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# IDENTIFICATION OF PLANT PARASITIC NEMATODES IN TRIPLOID AND TETRAPLOID BANANAS IN BRAZIL[1]

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**ABSTRACT** – Nematodes are important pathogens in banana plants, and the lack of resistant genotypes is the biggest challenge of the banana breeding programs. Little is known on the behavior of banana triploids and tetraploids developed by Embrapa regarding parasitism by plant-parasitic nematodes in field conditions. Embrapa Mandioca e Fruticultura experimental areas, naturally infested in five Brazilian states (Embrapa Acre - Acre, Embrapa Semiárido - Pernambuco, Embrapa Cerrados - Distrito Federal, Palmital - São Paulo and Epagri - Santa Catarina) were evaluated for the distribution and population levels of plant-parasitic nematodes in commercial cultivars and triploid and tetraploid genotypes in the final breeding stage. The root-knot nematodes (*Meloidogyne* spp.) were the most frequent in roots (40 - 100%) and soil (85.71 - 100%), with a detectable number of juveniles (J2) varying between genotypes (4 - 148 J2.250g$^{-1}$ roots, and 1 - 110 J2.100 cm$^{-3}$soil). Four esterase phenotypes were characterized: *M. incognita* (Est I1 = Rm: 1.0), *M. javanica* (Est J3 = Rm: 1.0; 1.25 and 1.40 and Est J2 = Rm: 1.0 and 1.40) and *M. arenaria* (Est A2 = Rm: 1.20 and 1.35), *M. javanica* (Est J3) was predominant. *Meloidogyne javanica* and *M. incognita* were predominant, however mixed infestations between species were found. The occurrence of *Meloidogyne* spp. was: *M. javanica* (68.26%), *M. incognita* (64.73%) and *M. arenaria* (16.81%). *Helicotylenchus multicinctus* and *Rotylenchulus reniformis* was the second most frequent group. *Radopholus similis*, *Scutellonema* sp., *Criconemoides* sp. and *Helicotylenchus* sp. presented themselves in low frequency and population levels in banana plants.

**Keywords**: *Musa* spp. Occurrence *Meloidogyne* spp. *Helicotylenchus* spp. *Rotylenchulus reniformis.*

## IDENTIFICAÇÃO DE FITONEMATOIDES EM BANANEIRAS TRIPLOIDES E TETRAPLOIDES NO BRASIL

**RESUMO** – Nematoides são importantes patógenos em bananeiras, e a inexistência de genótipos com resistência é o maior desafio dos programas de melhoramento genético da cultura. Pouco se conhece sobre o comportamento de bananeiras triploides e tetraploides desenvolvidas pela Embrapa quanto ao parasitismo por fitonematoides em campo. Áreas experimentais naturalmente infestadas da Embrapa Mandioca e Fruticultura em cinco estados brasileiros (Embrapa Acre - Acre, Embrapa Semiárido - Pernambuco, Embrapa Cerrados - Distrito Federal, Palmital - São Paulo e Epagri - Santa Catarina) foram avaliadas quanto a distribuição e níveis populacionais de fitonematoides em cultivares comerciais e genótipos triploides e tetraploides em fase final de melhoramento. Os nematoides das galhas (*Meloidogyne* spp.) foram os mais frequentes em raízes (40 - 100%) e solo (85,71 - 100%), com número detectável de juvenis (J2) variando entre genótipos (4 - 148 J2.250g$^{-1}$ raízes e 1 - 110 J2.100 cm$^{-3}$solo). Quatro fenótipos de esterase foram caracterizados: *M. incognita* (Est I1 = Rm: 1,0), *M. javanica* (Est J3 = Rm:1,0; 1,25 e 1,40 e Est J2 = Rm: 1,0 e 1,40) e *M. arenaria* (Est A2 = Rm:1,20 e 1,35), com predominância de *M. javanica* (Est J3). *Meloidogyne javanica* e *M. incognita* foram predominantes, entretanto infestações mistas entre as espécies foram encontradas. A ocorrência de *Meloidogyne* spp. foi: *M. javanica* (68,26%), *M. incognita* (64,73%) e *M. arenaria* (16,81%). *Helicotylenchus multicinctus* e *Rotylenchulus reniformis* foi o segundo grupo mais frequente. *Radopholus similis*, *Scutellonema* sp., *Criconemoides* sp. e *Helicotylenchus* sp. se apresentaram em baixa frequência e nível populacional nas bananeiras.

**Palavras-chave**: *Musa* spp. Ocorrência *Meloidogyne* spp. *Helicotylenchus* spp. *Rotylenchulus reniformis.*

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# INTRODUCTION

Bananas rank second in world fruit production, and along with rice, wheat and maize, they are considered the most important food sources worldwide (PERRIER et al., 2011). India leads in banana production in world rankings, accounting for 26.8% of bananas produced globally, followed by China, with 9.8%; Indonesia, with 6.3%; Brazil, with 5.9%; Ecuador, with 5.5%; and the Philippines, with 5.3% (FAOSTAT, 2017).

Although bananas are the second most produced crop in Brazil, few cultivars are available for commercial exploitation that are resistant or tolerant to pests and diseases. Banana plants are attacked by several plant pathogens, among them plant-parasitic nematodes. The burrowing nematode [*Radopholus similis* (Cobb, 1893) Thorne, 1949] has been the largest cause of banana crop damage in Brazil, although the root-knot nematodes (species of *Meloidogyne* Göldi, 1887) and the spiral nematode [*Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956] are the most frequent in banana-growing areas, however, little research on the economic losses incurred by banana farms from nematode damage has been conducted at the field level. Measures of chemical control used by large companies are not very applicable to small farmers, who represent the majority of Brazilian banana growers. Therefore, the use of resistant cultivars, either by selection within existing genetic resources or by the generation of new cultivars by hybridization, is considered the most efficient control measure (AMORIM et al., 2011).

Since 1995, several diploid, triploid and tetraploid banana genotypes have been evaluated under greenhouse conditions at Embrapa Mandioca e Fruticultura, in Cruz das Almas (BA) and Universidade de Brasília (DF) for resistance to *R. similis* and *Meloidogyne* spp. (COSTA; SILVA; ALVES, 1998; COSTA; RIBEIRO; LICHTEMBERG, 2003; COSTA; SILVA; ROCHA, 2000; COSTA, 2004; TEIXEIRA, 2007; MONTEIRO, 2011; SANTOS et al., 2010, 2013). The main goal of the field trials is to evaluate and validate the genotypes of interest regarding their agronomic and yield characteristics under varying environmental conditions, such as soil conditions, nematode occurrence, and climate. However, little is known about the frequency of plant-parasitic nematodes on triploids and tetraploids banana plants introduced and improved by Embrapa Mandioca e Fruticultura under field conditions. Based on this context, this study aimed to evaluate the occurrence of nematodes associated with the roots and soil of the triploids (AAA and AAB) and tetraploids (AAAA and AAAB) commercials and in the final stage of improvement in different experimental areas planted with banana genotypes of Embrapa Mandioca e Fruticultura.

