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Native-plant hosts of *Meloidogyne* spp. from Western Paraná, Brazil

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ABSTRACT

The present study was focused on the parasitism of Meloidogyne species on the roots of native nursery plants from the Atlantic forest. Native plants were selected from a commercial nursery in Western Paraná, searching for the natural infection of Meloidogyne. Also, the seeds of native plants were cultivated in sterile soil and inoculated with M. incognita. In both the experiments, the number of galls and number of eggs and J2 per root, allied to the reproduction factor of M. incognita on each inoculated plant were assessed. Natural infection by M. javanica was found on Cordia ecalyculata, Citharexyllum myrianthum and Aspidosperma subincanum and by M. incognita on Croton urucurana, Lonchocarpus muehlbergianus, Tabebuia impetiginosa and T. serratifolia. Meloidogyne incognita induced galls formation on Genipa americana, Schinus terebinthifolius and Rollinia mucosa after inoculation, which suggested that those plants could host this nematode in natural biomes. Nursery soil should be disinfested before seeding the native forest plants for reforestation purposes

Key words: Atlantic forest, native-plants, Root-knot nematodes

INTRODUCTION

Meloidogyne spp. are one of the most important plant-parasitic nematodes in the world, showing a wide distribution and a large host range. In Western Paraná, *Meloidogyne* species cause economical damages to different crops such as soybean (Roese et al. 2001; Franzener et al. 2005), cotton (Pires et al. 2008) and coffee (Portz et al. 2006), among others.

Western Paraná, located at Paraná state, south of Brazil, presents a semi-deciduous subtropical forest called "The Upper Paraná Atlantic Forest" in which most of native plants belong to the Atlantic forest biome (Di Bitetti et al. 2003). Despite being fragmented, Atlantic forest is still considered one of the hot spots for fauna and flora biodiversity for conservation purposes (Myers et al. 2000).

Regarding the Atlantic Forest only 12% remains intact. Therefore, the preservation of areas covered by the Atlantic forest as well as the implementation of reforestation programs in Brazil will aid to protect the forest biodiversity and to promote its sustainable development. A reforestation project involving the Climate, Community and Biodiversity Alliance (CCBA) and partnerships has started in 2008. This project aims to revive 1,000 hectares of Atlantic Forest located between the National Parks of Monte

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Pascoal and Pau Brasil. The forest recovery will also contribute to remove 360,000 tons of carbon over 30 years. Certainly, the success of this project will contribute to prioritize the actions towards the preservation and reforestation of the Atlantic Forest in Brazil. (Donsky and Boyer 2010).

The biodiversity of the fauna and flora of the Atlantic Forest has been well characterized but not that related to the nematodes, particularly root-knot nematodes. Surveys on *Meloidogyne* spp. have been carried out in Atlantic forest (Lima et al. 2005) reporting the presence of *M. incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* (= *M. mayaguensis*), and *M. exigua*. For other Brazilian biomes such as "cerrado" or brazilian savannah (Souza et al. 1994; Huang et al. 1996; Cares and Huang 1991; Cares and Huang 2008), *M. javanica* and *M. arenaria* race 2 were reported and for the Amazon forest (Cares and Huang 2008; Silva et al. 2008) *M. javanica* has also been reported. Currently, there is no information for the native forests from Western Paraná regarding to the parasitism of *Meloidogyne* spp.

The challenges found in the native areas when searching for root-plant parasites are mainly related to the mix of the roots at the rhizosphere of plants, which makes difficult to identify their putative hosts. As the Atlantic forest is composed of dense vegetation, different strategies were adopted in the present work to assess the parasitism of *Meloidogyne* on native plants: 1. A survey was carried out in a commercial nursery aiming to analyze roots of plants grown in non-sterile soil, as a way to search for the natural infection of *Meloidogyne* spp.; 2. Artificial inoculation was provided to the plants cultivated in sterile soil to evaluate *Meloidogyne* parasitism on the native plants.

