RESEARCH ARTICLE / *ARTIGO*

Defense responses to *Meloidogyne exigua* in resistant coffee cultivar and non-host plant

Rodrigo V. Silva[1,2], Rosângela D.L. Oliveira[1], Patrícia S. Ferreira[1], Aline O. Ferreira[1], Fabrício A. Rodrigues[1]

[1]Departamento de Fitopatologia, Laboratório de Nematologia, Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil; [2]Instituto Federal Goiano - Campus Morrinhos, Departamento de Agronomia, 75650-000, Morrinhos, GO, Brazil

Author for correspondence: Rosângela D. L. Oliveira, e-mail: rdlima@ufv.br

ABSTRACT

The resistance of coffee plants to *Meloidogyne exigua* is conferred by the gene *Mex-1*. However, the mechanisms of resistance still need to be clarified. Therefore, the penetration, development and reproduction of two populations of *M. exigua* (M1, isolated from the coffee plant and M2, from rubber tree) in susceptible (*Coffea arabica* 'Catuai') and resistant (*C. canephora* 'Apoatã') cultivars were studied. A greater quantity of J2 from M1 penetrated the susceptible cultivar, but there was no difference between the cultivars for M2. Although the resistant coffee cultivar formed some galls, the nematode did not reproduce. M2 did not induce the formation of galls or the production of eggs in either cultivars. Events related to hypersensitive reaction (HR) were observed as well as other defense responses of the coffee cultivar against *M. exigua,* which inhibited the formation of the feeding site, provoked emigration of the J2 and delayed or inhibited development and reproduction. The response of the non-host plant was more effective, because it did not allow development of the nematode or, consequently, its reproduction. It was concluded that the coffee cultivar's resistance to *M. exigua* is not restricted to HR, but rather to a set of defense responses, both constitutive and induced, expressed after nematode penetration, especially phenolic-like compounds.

Key words: *Coffea* spp., hypersensitive reaction, phenolic compounds, resistance, root-knot nematode.

INTRODUCTION

In Brazil and in Latin America, parasitism by root-knot nematodes, especially *Meloidogyne exigua*, is considered one of the main factors responsible for reduced productivity in *Coffea arabica* cultivars (Campos & Villain, 2005). Damage levels vary, but may reach a 45% reduction in productivity, as observed by Barbosa et al. (2004) in a plantation in the state of Rio de Janeiro. Losses arising from nematode infestation, leading to destruction of the affected plants and devaluation of the infested property, were reported by Campos et al. (1985).

Interaction between the host plant and sedentary endoparasitic nematodes, especially those belonging to the genus *Meloidogyne,* is highly specialized and complex (Williamson & Hussey, 1996). Events that involve stimulus for hatching, attraction, penetration of the host tissues, recognition of the susceptible tissue for induction of the feeding site, anatomic modification in the cells and alteration in the plant's gene expression are all fundamental for the success of the infection (Davis & Mitchum, 2005). The second-stage juvenile (J2), the mobile and infectious phase of root-knot nematodes, penetrates the meristematic region of the root (Endo, 1975; Mendes et al., 1977), and from there migrates through the cells of the cortex until reaching the differentiated vascular tissue. Here, it selects some cells of the parenchyma, in which it induces a series of changes in gene expression, in order to form an elaborate feeding site that will provide nutrients for its development and reproduction (Davis et al., 2008). The clearest morphological response in the host plant is the formation of root galls, which hamper absorption of water and nutrients (Kirkpatrick et al., 1991).

The use of resistant cultivars is the most economical and efficient way to replace infected and unproductive plantations. A resistant plant restricts or prevents the nematode's reproduction by activating defense mechanisms, which can be pre- or post-infective (Huang, 1985). Mechanisms of pre-infection limit penetration of J2, and they include pre-existing morphological factors or the production of root exudates that do not attract J2 or may repel them (Jatala & Russell, 1972; Huang, 1985). In post-infection mechanisms, the activation of physiological and molecular processes in the plant inhibits formation of the feeding site, and prevents or delays J2 development and/or reproduction of the adult female (Giebel, 1982; Huang, 1985; Anwar & McKenry, 2000). In general, the latter type of process is more effective in the control of species of *Meloidogyne*.

The main strategy for developing cultivars that are resistant to *M. exigua* is by transferring the resistance gene from *Coffea canephora* to *C. arabica* by means of back-

crosses. It is now known that resistance to this pathogen is conferred at least by one dominant gene known as *Mex-1* (Noir et al., 2003). However, the mechanisms of resistance to this nematode in the coffee cultivar still need to be clarified. Information of this type will be useful for research towards the development of *C. arabica* cultivars that are resistant to this nematode.