# MATERIALS AND METHODS

**Banana Genotypes**

In the years 2010/2011, eleven triploids (AAA and AAB) and nineteen tetraploids (AAAA and AAAB) (Table 1) genotypes from the Banana Germplasm Bank of Embrapa Mandioca e Fruticultura were evaluated for occurrence and population levels of plant-parasitic nematodes in five experimental areas, located in units belonging to Embrapa and State Research Institutions (Embrapa Acre - Rio Branco - AC; Embrapa Semiárido – Petrolina – PE; Embrapa Cerrados – Planaltina – DF; Experimental Area of Sustainable Agriculture (Middle Paranapanema Region) – Palmital – SP; Agricultural Research and Rural Extension Company of Santa Catarina/EPAGRI – Luis Alves - SC). The experimental design was a randomized block with three replicates, each block containing six clumps of each genotype, with a spacing of 2.5 m × 3.0 m. The number of genotypes evaluated varied according to the location.

**Root and soil samples from the rhizosphere of banana genotypes**

Samples of roots and soil were collected at a depth of 0 – 20 cm at four core points around the plants of each banana genotype distributed among the three blocks. After collecting individual samples, a composite sample composed of roots (500 g) and one of soil (300 $cm^3$) were obtained by pooling and mixing the individual samples, and these were sent to the Laboratory of Nematology/Plant Quarantine Laboratory of Embrapa Recursos Genéticos e Biotecnologia located in Brasília, DF.

**Extraction, quantification and identification of nematodes**

For nematode extraction, the root samples were divided into two parts of 250 g. The first part of the roots and the soil (100 $cm^3$) samples were used for extraction of nematodes following the methodologies of Coolen and D'Herde (1972) and Jenkins (1964), respectively. Nematodes extracted from both roots and soil were killed at 60 °C for 1 minute and fixed with 4% FAA (Formaldehyde/Acetic Acid/Ethyl Alcohol). The quantification of the total nematode population was performed using Peter's glass slide under light microscope. Specimens mounted as semipermanent slides in a drop of formalin 2%, sealed with histologic paraffin (HOOPER, 1990; TIHOHOD, 1993; PASCHOAL, et al., 1995) were prepared for microscopy and identified under a light microscope to the genus (MAI, 1975) and species levels (FORTUNER, 1991; JATALA, 1991; LOOF, 1991; CARES; ANDRADE, 2006).

The second part of the roots was ground in a blender for 1 minute in water, and the suspension of each sample obtained was inoculated onto tomato plants cv. Santa Cruz, which were maintained in a greenhouse for 45 days for multiplication and identification of the *Meloidogyne* species. From each sample, 36 females were extracted from tomato roots and submitted to vertical eletrophoresis (ESBENSHADE; TRIANTAPHYLLOU, 1985) in a 7% polyacrylamide gel prepared according to Carneiro and Almeida (2001). The characterization of *Meloidogyne* species was based on the revealed esterase phenotypes (CARNEIRO, ALMEIDA, CARNEIRO, 1996; CARNEIRO, ALMEIDA, QUENÉHERVÉ, 2000). Individual females of *M. javanica* (Est $J_3$) were used as the standard phenotype.

**Table 1**. Eleven triploid and nineteen tetraploid genotypes of banana plants from the Embrapa Mandioca e Fruticultura Germplasm Bank were evaluated in five experimental areas located in the states of Acre, Pernambuco, Distrito Federal, São Paulo, and Santa Catarina, Brazil in 2010/2011.

| Genotypes | Genomic composition | Origin | Genotypes | Genomic composition | Origin |
|---|---|---|---|---|---|
| BRS Conquista | AAB (C) | Brazil | BRS Platina | AAAB (H) | Brazil |
| Bucanero | AAAA (H) | Sta. Lucia | Galeo 18 | AAB (H) | Brazil |
| Caipira | AAA (C) | France | Pacovan | AAB (C) | Brazil |
| Calypso | AAAA (H) | Sta. Lucia | Pacovan Ken | AAAB (C) | Brazil |
| Enxerto 33 | AAB (C) | Brazil | Prata Anã | AAB (C) | Brazil |
| FHIA 02 | AAAB (H) | Brazil | Princesa | AAAB (H) | Brazil |
| FHIA 17 | AAAB (H) | Brazil | PV 7934 | AAAB (H) | Brazil |
| FHIA 18 | AAAB (H) | Brazil | PV 9401 | AAAB (H) | Brazil |
| FHIA 23 | AAAB (H) | Brazil | Thap Maeo | AAB (C) | Thailand |
| Garantida | AAAB (H) | Brazil | Tropical | AAB (H) | Brazil |
| Grande Naine | AAA (C) | Brazil | Vitória | AAB (C) | Brazil |
| Japira | AAAB (H) | Brazil | YB 4203 | AAAB (H) | Brazil |
| JV 42135 | AAAB (H) | Brazil | YB 4207 | AAAB (H) | Brazil |
| Maçã | AAB (C) | Brazil | YB 4217 | AAAB (H) | Brazil |
| Maravilha | AAAB (C) | Brazil | YB 4247 | AAAB (H) | Brazil |

C = Cultivar; H = Hybrid.

## RESULTS AND DISCUSSION

Using light microscopy, *Meloidogyne* juveniles (J2), *Helicotylenchus*, *Scutellonema* and *Criconemoides* (MAI, 1975) were morphologically identified to the genus level, whereas *Radopholus similis* (Cobb, 1893) Thorne, 1949 (LOOF, 1991; CARES; ANDRADE, 2006), *Rotylenchulus reniformis* Linford and Oliveira, 1940 (JATALA, 1991), and *H. multicinctus* (FORTUNER, 1991) were morphologically identified to the species level; *Meloidogyne* spp. were identified based on their esterase phenotypes (CARNEIRO, ALMEIDA, CARNEIRO, 1996; CARNEIRO, ALMEIDA, QUENÉHERVÉ, 2000). The quantification data for nematodes detected in the root and soil samples and the frequencies of each genera/species by experimental area are shown in Tables 2 and 3.

