Timor Hybrid, were introduced from the Coffee Rust Research Center (CIFC), in Oeiras, Portugal. The Timor Hybrid designation was given to the progenie of a coffee tree with a phenotype similar to *C. arabica,* originated from natural hybridization between *C. arabica* and *C. canephora,* reported in a plantation of the *C. arabica* cv. Typica on Timor Island, in 1917. The Timor Hybrid comprises coffee trees with high genetic variability that carry resistant genes to various biotic factors, such as RKN (BERTRAND et al., 1997, BETTENCOURT & FAZUOLI, 2008).

Considering that the plant resistance to RKN can be races and / or species specific (ROBERTS, 2002) and the occurrence of high intraspecific variability in populations of *M. exigua* coffee parasites (CARNEIRO & COFCEWICZ, 2008, MUNIZ et al., 2008), the objectives of this research were: i) to investigate the degree of parasitism of two races of *M. exigua* (races 1 and 2); ii) to compare the *M. exigua* assessment races 1 or 2 per plant in three holes of approximately 1 cm depth around the seedlings. After inoculation, the plants were maintained under greenhouse conditions at 24-29 °C, with the regular irrigation control and the use of vertical sticky traps for insect control. The experiment was arranged in a completely randomized design, with 10 replicates and single-plant plots.

**MATERIALS AND METHODS**

*Meloidogyne exigua* populations

The initial nematode populations were obtained from coffee roots parasitized by *M. exigua* collected from Campinas, SP (Est E2, race 1) and São Sebastião do Paraíso, MG (Est E1, race 2) and the race test was determined according to MUNIZ et al.(2008). There after, the nematodes were multiplied in pots containing plants of the susceptible *C. arabica* Catuai Vermelho IAC 144 under greenhouse conditions. Inoculum were obtained according to MUNIZ et al. (2008). Additionally, the final nematode population and reproduction factor (RF) was determined at four replicates from 21 Catuai

Plants

Forty-seven mother plants on F$_{3:4}$ or F$_{4:5}$ generation (Figure 1), derived by pedigree method from the crossing between Catuai Amarelo (CA) and Timor Hybrid (TH), were assessed to resistance to *M. exigua* races 1 and 2 in greenhouse conditions. The first combination resulting of the performed cross was designated H 419, from the hybridization between CA IAC 30 and TH CIFC 2570 (UFV 445-46), the second was designated H 514, from the cross between CA IAC 86 and TH CIFC 2570 (UFV 440-10) and the third, H 516, from the hybridization between CA IAC 86 and TH CIFC 2570 (UFV 446-08). From the combination H 419,14 progenies F$_{3:4}$ from the four coffee trees F$_i$ named H 419-3 (2), H 419-5 (5), H 419-6 (3) and H 419-10 (4) and 2 progenies F$_{4:5}$ from the F$_i$ H 419-5 were evaluated. Also, 13 progenies F$_{3:4}$ from the hybrid F$_i$ H 514-11 and 18 progenies F$_{4:5}$ from the hybrid F$_i$ H 516-2 were evaluated.

The cultivar Obatã IAC 1669-20 and the Sarchimor selection IAC 4361, both from the hybrid CIFC H 361-4 (Villa Sarchi x TH CIFC 832-2), the variety Laurentii col.5, and the selection C2258 e86 Ad of *C. canephora* were used as experimental checks. The cultivars Apoatã IAC 2258 of Coffea canephora and Catuai Vermelho IAC 144 of *C. arabica* were used as susceptibility and resistance checks, respectively.

**Experiment conduction**

Four months age seedlings of 47 progenies and six checks treatments were transplanted into 300 mL plastic pots containing a mixture of soil and sand 1:1 (v:v), autoclaved at 127 °C for two hours, and fertilized with simple superphosphate and with a controlled release complex fertilizer (NPK 16-8-12), supplying the soil with nutrients amount equivalent to 0.429 kg m-3 of N, 0.567 kg m-3 of P and 0.498 kg m-3 of K. After 30 days, the plants were inoculated with approximately 3,000 eggs + second-stage juveniles (J2) of *M. exigua* races 1 or 2 per plant in three holes of approximately 1 cm depth around the seedlings. After inoculation, the plants were maintained under greenhouse conditions at 24-29 °C, with the regular irrigation control and the use of vertical sticky traps for insect control. The experiment was arranged in a completely randomized design, with 10 replicates and single-plant plots.