A methodology with high but as yet barely explored potential among studies on incompatibility mechanisms in the pathogen-host interaction, especially for plant nematodes, is the use of populations ("races" or "biotypes") from the same pathogen species that differ in terms of their capacity to infect genotypes of the host and non-host plant. These studies may be useful in clarifying, at least in part, the mechanisms involved in coffee resistance to *M. exigua*.

To this end, the current study aimed to evaluate pre- and post-infection development of populations of *M. exigua,* which differ in their capacity to infect susceptible and resistant coffee cultivars.

MATERIAL AND METHODS

General information

The study was carried out in a greenhouse at the Department of Plant Pathology at the Universidade Federal de Viçosa (UFV), Minas Gerais state, at latitude 20° 45' 14" S and longitude 42° 52' 53" W. During the experimental period, the minimum and maximum air temperatures were, respectively, 19.6 and 28.3ºC.

Populations of *M. exigua*

Two populations of *M. exigua* were studied, which had already been characterized by Silva et al. (2007). Population 1 (M1) was collected from a coffee cultivar in the municipality of Manhuaçu, MG, and belonged to race 2 and esterase phenotype (EST) E2 (Rm 1.60 and 1.90); population 2 (M2) came from a rubber plant in São José do Rio Claro, MT, and belonged to race 3 and phenotype EST E1b (Rm 1.10 and 1.60). These populations were multiplied for about one year on *C. arabica* 'Catuaí Vermelho IAC 44' and on a rubber plant clone 'RRIM 600', respectively, in a greenhouse.

Coffee cultivars and plant inoculation

Seedlings from coffee 'Catuaí Vermelho IAC 44' (susceptible) and *C. canephora* 'Apoatã IAC 2258' (resistant) at the stage of three to four pairs of completely developed leaves were used in this study. Eggs of *M. exigua* were extracted using the method of Boneti & Ferraz (1981) and then incubated in deionized water in a growth chamber at 26ºC for second-stage juveniles (J2) to hatch. This procedure was carried out according to the Baermann's method (Baermann, 1917) with modifications, using a bowl instead of a funnel. Nematodes that hatched within 24 hours were discarded with the aim of standardizing the age of the J2. For four days J2 were collected daily and the

suspension was calibrated in a Peters counting chamber to reach the concentration of 500 J2 per mL of suspension. Then each plant was inoculated with 2000 J2, distributed in three openings, placed 1 cm from the stalk, at a depth of approximately 3 cm.

Forty-eight hours after inoculation, the plants were removed from the plastic pots and their roots were washed to eliminate any J2 that had not yet penetrated, in order to standardize the age of the J2 at two days. Next, each plant was transferred to a 2 L clay pot, and filled with previously sterilized soil and sand (2:1).

Analysis of penetration and development of *M. exigua*

First evaluation occurred before the plant transplanting, two days after inoculation (DAI). Three plants from each cultivar were collected every two days until the tenth day, and from then on they were collected at 15, 20, 30 and 40 DAI. The localization of the different development stages of the nematode in the roots was determined by *in situ* staining (Byrd et al., 1983) with some modifications. Roots that had been pre-washed in running water were dipped in a solution of sodium hypochlorite at 2% for 12 minutes. The roots were then washed again for 30 to 45 seconds to remove the excess sodium hypochlorite and placed in water for 15 minutes. The roots were transferred to a flask containing boiling 120 mL of water to which 4 mL staining solution (75 mL of distilled water, 25 mL of glacial acetic acid and 350 mg of acid fuchsin) and kept there for 30 seconds after boiling. After cooling to room temperature, the excess staining solution was removed in running water and the roots were heated to boiling point in 20 to 30 mL of acidified glycerol with 5 drops of hydrochloric acid (HCl 5N). Roots taken from the whole root system were spread in a glycerine film between two glass slides (7.5 × 2.5 cm) and analyzed under a light microscope (Carl Zeiss Axio Imager A1) to evaluate the number of nematodes within the roots and their development stage. During the evaluation period, the slides were covered with aluminum paper and stored in the refrigerator at 6ºC to avoid roots oxidation.

Reproduction analysis

At 50 DAI, the reproduction of the nematode was also evaluated on six plants in each treatment. To confirm the viability of the inoculum of populations M1 and M2, eggs production of these populations was determined on seedlings of the sweet pepper 'Early California Wonder' and the rubber plant clone RRIM 600. The pepper plants presented a mean number of 11,260 eggs and the rubber plant, 5,984 eggs.