MATERIALS AND METHODS

Search for *Meloidogyne* spp. on native plant seedlings from a commercial nursery

In order to detect *Meloidogyne* spp. infecting the native plants, a survey was carried out on the plant seedlings from a commercial nursery belonging to the Western Paraná State University. The assay was arranged in a completely randomized design containing 36 different treatments (plant species) and five replications. All the selected plants had the same age (one year old) and received a standard treatment in the nursery such as daily watering, ten granules of fertilizer N(4)-P(14)-K(8) applied to each plant every three months and fungicide application to control the shoot diseases when necessary. The seedlings were kept into a 3 Kg black plastic bag of 30 cm depth and 10 cm wide, filled with non-sterile soil.

The plant root systems were washed and analyzed for the presence of galls caused by *Meloidogyne* spp. From the galls, single females (ten from each root plant) were extracted and *Meloidogyne* species were identified by esterase phenotype, according to Esbenshade and Triantaphyllou (1985). Briefly, the extracted proteins from single females were run in a MGV-202 electrophoresis system (Biosystems). Esterase phenotypes were visualized on polyacrilamide gels 8% and stained with 0.1% fast blue RR and α-naftil acetate (Sigma) stains. The relative mobility (Rm) of esterase in the gels was calculated by the equation: migration distance of each band/distance of the front dye (bromophenol blue). The Rm provided information about the molecular weight of molecules according to their migration in gels. For *Meloidogyne* identification, *M. javanica* is usually used as a standard for comparison with other species. In this study, populations of *M. javanica* were found on the root system of some hosts and were used for comparison in the gels (Fig. 1).

In addition, the number of eggs and J2 stages were evaluated per root system. In this sense, the roots were washed in tap water, cut into small pieces and triturated in a blender for 30 seconds under low rotation, following Coolen and D'Herbe (1972). The reproduction factor (RF) was not considered at this first stage because the initial population was unknown.

Artificial inoculation of *M .incognita*

Artificial inoculation of *M. incognita* (Est I1) was accomplished on the 29 native plant species that did not demonstrate natural infection by *Meloidogyne* spp. (Table 1). This assay was conducted in a greenhouse with temperature and air humidity ranging from 11.1 to 31.7 ^{o}C and from 36.4 and 97.3 %, respectively. A completely randomized design with 29 treatments (plant species) and four replications was arranged for this experiment. One population of *M. incognita*, extracted from the roots of *Tabebuia impetiginosa*, was multiplied on tomato plants in a greenhouse and used as inoculum source. Each native-plant species was seeded in the plastic pots filled with 3 Kg of sterile soil. The soil was sterilized by autoclaving at 120 ^{o}C for 1 hour. A single plant,

three months of age, was used per plastic pot. The plants were inoculated with 5,000 eggs and second-stage juveniles (J2) of *M. incognita* and were evaluated after sixty days. The evaluation procedure involved the washing of each plant root to record the number of galls. The process of eggs and J2 extraction was performed as previously mentioned, following Coolen and D'Herbe's methodology (1972). The reproduction factor (RF) was calculated according to the formula RF = Pi/Pf, where the Pf represented the final and the Pi the initial population. In this study, the initial population was 5,000 eggs and J2, both considered as a single value to calculate the RF. The RF is used to measure the reproductive ability of nematodes on a given host. Therefore, hosts are considered resistant when RF≥1.0 and susceptible when RF≤1.0.

RESULTS AND DISCUSSION

From 36 native-plant species surveyed, seven were naturally infected by *M. incognita* (Est I1, Rm: 1.0) or *M. javanica* (Est J3, Rm: 1.0, 1.25 and 1.33) (Fig. 1). *Meloidogyne incognita* was found on the roots of *Lonchocarpus muehlbergianus*, *Tabebuia impetiginosa*, *Croton urucurana* and *Tabebuia serratifolia*, whereas *M. javanica* occurred on *Cordia ecalyculata*, *Cytharexyllum myrianthum* and *Aspidosperma subincanum* (Table 1). The average of galls on the roots of naturally-infected plants ranged from 8 to 129, while the number of eggs and\or J2 per root system ranged from 87 to 5,733 (Table 2). These plants could be considered new hosts for *M. incognita* and *M. javanica* since no previous records were found in the literature.

