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Artigos

Host status of plants from the Cerrado Biome to *Meloidogyne* spp.

Hospedabilidade de plantas do Bioma Cerrado a *Meloidogyne* spp.

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ABSTRACT

Among the Brazilian biomes, Cerrado is the second in territorial extension and diversity of plants and animal species. The Cerrado's biodiversity has been studied in terms of its flora and fauna, lacking data from microorganisms representing the microfauna, such as root-knot nematodes. *Meloidogyne* spp. are sedentary endoparasitic nematodes of plants. Its presence in soil covered with native cerrado vegetation, under preservation, is a strong indication that its permanence is due to the parasitism on native plants. This work aimed to study the host suitability of cerrado plants to *Meloidogyne* spp. Thirty-five plant species from the Cerrado biome, including grasses and trees, were selected for inoculation with four *Meloidogyne* isolates, three of them previously collected in a native Cerrado area under preservation (*Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne morocciensis*), and the fourth (*Meloidogyne paranaensis*) an exotic species in Cerrado. Five thousand eggs and eventual second-stage juveniles (J2) of each nematode species were inoculated per plant. The experiments were conducted with 35 treatments (native plants) and 5 replicates, having been repeated in time. The variables assessed were gall index and egg mass index, eggs and J2 per gram of root and reproductive factor (RF), as well as the symptomatology caused by the nematodes on the roots. The following plant species were considered as hosts by presenting mean of the Reproduction Factor (RF)>1.0: *Triplaris gardneriana* for *Meloidogyne javanica*, *Meloidogyne incognita*, and *Meloidogyne morocciensis*; *Andropogon bicornis* for *Meloidogyne javanica* and *Meloidogyne incognita* and *Copaifera langsdorffii* for *Meloidogyne incognita*. The following plant species were classified as potential hosts by developing RF>1.0 in at least one of the replicates: *Copaifera langsdorffii* for *Meloidogyne morocciensis*; *Esenbeckia leiocarpa* for *Meloidogyne javanica* and *Guibourtia hymenifolia* for *Meloidogyne incognita*. The other plant species were classified as non-hosts considering the conditions of this study. The inoculated plants showed varied symptoms on the roots, such as galls associated with egg masses, egg masses without galls, swelling with egg masses, cracks with egg masses without galls, and swelling with egg masses.

Keywords: Root-knot nematodes; Brazilian savanna; Native plants; Host plants

RESUMO

Dentre os biomas brasileiros, o Cerrado é o segundo em extensão territorial e em diversidade de espécies vegetais e animais. A biodiversidade do Cerrado tem sido estudada no que tange a sua flora e fauna, carecendo de dados de microorganismos representantes da microfauna, a exemplo dos nematoides de galhas radiculares. *Meloidogyne* spp. são nematoides endoparasitas sedentários de plantas. A sua presença em solo de áreas com vegetação nativa de cerrado, sob preservação, é um forte indício de que a sua permanência ocorre devido ao parasitismo de plantas nativas. Este trabalho objetivou estudar a hospedabilidade de plantas ocorrentes no bioma cerrado a *Meloidogyne* spp. Foram selecionadas trinta e cinco espécies vegetais desse bioma, incluindo espécies gramíneas e arbóreas, para estudo da hospedabilidade frente a três isolados de *Meloidogyne* coletados em área nativa de cerrado sob preservação (*Meloidogyne incognita*, *Meloidogyne javanica* e *Meloidogyne morocciensis*), além de uma espécie até então exótica ao cerrado (*Meloidogyne paranaensis*). Foram inoculados 5.000 ovos e eventuais juvenis de segundo estádio (J2) de cada espécie de nematoide por planta. Os experimentos foram conduzidos com 35 tratamentos (plantas nativas) e 5 repetições, tendo sido repetidos no tempo. As variáveis avaliadas foram índice de galhas e de massas de ovos, ovos e J2 por grama de raiz e fator de reprodução, além da sintomatologia causada pelos nematoides nas raízes. As seguintes espécies vegetais foram consideradas hospedeiras, com média do Fator de Reprodução (FR)>1,0: *Triplaris gardneriana* para *Meloidogyne javanica*, *Meloidogyne incognita* e *Meloidogyne morocciensis*; *Andropogon bicornis* para *Meloidogyne javanica* e *Meloidogyne incognita* e *Copaifera langsdorffii* para *Meloidogyne incognita.* As seguintes espécies vegetais foram classificadas como hospedeiras potenciais, com FR>1,0 para pelo menos uma das repetições: *Copaifera langsdorffii* para *Meloidogyne morocciensis*; *Esenbeckia leiocarpa* para *Meloidogyne javanica* e *Guibourtia hymenifolia* para *Meloidogyne incognita*. As demais espécies vegetais foram classificadas como não hospedeiras para as condições deste estudo. As plantas inoculadas apresentaram sintomas variados nas raízes como a presença de galhas associadas a massas de ovos, formação de massas de ovos sem galhas, rachaduras com massas de ovos e ausência de galhas e intumescimento com massas de ovos.