Histopathological analysis

Roots were sampled at 2, 4 and 6 DAI, with three replications per treatment, and for each evaluation period three root fragments (0.5 to 1.0 cm) were selected. For inclusion in the resin, the root sections were fixed in glutaraldehyde 2.5% prepared in sodium cacodylate buffer

0.1 M, pH 7.2, for 12 hours. Next, the samples were washed with the same buffer to remove the excess fixative, and post-fixed with osmium tetroxide at 1%. Dehydration was carried out in graded alcohol series (30, 50, 70, 80, 95 and 100%) twice, with each phase lasting 15 minutes. In a next step, samples were embedded in Spurr resin. After infiltration, the roots were arranged in flat plastic trays containing resin, and taken to be polymerized in the oven at 65ºC for 12 hours. Semi-thin transversal sections of the roots, measuring 0.5 to 1.5 μm in thickness, were obtained in a ultramicrotome and stained with toluidine blue 0.05% prepared in acetate buffer (pH 4.7) for observation under light microscope (Carl Zeiss Axio Imager A1).

Experimental design and statistical analysis

The experiment was installed in a completely randomized design in a factorial scheme of 2 x 2 (2 coffee cultivars x 2 populations of *M. exigua*) with three plants per each treatment for each evaluation time. Each experimental unit was composed of one plant. Data for reproduction and number of individuals in each evaluation time were submitted to analysis of variance and means tests using the program SAEG (2007).

RESULTS

There was a significant interaction between the factors nematode population and coffee cultivar, as well as a significant difference ($P \leq 0.05$) for the factors in isolation.

The number of individuals of *M. exigua* that penetrated the roots of the coffee plant, the development stage and the reproduction factor differed ($P \leq 0.05$) between the two cultivars (Table 1). Many J2 ($P \leq 0.05$) of the M1 population (isolated from the coffee plant) penetrated the roots of the susceptible Catuaí cultivar plants as compared with the resistant Apoatã cultivar. The same pattern happened for the M2 population (from the rubber plant) in the initial phase of infection, but it did not differ from 8 DAI onwards. Very few J3-J4 and females from the M2 population were found in this cultivar, even after the 25th day.

On the second and fourth DAI various agglomerates of J2 from M1 were observed at the tips of the final roots of 'Catuaí' (Figure 1A), which was not observed in the other treatments. From the sixth DAI, most of the J2 were found near the vascular bundle of the roots of these plants. The nematodes were perpendicular, parallel and tangential in relation to the vascular cylinder (Figure 1B). In 'Apoatã', the few J2 that managed to penetrate the roots were found in the first layers of cortical cells and, predominantly, parallel to the vascular bundle. In this period, there was a significant reduction ($P \leq 0.05$) in the number of nematodes in comparison to the first evaluation in the plants inoculated with M1. Root galls started to be evident at 10 DAI, and only in 'Catuaí' plants inoculated with M1. These were typical root galls, small, rounded, and located at the tips of the fine roots, but without necrosis. At about 30 DAI eggs production occurred. These were wrapped

in a gelatinous matrix and mainly located inside the root cortex. The first third-stage (J3) and/or fourth-stage (J4) juveniles in this population (18 specimens), already exhibiting 'sausage-shaped' tumescence in the body, were observed in this period and only on 'Catuaí' plants. Most of the nematodes reached complete development, i.e. the adult female stage, at 30 DAI, while in 'Apoatã' the few individuals that managed to reach the adult female stage were seen at 40 DAI. No nematodes were observed at J3 or J4 stages, or as adult females, on plants from either coffee cultivar, when the M2 population was inoculated.

The histopathological study showed various morphological alterations in the internal structure of the root when inoculated with *M. exigua* (Figure 2 A and B). The J2 penetrated the region of cellular lengthening, at the tips of the fine roots just next to the cap, and migrated intercellularly through the cortex in the direction of the vascular cylinder, where most of J2 individuals managed to establish a feeding site. After this, the nematode became more bulky and went through the development stages until reaching the stage of female adulthood. Although the J2 migration occurred in an intercellular manner, rupture of the cell wall and deformation of many cells could be observed, as well as cell disorganization in the vascular cylinder region (Figure 2 B and C). On the second DAI, the J2 could be found on the root surface and or in the first layers of cortical cells, because the J2 were in the penetration process and starting to migrate. During this period, no structural change could be seen in the root tissues. On the fourth DAI, it was observed that most of the nematodes were already near the vascular cylinder in the susceptible plants, while in the resistant plants there were few individuals. The first signs of the plant's defense response were observed from the fourth DAI onwards, both for the incompatible interaction (population M1 - 'Apoatã') and for the non-host plant response (population M2 – 'Catuaí' and 'Apoatã'). Near the body of the nematode root cells showing deformity and necrosis were seen, as well as condensation of the cytoplasm, and a large accumulation of material strongly stained with toluidine blue dye (Figure 2 C and D). From the fourth DAI, in the interaction of the M2 population with plants from the two coffee genotypes, decomposing nematodes were observed in the first cell layers, near the epidermis and on the cortex (Figure 2 F), but without signs of HR.

