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# Variability of *Meloidogyne exigua* on Coffee in the Zona da Mata of Minas Gerais State, Brazil[1]

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*Abstract:* Minas Gerais is the major coffee-producing state of Brazil, with 28% of its production coming from the region of Zona da Mata. Four major species of root-knot nematode attacking coffee (*Meloidogyne incognita, M. paranaensis, M. coffeicola,* and *M. exigua*) have been reported from Brazil. To determine the variability in *Meloidogyne* spp. occurring in that region, 57 populations from 20 localities were evaluated for morphological, enzymatic, and physiological characteristics. According to the perineal pattern, all the populations were identified as *M. exigua;* however populations from the municipality of São João do Manhuaçu exhibited patterns very similar to *M. arenaria.* The identity of all the populations was confirmed by the phenotypes of esterase, malate dehydrogenase, superoxide dismutase, and glutamate-oxaloacetate transaminase. Thirteen populations (22.8%) showed the typical one-band (E1) esterase phenotype, whereas the others (77.2%) had a novel two-band phenotype (E2). No intraspecies variability was found in any population. All populations were able to reproduce on tomato, pepper, beans, cacao, and soybean. Reproduction was greater on tomato and pepper than on coffee seedlings, the susceptible standard.

*Key words: Coffea arabica,* isozyme analysis, *Meloidogyne exigua,* root-knot nematode.

Coffee is a major agricultural commodity in the world market, and Brazil is the largest producer and exporter. Minas Gerais State produces almost half of the Brazilian coffee, with 28% produced in the region of Zona da Mata (CONAB, 2003). The eco-climatic conditions of this region favor production of fine and special-grade coffee with a higher market value. Root-knot nematodes (RKN), *Meloidogyne* spp., are among the most important pathogens for this crop. Due to the extensive distribution of RKN in coffee plantations and their high reproductive capacity, coffee productivity in Brazil has declined since the 1960s (Campos et al., 1990). In some cases this has led to abandoning of plantations (Carneiro, 1995). At present, the economically viable management practice is the use of RKN resistance, especially resistant rootstocks. The use of resistance demands knowledge of the RKN species and, in some cases, races present in the area. Information on occurrence and distribution of the root-knot nematodes in Minas Gerais dates back to the 1980s and is based mainly on morphological characters (Campos et al., 1987; Ferraz, 1980). The objective of this study was to use precise techniques of characterizing *Meloidogyne* spp. to update information on the RKN identity and distribution from the coffee plantations of the Zona da Mata region of Minas Gerais.

## Materials and Methods

Fifty-seven populations of *Meloidogyne* spp. were collected from the nematode-infested coffee plantations in 20 municipalities of the Zona da Mata region of Minas Gerais, Brazil (Table 1). Soil and root samples were collected from four points, to a depth of 30 cm from under the canopy of selected trees and pooled. The composite sample of approximately 500 g soil and 200 g roots was placed in plastic bags, labeled, and transported to the nematology laboratory. The eggs of *Meloidogyne* spp. were extracted from the roots according to Boneti and Ferraz (1981) and used to inoculate coffee seedlings (*Coffea arabica* L. cv. Catuaí) for nematode multiplication in greenhouse. The collected nematode populations were maintained on coffee in the greenhouse for 2 years.

At least 10 females from each population were prepared for perineal pattern analysis (Taylor and Netscher, 1974). Isozyme characterizations were conducted for esterase (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD), and glutamate-oxaloacetate transaminase (GOT). Milky-white, reproductive females were removed from galls on inoculated coffee seedlings and transferred to micro-centrifuge tubes containing 3.5 µL protein extraction buffer (Dalmasso and Bergé, 1978). Females of known greenhouse isolate of *M. javanica* were extracted from tomato roots (*Lycopersicon esculentum* cv. Santa Clara) and used as a reference standard. Electrophoresis was carried out in a continuous buffer system with 7% acrylamide running gel. The voltage was maintained at 100 V during the running period (Carneiro et al., 1996a). After electrophoresis, the gels were removed and placed in the appropriate reaction mixture to determine EST, MDH, SOD, and GOT activity (Alfenas et al., 1991). Enzyme phenotypes were designated by a letter suggestive of the species it specified and a numeral indicating the number of bands (Carneiro et al., 2000).