Palavras-chave: Nematoide das galhas; Savana brasileira; Plantas nativas; Plantas hospedeiras

1 INTRODUCTION

The Brazilian Cerrado boasts one of the richest biodiversities. It also presents agriculture in rapid expansion, contributing significantly to the growth of the Brazilian agribusiness and strength of the country's trade balance. This scenario is often challenged by the incidence of phytopathogens, especially phytoparasitic nematodes that reduce crop productivity. Economical losses caused by nematodes on the global agriculture are estimated in 157 billion dollars/yea*r (AB*AD et al., 2008; *ONKEN*DI et al., 2014) .

Among phytoparasitic nematodes, species of the genus *Meloidogyne* are the major cause of economic damage, especially *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria* in a tropical climate and *Meloidogyne hapla* in a temperate climate (ONKENDI *et al*., 2014). However, emerging species such as *Meloidogyne paranaensis* and *Meloidogyne enterolobii* have gained importance over the years due to their spread and damage to crops of increasing economic importance such as coffee and guava.

Gall-inducing nematodes of the genus *Meloidogyne* have been reported to occur in soils of cerrado areas under permanent preservation (SOUZA; DOLINSKI; HUANG, 1994; SILVA *et al*., 2013). According to Antes *et al*. (2012) and Silva *et al*. (2013), *Meloidogyne* species occur in low population density in soils occupied with native vegetation, surviving due to the interaction with plant species. According to the same authors, the low population density of nematodes in areas occupied with native vegetation can be explained by the diversity and the genetic variability of vegetation present in those areas. However, their infective and reproductive capacity on plants of natural biomes is still poorly understood. This work aimed to study the reproduction of isolates of *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne morocciensis* and *Meloidogyne paranaensis* in plant species of the Cerrado biome.

2 MATERIAL AND METHOD

2.1 Origin, identification, and maintenance of *Meloidogyne spp.*

The populations of *Meloidogyne* spp. used in this study were collected in Cerrado areas under permanent preservation in Brasília, Federal District, according to a survey conducted by Silva *et al*. (2013). *Meloidogyne paranaensis*, was obtained from the Embrapa-Cenargen collection. *Meloidogyne morocciensis* was collected in clean field phytophysiognomy of Brasília National Park, *Meloidogyne incognita* in gallery forest, and *Meloidogyne javanica* in the clean field at 'Água Limpa' farm of the University of Brasília. The *Meloidogyne* species mentioned above were confirmed based on the esterase

phenotypes, according to the methodology proposed by Alonso and Alfenas (1998) as follows: *Meloidogyne javanica* (EST-J3), *Meloidogyne incognita* (EST-I1), *Meloidogyne morocciensis* (EST-A3), and *Meloidogyne paranaensis* (EST-P1). The four nematode species were inoculated in tomato cv. Santa Clara and maintained in a greenhouse.

2.2 Plant seeds and seedlings

Seeds from tree plants, whose origin is unknown, were provided by the Nursery II of NOVACAP Company ('Companhia Urbanizadora da Nova Capital do Brasil'). For *Enterolobium gummiferum* (Tamboril), the seeds were collected from a single tree in a Cerrado area. Grasses were multiplied in pots by cultivating tillers collected in the field. Exsiccates were made for each botanical species and deposited at the Herbarium of the University of Brasília to confirm the species identity.The tree plants were transplanted from tubes to polyethylene bags with a capacity for 2500 cm^3 of a sterile substrate and the grasses were transplanted to plastic pots with a capacity for 3 L of the substrate, which was composed of soil and sand in a ratio of 1:1. The plants were kept in a greenhouse at the Biology Experimental Station of the University of Brasília until they reached 30 cm in height. A total of thirty-five plant species from the Cerrado biome were studied (Table 1).

