*Tropical Plant Pathology*, vol. 36, 3, 190-194 (2011)

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**SHORT COMMUNICATION / *COMUNICAÇÃO***

# Characterization of *Meloidogyne incognita* populations from São Paulo and Minas Gerais state and their pathogenicity on coffee plants

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**ABSTRACT**

*Meloidogyne incognita* is one of the most aggressive and harmful plant-parasitic nematodes attacking coffee plantations in Brazil. However, populations from Minas Gerais state (MG) do not incite disease on coffee plants as strongly as populations from São Paulo state (SP). This study aimed to compare the capacity to incite disease on coffee plants from SP and MG-populations based on penetration and post-infective development of second-stage juveniles ($J_2$) stage. Both populations were confirmed as *M. incognita* by using esterase phenotype I1 and species-specific PCR. Physiologically they were classified as race 2 by differential host test. Susceptible (*C. arabica* 'Catuaí Vermelho IAC 44') and resistant coffee seedlings (*C. canephora* 'Apoatã IAC 2258') were inoculated with $J_2$ and assessed for penetration and development from 2nd to 40th day after inoculation. Although the penetration rate of the $J_2$ from both populations was higher in susceptible than in resistant seedlings, the SP-population showed a higher penetration than the MG-population for both variables. Post-infective development proceeded only in individuals of the SP-population in susceptible seedlings. The incompatibility between the MG-population and coffee seedlings was evident at the penetration phase, which was also followed by post-penetration resistance factors leading to significant $J_2$ emigration, impeding nematode establishment.
**Key words:** *Coffea* spp., pathogenesis, resistance mechanisms, root-knot nematode, variability.

**RESUMO**

**Caracterização de populações de *Meloidogyne incognita* de São Paulo e Minas Gerais e sua patogenicidade em cafeeiro**

No Brasil, *Meloidogyne incognita* é considerado um dos fitonematóides mais agressivos e prejudiciais ao cafeeiro. Entretanto, populações desse nematóide, presentes em Minas Gerais, são incapazes de infectar esse hospedeiro. O objetivo deste trabalho foi caracterizar e avaliar a penetração e o desenvolvimento pós-infectivo de populações de *M. incognita* que diferem quanto à capacidade de infectar o cafeeiro. As duas populações foram confirmadas como *M. incognita* pelo fenótipo isoenzimático de esterase, I1, e PCR específico. Foram classificadas como raça 2 pela reação dos hospedeiros diferenciadores. Mudas de cafeeiro suscetível e resistente foram inoculadas com juvenis de segundo estádio ($J_2$) de cada população e avaliadas quanto à penetração e desenvolvimento do nematóide a partir do segundo até o quadragésimo dia após a inoculação. A penetração de $J_2$ da população de *M. incognita* de São Paulo foi maior do que a da população de Minas Gerais, tanto em cafeeiro suscetível (*C. arabica* 'Catuaí Vermelho IAC 44') quanto resistente (*C. canephora* 'Apoatã IAC 2258'). Como esperado, ocorreu maior penetração de $J_2$ das duas populações de *M. incognita* no cafeeiro suscetível. Nas avaliações posteriores, somente foi observado desenvolvimento pós-infectivo nos indivíduos pertencentes à população de *M. incognita* de São Paulo em cafeeiro suscetível. Os mecanismos que conferiram incompatibilidade entre as populações de *M. incognita* de Minas Gerais e o cafeeiro atuaram principalmente na fase de penetração, mas também foi acompanhada pela ação de fatores de resistência pós-penetração que ocasionaram uma significativa emigração de $J_2$ e impediram o estabelecimento do nematóide.
**Palavras-chave:** *Coffea* spp., nematóide-das-galhas, patogênese, resistência.