*Meloidogyne* spp. frequently occurred in root (40 - 100%) and soil (85.71 - 100%) samples across all areas investigated, with the detected number of nematodes varying among banana genotypes in the root (4 to 148 J2.250$g^{-1}$ roots) and soil (1 to 110 J2.100 $cm^{-3}$ soil) samples (Tables 2 and 3). In the experimental area of Embrapa Acre, it was observed that the cultivar FHIA 17 stood out from the other evaluated genotypes because of the absence of root-knot nematodes, both in its root and soil samples (Tables 2 and 3). Similar results to those of cv. FHIA 17 were found in the Epagri experimental area, with emphasis on the genotypes PV 7934 and BRS Conquista, where root-knot nematodes were not detected in either the root or soil samples. In the experimental areas of Embrapa Semiárido, Embrapa

Cerrados and Palmital, higher population levels of root-knot nematodes were detected, in both the root and soil samples, mainly in the Embrapa Semiárido, where these nematodes were detected in all genotypes and cultivars, with the highest J2 populations occurring in the cultivars FHIA 02 (148 J2.250g$^{-1}$ roots) and BRS Plantina (126 J2.250g$^{-1}$ roots and 102 J2.100 cm$^{-3}$ soil). In the experimental area of Embrapa Cerrados, root-knot nematodes were more abundant in soil than in roots (Tables 2 and 3), with the exception of the rhizosphere soil of the cultivar Japira. In Embrapa Cerrados, root-knot nematodes were not detected in the roots of the YB 4207 and PV 9401 genotypes, or the roots of the Bucanero, Pacovan, FHIA 17 and Maçã cultivars, but were present in all other varieties (Table 2). In the Palmital experimental area, root-knot nematodes were present in the roots and soil of all genotypes and cultivars examined, with the exception of cv. Bucanero, for which root-knot nematodes were absent from roots and present at only a very low population level in soil (1 J2.100 cm$^{-3}$ soil).

**Table 2**. Number and frequency of plant-parasitic nematodes detected per 250 g of roots of banana in samples from different experimental areas of Embrapa Mandioca e Fruticultura, located at Embrapa, Acre State (AC); Embrapa Semiárido, Pernambuco State (PE); Embrapa Cerrados, Federal District (DF) Palmital Middle Paranapanema Region (Sustainable Agriculture), São Paulo State (SP); and Agricultural Research and Rural Extension Company of Santa Catarina/ EPAGRI, State of Santa Catarina (SC) during the years 2010/2011.

| Genotypes | AC | | PE | | DF | | SP | | | | SC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *M*.sp. | *H.m* | *M*.sp. | *R.r* | *M*.sp. | *H*.sp. | *M*.sp. | *H.m* | *R.r* | *Sc*.sp. | *M*.sp. | *H.m* |
| BRS Conquista | - | - | - | - | - | - | - | - | - | - | 0 | 4 |
| BRS Platina | 0 | 0 | 126 | 4 | 5 | 0 | 14 | 0 | 5 | 0 | 18 | 0 |
| Bucanero | 0 | 0 | 81 | 0 | 0 | 0 | 0 | 9 | 4 | 0 | 18 | 0 |
| Caipira | 0 | 0 | 74 | 0 | 20 | 0 | 8 | 7 | 0 | 1 | 0 | 0 |
| Calypso | 0 | 0 | 88 | 0 | - | - | - | - | - | - | - | - |
| Enxerto 33 | 4 | 4 | - | - | - | - | - | - | - | - | - | - |
| FHIA 17 | 0 | 0 | 74 | 0 | 0 | 0 | 27 | 1 | 0 | 0 | 11 | 14 |
| FHIA 18 | 7 | 0 | 95 | 0 | 20 | 0 | 21 | 1 | 1 | 0 | 0 | 0 |
| FHIA 2 | 4 | 0 | 148 | 0 | 10 | 0 | 54 | 7 | 0 | 0 | 0 | 0 |
| FHIA 23 | 4 | 0 | 77 | 0 | - | - | - | - | - | - | - | - |
| Galeo 18 | 8 | 0 | 105 | 0 | 5 | 0 | 39 | 11 | 0 | 0 | 0 | 4 |
| Garantida | 0 | 28 | 63 | 0 | 5 | 0 | 14 | 4 | 4 | 0 | 4 | 0 |
| Grande Naine | 18 | 0 | 84 | 0 | 60 | 0 | 21 | 11 | 4 | 0 | 0 | 0 |
| Japira | 4 | 0 | 95 | 0 | 5 | 0 | 10 | 11 | 0 | 1 | - | - |
| JV 42135 | 0 | 0 | 77 | 0 | - | - | - | - | - | - | - | - |
| Maçã | 0 | 0 | 88 | 0 | 0 | 0 | - | - | - | - | - | - |
| Maravilha | 0 | 0 | 98 | 0 | 55 | 0 | 32 | 4 | 0 | 0 | 4 | 14 |
| Pacovan | - | - | 77 | 0 | 0 | 0 | 17 | 1 | 4 | 0 | 46 | 0 |
| Pacovan Ken | 0 | 0 | 98 | 0 | 30 | 0 | 10 | 26 | 0 | 0 | 7 | 7 |
| Prata Anã | 0 | 0 | 95 | 0 | 30 | 0 | 11 | 1 | 1 | 0 | 0 | 0 |
| Princesa | 4 | 4 | 105 | 0 | - | - | - | - | - | - | - | - |
| PV 7934 | 0 | 0 | 63 | 0 | 5 | 15 | - | - | - | - | 0 | 0 |
| PV 9401 | - | - | - | - | 0 | 0 | 6 | 9 | 10 | 0 | 4 | 0 |
| Thap Maeo | 0 | 0 | 91 | 0 | 5 | 0 | 12 | 7 | 0 | 0 | 0 | 7 |
| Tropical | 14 | 0 | 93 | 0 | 10 | 0 | 11 | 11 | 7 | 0 | 4 | 7 |
| Vitória | - | - | - | - | 20 | 0 | - | - | - | - | - | - |
| YB 4203 | 0 | 0 | 42 | 4 | 30 | 0 | 5 | 4 | 1 | 0 | 0 | 0 |
| YB 4207 | - | - | - | - | 0 | 0 | 35 | 12 | 0 | 0 | 0 | 0 |
| YB 4217 | 0 | 0 | - | - | - | - | - | - | - | - | - | - |
| YB 4247 | 11 | 0 | - | - | - | - | - | - | - | - | - | - |
| Frequency (%) | 40 | 8 | 100 | 8 | 72.72 | 4.54 | 94.73 | 94.73 | 52.63 | 10.52 | 45 | 35 |
| | (25) | (25) | (23) | (23) | (22) | (22) | (19) | (19) | (19) | (19) | (20) | (20) |

*M*.sp. = unidentified *Meloidogyne* species; *H.m* = *Helicotylenchus multicinctus*; *R.s* = *Radopholus similis*; *R.r* = *Rotylenchulus reniformis*; *H*.sp. = unidentified *Helicotylenchus* species; *Cr*.sp. = unidentified *Criconemoides* species; *Sc*.sp. = unidentified *Scutellonema* species; (-) genotypes not evaluated. In parentheses, number of genotypes evaluated.