Table 1 – Botanical species from the Cerrado biome evaluated for hostability to *Meloidogyne* spp.

Scientific name	Common name	Type	Family
Albizia niopoides (Benth.) Burkart	Angico-branco	Tree	Fabaceae
Anacardium humili St. Hilaire	Cajuí	Tree	Anacardiaceae
Anadenanthera macrocarpa (Benth.) Brenan	Angico-preto	Tree	Fabaceae
Astronium flaxinifolium Schott	Gonçalo-Alves	Tree	Anacardiaceae
Andropogon bicornis L.	Andropogon	Grass	Poaceae
Calophyllum brasiliense Cambess.	Landim	Tree	Calophyllaceae
Copaifera langsdorffii Desf.	Copaíba	Tree	Fabaceae
Cybistax antisyphilitica Mart.) Mart.	Ipê-verde	Tree	Bignoniaceae
Dalbergia miscolobium Benth.	Caviúna	Tree	Fabaceae

To be continued ...

Table 1 – Conclusion

Scientific name	Common name	Type	Family
Jacaranda mimosifolia D. Don	Jacarandá-Mimoso	Tree	Fabaceae
Echinolaena inflexa (Poir.) Chase.	Capim-flechinha	Grass	Poaceae
Elionurus muticus (Spreng.) Kuntze.	Capim-cheiroso	Grass	Poaceae
Enterolobium gummiferum (Mart.) J.F. Macbr.	Tamboril	Tree	Fabaceae
Eriochrysis filiformis (Hack.) Filg.	-	Grass	Poaceae
Eriotheca pubescens Mart. & Zucc.	Paineira-loira	Tree	Malvaceae
Esenbeckia leiocarpa Engl.	Guarantã	Tree	Rutaceae
Eugenia tomentosa Camb.	Cabeludinha	Tree	Myrtaceae
Guibourtia hymenifolia (Moric.) J. Leonard	Copaibeira	Tree	Fabaceae
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Ipê-roxo	Tree	Bignoniaceae
Handroanthus caraíba (Mart.) Mattos.	Ipê-caraíba	Tree	Bignoniaceae
Handroanthus chrysotrichus (Mart. ex DC.)	Ipê-tabaco	Tree	Bignoniaceae
Handroanthus serratifolius (Vahl) S. O. Grose	Ipê-amarelo	Tree	Bignoniaceae
Hymenaea courbarilvar. stilbocarpa L.	Jatobá	Tree	Fabaceae
Inga fagifolia Benth.	Ingá-mirim	Tree	Fabaceae
Inga heterophylla Willd.	Ingá-colar	Tree	Fabaceae
Kielmeyera coriaceae Mart. & Zucc.	Pau-santo	Tree	Calophyllaceae
Kielmeyera speciosa A. St.-Hil.	Pau-santo	Tree	Calophyllaceae
Myracrodruon urundeuva Fr. All.	Aroeira preta	Tree	Anacardiaceae
Paspalum pilosum Lam.	Paspalum	Grass	Poaceae
Paspalum plicatulum Michx.	Plicatulum	Grass	Poaceae
*Physocalymma scaberrimum*Pohl	Pau-de-rosas	Tree	Litraceae
Pseudobombax tomentosum (Mart.) A. Robyns	Imbiruçu	Tree	Malvaceae
Stryphnodendron adstringens (Mart.) Coville	Barbatimão	Tree	Fabaceae
Terminalia tomentosum L.	Piúna	Tree	Combretaceae
Triplaris gardneriana Wedd.	Pajeú	Tree	Polygonaceae

Source: Authors (2019)

2.3 Physical-chemical and biological analysis of the substrate

The chemical analysis of the substrates used in the greenhouse tests was carried

out by the company 'Solocria Laboratório Agropecuário LTDA', located in Goiânia – Goiás state. Soil samples were collected from the rizosphere of grasses during tiller collection and analyzed for the presence of phytoparasitic nematodes, according to the methodology proposed by Jenkins (1964).