The M1 population of *M. exigua* induced the formation of many galls (76 galls/root system on average) and the production of a large number of eggs (5460/root) in plants from the susceptible cultivar, at 50 DAI. Although there were a small number of galls, on average only 3 galls, on the resistant cultivar plants, no eggs could be found. M2 individuals did not induce the formation of galls or production of eggs in either cultivar.

DISCUSSION

From the results of this study, it could be noted that penetration, subsequent development and reproduction

TABLE 1 - Number of nematodes at various developmental stages of *Meloidogyne exigua* populations from coffee (M1) and rubber tree (M2) on roots of coffee cultivars 'Catuaí Vermelho IAC 44' (susceptible) and 'Apoatã IAC 2258' (resistant) from 2 to 40 days after inoculation (DAI)

CULTIVARS	STAGES	2 DAI		4 DAI		6 DAI		8 DAI		10 DAI	
		M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Catuaí	J2	186 aA	132 bA	190 aA	99 bA	163 aA	102 bA	146 aA	87 bA	149 aA	55 bA
Apoatã	J2	104 aB	89 bB	88 aB	84 aA	62 aB	73 aB	32 bB	74 aA	18 bB	15 aA

Cont.

CULTIVARS	STAGES	15 DAI		20 DAI		25 DAI		30 DAI		35 DAI		40 DAI	
		M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Catuaí	J2	41	38	22	32	21	28	26	22	9	6	6	0
	J3 e J4	77	0	108	0	88	5	33	1	18	0	16	0
	Female	0	0	6	0	39	0	86	0	105	0	99	0
	Total	118 aA	38 bA	136 aA	32 bA	148 aA	33 bA	145 aA	23 bA	132 aA	6 bA	121 aA	0 bA
Apoatã	J2	16	35	10	33	8	26	3	22	0	8	0	0
	J3 e J4	0	0	6	0	4	0	4	1	3	0	0	0
	Female	0	0	0	0	0	0	0	0	2	0	4	0
	Total	16 bB	35 aA	16 bB	33 aA	12 bB	26 aA	7 bB	23 Aa	5 aB	8 aA	4 aB	0 aA