**Resistance and degree of parasitism of two races of *M. exigua* assessment**

Plant response from all 47 progenies and checks (Table 1) was evaluated 160 days after inoculation, using the gall index (GI), according to SASSER et al. (1984). Additionally, the final nematode population and reproduction factor (RF) was determined at four replicates from 21 Catuai
Amarelo X Timor Hybrid germplasm and six check plants (Table 2). The final population estimated was obtained by counting the nematodes in Peters' slides under a light microscope, quantifying the eggs and juveniles of both *M. exigua* races extracted from the entire roots of each plant. The RF was determined according to OOSTENBRINK (1966).

The similarity between the responses of each genotype to infection by races 1 and 2 of *M. exigua* was assessed by the following parameters: Accuracy of method (AM), calculated from sum of simultaneously resistant plants or simultaneously susceptible to the races 1 and 2 of *M. exigua* divided by total number of plants evaluated; False positive rate (FPR), calculated by dividing the number of resistant plants to race 1, but susceptible to race 2 of *M. exigua*, by total number of plants evaluated; False negative rate (FNR), calculated by division of number of susceptible plants to race 1, but resistant to race 2 of *M. exigua*, by total number of plants evaluated; Total rate error (TRE), calculated from sum of resistant plants to race 1, but susceptible to race 2 and the number of susceptible plants to race 1, but

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Table 1 - Average values of the gall index (GI), percentage of plants with GI less than or equal to two (GI ≤ 2) and classification (C), according to Oostenbrink (1966), S = susceptible and R = resistant, 160 days after the inoculation with Meloidogyne exigua races 1 and 2.

| Germplasm | -----Race 1----- | -----Race 2----- | Germplasm | -----Race 1----- | -----Race 2----- |
|-----------|------------------|------------------|-----------|------------------|------------------|
|           | GI               | GI ≥ 2           | C         | GI               | GI ≥ 2           | C         |
| Apoatã IAC 2258 | 0 100 R 0 100 R | CV 144        | 4.4 0     | S 4.3            | 0 S |
| C 2258 c86 Ad* | 0 100 R 0 100 R |               |           |                  |                  |
| Laurentii col.5 | 0 100 R 0 100 R |               |           |                  |                  |
| Sarchimor IAC 4361 | 0 100 R 0 100 R |               |           |                  |                  |
| Obatã IAC 1669-20 | 3.4 0 S 3.7 0 S |               |           |                  |                  |

H516-3-1-1-14 | 0 100 R 0 100 R | H419-5-2-1-14 | 1.4 60 R 0.6 80 R |
H419-3-1-3-8 | 0 100 R 0 100 R | H419-5-3-3-8 | 0.9 70 R 0.3 90 R |
H419-6-3-2-9 | 0 100 R 1.2 60 R | H419-5-4-5-2 | 0 100 R 0 100 R |
H419-6-3-6-5 | 0 100 R 0 100 R | H419-5-4-5-10 | 0 100 R 0 100 R |
H419-6-3-6-10 | 0 100 R 0 100 R | H419-5-5-5-4-1 | 3.8 0 S 3.4 10 S |
H419-5-5-5-4-10 | 3.4 0 S 3.7 0 S | |
H516-2-1-1-18-1 | 3.9 0 S 2.6 20 S | H419-10-2-1-17 | 0 100 R 0 100 R |
H516-2-1-1-18-2 | 3.9 0 S 3.2 0 S | H419-10-5-1-15 | 0 100 R 0 100 R |
H516-2-1-1-18-3 | 4.1 0 S 3.8 0 S | H419-10-6-2-1 | 0 100 R 0 100 R |
H516-2-1-1-18-4 | 3.5 0 S 3.7 0 S | H419-10-5-1-15 | 0 100 R 0 100 R |
H516-2-1-1-18-5 | 3.0 0 S 3.5 0 S | |
H516-2-1-1-18-6 | 3.7 0 S 3.2 0 S | H514-11-5-5-1 | 0 100 R 0 100 R |
H516-2-1-1-18-7 | 4.4 0 S 2.6 20 S | H514-11-5-5-4 | 0 100 R 0 100 R |
H516-2-1-1-18-8 | 4.1 0 S 3.7 0 S | H514-11-5-5-5 | 0 100 R 0 100 R |
H516-2-1-1-18-9 | 3.4 0 S 2.7 20 S | H514-11-5-5-8 | 0 100 R 0 100 R |
H516-2-1-1-18-10 | 3.6 0 S 3.2 10 S | H514-11-5-5-9 | 0 100 R 0 100 R |
H516-2-1-1-18-12 | 3.6 0 S 3.1 0 S | H514-11-5-5-11 | 0 100 R 0 100 R |
H516-2-1-1-18-13 | 4.2 0 S 3.6 10 S | H514-11-5-5-12 | 0 100 R 0 100 R |
H516-2-1-1-18-15 | 4.0 0 S 3.3 0 S | H514-11-5-5-13 | 0 100 R 0 100 R |
H516-2-1-1-18-16 | 4.1 0 S 3.7 0 S | H514-11-5-5-15 | 0 100 R 0 100 R |
H516-2-1-1-18-17 | 2.9 30 S 3.4 30 S | H514-11-5-5-16 | 0 100 R 0 100 R |
H516-2-1-1-18-18 | 4.0 0 S 3.4 20 S | H514-11-5-5-20 | 0 100 R 0 100 R |