2.4 Reaction of Cerrado plants to *Meloidogyne spp.*

2.4.1 Experimental design, inoculum and evaluated variables

Two tests were carried out at different times for each plant species, being them established in a completely randomized design (CRD), with five replications. Single plants were inoculated with five thousand eggs and eventual J2 of each *Meloidogyne* species. The inoculated plants were kept on separate benches to avoid contamination. The tomato cv. 'Santa Clara' was used as a control treatment due to the high susceptibility to *Meloidogyne* spp. The plant species used in the tests were free from nematode contamination.

2.4.2 Root staining and symptomatology

Plant shoots were separated from the roots, washed in tap water and immersed in acid fuchsin solution 0.1% for seven minutes to color the egg masses, according to the methodology proposed by Silva, Santos and Ferraz (1988). Egg masses were counted using a light microscope (DICKSON; STRUBLE, 1965). Symptoms produced by the nematodes on plant roots were described and photo-documented.

2.4.3 Egg Mass Index, Gall Index, Number of Eggs and / or J2 per Gram of Root and Reproduction Factor

To determine the EMI (Egg Mass Index), the Taylor and Sasser scale (1978) was used with grades ranging from 0 to 5, in which: 0 = no egg masses; 1 = presence of 1 to 2 egg masses; 2 = 3 to 10 egg masses; 3 = 11 to 30 egg masses; 4 = 31 to 100 egg masses and 5 = more than 100 egg masses.

The GI was found by counting the number of galls under a light microscope, in the entire root system of each plant/replicate, using a scale from 1 to 5, according to Taylor and Sasser (1978), in which: 0 = absence of galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls and 5 = more than 100 galls.

The number of eggs and/or J2 per gram of root (NOJ2GR) was obtained by the ratio of total eggs and J2 extracted from the roots and the root mass. The Reproduction Factor (FR) for the different plant species was determined according to Taylor and Sasser (1978). For this, the equation RF = FP / IP was used, where FP = final population and IP = initial population, according to Oostenbrink (1966). IP consisted of the inoculum extracted from tomato roots, quantified and calibrated to contain 5000 eggs and / or juveniles per ml of the suspension. FP was quantified based on J2 present in the soil plus eggs and J2 present in the roots.

2.4.4 Extraction of eggs and J2 from roots

The extraction of eggs and J2 from plant roots was carried out according to the technique adapted from Hussey and Barker (1973) with the roots being cut into pieces of about 1 to 2 cm, crushed with the use of a domestic blender in sodium hypochlorite solution with 0.5% active chlorine, at low speed for 30 s. The crushed was poured into overlaped sieves of 0.355 mm and 0.025 mm. The content of the 0.025 mm sieve was collected with the aid of a pissette. The suspension was cleaned in sucrose solution through the centrifugal flotation technique (JENKINS, 1964). Samples were centrifuged at 650 g for 5 min and the supernatant was discarded. Afterwards, sucrose solution was added, at a concentration of 454 $g.L^{-1}$, and the sample was centrifuged for 1 min. at 650 g. The suspension was poured through a 0.025 mm sieve and collected in a Becker (maximum 40 ml) with the aid of a pissete.