**Table 3**. Number and frequency of plant-parasitic nematodes detected per 100 $cm^3$ of soil in samples collected in banana plant rhizosphere from different experimental areas of Embrapa Mandioca e Fruticultura, located at Embrapa, Acre State (AC); Embrapa Semiárido, Pernambuco State (PE); Embrapa Cerrados, Federal District (DF); Palmital Middle Paranapanema Region (Sustainable Agriculture), São Paulo State (SP); and Agricultural Research and Rural Extension Company of Santa Catarina/ EPAGRI, State of Santa Catarina (SC) during the years 2010/2011.

| Genotypes | AC | | | PE | | DF | | | | SP | | | | | SC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *M*.sp. | *H.m* | *R.s* | *M*.sp. | *R.r* | *M*.sp. | *H*.sp. | *R.r* | *Cr*.sp. | *M*.sp | *H.m* | *R.r* | *Sc*.sp | *R.s* | *M*.sp | *H.m* |
| BRS Conquista | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | 11 |
| BRS Platina | 4 | 7 | 0 | 102 | 14 | 70 | 0 | 0 | 0 | 7 | 4 | 0 | 0 | 0 | 32 | 7 |
| Bucanero | 7 | 7 | 0 | 66 | 28 | 25 | 0 | 0 | 0 | 1 | 7 | 0 | 0 | 0 | 53 | 0 |
| Caipira | 4 | 11 | 0 | 11 | 41 | 55 | 0 | 0 | 0 | 37 | 7 | 0 | 0 | 0 | 56 | 0 |
| Calypso | 25 | 4 | 0 | 7 | 49 | - | - | - | - | - | - | - | - | - | - | - |
| Enxerto 33 | 25 | 17 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| FHIA 17 | 0 | 0 | 0 | 14 | 56 | 75 | 0 | 0 | 0 | 65 | 1 | 0 | 0 | 0 | 28 | 7 |
| FHIA 18 | 53 | 4 | 0 | 35 | 11 | 80 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 28 | 14 |
| FHIA 2 | 18 | 7 | 4 | 48 | 101 | 75 | 0 | 0 | 0 | 48 | 4 | 0 | 0 | 15 | 25 | 11 |
| FHIA 23 | 46 | 7 | 0 | 46 | 49 | - | - | - | - | - | - | - | - | - | - | - |
| Galeo 18 | 24 | 8 | 0 | 37 | 34 | 35 | 0 | 0 | 0 | 49 | 0 | 0 | 0 | 0 | 67 | 7 |
| Garantida | 21 | 46 | 4 | 53 | 11 | 45 | 0 | 0 | 0 | 25 | 18 | 0 | 0 | 1 | 63 | 14 |
| Grande Naine | 39 | 7 | 28 | 60 | 13 | 35 | 15 | 0 | 0 | 11 | 1 | 0 | 0 | 0 | 32 | 11 |
| Japira | 35 | 0 | 0 | 3 | 84 | 0 | 0 | 0 | 0 | 25 | 31 | 0 | 0 | 0 | - | - |
| JV 42135 | 11 | 7 | 0 | 49 | 53 | - | - | - | - | - | - | - | - | - | - | - |
| Maçã | 11 | 0 | 0 | 12 | 90 | 30 | 5 | 0 | 0 | - | - | - | - | - | - | - |
| Maravilha | 32 | 14 | 0 | 81 | 11 | 10 | 0 | 0 | 0 | 51 | 0 | 0 | 0 | 0 | 46 | 11 |
| Pacovan | - | - | - | 81 | 11 | 110 | 0 | 0 | 0 | 21 | 7 | 0 | 0 | 0 | 70 | 0 |
| Pacovan Ken | 18 | 4 | 0 | 32 | 28 | 70 | 0 | 0 | 0 | 15 | 3 | 0 | 0 | 0 | 28 | 14 |
| Prata Anã | 4 | 0 | 0 | 35 | 49 | 70 | 0 | 0 | 0 | 25 | 4 | 11 | 0 | 0 | 56 | 11 |
| Princesa | 35 | 4 | 0 | 40 | 62 | - | - | - | - | - | - | - | - | - | - | - |
| PV 7934 | 21 | 7 | 0 | 56 | 42 | 30 | 0 | 10 | 0 | - | - | - | - | - | 0 | 0 |
| PV 9401 | - | - | - | - | - | 5 | 0 | 0 | 0 | 33 | 3 | 0 | 0 | 0 | 46 | 0 |
| Thap Maeo | 32 | 11 | 21 | 42 | 32 | 65 | 5 | 0 | 0 | 14 | 17 | 0 | 1 | 0 | 42 | 18 |
| Tropical | 11 | 25 | 4 | 49 | 72 | 15 | 0 | 0 | 0 | 7 | 28 | 14 | 0 | 0 | 18 | 14 |
| Vitória | - | - | - | - | - | 35 | 0 | 0 | 5 | - | - | - | - | - | - | - |
| YB 4203 | 4 | 11 | 0 | 14 | 84 | 25 | 0 | 0 | 5 | 32 | 11 | 0 | 0 | 0 | 60 | 0 |
| YB 4207 | - | - | - | - | - | 35 | 0 | 0 | 0 | 50 | 3 | 0 | 0 | 0 | 11 | 11 |
| YB 4217 | 18 | 11 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| YB 4247 | 0 | 18 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Frequency (%) | 92 | 84 | 20 | 100 | 100 | 95.45 | 13.63 | 4.54 | 9.09 | 100 | 84.21 | 10.52 | 5.26 | 10.52 | 90 | 70 |
| | (25) | (25) | (25) | (23) | (23) | (22) | (22) | (22) | (22) | (19) | (19) | (19) | (19) | (19) | (20) | (20) |

*M*.sp. = unidentified *Meloidogyne* species; *H.m = Helicotylenchus multicinctus*; *R.s = Radopholus similis*; *R.r = Rotylenchulus reniformis*; *H*.sp. = unidentified *Helicotylenchus* species; *Cr*.sp. = unidentified *Criconemoides* species; *Sc*.sp. = unidentified *Scutellonema* species; (-) genotypes not evaluated. In parentheses, number of genotypes evaluated

The identification of *Meloidogyne* species by their esterase phenotypes allowed us to evidence four esterase profiles of the three most common *Meloidogyne* species previously associated with banana plants: *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 ($I_1$) one band of esterase activity (Rm:1.0); *M. javanica* (Treub, 1885) Chitwood, 1949 ($J_3$) three bands (Rm:1.0; 1.25 and 1.40) and ($J_2$) two bands (Rm:1.0 and 1.40) of esterase activity; and *M. arenaria* (Neal, 1889) Chitwood, 1949 ($A_2$) two bands of esterase activity (Rm:1.20 and 1.35)(Figure 1).