Brazil is the largest producer and exporter of coffee (*Coffea arabica* L.) in the world. Minas Gerais state is responsible for approximately half (1.28 million tons in 2008-09) of the total Brazilian production (Conab 2008). The state is also known for the excellent quality of its coffee, fetching higher prices, especially on international markets. Plant diseases are among the major constraints that reduce coffee production. Nematode attack reduces yield and in some cases even results in abandoning the plantation (Carneiro, 1995). In Brazil, *Meloidogyne incognita* (Kofoid and White) Chitwood is considered one the most harmful plant nematode species affecting coffee plantations. This nematode is difficult to control, not only because of its aggressiveness, infecting the main root of the coffee plants (Lordello & Mello Filho, 1970), but also due to its wide host range (Roberts, 1995) and the

Part of the PhD Thesis of the first author. Universidade Federal de Viçosa. 2006.

existence of several physiological races (Hartman & Sasser, 1985).

The four physiological races of *M. incognita* which are prevalent in Minas Gerais (MG) attack a number of crops and weeds, but curiously do not infect coffee plants (Oliveira et al., 2009). This is directly contrary to what occurs in the states of São Paulo and Paraná, where this nematode is highly damaging to coffee plantations (Campos & Villain, 2005). The reasons explaining this phenomenon are unknown, especially because the main coffee cultivars planted in MG, such as Catuaí and Mundo Novo, are susceptible to all these races and are also planted in these two states.

Plant resistance can be expressed during penetration, post-infection development and/or during the reproductive phase of the nematode (Anwar et al., 1994). The second-stage juveniles ($J_2$) of *Meloidogyne* spp. can penetrate similarly resistant or susceptible plants (Schneider, 1991), or can be either higher in susceptible plants (Lawrence & Clark, 1986) or resistant plants (Herman et al., 1991). Post-penetration development is affected by physiological processes of the host that either prevent or delay development of $J_2$ or limit its reproduction (Anwar et al., 1994). The following study was done to evaluate the penetration and post-penetration development and the pathogenicity of *M. incognita* populations from São Paulo (SP) and Minas Gerais (MG) on coffee seedlings.

Two populations of *M. incognita*, one from okra plants (*Hibiscus esculentus*) from MG and the other from (*Coffea arabica* 'Catuaí Vermelho IAC 44') from SP, were multiplied for 120 days on tomato (*Lycopersicon esculentum* 'Santa Cruz Kada') and coffee (*C. arabica* 'Catuaí Vermelho IAC 44') respectively. Their identity was confirmed by morphological (perineal pattern as in Taylor & Netscher, 1974) and physiological characteristics (differential host test as in Hartman & Sasser, 1985), isozyme analysis (Ornstein 1964; Davis, 1964), and species-specific PCR (Randig et al., 2002). PCR products were analyzed by electrophoresis through 1.4% agarose gel and staining with ethidium bromide.

Preliminary identification as *M. incognita* race 2 was based on female perineal pattern morphology and on the reaction of the populations in the North Carolina differential host test. The results of the differential host test showed that tomato, pepper, watermelon and tobacco were good hosts with a gall and egg mass index = 5. Biochemical studies, based on the phenotypes of EST, MDH, SOD and GOT, revealed that these populations have the same profile: I1, N1, I2 and N1, respectively (Figures 1 A, B, C and D). Protein extracts from *M. javanica* were used as a reference population. Although the EST I1 phenotype is the most common in *M. incognita* (Esbenshade & Triantaphyllou, 1985), two other phenotypes (I2 and S1) have been detected in Brazilian *M. incognita* populations, without bearing any relation to their capacity to parasitize coffee or any other host (Carneiro et al., 2000; Oliveira et al., 2006). The isozyme profiles of the

