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# DISTRIBUTION OF *Meloidogyne enterolobii* IN GUAVA ORCHARDS IN THE STATE OF CEARÁ, BRAZIL[1]

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**ABSTRACT** - Among the factors negatively impacting guava (*Psidium guajava*) crop in Brazil, one of the most important is the root-knot nematode, *Meloidogyne enterolobii*, which may cause considerable yield losses and even the cessation of guava cultivation in some areas. In addition to affecting guava, the pathogen has been reported as a parasite in various other crops, among them both oleraceous and ornamental crops, as well as in spontaneous vegetation. The aim of this study was to verify the occurrence of *M. enterolobii* in plants collected in guava orchards in different counties of Ceará state, identified through electrophoresis with the isoenzyme esterase, and to observe its infecting behavior into *Meloidogyne* differentiating plants. Fifty root samples from guava, tasselflower (*Emilia fosbergii*), and jurubeba (*Solanum paniculatum*), were collected in 13 counties from eight micro-regions in the state of Ceará. In all analyzed samples, only esterase phenotype M2 (Rm: 0.6; 0.9), characteristic of *M. enterolobii*, was detected, showing that the nematode is widespread in orchards throughout the state, where is affecting these fruit tree, and that it is also able to parasitizing plants of the spontaneous vegetation. Based on the results, this nematode currently constitutes a serious threat to guava plantations in Ceará, and effective control mechanisms are crucial to prevent the spread of this pathogen to other, still unaffected, areas.

**Keywords**: Root-knot nematode. *Psidium guajava*. Electrophoresis. Esterase.

## DISPERSÃO DE *Meloidogyne enterolobii* EM POMARES DE GOIABEIRAS EM MUNICÍPIOS DO ESTADO DO CEARÁ

**RESUMO** - Dentre os problemas que afetam a cultura da goiabeira (*Psidium guajava*) no Brasil, destaca-se o nematoide das galhas *Meloidogyne enterolobii,* o qual pode provocar consideráveis perdas na produção e tornar inviáveis áreas para o cultivo da fruteira. Além da goiabeira, o patógeno já foi relatado parasitando diversas outras culturas dentre olerícolas, ornamentais, como também em plantas de vegetação espontânea. Objetivou-se, com este trabalho, verificar a dispersão de *M. enterolobii* em plantas coletadas em pomares de goiabeiras em diferentes municípios do Ceará, empregando na identificação a técnica da eletroforese com a isoenzima esterase e observar o comportamento do patógeno em espécies diferenciadoras de *Meloidogyne.* Analisaram-se aproximadamente 50 amostras de raízes obtidas de goiabeiras, de falsa serralha (*Emilia fosbergii*) e de jurubeba (*Solanum paniculatum*) coletadas em 13 municípios pertencentes a oito microrregiões do estado. Em todas as amostras analisadas detectou-se apenas o fenótipo de esterase M2 (Rm: 0,6; 0,9) característico de *M. enterolobii,* indicando que o nematoide está disseminado no estado afetando a fruteira e que pode permanecer na área parasitando plantas da vegetação espontânea. Com as informações obtidas, verificou-se que este nematoide constitui, atualmente, uma séria ameaça aos cultivos da goiabeira no Ceará e que práticas efetivas de controle são requeridas para evitar a dispersão desse patógeno em áreas ainda isentas do fitoparasita.

**Palavras-chave**: Nematoide das galhas. *Psidium guajava*. Eletroforese. Esterase.

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# INTRODUCTION

Guava (*Psidium guajava* L.), a species of the Myrtaceae family, is widely grown in almost all tropical and subtropical regions of the world and well adapted to different environmental conditions (GONZAGA-NETO et al., 2001). In northeastern Brazil, the main growing states are Pernambuco, Bahia, and Ceará. According to the Instituto Brasileiro de Geografia e Estatisitica (IBGE), in 2014, the area cultivated with guava in Brazil was 15,831 hectares, with a production of 359,349 tons, and the harvested area of guava in Ceará was 1,515 ha, with a production of 18,936 tons, including harvests from irrigated areas in the regions of Baixo Jaguaribe, Acaraú, and Cariri and conventional crops in several municipalities (ADECE, 2013; IBGE, 2014).