Of the 29 plant species artificially-inoculated with *M. incognita*, only *Genipa Americana*, *Schinus terebinthifolius*, and *Rollinia mucosa* presented susceptibility to this nematode species. Variations were observed in the reproductive factors as a result of artificial inoculation of *M. incognita* on *Genipa Americana* (RF average = 0.45), *Schinus terebinthifolius* (RF average = 1.73) and *Rollinia mucosa* (RF average = 1.2). From these plants, *Schinus terebinthifolius* and *Rollinia mucosa* showed RF≥1.0 and could be considered as good hosts for this nematode.

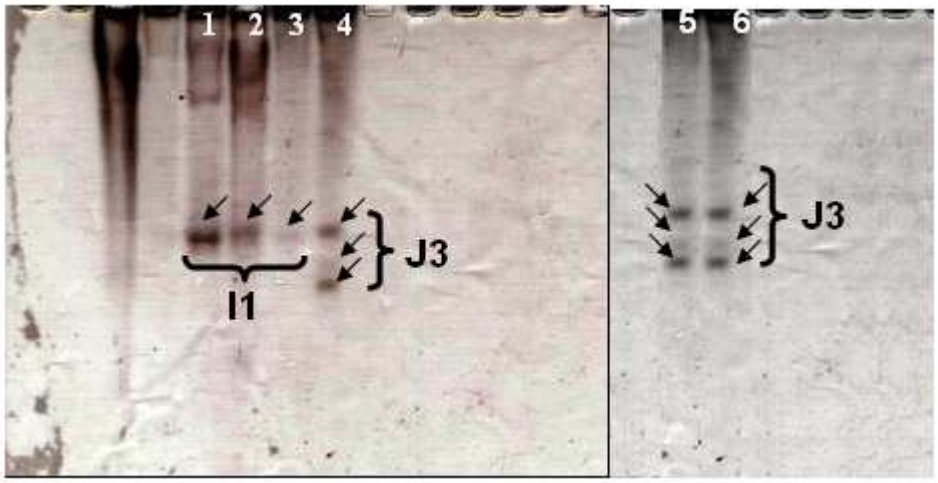

Figure 1 - Esterase phenotypes of *M. incognita* (I1) and *M. javanica* (J3) displayed in two polyacrylamide gels: Gel 1: *Lonchocarpus muehlbergianus* (1), *Croton urucurana* (2), *Tabebuia impetiginosa* (3), *Cordia ecalyculata* (4); Gel 2: *Cytharexyllum myrianthum* (5) and *Aspidosperma subincanum* (6).

Table 1 - Seedlings of native plants collected from the commercial nursery of the Western Paraná State University and assessed for infection of *Meloidogyne* spp.