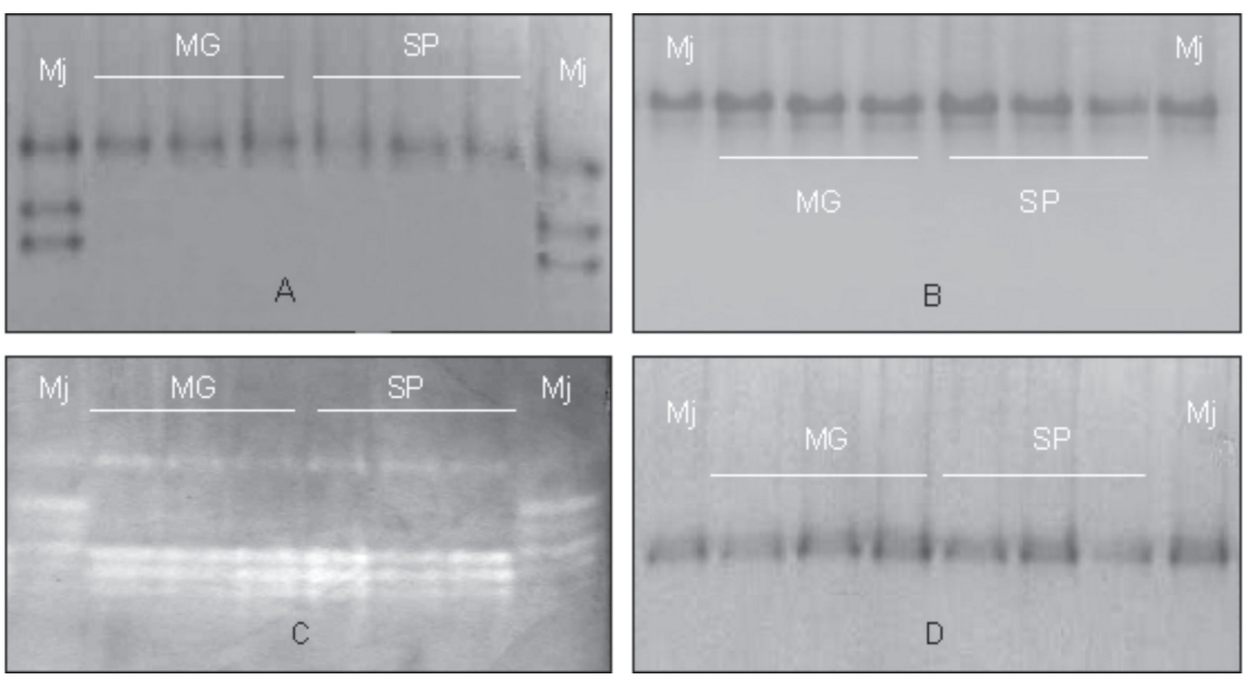


**FIGURE 1 -** Isozymatic phenotypes of the populations of *Meloidogyne incognita* from Minas Gerais MG and São Paulo SP. Phenotypes: A - Esterase (I1); B - Malate dehydrogenase (N1); C - Superoxide dismutase (I2) and D - Glutamate-oxaloacetate transaminase (N1). Mj: Phenotype of *M. javanica* used as a comparative standard.

MG- and SP-populations confirmed the previous diagnosis of the species.

The use of the primer pair SCAR-coffee (inc-K14-R and inc-K14-F) specific for *M. incognita* generated a DNA fragment of 399 base pairs (Figure 2). This amplified product was similar to that reported by Randig et al. (2002) for *M. incognita* populations from coffee, confirming the identification of the two populations used in this study. Coffee seedlings of *Coffea arabica* 'Catuaí Vermelho IAC 44' (susceptible) and *C. canephora* 'Apoatã IAC 2258' (resistant) were evaluated, considering penetration and post penetration development of *M. incognita*. Coffee seedlings were kept in growth chambers at 26ºC and single seedlings were inoculated with 10,000 $J_2$ when they reached two pairs of permanent leaves. To standardize the infection age, the inoculated seedlings were removed from the substrate after 48 hours and the roots thoroughly washed to remove $J_2$ that had not penetrated yet. The seedlings were replanted in 500 mL plastic cups filled with sand-soil mixture (1:2) previously treated with methyl bromide (100 mL/$m^3$). Inoculum viability was confirmed by inoculating 2,000 $J_2$ of either population on tomato seedlings (Santa Cruz 'Kada').