Several genera and species of nematodes such as *Meloidogyne* (*M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood) and *Radopholus* sp., *Rotylenchulus reniformis* Linford and Oliveira, *Helicotylenchus nannus* Steiner, and *Aphelenchus avenae* Bastian, are reported in association with guava plants (MOREIRA; SHARMA, 2001). In addition, other species belonging to at least 16 genera have been reported (SILVA, 2009).

In Brazil, *M. enterolobii* Yang and Eisenback was the most frequent species affecting guava crops, threatening the country´s guava industry (CHARCHAR et al., 2009). The first report in Brazil in 2001 was to the cities of Petrolina, Curaçá, and Maniçoba-BA, with severe damage to commercial guava plantations caused by *M. mayaguensis* Rammah and Hirschmann, 1988 (CARNEIRO et al., 2001).

The specie *M. enterolobii* was first described in roots of *Enterolobium contortisiliquum* L. in South China (YANG; EISENBACK, 1983), while *M. mayaguensis* was reported first on eggplants (*Solanum melongena* L.) in Puerto Rico (RAMMAH; HIRSCHMANN, 1988). Although *M. mayaguensis* was considered then as a new species, studies conducted by Xu et al. (2004) involving morphology, host range, isozyme phenotypes (esterase and malate dehydrogenase), and *mt*DNA sequence analysis made it possible to clarify that both were the same species of nematode.

Following the first report of *M. enterolobii* in guava plants in the states of Pernambuco and Bahia (CARNEIRO et al., 2001), this nematode was also found on guava plants in Rio de Janeiro (LIMA et al., 2003), Ceará (TORRES et al., 2005), São Paulo (ALMEIDA et al., 2006), Paraná (CARNEIRO et al., 2006), Piauí (SILVA et al., 2006), Espírito Santo (LIMA et al., 2007), Minas Gerais (OLIVEIRA et al., 2007), Maranhão (SILVA et al., 2008), Santa Catarina and Rio Grande do Sul (GOMES et al., 2008), Goiás (SIQUEIRA et al., 2009), and Tocantins (CHARCHAR et al., 2009). Since then, this pathogen has been introduced into new guava orchards and has rapidly spread all over the country, compromising myrtaceous crops and contributing to yield reductions and even complete crop losses in infested areas (PEREIRA et al., 2009). In the São Francisco Valley, in the Northeast, crop losses caused by *M. enterolobii* in guava orchards range from reduced seedling development to the death of adult plants; in severe cases, whole orchards have been eradicated in the fourth year after planting (TORRES et al., 2007).

The nematode *M. enterolobii* was first reported in 2004 in the guava variety 'Paluma', the most common variety in commercial orchards at Limoeiro do Norte county (TORRES et al., 2005) in the eastern region of the state. According to the authors, the orchards were established from seedlings from Pernambuco. Nematode species identification was performed by analysis of esterase phenotype. In 2011, this nematode was reported in orchards in the city of Barbalha, in the southern of Ceará state, with considerable infestation and high losses in fruit production (MOURA et al., 2011).

Considering the significance of nematode infections in guava plants and the rapid spread of *M. enterolobii* in Brazil, the objectives of this study were to investigate the distribution of this nematode species in guava trees and weeds in orchards in the state of Ceará and to observe its infecting behavior into *Meloidogyne* differentiating plants.

# MATERIAL AND METHODS

### Field sampling

Root samples were collected in guava orchards during 2012 and 2013. Approximately 40 plants exhibiting infection symptoms were collected at 21 orchards from 13 counties throughout the state of Ceará, belonging to eight different micro-regions (Figure 1). Georeferenciated locations were as following: Limoeiro do Norte - 5°08"37,82"S and 38°05'06,12"O, Quixeré 5º04'06,49"S and 37° 59'36,86"O (Microrregião do Baixo Jaguaribe), Barbalha - 7°17'52,02"S and 39°18'09,62"O, Crato - 7°13'44,87"S and 39°24'46,84"O, Juazeiro do Norte - 7°13'37,40"S and 39°18'43,64"O and Missão Velha - 7°14'58,53"S and 39°08'25,10"O (Cariri region), Cascavel - 4°07'56,76"S and 38°14'49,84"O (Cascavel region), Fortaleza - 3°43'54,85"S and 38° 31'37,12"O and Guaiuba - 4°02'31,75"S and 38° 38'01,33"O (Fortaleza region), Acaraú - 2° 53'14,88"S and 40°07'18,33"O (Camocim and Acaraú region), Pentecoste - 3°47'32,18"S and 39° 16'11,54"O (Médio Curu region), Mauriti - 7° 22'58,09"S and 38°46'18,11"O (Barro region), and Pacajus - 4°10'22,61"S and 38°27'39,19"O (Pacajus

region)". In addition to root galls, sampled plants showed reduced growth, mineral deficiency, yellowing and detaching of leaves.