The species *M. javanica* and *M. incognita* were predominant. However, mixed infestations of these species were found in Embrapa Acre, Embrapa Cerrados, and Palmital (SP); these sometimes co-occurred with *M. arenaria*, which showed low incidence (Table 4). Co-infestations only occurred in some cultivars, i.e., FHIA 23, Maçã, Prata Anã, Thap Maeo, Maravilha and Bucanero, and in the genotype PV 7934 (Table 4). *Meloidogyne javanica* was prevalent across all genotypes, with average values of occurrence in Embrapa Cerrados (91.78%), Palmital (91.74%) and Embrapa Semiárido (67.70%). We note that *Meloidogyne javanica* comprised 100% of the nematodes occurring on most genotypes in Palmital, except for cv. Pacovan and the genotype YB 4203, which had mixed infections with a predominance of *M. incognita*. A predominance of *M. incognita* over *M. javanica* was observed only at Embrapa Acre (92.31%) and Epagri (74.07%) (Table 4). The results showed that *M. javanica* was the predominant *Meloidogyne* species in banana plants, followed by *M. incognita* and *M. arenaria*.

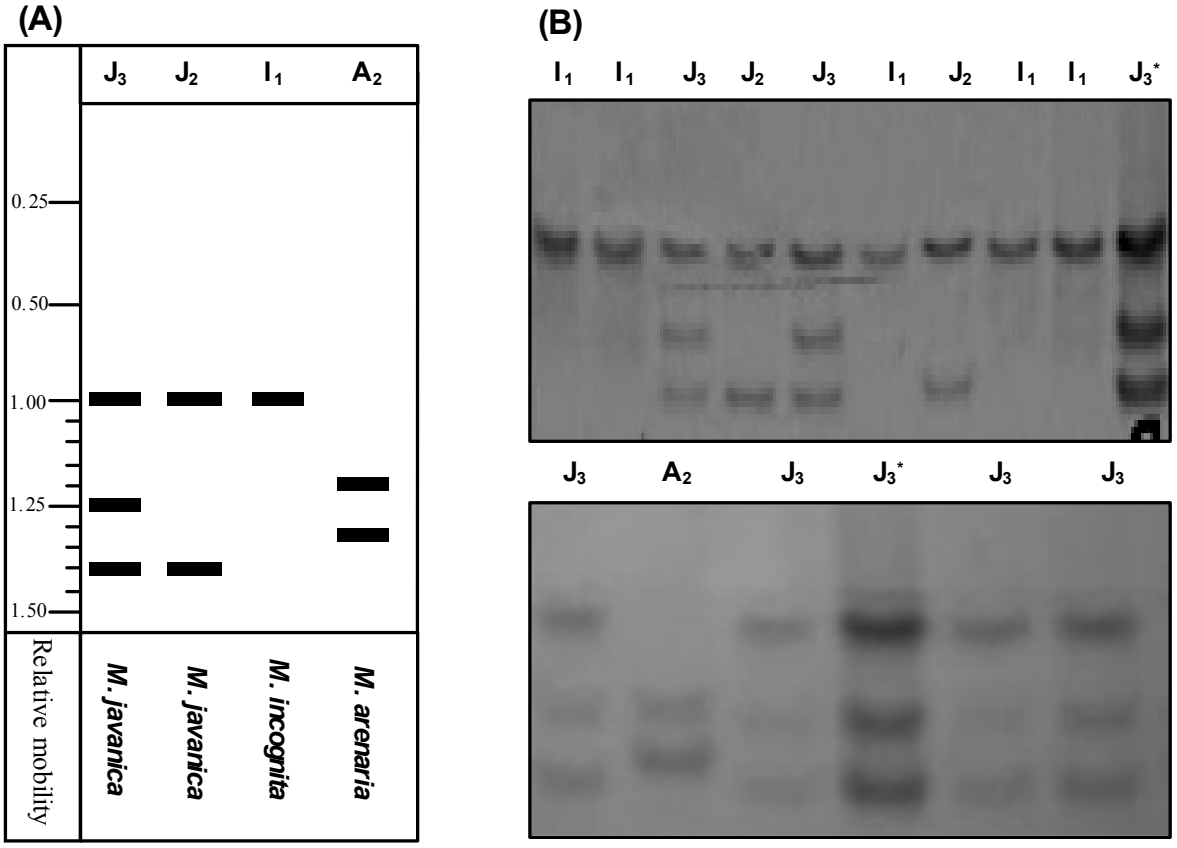


**Figure 1**. (A) Esterase band patterns (Est) detected for *Meloidogyne* species and their relative mobility from banana samples collected in areas of experimental fields of Embrapa, Acre State; Embrapa Semiárido, Pernambuco State; Embrapa Cerrados, Federal District; Palmital Middle Paranapanema Region (Sustainable Agriculture), São Paulo State; and Agricultural Research and Rural Extension Company of Santa Catarina / EPAGRI, State of Santa Catarina during the years 2010/ 2011. (B) Polyacrylamide gels (7%) showing patterns of esterase bands for *Meloidogyne* females in the banana genotypes evaluated in this study. ($J_3$*) *M. javanica* standard used.

The spiral nematodes *Helicotylenchus multicinctus* and the reniform nematode *Rotylenchulus reniformis* were the next most prevalent detected species. The frequency of *H. multicinctus* in the root samples ranged from 8 to 94.73% and their population sizes ranged from 4 to 26 specimens.250g$^{-1}$ roots, with the highest prevalences in Palmital-SP. The highest frequencies in soil samples were observed in Palmital-SP (84.21%), Embrapa Acre (84%), and Epagri-SC (70%) with population sizes ranging from 4 to 46 specimens.100 cm$^{-3}$ soil. For *R. reniformis*, its frequency was variable, ranging from 8 to 52.63% in the roots and from 4.54% to 100% in soil samples. *Rotylenchulus reniformis* was most commonly observed among the genotypes in Embrapa Semiárido soils and Palmital roots. *Rotylenchulus reniformis* population sizes varied among the samples, ranging from 4 to 10 specimens.250g$^{-1}$ roots and from 11 to 101 specimens.100 cm$^{-3}$ soil. At Embrapa Semiárido, *R. reniformis* was detected only in the roots of the genotypes YB 4203 and BRS Platina (Table 2). However, in the rhizosphere soil samples of all genotypes, was detected in large quantities of *R. reniformis* (Table 3). At Embrapa Cerrados, *R. reniformis* was only detected in rhizosphere soil samples of the genotype PV 7934.