*Clone selected in Adamantina, São Paulo State, Brazil, †Cultivar Catuai Vermelho IAC 144.

RESULTS AND DISCUSSION

The cultivar Catuai Vermelho IAC 144 was efficient in the multiplication of M. exigua, proving the viability of the inoculum used, since the GI values were 4.4 and 4.3 (Table 1) and RF were 5.1 and 3.1 (Table 2), respectively, for M. exigua races 1 and 2. Conversely, the mother plants of C. canephora, including the cultivar Apoatã IAC 2258, C 2258 c86 Ad and Laurentii col.5; and C. arabica X C. canephora Sarchimor IAC 4361 showed low nematode reproduction rates, confirming the resistance of this species to RKN (CURI et al., 1970, BERTRAND et al., 2001).

According to the results for GI and RF of both resistant and susceptible checks of coffee
trees, it was observed a close similarity of the performance between the adopted methodologies to evaluate the resistance. Similarly, the same progenies classified as resistant based on GI (Table 1) were classified at this same category by RF (Table 2), resulting in four check plants and 8 Catuai Amarelo X Timor Hybrid germoplasm resistant to both *M. exigua* race 1 and 2.

Galls formed by RKN are not essential to nematode development and reproduction and in some cases are only good indicators of the reactions in affected tissues, since resistant plants can present galls in the absence of nematode reproduction and susceptible plants not always present galls (LORDELLO, 1984; MOURA, 1997; ROBERTS et al., 1998). However, we found a close relationship

Table 2 - Average values of the reproduction factor (RF) and classification of coffee trees (C) 160 days after inoculation with 3,000 eggs + J2 of *Meloidogyne exigua* races 1 and 2.