Additionally, ten samples of frequently found weeds in several areas (*Emilia fosbergii* Nicolson and *Solanum paniculatum* L.) from Acaraú, Barbalha, Cascavel, Crato, Missão Velha, Pacajus, and Pentecostes were included in this study. All sampled plant materials were taken to the laboratory for labeling, examining, and testing.

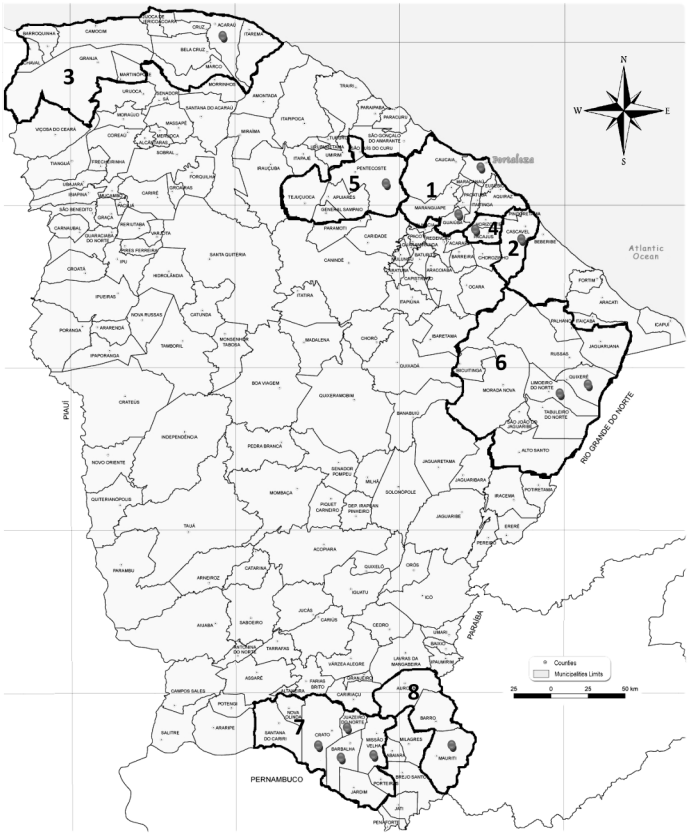


**Figure 1**. Ceará State map highlighting the regions where root samples were collected: 1- Fortaleza; 2- Cascavel; 3- Litoral Camocim and Acaraú; 4- Pacajus; 5-Médio Curu; 6- Baixo Jaguaribe; 7- Cariri; 8- Barro.

**Nematode extraction and inoculation**

Eggs and second stage juveniles (J2) were extracted from sampled galls by the Coolen and D'Herde technique (1972), which uses 20 and 400 mesh sieves and centrifuging in 45% sucrose solution. The obtained suspension was calibrated in a Peters chamber under microscopic stereoscopy to determine egg concentration (eggs/ml) for inoculation into Coleus (*Solenostemon scutellarioides* L.) seedlings grown in autoclaved soil in order to maintain *M. enterolobii* populations for further studies. Subsequent to inoculation, plants were kept in a greenhouse at 29 $\pm$ 4ºC.

**Isoenzymatic characterization of the *Meloidogyne* population**

Six to seven milk-whitish females in the oviposition stage were taken from each root sample, with a total of 300 individuals in each analysis. Females obtained from different parts of the roots were transferred to individual wells containing 15 µL of prepared solution for protein extraction (20% sucrose, 2% Triton X-100, 0.01% bromophenol blue, and 78% of distilled water). Then, 10 µL of each protein extract obtained were applied to the cavity polyacrylamide gel. The standard sample consisted of protein extracts of the species *M. javanica*, which were distributed in at least one of the cavities of each gel (ALFENAS; BRUNE, 2006).