The burrowing nematode (*Radopholus similis*) was rare across all experimental areas. It was not detected in the roots of any of the genotypes and was detected in only a few soil samples in Embrapa Acre (20%) and Palmital (10.52%) that were associated with the genotypes Garantida, Thap Maeo, Grande Naine, Tropical, and FHIA 2, ranging in detectable population levels from 1 to 28 specimens.100 cm$^{-3}$ soil (Table 2 and 3). Other spiral nematodes were also detected in low percentages in the experimental areas of Embrapa Cerrados and Palmital in the roots and soil samples, e.g., *Helicotylenchus* sp. (15 specimens.250g$^{-1}$ roots in the PV 7934 genotype), and 5 to 15 specimens.100 cm$^{-3}$ soil in the Thap Maeo, Grande Naine and Maçã cultivars), and *Scutellonema* sp. (1 specimen.250g$^{-1}$ roots and per 100 cm$^3$ of soil in the Caipira, Japira and Thap Maeo cultivars). The ring nematode (*Criconemoides* sp.) was detected only in soil samples associated with cv. Vitória and the genotype YB 4203 (2 specimens.100 cm$^{-3}$ soil).

**Table 4**. Occurrence of *Meloidogyne* species in different banana genotypes from five experimental areas of Embrapa Mandioca e Fruticultura: Embrapa, Acre State (AC); Embrapa Semiárido, Pernambuco State (PE); Embrapa Cerrados, Federal District (DF); Palmital Middle Paranapanema Region (Sustainable Agriculture), São Paulo State (SP); and Agricultural Research and Rural Extension Company of Santa Catarina / EPAGRI, State of Santa Catarina (SC) during the years 2010/ 2011.

| Genotypes | AC | | | PE | | DF | | | SP | | | SC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mj* | *Mi* | *Ma* | *Mj* | *Mi* | *Mj* | *Mi* | *Ma* | *Mj* | *Mi* | *Ma* | *Mj* | *Mi* |
| BRS Conquista | * | * | * | * | * | * | * | * | * | * | * | - | 100 |
| BRS Platina | 0 | 0 | 0 | 85.7 | 14.3 | 19.4 | 80.6 | - | 100 | - | - | 0 | 0 |
| Bucanero | 44.4 | 55.6 | - | 67.9 | 32.1 | 69.4 | 27.8 | 2.8 | 100 | - | - | - | 100 |
| Caipira | 0 | 0 | 0 | 100 | - | 11.1 | 88.9 | - | 100 | - | - | 16.7 | 83.3 |
| Calypso | - | 100 | - | 60.7 | 39.3 | * | * | * | * | * | * | * | * |
| Enxerto 33 | - | 100 | - | * | * | * | * | * | * | * | * | * | * |
| FHIA 17 | 0 | 0 | 0 | 35.7 | 64.3 | 47.2 | 52.8 | - | 100 | - | - | - | 100 |
| FHIA 18 | 0 | 0 | 0 | 60.7 | 39.3 | 83.3 | 16.7 | - | 100 | - | - | 0 | 0 |
| FHIA 2 | 0 | 0 | 0 | 64.3 | 35.7 | 100 | - | - | 100 | - | - | 94.4 | 5.6 |
| FHIA 23 | - | 66.7 | 33.3 | 100 | - | * | * | * | * | * | * | * | * |
| Galeo 18 | - | 100 | - | 46.4 | 56.6 | 100 | - | - | 100 | - | - | 0 | 0 |
| Garantida | 0 | 0 | 0 | 28.6 | 71.4 | 100 | - | - | 100 | - | - | 72.2 | 27.8 |
| Grande Naine | 0 | 0 | 0 | 67.9 | 32.1 | 100 | - | - | 100 | - | - | 100 | - |
| Japira | 11.1 | 88.9 | - | 89.3 | 10.7 | 100 | - | - | 100 | - | - | * | * |
| JV 42135 | 0 | 0 | 0 | 57.1 | 42.9 | * | * | * | * | * | * | * | * |
| Maçã | 11.1 | 88.9 | - | 78.6 | 21.4 | 25 | 30.6 | 44.4 | * | * | * | * | * |
| Maravilha | - | 100 | - | 85.7 | 14.3 | 97.2 | - | 2.8 | 100 | - | - | 11.1 | 88.9 |
| Pacovan | * | * | * | 71.4 | 28.6 | 91.7 | 8.3 | - | 5.6 | 88.8 | 5.6 | 0 | 0 |
| Pacovan Ken | 0 | 0 | 0 | 57.1 | 42.9 | 83.3 | 16.7 | - | 100 | - | - | - | 100 |
| Prata Anã | - | 100 | - | 60.7 | 39.3 | 83.3 | 8.3 | 8.3 | 100 | - | - | 66.7 | 33.3 |
| Princesa | - | 100 | - | 85.7 | 14.3 | * | * | * | * | * | * | * | * |
| PV 7934 | - | 100 | - | 78.6 | 21.4 | 91.7 | 5.6 | 2.7 | * | * | * | 0 | 0 |
| PV 9401 | * | * | * | * | * | 94.4 | 5.6 | - | 100 | - | - | 50 | 50 |
| Thap Maeo | - | 100 | - | 67.9 | 32.1 | 88.9 | 2.8 | 8.3 | 100 | - | - | - | 100 |
| Tropical | - | 100 | - | 100 | - | 97.2 | 2.8 | - | 100 | - | - | 100 | - |
| Vitória | * | * | * | * | * | 91.7 | 8.3 | - | * | * | * | * | * |
| YB 4203 | 0 | 0 | 0 | 92.9 | 7.1 | 88.9 | 11.1 | - | 37.5 | 62.5 | - | - | 100 |
| YB 4207 | * | * | * | * | * | 77.8 | 22.2 | - | 100 | - | - | 100 | - |
| YB 4217 | 0 | 0 | 0 | * | * | * | * | * | * | * | * | * | * |
| YB 4247 | 0 | 0 | 0 | * | * | * | * | * | * | * | * | * | * |
| **Occurrence (%)** | 22.20 | 92.31 | 33.3** | 67.70 | 33.00 | 91.78 | 48.63 | 11.55 | 91.74 | 75.65 | 5.6** | 67.90 | 74.07 |

(*)Genotypes not evaluated.; (0) genotypes evaluated, but females absent; (-) species absence; *Mi = M. incognita*; *Mj = M. javanica*; *Ma = Meloidogyne arenaria*. The numbers represent the percentages of each species in the genotypes per 36 evaluated females. **Average of a single observation on the genotype.