Means followed by the same lowercase number in a line and uppercase letter in a column do not differ among themselves by Tukey test at 5% probability.
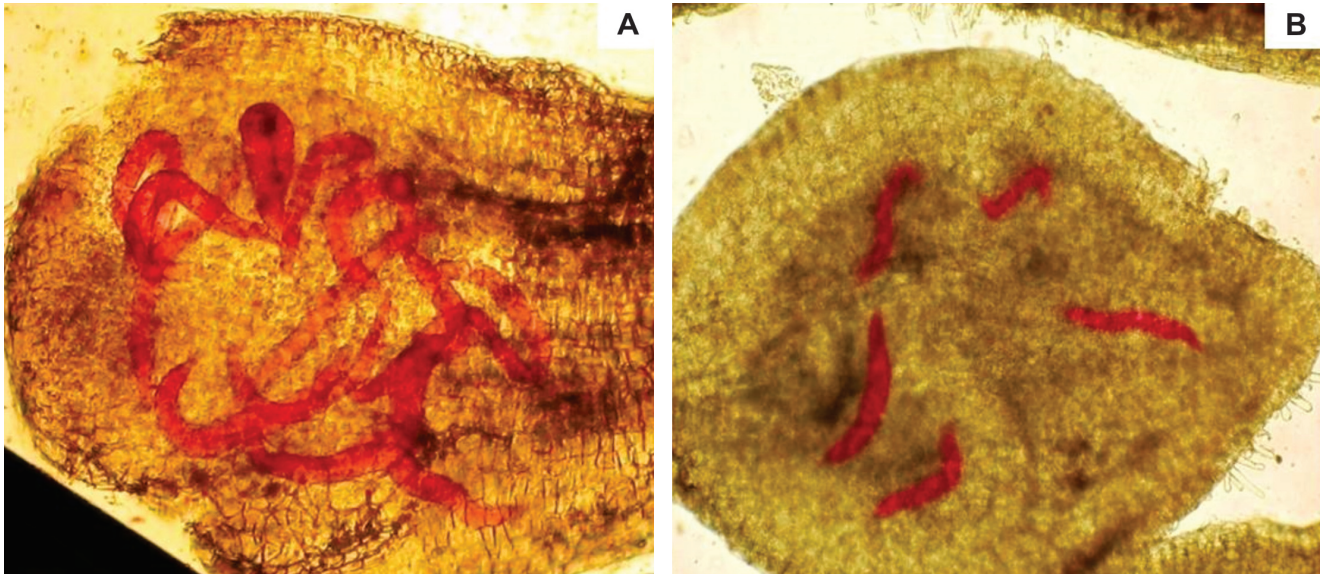
FIGURE 1 - Juveniles of *Meloidogyne exigua* on roots of coffee cultivar 'Catuaí Vermelho IAC 44'. **A.** crowding of J2 at root tip; **B.** juveniles in various positions in the vascular cylinder after establishment of feeding site.
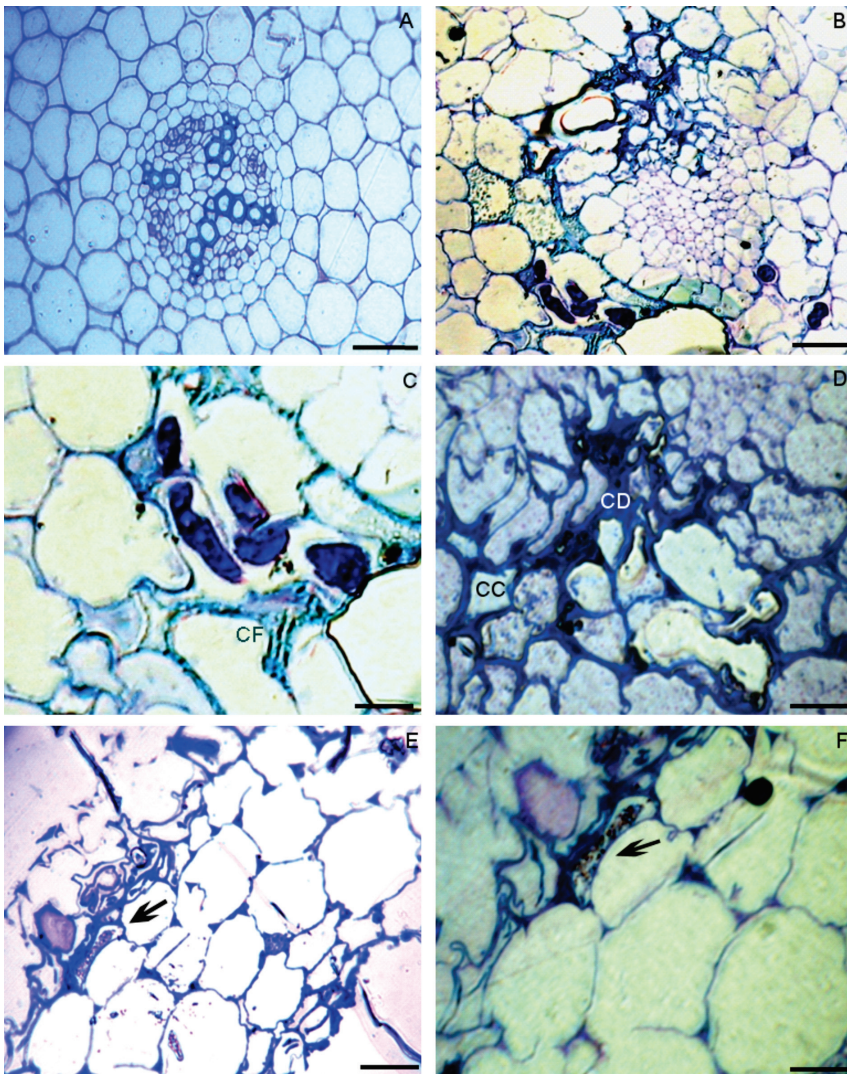