The set of differential hosts for *Meloidogyne* host races (Hartman and Sasser, 1985) plus onion (*Allium cepa* cv. Baia Periforme), cacao (*Theobroma cacao* clone SIC 23), field beans (*Phaseolus vulgaris* cv. Carioca), and soybean (*Glycine max* cv. FT Cristalina) were used for physiological characterization of *Meloidogyne* spp. populations. Coffee cv. Catuaí was used as the susceptible standard. The seeds of each species were sown in separate trays

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TABLE 1. Esterase phenotypes of *Meloidogyne exigua* populations collected from coffee plantations in municipalities of Zona da Mata of Minas Gerais. E1 is a single-band phenotype with Rm of 1.60; E2 is a two-banded phenotype with bands at Rm 1.60 and 1.90.

| Municipality | Number of populations | Number of populations with phenotype | |
|---|---|---|---|
| | | (E1) | (E2) |
| Alto Jequitibá | 3 | 1 | 2 |
| Araponga | 4 | 2 | 2 |
| Caiana | 2 | 0 | 2 |
| Canaã | 4 | 0 | 4 |
| Carangola | 2 | 1 | 1 |
| Divino | 2 | 0 | 2 |
| Espera Feliz | 3 | 1 | 2 |
| Faria Lemos | 2 | 0 | 2 |
| Fervedouro | 2 | 0 | 2 |
| Lajinha | 2 | 0 | 2 |
| Manhuaçu/Realeza | 12 | 3 | 9 |
| Manhumirim | 1 | 0 | 1 |
| Miradouro | 1 | 0 | 1 |
| Miraí | 2 | 1 | 1 |
| Muriaé | 2 | 1 | 1 |
| São Francisco do Glória | 2 | 1 | 1 |
| São Miguel do Anta | 3 | 1 | 2 |
| Santana do Manhuaçu | 1 | 0 | 1 |
| São João do Manhuaçu | 5 | 1 | 4 |
| Viçosa | 2 | 0 | 2 |
| Total | 57 | 13 | 44 |

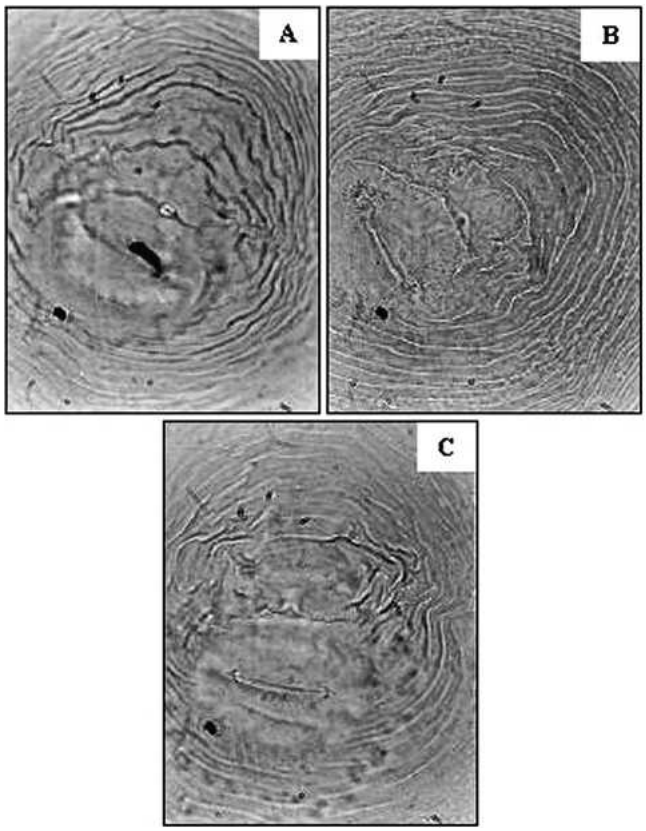


FIG. 1. Perineal patterns in *Meloidogyne* spp. populations collected from coffee plantations of Zona da Mata of Minas Gerais, Brazil. A,B) Typical perineal patterns of *M. exigua* found in the majority of populations evaluated. C) The perineal patterns found in some populations from the municipality of São João do Manhuaçu.

containing methyl bromide-treated sand, and the seedlings at four-leaf stage were transplanted to 3-liter clay pots containing methyl bromide-treated substrate (soil: sand, 2;1, v/v). The seedling were inoculated 2 days after transplant using 5,000 eggs/seedling of either of the 10 *M. exigua* populations (5 E1 and 5 E2 esterase phenotype) from each municipality. The experiment was a completely randomized factorial design (11 plant species × 10 populations) with six replications. The number of eggs per root system was determined 60 days after inoculation. The egg number was used to determine the nematode reproduction factor (Oostenbrink, 1966). The data were transformed to $\sqrt{x}$, and the means were compared using Tukey's test ($P = 0.05$).