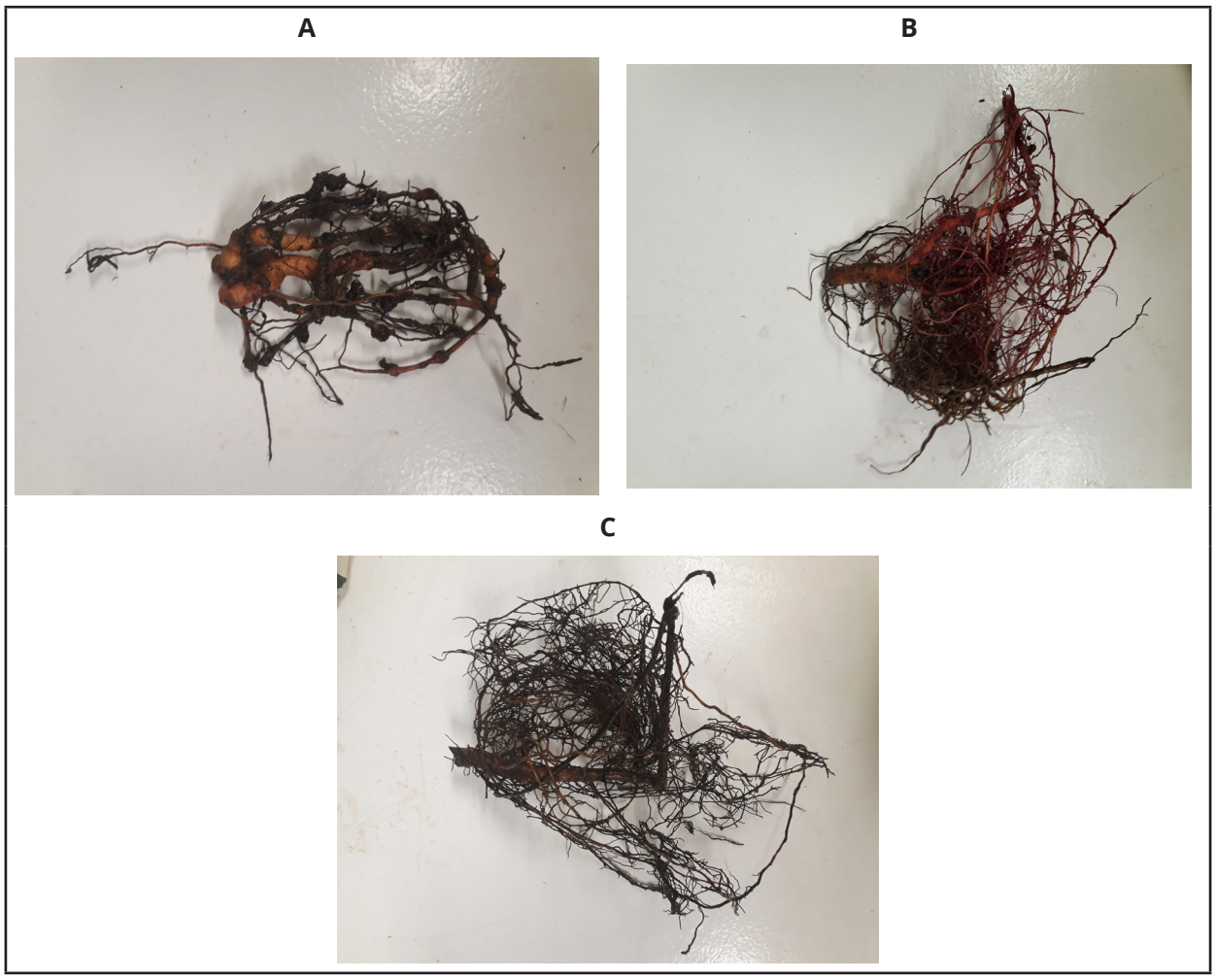
Aliquots of 150 cm3 of soil were homogenized in plastic container with tap water, stirred for 15 s and poured into overlaped sieves of 0.355 mm and 0.020 mm. The content of the 0.020 mm sieve was collected with the aid of a pissette and the sample was cleaned by the Jenkins technique (1964).

After processing the soil and the roots, eggs and J2 were determined from readings in triplicate, using a Peters' slide, with the aid of a light microscope.

2.4.5 Classification of plant-species based on the reproductive rate of nematodes

The plant-species were classified into different groups, according to the reproductive rate of the nematodes, as follows: a) Hosts: Average of nematodes with RF>1.0, presence of many galls on the roots, cracks and egg masses (Figure 1A); b) Potential hosts: RF>1.0 in at least one of the replicates, but with an average of RF with few galls or cracks and few egg masses on the roots (Figure 1B); c) Non-hosts: RF = 0, without apparent symptoms and absence or occasional induction of egg masses and/or galls (Figure 1C).