For species identification, was used the discontinuous method of vertical electrophoresis in polyacrylamide gels whose bis-acrylamide concentrations were 7.5% (2.5ml bis-acrylamide, 1.88 ml of tris-HCl, pH 8.8, 45 µL of ammonium persulfate, 10 µL TEMED, and 5.75 ml of distilled water) and 4.0 % (500 µL bis- acrylamide, 1.25 ml of tris-HCl, pH 6.8, 45 µL of ammonium persulfate, 10 µL TEMED, and 3.10 ml of distilled water) in the separator and stacking gels, respectively. The procedure was carried out inside a refrigerator at 4°C under 80V in the stacking race (30-40 minutes), with 200 V for the separation step in the running gel (40-60 minutes). After the specified time, the gels were transferred to a developing solution of enzyme esterase (100 ml of 0.05M potassium phosphate buffer pH 6.0, 100 mg of Fast Blue RR Salt, and

4.5 ml of α -naftilacetato 1%) and kept at 37°C for 30 minutes. The gels were then washed in distilled water and transferred to fixative solution (45% methanol, 9% acetic acid, and 45% distilled water) and kept for 20 minutes at 37°C. After staining, fixing, washing (for proper interpretation of bands), and drying, was used the frame technique with cellophane (ESBENSHADE; TRIANTAPHYLLOU, 1990; ALFENAS; BRUNE 2006).

**Morphological characterization of *Meloidogyne* species**

Alongside the enzymatic analysis, we proceeded to the observation of the perineal configuration, using 35 to 40 females taken from the roots of guava trees. Cuts at the perineal region, made with a scalpel under a stereomicroscope, were examined under an optical microscope, aiming to identify species based on standards of perineal settings already described for *Meloidogyne* (HUNT; HANDOO, 2009). Perineal configuration examination was not performed for females from *Emilia fosbergii* and *Solanum paniculatum* roots, which were only used the isoenzyme analysis.

**Physiological characterization of *Meloidogyne* populations**

Only *Meloidogyne enterolobii* populations from guava identified by electrophoresis were inoculated in differentiating plant species (HARTMAN; SASSER, 1985) in order to compare the reactions of plants inoculated with the species *M. incognita, M. javanica, M. arenaria* and *M. hapla*. Seedlings of tobacco (*Nicotiana tabacum* L. 'NC 95'), pepper (*Capsicum frutescens* L. 'Early California Wonder'), cotton (*Gossypium hirsutum* L. 'Deltapine 16'), watermelon (*Citrullus lanatus* (Tunb.) Matsum. and Nakai 'Charleston Gray'), peanut (*Arachis hypogaea* L. 'Florunner'), and tomato (*Solanum lycopersicon esculetum* L. 'Santa Clara') (control) were inoculated with 4,000 eggs/J2 per plant. Inoculated differentiating plants remained for 60 days in a greenhouse (29 ± 4°C) for evaluation of the presence or absence of root galls.

## RESULTS AND DISCUSSION

*Meloidogyne enterolobii* was the only species found in guava trees grown in 13 municipalities in Ceará. The esterase profile was typical of *M. enterolobii* (M2), with two very obvious main bands (Rm: 0.6, 0.9) and two faint secondary bands (Figure 2), as also described in Carneiro et al. (2000). A similar number of bands was observed by Silva and Oliveira (2010) with *M. enterolobii,* but using samples containing at least three females per well. When the protein extract was originated from a single female, only the two main bands were visible for *M. enterolobii* (SILVA; OLIVEIRA, 2010).