FIGURE 2 - Thin sections *Coffea canephora* 'Apoatã IAC 2258' roots stained with toluidine blue at 6 days after inoculation with *Meloidogyne exigua*: A-D – population of *M.exigua* from coffee (M1) and E-F – from rubber tree (M2). **A.** vascular cylinder of healthy root; **B.** vascular cylinder of root with nematode showing cell disorganization; **C.** deformed cells containing phenolic-like compounds (CF); **D.** details of hypersensitivity reaction (HR), cell death (CD), condensation of cytoplasm (CC); **E-F**. degraded nematodes on epidermis and parenchyma with no evidence of HR (arrowed). Bars: A = 100 µm; B = 40 µm; E = 30 µm C, D and F = 15 µm.

of *M. exigua* were affected by the coffee genotype. Penetration, change of phase, induction of symptoms and histological changes, and the reproduction of population M1*,* on a susceptible host ('Catuaí') were similar to what was observed in previous studies (Mendes et al., 1977; Lima et al., 1985; Anthony et al., 2005). The root region where second stage juveniles had penetrated and their intercellular migration through the cortex until next to endoderm follow the findings of Mendes et al. (1977) with *M. exigua* in coffee 'Mundo Novo'.

A complete development of the females without the presence of any male on the root was observed by the 30th day. This observation demonstrates that the roots were fit to meet the nutritional needs of the nematodes, especially females, and that the plant was not suffering extreme stress as reported by Van Gundy (1985), what would provoke formation of males. Reproduction in *M. exigua* was observed from one month, when eggs production was detected. This is possible because although *M. exigua* reproduces by anfimixis, this species uses meiotic parthenogenesis optionally when males are absent (Triantaphyllou, 1985).

Not all juveniles that penetrated the roots of the susceptible coffee plant could reach female adulthood. In this study, about 30% of J2 continued to be vermiform on the root cortex and were incapable of inducing formation of giant cells. This can be explained because the body energy of the J2, principally the lipid content accumulated during embryonic development (Lee & Atkinson, 1977), makes it possible to penetrate but not establish a feeding site. Consider that the age of J2 at the inoculation's day was four days old and during this time they used part of your body's lipid reserves to survive. To be successful in the infective process during pathogenesis, the J2 of *Meloidogyne* spp. need at least 50% of lipids in their body content (Van Gundy et al., 1967).

In the incompatible interaction, involving the M1 population and the resistant cultivar Apoatã, there was a drastic reduction in the quantity of nematodes within the roots from 8 days after penetration onwards. It was considered that there was emigration of the J2 of *M. exigua*, in response to the coffee cultivar's mechanisms of resistance. According to Huang (1985), in some resistant plants there may not be enough nutrients or other essential substances for the establishment of the nematode, which would result in emigration and/or developmental delay. Based on this observation, it is plausible that besides the hypersensitivity response (HR), other plant defense mechanisms could be envolved, which do not necessarily caused the J2 death, but prevent it from establishing a feeding site. The phenomenon of emigration as a result of the plant defense response was also previously observed in 'Apoatã' cultivar inoculated with *M. incognita* (Oliveira, 2006).

Characterization of the coffee plant's resistance gene to *M. exigua,* known as *Mex-1* (Noir et. al, 2003), and studies on inherited resistance reinforce the hypothesis that this interaction follows the model of Flor's gene-to-gene theory (Flor, 1971). In addition, the results from studies by Rodrigues et al. (2000) and Anthony et al. (2005) corroborate this information, because these observed cellular alterations, such as HR-type reaction in the resistant cultivar IAPAR 59. In the tissues, where the second-stage juveniles tried to establish their feeding site, there was condensation of the cytoplasm, an increase in the size of the nucleus and retraction of the plasmatic membrane of the cell wall (Anthony et al., 2005), like in the present study. Differential contrast with toluidine blue made it possible to view a large quantity of dark blue-colored material near the decomposing body of the nematode, a clear indication that phenolic compounds played a role as defense response of the coffee plant to *M. exigua*.

Previous studies involving the incompatible nematode x host interaction have shown that the HR is one of the main defense mechanisms of the plant. The level of hypersensitivity, the time of its occurrence and the final destination of the nematode depend on the pathogen-host combination (Canto-Saenz & Brodie, 1987). This reaction took place after 12 hours of inoculation of roots of tomato plants with *M. incognita* juveniles (Dropkin, 1969), while in the hybrid rootstock Citrumelo 'Swingle' (*Citrus paradisi* x *Poncirus trifoliata*) inoculated with *Tylenchulus semipenetrans*, the HR took more than two weeks to occur (Kaplan, 1981). In the association *M. exigua*-coffee cultivar the time it takes for the HR reaction to start is still not known, and in the current study the first evidence of HR were observed from the fourth day after inoculating J2.