The experiment was carried out in a completely randomized factorial design 2 x 2 x 11 (2 populations of *M. incognita* x 2 coffee species x 11 evaluation periods) and three replications. During the initial 10 days, three seedlings from each treatment were harvested, at a 2-day interval followed by a 5-day interval until 40 days after inoculation. The roots were stained with acid fuchsin (Byrd et al., 1983). The number of nematodes and their developmental stage inside the roots was evaluated. The treatment means were compared by Tukey's test ($\alpha = 0.05$).

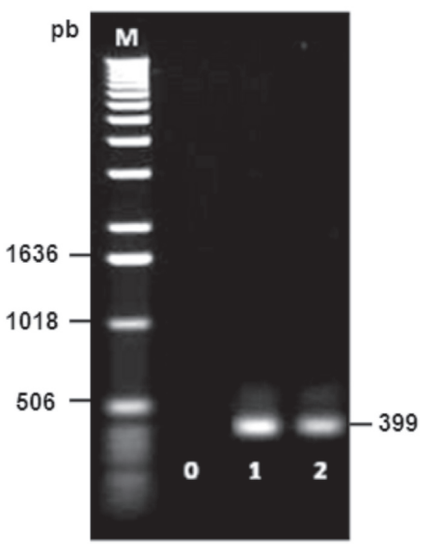


**FIGURE 2 -** Profile of the amplification of egg DNA of the populations of *Meloidogyne incognita* from Minas Gerais (1) and São Paulo (2) using the SCAR-coffee primers (inc-K14-R and inc-K14-F), specific for this species. M: DNA size standard 1 kb-DNA-ladder (Invitrogen), pb: base pair; 0: negative control, reaction control devoid of DNA template.

Populations of M. *incognita* from MG and SP showed a reproduction factor of 12.4 and 16.5, respectively, on tomato roots, thus confirming the inoculum viability. In the susceptible and resistant cultivars, $J_2$ penetration by the SP-population, pathogenic on coffee, was greater ($P \leq 0.05$) than by the MG-population (Figure 3). However, the penetration rate of both populations was higher in the susceptible than in the resistant cultivar ($P \leq 0.05$) (Table 1). Four days after inoculation, the number of nematodes inside the roots of resistant coffee plants declined significantly ($P \leq 0.05$), independent of the population. Similar results were found in the susceptible cultivar inoculated with the MG-population ($P \leq 0.05$). Eight days after inoculation, no nematode from the MG-population was detected in the roots of the resistant cultivar, and the same result occurred in the susceptible cultivar 10 days after inoculation (Table 1). Complete post-penetration development of the nematode was observed in the susceptible cultivar inoculated with the SP-population. Only vermiform $J_2$ were encountered in the root tissues up to six days after inoculation, and over the following two days, 68% of $J_2$ had enlarged, indicating the establishment of a feeding site (giant cells). On the 15$^{th}$ day after inoculation, about 54% of the individuals were at $J_3$ or $J_4$ stage. The presence of females at the initial posture stage was observed on the 25$^{th}$ day after inoculation (Table 1).

Penetration of the MG-population $J_2$ in the susceptible and resistant cultivars occurred, on average, 89.5 and 92.7% less than that of the SP-population, respectively. Thus, the incompatibility shown by the MG-population can be partially explained by the existence of plant resistance mechanisms that reduce nematode penetration, such as root exudates that either do not attract or repel the $J_2$ of this population (Potenza et al., 1996). The $J_2$ are attracted to host roots in response to the signals emitted by the plant in the form of organic compounds such as exudates, secretions and mucilage, which are perceptible to the sensorial organs of $J_2$, such as amphids and labial papillae (Prot, 1980). The occurrence of intra-specific variability may explain the differences in penetration by the $J_2$ from the two populations.

The reduction in penetration rate of root-knot nematode $J_2$, as a mechanism of resistance, has been observed in coffee. Anthony et al. (2005) found 28% less penetration of *M. exigua* $J_2$ on resistant cultivar ('Iapar 59') compared to the susceptible ('Caturra'). Araya & Caswell-Chen (1995) reported cultivars 'Caturra' and 'Catuaí' as non-hosts of *M. javanica* populations from California, inferring that the non-establishment of this nematode was due to low $J_2$ penetration. Only 2% of the inoculated $J_2$ penetrated the roots of these cultivars.