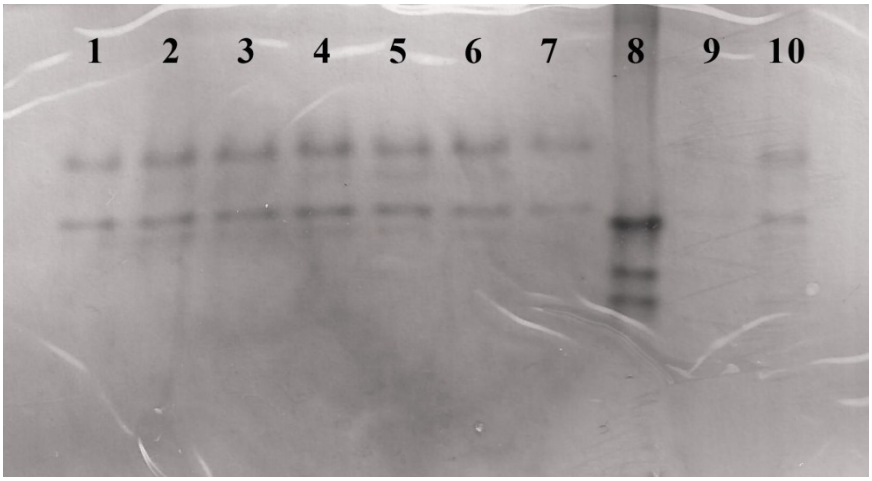


**Figure 2**. Esterase phenotypes of *Meloidogyne enterolobii* populations from plants collected in different municipalities in the state of Ceará. Guava (1 to 5), jurubeba (6 and 7), *M. javanica* (8), tasselflower (9 and 10).

Visibility of the secondary bands depends on esterase concentration in the female. However, in some cases, even visibility of the major bands may require more than one female per well of the gel (CARNEIRO et al., 2001). The tests conducted in this work used protein extract from a single female per well in the gel, however, it allowed visualization of the four bands of *M. enterolobii*.

Light microscopic analysis of the perineal patterns obtained from females removed from guava roots alone was not conclusive for the definition of species because of their variations. The patterns exhibited a dorsal arch close to trapezoidal with curling incisures, with the region near the tail devoid of lines, lacking the rounded or oval shape pattern typical for this species. The dorsal arch, however, seems not to be much different from that of *M. enterolobii*, described as moderately to high, often round, in some cases almost squared (YANG; EISENBACK, 1983). Similar observations were

reported by Torres et al. (2005), which failed to identify the species of *Meloidogyne* from guava roots based on perineal patterns, since the observed characteristics resembling *M. incognita* were also atypical. Variability of perineal patterns was also reported by Gomes et al. (2008) from females obtained from tobacco and guava parasitized by *M. enterolobii*, which was later identified by analysis of esterase profile. In another study by Silva and Oliveira (2010), the perineal patterns of females from guava roots showed variability, not allowing the identification of the examined species. In the report of the first occurence of *M. enterolobii* on guava plants and on *Byrsonima cydoniifolia* A. Juss (a wild fruit plant of the Myrtaceae family, commonly found in Brazil) in the state of Mato Grosso, in addition to perineal patterns, they considered the labial region of males and isoenzyme phenotype esterase for species identification (PAES et al., 2012). In this work the labial region of males was not analyzed and due to the variations observed in the perineal settings, the analysis of esterase profiles was considered as a safe method for identification of the species present in all plant material.

The results presented here showed that *M. enterolobii* was found in all studied regions, suggesting its rapid spread in the state, since it was first reported in 2004 in Limoeiro do Norte county, Baixo Jaguaribe region (TORRES et al., 2005), followed by another report in 2011 in the county of Barbalha, Cariri region (MOURA et al., 2011). Apart from these two locations, in the guava orchards studied in 2012 and 2013 it had not yet been carried out sampling of guava roots aiming the detection of nematodes.

The nematode *M. enterolobii* strongly reduces crop yield by weakening the plants in the orchard, making the planting impracticable after the fourth year of cropping (MOREIRA et al., 2003). Pereira et al. (2009) studied the impact caused by *M. enterolobii* parasitism on guava crops in five Brazilian states, including Ceará, and estimated that the direct damage caused by this nematode was R$ 112.7 million, in addition to unemployment of thousands of workers as a result of guava production decline five years after the establishment of orchards.

Although there are reports of the occurrence of *M. enterolobii* in areas with native vegetation or in preserved areas of the Atlantic Forest, as documented for Rio de Janeiro by Lima et al. (2005), in Ceará, the marketing of infected seedlings may be the primary source of introduction and spread into guava orchards. In the state of Mato Grosso, introduction of *M. enterolobii* into various locations occurred through guava and muricizeiro (*Byrsonima cydoniifolia)* infected seedlings, causing the loss of more than 80% of the acquired plants and eradication of all plants from the nursery. This incidence underlines the importance of acquisition of nematode-free plant propagating substrate (PAES et al., 2012). Gomes et al. (2008) reported that the introduction of *M. enterolobii* in the state of Rio Grande do Sul possibly occurred through infected guava plants from São Paulo. Torres et al. (2005) pointed out that the introduction of *M. enterolobii* into the state of Ceará occurred through infected seedlings from Pernambuco. Torres et al. (2007) also reported that seedlings from the county of Assu, Rio Grande do Norte produced in soil from areas with native vegetation resulted in infested orchards. Thus, considering that the spread of *M. enterolobii* in and within states may occur through guava seedlings (Torres et al., 2007), the production and marketing of these plants should occur with greater rigor or even using commercial soil-less substrate. Once introduced within an area, nematodes spread to neighboring areas by movement of land and water from rain or irrigation.