| Germplasm                | Race 1 |   | Race 2 |   |
|--------------------------|--------|---|--------|---|
|                          | RF     | C’|        | RF | C’|
| Catuai Vermelho IAC 144  | 5.1    | S | 3.1    | S  |
| C. arabica               |        |  |        |    |
| Apoatã IAC 2258          | 0      | R | 0      | R  |
| C 2258 c86 Ad”           | 0      | R | 0      | R  |
| Laurentii Col.5          | 0      | R | 0      | R  |
| C. canephora             |        |  |        |    |
| Sarchimor IAC 4361       | 0.06   | R | 0      | R  |
| Obatã IAC 1669-20        | -      | - | 4.4    | S  |
| Catuai Amarelo X Timor Hybrid |      |   |        |    |
| H419-3-1-1-14            | 0      | R | -      | -  |
| H419-3-1-3-8             | 0.04   | R | -      | -  |
| H419-5-2-1-14            |        |  | 0.05   | R  |
| H419-5-4-5-2             | 0.01   | R | -      | -  |
| H419-5-4-5-10            | 0      | R | 0.01   | R  |
| H419-6-3-2-9             | 0.05   | R | 0.01   | R  |
| H419-6-3-6-5             | 0.02   | R | 0      | R  |
| H419-10-5-1-15           | 0.01   | R | 0      | R  |
| H419-10-6-2-1            | 0.01   | R | 0      | R  |
| H419-10-6-2-12           | 0      | R | 0      | R  |
| H514-11-5-5-1            | 0.01   | R | 0      | R  |
| H514-11-5-5-4            | 0.01   | R | 0      | R  |
| H514-11-5-5-7            |        |  | 0      | R  |
| H514-11-5-5-7”           |        |  | 0      | R  |
| H514-11-5-5-9            | 0      | R | -      | -  |
| H514-11-5-5-12           | 0.03   | R | -      | -  |
| H514-11-5-5-14           |        |  | 0      | R  |
| H514-11-5-5-14”          |        |  | 0      | R  |
| H514-11-5-5-16           | 0      | R | -      | -  |
| H514-11-5-5-21           | 0      | R | -      | -  |
| H514-11-5-5-22           | 0      | R | -      | -  |

*Classification of coffee trees according to Oostenbrink (1966), S = susceptible and R = resistant. **Clone selected in Adamantina, São Paulo State, Brazil.
between GI and RF. Therefore, selection of coffee plants aiming resistance to *M. exigua* under greenhouse conditions through GI can be a suitable methodology to evaluate a large number of plants in a short period of time, as it is faster, less laborious and less expensive process than RF.

From the 47 mother plants evaluated to access the resistance to *M. exigua* races 1 and 2, 27 progenies (57.4%) were classified as resistant to both races, with GI ranging from 0.0 to 1.4 and 20 progenies (42.6%) were susceptible with GI from 2.6 to 4.4 (Table 1). The data also indicated no differences in virulence between the nematode populations, since the progenies showed similar reactions to resistance or susceptibility in relation to the *M. exigua* race 1 and 2. There was only a higher reproduction of *M. exigua* race 1 in the cultivar Catuaí Vermelho compared to *M. exigua* race 2, confirmed by the RF values of 5.1 and 3.1, respectively (Table 2), as previously observed by MORERA & LOPEZ (1988).

The responses of each coffee genotype to infection by races 1 and 2 of *M. exigua* are showed in table 3. The AM calculated with the data from the comparative analysis of the reaction of coffee trees to infection by races 1 and 2 of *M. exigua* was 100% when considering the total plant population of the experiment, as well as, in a group isolated from plants of the ‘Catuaí Amarelo x Timor Hybrid’ progenies. As a result of the maximum observed accuracy, the FPR, FNR and TRE were equal to zero.

The calculated $\chi^2$ values were significant for the RF of the total plant population and for the GI of ‘Catuaí Amarelo x Timor Hybrid’ progenies and also to the total plant population of the experiment. Thus, it indicates the existence of an association between the reactions of plants to races 1 and 2 of *M. exigua*. The Yule’s association coefficients, equal to 1, revealed the positive high-intensity association between observations, allowing us to say that there are no differences in virulence between nematode populations.

Based on biochemical characterization of 57 populations of *M. exigua* race 1 from coffee plantations in Minas Gerais State, OLIVEIRA et al. (2005b) found the typical E1 esterase phenotype in 13 populations of *M. exigua*, while most populations (77.2%) exhibited the E2 phenotype. Despite these differences in isoenzymatic patterns, no intraspecific physiological variability was observed in the populations studied by the authors, since there were no significant differences between nematode reproduction rate in the host plants tested: coffee, peppers, tomatoes, beans, cocoa and soybeans. The *M. exigua* phenotypes E1 and E2 have also been identified in Brazilian coffee plantations by several authors (ESBENSHADE & TRIANTAPHYLLOU, 1990, OLIVEIRA et al., 2005a) while E3 phenotype was reported by MUNIZ et al. (2008).