Intermediate or incomplete resistance of the coffee cultivar to *M. exigua* was recently suggested by Alpizar et al. (2007), who observed different reproductive rates in resistant and susceptible pure lines and in the hybrids obtained from these crosses. The heterozygote lines for the resistance gene provided greater nematode reproduction than the homozygote lines. The authors concluded that the *Mex-1* gene may have an incomplete dominance expression, allowing penetration, but preventing reproduction of the nematode.

In the current study, fewer J2 from the M1 population penetrated the resistant cultivar. At 2 DAI, approximately 44% fewer J2 had penetrated the roots of 'Apoatã' than that of 'Catuaí', indicating that either the roots of the resistant cultivar were less attractive to the J2 of *M. exigua* or some physical or chemical characteristics in the epidermis cell wall of 'Apoatã' make penetration difficult. It seems that additionally to those mentioned above other defense responses of the plant, perhaps be more important than these, are active during pathogenesis, especially provoking the emigration of J2. It is remarkable that at 10 DAI the number of J2 observed in roots of resistant cultivar dropped to only 17.3% of that viewed at 2 DAI. It was also observed that some individuals developed, albeit more slowly and only reached the adult female phase at about 40 DAI. Even so, reproduction of the nematode did not take place, since no eggs were found. It is thought that compounds connected

to plant defense response are involved in this interaction, as reported by Silva et al. (2010). These authors observed an increase in the activities of peroxidases, polyphenoloxidases and phenylalanine-ammonia-lyases as well as a higher concentration of lignin and phenolic compounds in the roots of the resistant coffee cultivar infected by *M. exigua*.

Although coffee is the *M. exigua* type host, and penetration of J2 did occur, there was no development and reproduction of the M2 population originating from the rubber tree. Previous studies have revealed the incapacity of this biotype to induce gall formation and egg production in coffee plants (Santos, 1997; Carneiro & Almeida, 2000; Silva et al., 2007), independent of the genotype of the coffee cultivar analyzed (Silva et al., 2007). This population did not evolve to other development stages beyond J2 and disappeared from the roots in the first weeks after penetration, but the non-host plant's response (coffee-M2) was different from that of the resistant plant. On the non-host plant no evidence of HR was seen, and this plant was more effective in inhibiting the establishment of a feeding site and consequent development and reproduction of the nematode.

It was concluded that the coffee cultivar's resistance to *M. exigua* is not only due to HR, as previous studies have suggested. Instead, a set of defense responses, which are both constitutive and induced after nematode penetration, inhibit feeding site formation, provoke J2 emigration or inhibit nematode development and reproduction.

ACKNOWLEDGEMENTS

REFERENCES

Alpizar E, Etienne H, Bertrand B (2007) Intermediate resistance to *Meloidogyne exigua* root-knot nematode in *Coffea arabica*. Crop Protection 26:903-910.

Anwar SA, Mckenry MV (2000) Penetration, development and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* spp. Nematropica 30:9-17.

Anthony F, Topart P, Martinez A, Silva M, Nicole M (2005) Hypersensitive-like reaction conferred by the *Mex-1* resistance gene against *Meloidogyne exigua* in coffee. Plant Pathology 54:476-482.

Baermann G (1917) Eine einfache methode zur auffindung von ankvlostomum (nematoden) larven in erdproben. Natuurkunndig Tijdschrift voor Nederlandsch Indie 57:131-137.

Barbosa DHSG, Vieira HD, Souza RM, Viana AP, Silva CP (2004) Field estimates of coffee yield losses and damage threshold by *Meloidogyne exigua*. Nematologia Brasileira 28:49-54.

Boneti JIS, Ferraz S (1981) Modificação do método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* de raízes de cafeeiro. Fitopatologia Brasileira 6:553.

Byrd JDW, Kirkpatrick J, Barker KR (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

Campos VP, Lima RD, Almeida VF (1985) Nematóides parasitas do cafeeiro. Informe Agropecuário 11:50-58.

Campos VP, Villain L (2005) Nematode parasites of coffee and cocoa. In: Luc M, Sikora RA, Bridge J (Eds.) Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford UK. CAB International. pp. 529-579.

Canto-Saenz M, Brodie BD (1987) Comparison of compatible and incompatible potato to *Meloidogyne incognita*. Journal of Nematology 19:218-221.

Carneiro RMDG, Almeida MRA (2000) Caracterização isoenzimática e variabilidade intraespecífica dos nematóides de galhas do cafeeiro no Brasil. In: I Simpósio de Pesquisa dos Cafés do Brasil. Poços de Caldas, MG. pp. 280-282.