Root-knot nematodes (*Meloidogyne* spp.) were the most abundant nematodes detected in the roots and soils of the genotypes we studied across all experimental areas, indicating these banana genotypes were good hosts for this nematode and confirming previous reports that banana plants are good multipliers of *Meloidogyne* spp. (MOREIRA, 1995; COSTA et al., 1997; COSTA; SILVA; ALVES, 1998; COSTA; SILVA; ROCHA, 2000; COSTA; RIBEIRO; LICHTEMBERG, 2003; PINTO et al., 2005; TEIXEIRA, 2007).

In this study the bands patterns of esterase activity detected for *M. javanica* (Est $J_3$, Est $J_2$), *M. incognita* (Est $I_1$) and *M. arenaria* (Est $A_2$), were compatible with the results obtained by Cofcewicz et al. (2004a). Cofcewicz et al. (2004a) analyzed samples of banana cultivars from different commercial areas (Grande Naine, Prata Anã, Maçã and Pacovan) across several Brazilian regions, and detected the phenotypes of *M. javanica* $J_2$ (Rm: 1.0 and 1.40) in mixed populations with *M. javanica* ($J_3$). According to Rajasekhar, Ganguly and Dasgupt (1990) and Carneiro, Almeida and Carneiro (1996), these phenotypes ($J_2$ = Rm: 1.0 and 1.25 and $J_2$ = Rm: 1.0 and 1.40) can be stable or not, being considered atypical patterns, since these populations did not remain stable for a long period in a greenhouse in tomato plants, maintaining only the $J_3$ phenotype after the purification steps. The poor physiological conditions of individual females in banana roots can be considered the reason for the absence of a band in the esterase profile leading to the production of the atypical phenotypes $J_2$ (COFCEWICZ et al., 2004a). *Meloidogyne incognita* ($I_1$) identified in the present study, was also observed by Cofcewicz et al. (2004a), but was accompanied by the additional phenotype $I_2$ when they evaluated the occurrence of root-knot nematodes among 25 commercial banana growing areas in Brazil. $I_2$ was absent in this study.

In samples of banana plants, Cofcewicz et al. (2004a) detected *M. javanica*, *M. incognita*, *M. arenaria*, and *Meloidogyne* spp. in proportions of 61.7%, 32.2%, 4.3% and 1.8%, respectively, in a study in which samples from 25 areas cultivated with different commercial banana cultivars were evaluated, with a predominance of the cultivars

Grande Naine and Pacovan, growing in the main banana producing regions of Brazil, where 20 of them had mixed species populations, and in the other five, single species were identified as *M. javanica* or *M. incognita*. In current study, the species described in Cofcewicz et al. (2004a) were present, but the most common species associated with banana triploids and tetraploids were *M. javanica* (68.26%), *M. incognita* (64.73%) and *M. arenaria* (16.81%).

Our evaluation in naturally infested fields demonstrated that the genotypes under consideration are hosts for the root-knot nematodes. However, the suitability of the banana genotypes as root-knot nematode hosts may vary depending on the species of *Meloidogyne* (CLAUDIO; DAVIDE, 1967; DINARDO–MIRANDA; TEIXEIRA, 1996; VILAS BOAS et al., 2002; COFCEWICZ et al., 2004b; TEIXEIRA, 2007) and the environmental conditions inherent to each location (ARAYA; VARGAS; CHEVES, 1999; DAVIDE, 1980; MACSORLEY; PARRADO, 1981; KASHAIJA et al., 2004; RIBEIRO et al., 2006). Both species *M. javanica* and *M. incognita* were detected, with each species occurring at a rate of up to 100% in certain genotypes. In other genotypes, these two species co-occurred, but in unequal ratios. Likewise, in Palmital (SP), almost all genotypes had isolated infections of *M. javanica*, but *M. incognita* was the predominant species only in the cultivar Pacovan and YB 4203 genotype. However, at Embrapa Acre, *M. incognita* was predominant. *Meloidogyne javanica* was identified in only three genotypes (Bucanero, Japira and Maçã), while *M. arenaria* was detected only in the hybrid FHIA 23. One explanation for the variation in the behavior of these species among banana genotypes is that aggressiveness is reported to differ among populations of the same *Meloidogyne* species (SASSER, HARTMAN, CARTER, 1987; ROBERTS, 1995; BLOK et al., 1997). *Meloidogyne javanica* has been reported as a species with low intraspecific variability. Studies by Cofcewicz et al. (2004a) with seven *M. javanica* populations parasitizing banana plants from different regions in Brazil revealed an intraspecific variability of 29.1%, which is still considered low. Even so, it can be hypothesized based on its observed population sizes, that *M. javanica* populations from Embrapa Semiárido, Embrapa Cerrados, and Palmital, were more aggressive to the banana genotypes than that of Embrapa Acre, whereas the Epagri population exhibited intermediate aggressive behavior. Another factor to be considered in the present study in relation to the behavior of banana genotypes between different locations is the level of natural infestation of these areas by each of the respective nematode species. In some situations, for example, *Meloidogyne* sp. were observed to be absent in the roots and soil of cultivar FHIA 17 (Embrapa Acre) and genotype PV 7934 (Epagri), while there was non-development of females in the roots of tomato plants that had been inoculated with the suspension of ground roots originating from these genotypes. However, in seemingly similar situations with other cultivars, in which nematodes were not detected in their roots, a large number of female nematodes developed in the roots of tomato plants that had been inoculated with the ground-root slurries of these cultivars; this was the case for the cultivars Grande Naine, Thap Maeo, and others, demonstrating that these nematodes were present, in the form of eggs, in the inoculum. However, for the other locations, the high levels of J2 juveniles in the roots and soil made them readily observable that these banana genotypes are favorable hosts to the root-knot nematodes. Therefore, experiments like this only show the hostability of banana genotypes, and it is not possible to evaluate their resistance or susceptibility to each nematode species.