Regarding the coffee plants derived from Villa Sarchi x Timor Hybrid, the selection Sarchimor IAC 4361 (C 1669-13) showed resistance to *M. exigua*, presenting 100% of their descendants with GI = 0 and FR = 0. Conversely, the cultivar Obatã IAC 1669-20 showed susceptible, with GI mean of 3.7 and RF mean of 4.4 and all progeny showed GI > 2. Although, both coffee plants were derived from TH CIFC 832/2, which is a source of resistance to *M. exigua* (BERTRAND et al., 2000), it is assumed that the cultivar Obatã IAC 1669-20, being from a

Table 3 - Estimation of parameters related to the reactions of coffee trees to infection by races 1 and 2 of the nematode *Meloidogyne exigua*, assessed by gall index (GI) and reproduction factor (RF).

| Parameter                              | ‘Catuaí Amarelo x Timor Hybrid’ progenies | --------- | Total Plant Population--------- |
|----------------------------------------|------------------------------------------|----------|-------------------------------|
|                                        | GI | FR | GI | FR |
| Accuracy of method (AM) ($\gamma_0$)   | 100| 100| 100| 100|
| False positive rate (FPR) ($\gamma_0$) | 0  | 0  | 0  | 0  |
| False negative rate (FNR) ($\gamma_0$) | 0  | 0  | 0  | 0  |
| Total rate error (TRE) ($\gamma_0$)   | 0  | 0  | 0  | 0  |
| Pearson chi-square ($\chi^2$)          | 470*| -  | 530*| 140*|
| Yule association coefficient (Q)       | 1  | 1  | 1  | 1  |

(-) Not calculated values; * significantly at 5% probability.
more advanced generation, may have lost resistance genes since it was selected for agronomic traits and resistance to the coffee rust caused by H. vastatrix.

From the 16 progenies of H 419, 14 (87.5%) showed resistance, especially those from prefixes H 419-3-1, H 419-5-4-5, H 419-6-3-6 and H 419-10, that presented all the progenies free of galls. The progenies of the 13 mother plants derived from H 514-11-5-5 presented the GI mean of 0.0, while all the 18 mother plants of H 516 were considered susceptible. It was observed that the alleles that govern the expression of M. exigua resistance present in C. canephora were effectively transferred to some Timor Hybrid selections, among them the introductions UFV 445-46 and UFV 440-10. Those introductions originated; respectively, the populations derived from the combinations H 419 and H 514, since they presented progenies with reactions similar to resistant parents. This result is of great interest as some sources of resistance to M. exigua present in other Coffea species are difficult to transfer and almost total loss of resistance may occur during backcrossing to C. arabica (FAZUOLI et al., 1977).

Our results showed that resistance to M. exigua reported in coffee plants derived from Timor Hybrids is consistent with previous reports from FAZUOLI et al. (1977), GONÇALVES & PEREIRA (1998), SILVAROLLA et al. (1998), BERTRAND et al. (2000), SALGADO et al. (2002). Assuming that an interspecific origin (C. arabica x C. canephora) of the Timor Hybrid (BETTENCOURT & FAZUOLI, 2008) and the monogenic control of resistance to M. exigua is conferred by a dominant gene (NOIR et al., 2003), our results identified that a homozygous population in H 514 was reached early in the F2 or F3 generation, since 100% of the 14 F3:4 progenies evaluated proved H 514 was reached early in the F2 or F3 generation, our results identified that a homozygous population in the studied nematode populations, since the progenies showed similar resistance reactions to the M. exigua races 1 and 2. All the genotypes that were classified as resistant or susceptible by RF were similarly classified by GI, showing a close relationship between both methodologies. Our results also showed that most of the evaluated germplasm was very promising in relation to the development of new Arabica coffee cultivars with resistance to nematode M. exigua.

CONCLUSION

There was no difference in virulence between the studied nematode populations, since the progenies showed similar resistance reactions to the M. exigua races 1 and 2. All the genotypes that were classified as resistant or susceptible by RF were similarly classified by GI, showing a close relationship between both methodologies. Our results also showed that most of the evaluated germplasm was very promising in relation to the development of new Arabica coffee cultivars with resistance to nematode M. exigua.

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AUTHORS’ CONTRIBUTIONS

Conceptualization: AAP, WG and OGF. Data acquisition: WG, LBC and BJRF. Design of methodology and data analysis: WG, CMGO and OGF. WG and OGF prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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