## RESULTS

The perineal patterns for 91% of the populations were typical of *M. exigua* (Fig. 1A,B), with a slightly plane low dorsal arc, thick and well-spaced striae, nonperceptible lateral lines normally, demarked by either bent or interrupted striae. However, females of the populations collected from São João do Manhuaçu had perineal patterns similar to those of *M. arenaria* (Fig. 1C), with a rounded dorsal arc, the lateral lines forming a shoulder, yet thick and well-spaced striae as in *M. exigua.* These configurations were considered as atypical intra-species variation of *M. exigua.*

The typical EST phenotype of *M. exigua,* called E1, presents relative mobility (Rm) of 1.60. This phenotype was found in only 22.8% of the populations (Fig. 2A), whereas the remaining populations (77.2%) showed a new phenotype (designated E2), which was widely distributed in the sampled areas (Fig. 3). This phenotype consists of a weak (Rm 1.60) and a strong band (Rm 1.90) (Fig. 2B). The populations from the municipality of São João do Manhuaçu had the perineal patterns similar to that of *M. arenaria,* but with an *M. exigua* EST phenotype, e.g., four of the five populations had the E2 phenotype and one population had the E1 phenotype (Table 1). No polymorphism was observed for MDH, SOD, and GOT phenotypes (Figs. 4, 5, and 6). Characterization using these enzymes confirmed the diagnosis of each population as *M. exigua.* Phenotype N1 (Rm 1.00) for MDH (Fig. 4), E1 (Rm 1.10) for GOT (Fig. 5), and the phenotype N3 (Rm 1.25; 1.30 and 1.40) for SOD (Fig. 6) were typically exhibited.


FIG. 2. Esterase phenotypes of *Meloidogyne exigua* populations. A) A single-band phenotype (E1). B) Double-band phenotype (E2). J3 = Esterase phenotype of *M. javanica* used as standard for comparisons.

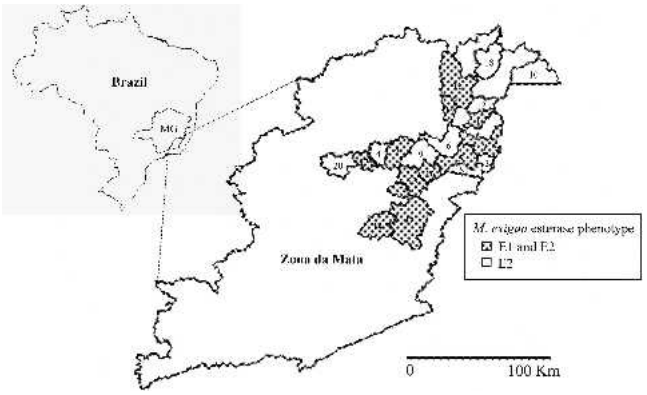


FIG. 3. Zonal distribution of E1 and E2 esterase phenotypes of *Meloidogyne exigua* populations in coffee plantations in 20 municipalities of Zona da Mata, Minas Gerais (MG), Brazil. For the name of the municipalities see Table 1.

No physiological variability on the differential hosts was detected among the populations ($P > 0.05$) (Table 2). The plant species × populations interaction was not significant for number of eggs produced ($P > 0.05$). Onion, watermelon, cotton, tobacco, and peanut were non-hosts of *M. exigua,* with reproduction factors of zero (data not shown).

## DISCUSSION

As in many other *Meloidogyne* species, reproduction in *M. exigua* occurs through parthenogenesis, and yet intra-species variability in morphological and physiological characters has been reported (Eisenback and Triantaphyllou, 1991; Lopes, 1985; Santos et al., 1992). The *M. exigua* populations of São João do Manhuaçu were atypical because their perineal patterns were similar to that of *M. arenaria,* which could lead to erroneous identification if based solely on this characteristic. However, the EST phenotypes confirmed each population as *M. exigua.* The perineal patterns had been the main taxonomical characteristic to identify species of *Meloidogyne* but now are used primarily to clarify doubts originating from the isoenzyme phenotypes.