Among spiral nematodes, *H. multicinctus* was the one that stood out most in experimental areas, not being detected in Embrapa Semiárido and Embrapa Cerrados experimental areas. An unidentified *Helicotylenchus* species was detected in Embrapa Cerrados. In Acre, *H. multicinctus* had already been reported by Cavalcante et al. (2002) in association with *H. dihystera* (Cobb, 1893) Sher, 1961 on banana roots (245 specimens.$100g^{-1}$ roots). In another survey carried out on bananas in Acre, the occurrence (100%) of *H. babensae* Elmiligy, 1970 was detected (CAVALCANTE; SHARMA; CARES, 2005). According to Costa-Manso et. al. (1994) other species have been reported in association with banana plants in Brazil (*H. californicus* Sher, 1966, *H. cavenessi* Sher, 1966, *H. crenacauda* Sher, 1966, *H. erytrinae* Zimmermann, 1904 *H. flatus* Roman, 1965, *H. lobus* Sher 1966, *H. pseudorobustus* (Steiner, 1914) Golden, 1956, *H. retusus* Siddiqi and Brown, 1964 and *H. serenus* Siddiqi, 1963).

Currently, *R. reniformis* is not considered a relevant crop pest for banana cultivation, however, it can become an important nematode, due to the increase in its population density in banana rhizosphere. *R. reniformis* was recovered from soil samples in greater numbers in areas of Embrapa Semiárido, in some soil samples in the Palmital area in São Paulo, and in the rhizosphere soil of the genotype PV 7934 in Embrapa Cerrados. This fact can be explained by the biological nature of the reniform nematode, which completes its life cycle in the soil. It was only observed in the roots of the genotypes BRS Platina and YB 4203 in Embrapa Semiárido and in the genotypes Garantida, Bucanero, FHIA 18, Pacovan, Grande Naine, Prata Anã, YB 4203, Tropical, BRS Platina, PV 9401 and Maçã in Palmital - SP, suggesting this nematode has a greater affinity for these respective banana genotypes. The reniform nematode had previously been reported in the state of Pernambuco in banana

roots by Moura (1972) and Zem and Lordello (1983). The results obtained in this study are in agreement with Ritzinger et al. (2007), who detected *R. reniformis* in a survey of plant-parasitic nematodes in banana plantations located in the irrigated perimeters of the municipalities of Petrolina -PE and Juazeiro-BA, where the authors found a positive correlation between the frequency of *R. reniformis* and *H. multicinctus* in roots of the banana cv. Pacovan.

The nematodes found most frequently in banana crops sampled worldwide belong to the genera *Helicotylenchus*, *Meloidogyne* and *Rotylenchulus* (CARLIER; WAELE, D; ESCALANT, 2003). Similar results found in a survey of plant-parasitic nematodes in banana plantations in Brazil reported high frequencies of individuals of these genera (ZEM; LORDELLO, 1984; NEVES; DIAS; BARBOSA, 2009; LIMA et al., 2013; VELOZO, 2018), being specimens of *Helicotylenchus* the most common among soil samples.

*Radopholus similis*, the nematode of greatest economic importance for the banana crop, (O'BANNON, 1977; GOWEN, 1979; TARTE; PINOCHET, 1981) was only present at low abundances in this study and only in the experimental areas of Embrapa Acre and Palmital in São Paulo. In Brazil, the species was first reported in banana plantations in the coastal region of São Paulo (CARVALHO, 1959), and years later in other Brazilian states by several authors (PEREIRA; FIGUEIREDO JUNIOR; HUSSNI, 1960; SHARMA, 1974a, b; ZEM, 1978; ZEM; ALVES, 1978; ZEM; BARREIRA; TEIXEIRA, 1980; ZEM, 1982; HUANG et al., 1982; ZEM; LORDELLO, 1983, 1984; COSTA-MANSO et al., 1994). Despite the importance of the species to many banana plantations around the world, studies conducted in Brazil show that *R. similis* is still sporadic in most growing regions (OLIVEIRA, 2013). Studies by Cavalcante et al. (2002) and Cavalcante, Sharma and Cares (2005) in areas planted with different banana cultivars in the state of Acre, did not detect *R. similis*. According to these author's personal information, the experimental area surveyed in the present study had already been cultivated with banana before the implementation of the current experiment, which may explain the presence of the burrowing nematode in the experimental field at Embrapa Acre.

Other secondary plant-parasitic nematodes of banana plants were also detected in low numbers, such as *Criconemoides* sp. and *Scutellonema* sp. Nematodes in the genus *Criconemoides* have been reported in banana plantations in the state of Acre by Cavalcante, Sharma and Cares (2005) and confirmed by Nogueira et al. (2013) in Bonal and Senador Guionard in banana plantation soils. Nematodes of the genera *Criconemoides* and *Scutellonema* have also been observed in banana plants (*Musa* spp., Group AAB) in Nigeria by (SPEIJER; ROTIMI; WAELE, 2001). Other banana-related species, as revised by Costa-Manso et al. (1994), with the synonymy of *Criconema* (*C. azani*) and *Criconemella* (*C. curvata* Raski, 1952, *C. onoensis* Luc, 1959, *C. ornata* Raski, 1958, *C. peruensiformis* (De Grisse, 1967) Luc, 1970 and *C. xenoplax* Raski, 1952) were reported in Brazil. In Santo Antonio do Amparo (MG), the nematode *Criconemella* sp. and, in São Vicente Ferrer (PE), the nematode *Sculellonema* spp. were detected in banana plants by (SOUZA; MAXIMINIANO; CAMPOS, 1999). The species *Scutellomema magniphasmum* Sher, 1965 have been reported to be associated with banana plantations in Malawi in Africa by Saka and Siddiqi (1979). *Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958 also has been reported in association with banana plants in Brazil (COSTA-MANSO et al., 1994).

## CONCLUSIONS

The nematode genera with the highest frequency and population levels detected in the banana genotypes belong to the genera *Meloidogyne*, *Helicotylenchu*s and *Rotylenchulus*. The root-knot nematodes were predominant in all banana genotypes in the experimental areas evaluated. Four esterase phenotypes were characterized for *Meloidogyne*: *M. incognita* (Est $I_1$), *M. javanica* (Est $J_3$ and $J_2$) and *M. arenaria* (Est $A_2$), predominantly for *Meloidogyne javanica* (Est $J_3$). In addition to the root-knot nematodes, the spiral nematode (*Helicotylenchus multicinctus*) and the reniform nematode (*Rotylenchulus reniformis*) were the most detected in soil samples. *Radopholus similis, Scutellonema* sp., *Criconemoides* sp. and *Helicotylenchus* sp. were plant-parasitic nematodes that showed low frequencies and population levels among banana genotypes.

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