Davis EL, Mitchum MG (2005) Nematodes: sophisticated parasites of legumes. Plant Physiology 137:1182-1188.

Davis EL, Hussey RS, Mitchum MG, Baum TJ (2008) Parasitism proteins in nematode-plant interactions. Current Opinion in Plant Biology 11:360-366.

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

Endo BY (1975) Pathogenesis of nematode-infected plants. Annual Review of Phytopathology 13:218-238.

Flor HH (1971) Current status of gene-for-gene concept. Annual Review of Phytopathology 9:275-296.

Giebel J (1982) Mechanism of resistance to plant nematodes. Annual Review of Phytopathology 20:257-279.

Huang CS (1985) Formation, anatomy and physiology of giant cells induced by root-knot nematodes. In: Sasser JN, Carter CC (Eds.) An Advanced Treatise on *Meloidogyne*. Vol. 1: Biology and Control. NCSU and USAID Cooperative Publication, Raleigh, NC, U.S.A. pp. 155-164.

Kaplan DT (1981) Characterization of citrus rootstock responses to *Tylenchulus semipenetrans* (Cobb). Journal of Nematology 13: 92-498.

Jatala P, Russell CC (1972) Nature of sweet potato resistance to *Meloidogyne incognita* and the effects of temperature on parasitism. Journal of Nematology 4:1-7.

Kirkpatrick TL, Oosterhuis DM, Wullschleger SD (1991) Interaction of *Meloidogyne incognita* and water stress in two cotton cultivars. Journal of Nematology 23:462-467.

Lee DL, Atkinson HJ (1977) Physiology of Nematodes. New York Columbia University. 215p.

Lima RD, Ferraz S (1985) Biologia de *Meloidogyne exigua* II. Desenvolvimento pós-embriogênico em cafeeiro 'Mundo Novo'. Revista Ceres 32:349-361.

Mendes BV, Ferraz S, Shimoya C (1977) Observações histopatológicas de raízes de cafeeiro parasitadas por *Meloidogyne exigua* Goeldi, 1887. Nematologia Brasileira 2:208-229.

Noir S, Anthony F, Bertrand B, Combes MC, Lashermes P (2003) Identification of a major gene (*Mex-1*) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. Plant Pathology 52:97-103.

Oliveira DS (2006) Patogenicidade de populações de *Meloidogyne incognita*, proveniente de Minas Gerais e São Paulo, ao cafeeiro. D. S. Thesis. Universidade Federal de Viçosa. Viçosa, MG, Brazil.

Rodrigues ACFO, Abrantes IMO, Melillo MT, Blevezacheo T (2000) Ultrastructural response of coffee roots to root-knot nematodes, *Meloidogyne exigua* and *M. megadora*. Nematropica 30:201-210.

SAEG (2007) Sistema para Análises Estatísticas. Versão 9.1: Fundação Arthur Bernardes - UFV - Viçosa, MG.

Santos JM (1997) Estudo das principais espécies de *Meloidogyne* Goeldi que infectam o cafeeiro no Brasil com descrição de *Meloidogyne goeldi* sp. n. D. S. Thesis. Universidade Estadual de São Paulo. Botucatu, SP, Brazil.

Silva RV, Oliveira RDL, Pereira AA, Sêni DJ (2007) Respostas de genótipos de *Coffea* spp. a diferentes populações de *Meloidogyne exigua*. Fitopatologia Brasileira 32:205-212.

Silva RV, Oliveira RDL, Nascimento KJT, Rodrigues FA (2010) Biochemical responses of coffee resistance against *Meloidogyne exigua* mediated by silicon. Plant Pathology 59:586-593.

Triantaphyllou AC (1985) Cytogenetics, citotaxonomy and phylogeny of root-knot nematodes. In: Sasser JN, Carter CC (Eds.) An Advanced Treatise on *Meloidogyne*. Vol. 1: Biology and Control. NCSU and USAID Cooperative Publication, Raleigh, NC, U.S.A. pp. 113-126.

Van Gundy SD, Byrd AF, Wallace HR (1967) Aging and starvation in juvenile of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. Phytopathology 57:559-571.

Van Gundy SD (1985) Ecology of *Meloidogyne* spp. - emphasis on environmental factors affecting survival and pathogenicity. In: Sasser JN, Carter CC (Eds.) An Advanced Treatise on *Meloidogyne*. Vol. 1: Biology and Control. NCSU and USAID Cooperative Publication, Raleigh, NC, U.S.A. pp. 177-182.

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. Plant Cell 8:1735-1745.