Emigration of infective forms of *M. incognita* due to resistance mechanisms of the host plant was reported for cotton and soybean (McClure et al., 1974; Herman

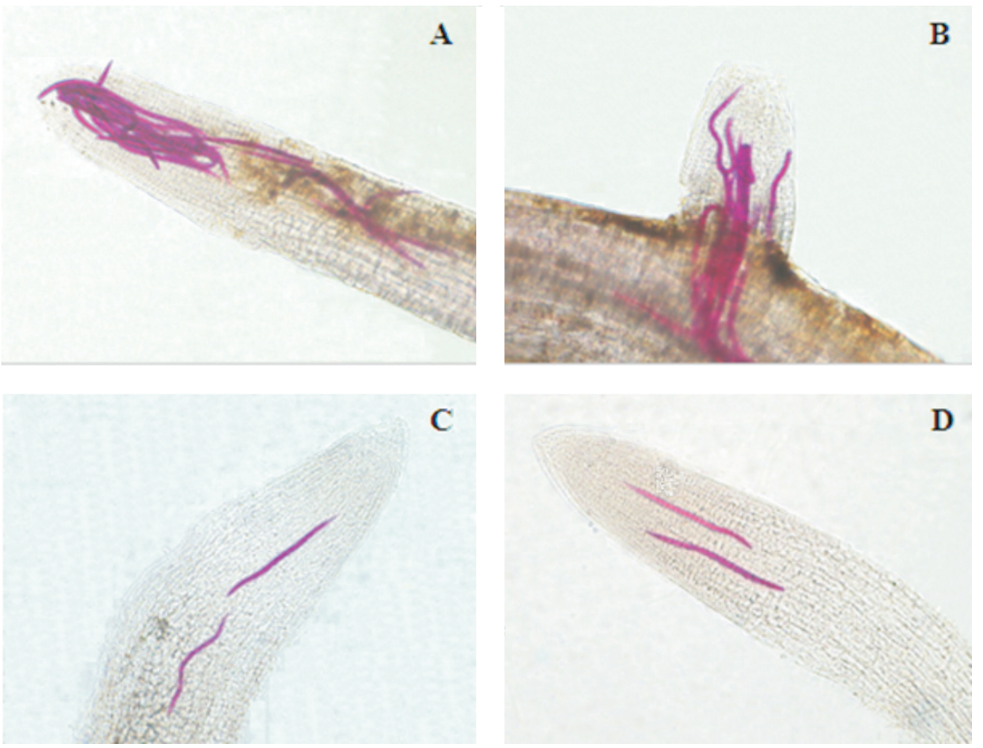


**FIGURE 3** - *Meloidogyne incognita* second-stage juveniles in coffee roots two days after inoculation. A and B: population from São Paulo on 'Catuaí Vermelho IAC 44' (susceptible) and Apoatã IAC 2258' (resistant), respectively; C and D: population from Minas Gerais on 'Catuaí' (susceptible) and 'Apoatã' (resistant), respectively.

et al., 1991). This could explain the reduced number of nematodes found four days after inoculation, in the roots of the resistant cultivar independently of the population, and in the susceptible cultivar inoculated with the MG-population (non-pathogenic). The mechanisms that conferred this incompatibility between the *M. incognita* population from Minas Gerais and coffee seem to act essentially at the penetration phase, but also at post-penetration, which caused a significant emigration of $J_2$ and did not allow the nematode to become established.

The morphological, physiological, biochemical and molecular data obtained for the characterization and identification of the MG and SP-populations demonstrated that these root- knot nematode populations belong to the same species, *M. incognita*. Thus, the observed difference in pathogenicity on coffee cannot be attributed to an erroneous identification of the populations. These results strongly indicate that the MG-population of *M. incognita*, incapable of infecting coffee, belongs to a different biotype from those found in other coffee-growing areas of Brazil, where this species is a limiting factor for coffee cultivation. To avoid high losses in coffee plantations of Minas Gerais, implementation of state control measures are warranted to prevent the introduction of *M. incognita* populations that are pathogenic on coffee.

## ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for granting scholarships. This research was partially supported by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café.

## REFERENCES

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.

Anwar SA, Trudgill DL, Phillips MS (1994) The contribution of variation in invasion and development rates of *Meloidogyne incognita* to host status differences. Nematologica 40:579-586.

Araya M, Caswell-Chen EP (1995) *Coffea arabica* cvs. Caturra and Catuaí nonhosts to a California isolate of *Meloidogyne javanica*. Nematropica 25:165-171.

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, L Villain (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. CABI Publishing. pp. 529-579.

Carneiro RG (1995) Reação de progênies de café 'Icatu' a *Meloidogyne incognita* raça 2, em condições de campo. Nematologia Brasileira 19:53-56.

Carneiro RMDG, Almeida MRA, Quénéhervé P (2000) Enzyme phenotypes of *Meloidogyne* spp. populations. Nematology 2:645-654.

CONAB. Previsão da safra brasileira de café 2008/2009, primeira estimativa. http://www.conab.gov.br (20 de janeiro, 2008).

Davis BJB (1964) *Disk electrophoresis*. II. Method and application to human serum proteins. Annals of the New York Academy of Sciences 121:404-427.

Esbenshade PR, Triantaphyllou AC (1985) Electrophoretic methods for the study of root-knot nematodes enzymes. In: Barker KR, Carter CC, Sasser JN (Eds.) An Advanced Treatise on *Meloidogyne.* Raleigh NC. North Carolina State University Graphics. pp. 115-123.

Hartman KM, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (Eds.) An Advanced Treatise on *Meloidogyne.* Raleigh NC. North Carolina State University Graphics. pp. 69-77.

Herman M, Hussey RS, Boerma HR (1991) Penetration and development of *Meloidogyne incognita* on roots of resistant soybean genotypes. Journal of Nematology 23:155-161.

Lawrence GW, Clark CA (1986) Infection and morphological development of *Meloidogyne incognita* in roots of susceptible and resistant sweet potato cultivars. Plant Disease 70:545-547.

Lordello LGE, Mello Filho AT (1970) Mais um nematóide ataca o cafeeiro. Revista de Agricultura 45:102.

McClure MA, Ellis KC, Nigh EL (1974) Post-infectional development and histopathology of *Meloidogyne incognita* in resistant cotton. Journal of Nematology 6:21-26.

Oliveira DS, Oliveira RDL, Gonçalves W (2006) Fenótipo S1 de esterase em *Meloidogyne incognita* no Brasil. Fitopatologia Brasileira 31:207.

Oliveira DS, Oliveira RDL, Silva DG, Silva RV (2009) Falha na adaptabilidade (Fitness) de *Meloidogyne incognita* ao cafeeiro. Nematologia Brasileira 33:207-211.

Ornstein L (1964) Disc Electrophoresis. I. Background and Theory. Annals of the New York Academy of Sciences 121:321-349.

Potenza CL, Thomas SH, Higgins EA, Sengupta-Goplan C (1996) Early root response *Meloidogyne incognita* in resistant and susceptible alfalfa cultivars. Journal of Nematology 28:475-484.

Prot JC (1980) Migration of plant-parasitic nematodes toward plant roots. Revue de Nématologie 3:305-318.

Randig O, Bongiovanni M, Carneiro RMDG, Castagnone-Sereno P (2002) Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. Genome 45:862-870.

Roberts PA (1995) Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annual Review of Phytopathology 33:199-221.

Schneider SM (1991) Penetration of susceptible and resistant tobacco cultivars by *Meloidogyne* juveniles. Journal of Nematology 23:225-228.

Taylor DP, Netscher C (1974) An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematropica 20:268.

*TPP 141 - Received 22 June 2010 - Accepted 30 June 2011*
*Section Editor: Regina M.D.G. Carneiro*