Plant species	Common Name/Family	Natural host*	Inoculated hosts**
Adenanthera pavonina L.	Red sandalwood tree/ Fabaceae Mimosoideae		
Albizia polycephala (Benth.) Killip ex Record.	Farinha-seca/Fabaceae Mimosoideae		
Anadenanthera macrocarpa (Benth.) Brenan	Angico Vermelho/ Fabaceae Mimosoideae		
Aspidosperma subincanum Mart.#	Guatambu/Apocynaceae	*M. javanica*	
Bauhinia longifolia (Bong.) Steud.	Pata de vaca/ Fabaceae Caesalpinoideae		
Cabralea canjerana (Vell.) Mart.	Canjerana/Meliaceae		
Caesalpinia ferrea Mart. Ex. Tul. var. *leiostachya* Benth.	Iron Wood/Fabaceae Caesalpinoideae		
Cedrela fissilis Vell.	Cedro/Meliaceae		
Chorisia speciosa A. St.-Hil.	Paineira/Bombacaceae		
Cordia ecalyculata Vell.#	Café de bugre/Boraginaceae	*M. javanica*	
Cordia trichotoma (Vell.) Arrab. ex Steud.	Frei Jorge 1/Boraginaceae		
Croton urucurana Boill.#	Dragon's blood/Euphorbiaceae	*M. incognita*	
Cytharexyllum myrianthum Cham#	Taruma Branco/Verbenaceae	*M. javanica*	
Enterolobium contortisiliquum (Vell.) Morong	earpod tree/Fabaceae Mimosoideae		
Eugenia involucrata DC.	Cherry of the Rio Gande/ Myrtaceae		
Eugenia uniflora L.	Brazilian cherry/Myrtaceae		
Genipa americana L.#	Genipap/Rubiacea		*M. incognita*
Inga laurina	Ice cream bean/Fabaceae Mimosoideae		
Jacaranda micrantha Cham	Jacaranda/Bignoniaceae		
Lonchocarpus muehlbergianus Hassl.#	Rabo de Bugio/Fabaceae Faboideae	*M incognita*	
Luehea paniculata Mart.	Açoita-cavalo/Tiliaceae		
Maclura tinctoria (L.) D. Don ex Steud.	Dyer's mulberry; Fustic mulberry/ Moraceae		
Myrcianthes pungeus (Berg) Legr.	Guabiju/Myrtaceae		
Myrocarpus frondosus Allemao	Cabreuva/Fabaceae Faboideae		
Parapiptadenia rigida (Benth.) Brenan	Angico gurucaia/Fabaceae Mimosoideae		
Patagonula americana L.	Guajuvira/Boraginaeae		
Peltophorum dubium (Spreng.)Taub.	Canafístula/Fabaceae Caesalpinoideae		
Prunus sellowii Kochne	Pessegueiro Bravo/Rosaceae		
Psidium cattleianum Sabine	Strawberry guava/Myrtaceae		
Pterogyne nitens Tul.	Amendoim bravo/Fabaceae Caesalpinoideae		
Rollinia mucosa (Jacq.) Baill.#	Biriba/Annonaceae		*M. incognita*
Ruprechtia laxiflora Meisn.	Marmeleiro/Polygonaceae		
Schinus terebinthifolius Raddi.	***Brazilian*** Pepper-tree/ Anacardiaceae		*M. incognita*
Tabebuia heptaphylla (Vell.) Tol.	Ipê Rosa/Bignoniaceae		
Tabebuia impetiginosa (Mart. Ex DC.) Standl	Taheebo; Pau d'Arco tree/ Bignoniaceae	*M. incognita*	
Tabebuia serratifolia (Vahl.) Nich.	Yellow poui/Bignoniaceae	*M. incognita*	

*Average of 5 replicates per treatment (host plant); **Average of 4 replicates per treatment (host plant); #Plants not inoculated with *M.incognita*.

Table 2 - Native plants naturally infected by *Meloidogyne incognita* and *M. javanica* according to the esterase phenotype (Est). Population assessments based on number of galls, number of eggs and J2 per root system.

Common name	Number of galls	Eggs and J2**	Nematode#
*Lonchocarpus muehlbergianus**	61	680	*M. incognita* Est (I1)
Tabebuia impetiginosa	8	86	*M. incognita* Est (I1)
Cordia ecalyculata	10	226	*M. javanica* Est (J3)
Cytharexyllum myrianthum	129	5.733	*M. javanica* Est (J3)
Aspidosperma subincanum	23	466	*M. javanica* Est (J3)
Croton urucurana	35	333	*M. incognita* Est (I1)
Tabebuia serratifolia	54	760	*M. incognita* Est (I1)

*****Average of 4 replicates per treatment (host plant). **Missing 10 females per root used for *Meloidogyne* identification by esterase phenotype; #RF was not calculated due to the lack of information about the initial population.

Meloidogyne incognita and *M. javanica* are the most widespread nematodes in the cultivated lands of Western Paraná, Brazil, infecting the main crops (Roese et al. 2001; Franzener et al. 2005; Portz et al. 2006; Pires et al. 2008). In this survey, these nematodes were also found on the roots of native-plant seedlings which have been commonly used for reforestation.