Being subjective, perineal patterns have contributed to the description of a large number of species in this genus. A good example of subjectivity and low confidence of this character is the description of *M. paranaensis,* which was detected in Paraná, Brazil, and is aggressive on coffee. The perineal pattern led to an initial identification as *M. incognita,* despite difference in symptoms and aggressiveness on coffee. Due to the lack of evaluation of more precise characters, it was designated as *M. incognita* biotype IAPAR for 22 years. In 1996 this biotype was re-evaluated and described as a new species on the basis of morphological and morphometric characteristics, the response of differential hosts, and, principally, EST phenotype (Carneiro et al., 1996b).

In the past, new species of *Meloidogyne* were described erroneously on the bases of small morphological variations, including in the perineal pattern. It is now well established that such intra-species variation is common. Many erroneously identified species that were later considered as synonyms of well-characterized species include *M. acrita, M. elegans,* and *M. inornata* (which are considered as synonyms of *M. incognita*), *M. bauruensis* and *M. lordelloi* (as synonyms of *M. javanica*), and *M. thamesi* (as a synonym of *M. arenaria*) (Eisenback and Triantaphyllou, 1991). Thus, morphological characteristics should be examined in a large number of specimens to determine the range of variation, which would minimize errors or doubts in identification of a population as a known species or as a new species.

The EST was used to characterize 57 populations because this enzyme shows the highest degree of polymorphism and specificity for the main species of the root-knot nematode (Carneiro et al., 2000; Esbenshade and Triantaphyllou, 1985). Of the two EST phenotypes found among the populations, the phenotype E1 of *M. exigua* is reported to be widely distributed in Brazilian coffee plantations (Naves et al., 2001; Santos and Triantaphyllou, 1992), but in this study it was found only in 22.8% of the populations collected from Zona da Mata of Minas Gerais. The new phenotype, E2, despite showing the same relative mobility values as E1b phenotype described by Carneiro et al. (2000), differs in the intensity of bands, where the band of greater intensity has an Rm of 1.90 (compared to the Rm of 1.60 in E1 phenotype). With the discovery of a new EST phe-

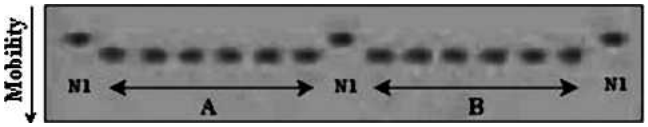


FIG. 5. Glutamate oxaloacetate transminase (GOT) phenotype of *Meloidogyne exigua* populations. A) Populations with E1 esterase phenotype. B) Populations with E2 esterase phenotype. N1 = *M. javanica* phenotype used standard.

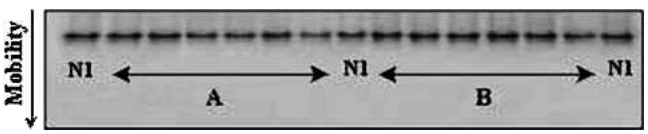


FIG. 4. Malate dehydrogenase (MDH) phenotype of *Meloidogyne exigua* populations. A) Populations with E1 esterase phenotype. B) Populations with E2 esterase phenotype. N1 = *M. javanica* phenotype used as standard.

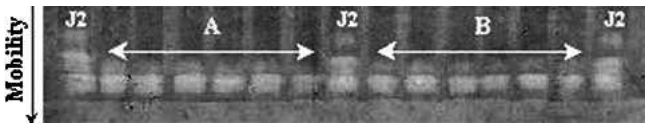


FIG. 6. Superoxide dismutase (SOD) phenotype of *Meloidogyne exigua* populations. A) Populations with E1 esterase phenotype. B) Populations with esterase E2 phenotype. N1 = *M. javanica* phenotype used as standard.

TABLE 2. Number of eggs per root system and the reproduction factor (RF) of different hosts inoculated with *Meloidogyne exigua* populations of E1 (1, 2, 3, 4, and 5) or E2 (6, 7, 8, 9, and 10) esterase phenotype collected from coffee plantations of Zona da Mata of Minas Gerais.