According to the visited growers in Cariri region (Crato, Juazeiro, Barbalha, and Missão Velha), there is a lack of information about the importance of root-knot nematodes, spreading mechanisms within orchards, and effective control practices. Therefore, in some Cariri settlements, there has been a reduction in the guava area and even a total replacement of guava by other fruit crops such as banana (*Musa* sp.). Such procedures have been repeated in other producing areas of the state with different fruit trees, such as papaya (*Carica papaya* L.), also susceptible to this nematode species. The report presented in this paper results from sampling in orchards from eight geographically different regions, but no attempt has been made to systematize the surveys as to specify size or percentage of *M. enterolobii* infested area.

*Meloidogyne enterolobii* was found parasitizing weed plants of tasselflower (*Emilia fosbergii*) and/or jurubeba (*Solanum paniculatum*) in guava orchards of seven sampled counties. In Jaboticabal - SP and São João da Barra - RJ, *M. enterolobii* was reported in seven and ten species of weeds, respectively, present in guava crops (SOUZA et al., 2006; ALMEIDA et al., 2011).

The reaction of the *M. enterolobii* population studied here was similar to that of *M. incognita* race 2 (HARTMAN; SASSER 1985) (Table 1): presence of galls on the roots of tobacco, pepper, watermelon, and no galls on cotton and peanut roots. Similar observations races of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* may not contribute to the physiological characterization of *M. enterolobii*.

**Table 1**. Reaction of differentiating host plants inoculated with *Meloidogyne enterolobii* from guava roots sampled in 2014 from different counties of Ceará state.

| County | Host plant | | | | | |
|---|---|---|---|---|---|---|
| | Cotton | Tobacco | Pepper | Watermelon | Peanut | Tomato |
| Acaraú | - * | + | + | + | - | + |
| Barbalha | - | + | + | + | - | + |
| Cascavel | - | + | + | + | - | + |
| Crato | - | + | + | + | - | + |
| Pacajus | - | + | + | + | - | + |
| Pentecoste | - | + | + | + | - | + |

*(-) no galls; (+) galls (HARTMAN; SASSER, 1985).

The results presented in this study suggest that within a few years, *M. enterolobii* could severely impact the guava commercial exploitation in the state of Ceará, similarly of what has been reported for the city of Barbalha (MOURA et al., 2011), by reducing guava growing area, either caused by replacements by other fruit crops or by economic infeasibility as a consequence of the rapid spread of nematode and climatic conditions of region favorable to the pathogen.

The most recommended practices to control root-knot nematodes involve the acquisition of seedlings from certified nurseries, rotation with non-host plants, consortium with antagonistic plants, biological control, elimination of natural vegetation from the area, and fumigant nematicide application, which should be considered both to prevent the introduction of nematode in pathogen-free areas and to reduce infestation in cultivated areas (SILVA, 2009; SOUZA et al., 2006). The adoption of quarantine measures to prevent the movement of infested plant material may be considered (CARNEIRO et al., 2001).

The control of *M. enterolobii* in guava in Brazil has been very difficult because of failures in the adoption of the aforementioned practices, the lack of registered nematicides to be applied to guava and the lack of enforcement in compliance with the legislation to inspect seedlings for pathogen presence (GOMES et al., 2008). Nevertheless, research has been conducted for the selection of guava rootstocks resistant to root-knot nematodes, which can effectively contribute to pathogen control in orchards, restoring the guava orchards already considered infeasible.

## CONCLUSIONS

*Meloidogyne enterolobii* was the only species of the genus *Meloidogyne* detected in guava and was distributed in all surveyed orchards surveyed in the state of Ceará, Brazil.

*Emilia fosbergii* and *Solanum paniculatum* are hosts of *M. enterolobii* in guava orchards in the state of Ceará, Brazil.

The behavior of differential host plants inoculated with *M. enterolobii* is similar to the reaction that induced by *M. incognita* race 2.

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