Tabebuia serratifolia and *T. impetiginosa* had been previously reported as the hosts of *M. javanica* and *M. arenaria* (Ferreira 1989; Mendes and Cardoso 1978). However, in this work, only *M. incognita* was detected infecting these plants. Close-related plants, such as those belonging to a same genus or family, presented different reactions when inoculated with *M. incognita*. In the genus *Tabebuia* (Bignoniaceae), *M. incognita* infected *T. impetiginosa* and *T. serratifolia* but not *T. heptaphylla* and in the Fabaceae/Faboidea, *M. incognita* infected *Lonchocarpus muehlbergianus* but not *Myrocarpus frondosus*. Moreover, considering the susceptible treatments (native plants) *M. incognita* was not virulent to all the replicates. Therefore, the different reactions found on replicates of a same plant species or on plants belonging to a same family or genus might indicate genetic diversity among the native plants within preserved areas.

Meloidogyne incognita was also detected in the soil samples collected at the rhizosphere of *Miconia cinnamonifolia* Naud. and *Sclerolobium* sp. from the Atlantic forest, whilst *M. javanica* was detected at the rhizosphere of several other native plants (Lima et al. 2005). The same authors also reported *M. javanica* at the rhizosphere of *Eugenia leitonni (*myrtaceae) in the Atlantic forest. However, in this study, native species belonging to the Myrtaceae family did not present infection for *M. javanica* or for *M. incognita*, even after *M. incognita* inoculation.

The parasitism of *M. incognita* and *M. javanica* was also obtained by seedlings inoculation of *Pinus* and *Eucalyptus* as reported by Ferraz and Lordello (1982) and Ferraz (1986). Other hosts for *M. incognita* included *Hevea brasiliensis* (Muell.) Arg. (Freire 1976) and *Psidium guajava* L. (Piccinin et al. 2005): the latter was also parasited by *M. mayaguensis* Rammah and Hirschmann**,** 1988 (Carneiro et al. 2001, Torres et al. 2004; Torres et al. 2005, and Silva et al. 2006).

Another host for *Meloidogyne* was *Luehea paniculata* from the Atlantic forest biome, which was found in association with *M. exigua* in the soil samples collected at its rhizosphere. In this work, this plant was not infected by both *M. incognita* and *M. javanica*. This suggested that *L. paniculata* might host *M. exigua* but not *M. incognita* and *M. javanica*.

In the biome Cerrado, Huang et al. (1991) detected natural infection of *M. javanica* and *M. arenaria* on the roots of *Dimophandra mollis* Benth. (Fabaceae) and *Cybianthus gardneri* A. DC. (Myrsinaceae), respectively. In native cerrado vegetation, Huang et al. (1996) reported a non-identified species of *Meloidogyne* from the soil samples collected at the rhizosphere of *Pterodon pubescens* Benth, 1860.

So far, the methodologies employed for *Meloidogyne* surveys on the native forests were mostly focused on collecting the soil samples from random sites and using bait plants such as tomato (*Solanum lycopersicum* L.) as an alternative to reproducing and identifying *Meloidogyne* species present in the soil (Huang et al. 1996; Souza et al. 1994; Lima et al. 2005). These methodologies previously used were successfully applied to the study of *Meloidogyne* diversity in different types of vegetation. However, they did not present

sufficient accuracy to associate *Meloidogyne* species with their putative hosts. Silva et al. (2008) were not able to identify a putative host for *M. exigua* by collecting the roots from the sites covered by the native Amazon forest. These results could be mainly associated with the high density of plant roots present at the collecting sites, which made the process of plant-nematode association very difficult. Therefore, the approach employed in this work could be useful when searching for plant-hosts of *Meloidogyne* in native biomes.

Nevertheless, the detection of *Meloidogyne* spp., as shown in this study, on the seedlings of native plants used for reforestation was a sign that more stringent protocols would be needed for soil sterilization (e.g. fumigation, solarization, or some additional treatment) prior to the propagation of these plants. Moreover, the use of sterile synthetic substrate for seedlings production, would also contribute to the sanity of the plants. Such approaches would strongly contribute to reducing the introduction and dissemination of *Meloidogyne* spp. and other plant pathogens in reforestation areas as well as it would reduce the environmental imbalances within the preserved areas.

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