| Populations | | Host | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tomato | Pepper | Coffee | Field beans | Cacao | Soybean |
| 1 | Eggs[1] | 238.8a | 193.2b | 93.3c | 57.1d | 29.5e | 18.8e |
| | RF[1] | 11.4a | 7.6b | 1.8c | 0.7d | 0.2e | 0.1e |
| 2 | Eggs | 276.1a | 182.7b | 86.8c | 44.9d | 27.0e | 17.7e |
| | RF | 15.3a | 6.8b | 1.6c | 0.5d | 0.2e | 0.1e |
| 3 | Eggs | 279.7a | 202.0b | 80.8c | 52.9d | 31.1e | 16.1e |
| | RF | 16.6a | 8.2b | 1.5c | 0.6d | 0.2e | 0.1e |
| 4 | Eggs | 215.4a | 172.1b | 88.1c | 55.3d | 30.8e | 15.8e |
| | RF | 9.5a | 6.0b | 1.2c | 0.7d | 0.2e | 0.1e |
| 5 | Eggs | 218.3a | 186.0b | 97.0c | 48.0d | 30.1e | 15.4e |
| | RF | 9.7a | 7.2b | 1.9c | 0.5d | 0.2e | 0.1e |
| 6 | Eggs | 254.9a | 176.0b | 84.5c | 51.8d | 31.2e | 19.1e |
| | RF | 13.1a | 6.2b | 1.4c | 0.5d | 0.2e | 0.1e |
| 7 | Eggs | 222.6a | 184.7b | 79.7c | 58.5d | 30.1e | 15.5e |
| | RF | 10.1a | 6.9b | 1.3c | 0.7d | 0.2e | 0.1e |
| 8 | Eggs | 230.3a | 170.6b | 92.4c | 64.8d | 30.9e | 17.5e |
| | RF | 10.7a | 5.8b | 1.7c | 0.7d | 0.2e | 0.1e |
| 9 | Eggs | 219.8a | 205.9b | 87.0c | 57.1d | 30.9e | 18.0e |
| | RF | 9.8a | 8.6b | 1.5c | 0.7d | 0.2e | 0.1e |
| 10 | Eggs | 238.5a | 193.3b | 98.8c | 54.3d | 31.1e | 18.1e |
| | RF | 11.5a | 7.5b | 1.9c | 0.6d | 0.2e | 0.1e |

[1] Mean of six replicates. The number of eggs per root system data transformed to $\sqrt{x}$. Means followed by the same letter within a line are not significantly different (Tukey, $P > 0.05$).

notype it is now possible to detect four phenotypes of *M. exigua:* E1 (Rm 1.60), Ela (Rm 1.10 and 1.60), E1b (Rm 1.60 and 1.90), and E2 (Rm 1.60 and 1.90). The occurrence of more than one phenotype for the same enzyme is known for other species of *Meloidogyne. Meloidogyne arenaria,* for example, has phenotypes with one, two, and three bands called A1, A2, and A3, respectively (Esbenshade and Triantaphyllou, 1985). A two-band EST phenotype (I2), with Rm 1.00 and 1.12, was reported for *M. incognita,* which until then was identified only by the phenotype I1 (Santos and Triantaphyllou, 1992; Carneiro et al., 1996a).

Because of the limited use of isozyme electrophoresis, there is little information about the isozymatic variability within the species of *Meloidogyne.* With wider use of this technique, the discovery of new EST phenotypes will allow for more precise and less subjective identification of species.

The analysis of other enzymes did not detect variability among the populations but were important for confirming the diagnoses of the *M. exigua,* because all populations examined had isozyme phenotypes typical of this species. The MDH phenotype N1 (Rm 1.00) was found in all the populations. Although not a specific phenotype, this enzyme is important for differentiating *M. exigua* from *M. naasi* with phenotype N1a (Rm 1.40), which has greater mobility than *M. exigua* (Esbenshade and Triantaphyllou, 1985). All populations examined showed the GOT phenotype E1 (Rm 1.10). In *M. exigua* populations of São Paulo and Minas Gerais, Brazil, the SOD phenotype N3 (Rm 1.25, 1.30, and 1.40) was reported by Carneiro et al. (2000). Through perineal patterns or by isozyme analyses, all 57 populations of the root-knot nematode from the coffee plantations of Zona da Mata-Minas Gerais were characterized as *M. exigua,* despite variability in perineal patterns in populations from São João do Manhuaçu. The EST analysis also showed occurrence of two phenotypes in the populations, but without any relationship between the two types of variability exhibited by *M. exigua.* There was also no relationship with the reproduction capacity on different hosts, because all phenotypes produced a similar number of eggs/plant. Such lack of relationship between races and the isoenzymatic phenotypes also has been reported in *M. incognita* and other species with physiological races (Carneiro et al., 2000; Janati et al., 1982).

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