Figure 1 – Root system of plant species classified as host, potential host and non-host of *Meloidogyne* spp.: A- *Triplaris gardneriana*, host; B - *Copaifera langsdorffii*, potential host; C - *Terminalia tomentosum*, non-host



Source: Authors (2019)

2.5 Identification of *Meloidogyne* species by the esterase phenotype

Milky-white females were extracted from tomato roots under a stereomicroscope, placed in 0.5 ml microtubes containing extraction buffer solution (20% sucrose, 2% Triton X-100, 0.01% bromophenol blue and 78% distilled water) and kept on ice. 10 µl of each sample were placed, with the aid of a micropipette, in wells of 8% polyacrylamide gel. The standard sample consisted of *Meloidogyne javanica* females with a J3 phenotype, as described by Alonso *et al*. (1999). The race was conducted in a Vertical Electrophoresis System LCV 10X10 (Loccus Biotechnology) for 1 hour at 200 volts, with the tank being kept refrigerated at 4 ºC. After the run, the gel was immersed in a developer solution for esterase (100 mL of 0.05M potassium phosphate buffer pH 6.0, 100 mg of Fast Blue RR Salt and 4.5 mL of 1% alpha-naphthylacetate) and maintained at 37 ºC for 30 minutes. Then, the gel was washed in distilled water and transferred to a fixative solution (45% methanol, 9% acetic acid and 45% distilled water), where it remained for 20 minutes in an oven at 37 ºC. The enzymatic phenotypes were determined based on the number, position and intensity of the bands (ESBENSHADE; TRIANTAPHYLLOU, 1985; 1990).

3 RESULTS AND DISCUSSION

3.1 Nematological analysis of soil, physical-chemical analysis of the substrate and confirmation of the identity of *Meloidogyne* spp.

Nematological analysis of soil collected from the rizosphere of tillers of the *Andropogon bicornis* grasses confirmed the absence of *Meloidogyne* J2 forms. Also, the analysis of tiller roots of the *Andropogon bicornis* grasses indicated the absence of egg masses. The Sandy texture of the substrate used in the first assay had a clay - silt-sandy ratio of 26% - 7% - 67% and in the second assay 21% - 5% - 74%. These data confirm that the texture of the substrate favored soil movement and infection of *Meloidogyne* spp. in the inoculated plants (RINALDI; NUNES; MONTECELLI, 2014).

According to the chemical analysis, the substrate used for the greenhouse tests showed low fertility (Table 2) and cover fertilizations with 2 g of N-P-K 4-14-8/pot were used. The *Meloidogyne* species showed the following phenotypes for the isoenzyme esterase: *Meloidogyne javanica* (EST-J3), *Meloidogyne incognita* (EST-I1), *Meloidogyne morocciensis* (EST-A3) and *Meloidogyne paranaensis* (EST-P1).

Table 2 – Chemical characterization of substrates used in greenhouse tests

Sample	cmolc.dm^{-3}					mg.dm^{-3}					g.dm^{-3}	
	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	K^{+}	P (Mehlich)	Zn^{+}	CTC (T)	V (%)	m (%)	MO	pH $CaCl_2$
Subst.[1]	1,7	0,2	0	1,3	0,1	11,6	4,1	3,31	60,7	0	10	5,7
Subst.[2]	1,7	0,2	0	1	0,1	7,7	5,2	2,97	66,3	0	8	6,1

Source: Authors (2019)

In where: [1]Substrate used in the 1st test in a greenhouse; [2]Substrate used to repeat the test in a greenhouse. Where: Ca^{2+} - exchangeable calcium; Al^{3+}: Exchangeable aluminum; K^{+} - Exchangeable potassium; H + Al - Potential acidity; P (Mehlich - Phosphorus extracted by the Mehlich method; Zn^{2+}: Exchangeable zinc; CTC (t) - Total cation exchange capacity; Mg^{2+} - Exchangeable magnesium; m (%) - Saturation by aluminum; pH $CaCl_2$ - pH in calcium chloride; MO - Organic matter.

3.2 Reaction of cerrado plants to *Meloidogyne* spp.

There was variation between the tests regarding the plant response to *Meloidogyne* spp. The reaction of inoculated plants was more conclusive in the first experiment than in the second. Tomato plants used as susceptibility pattern showed RFs>1.0 for the three *Meloidogyne* species tested but also with higher reprocutive rates in the first experiment (Table 3).

The evaluated species that behaved as non-hosts were: Albizia niopoides, Anadenanthera macrocarpa, Anacardium humili, Astronium fraxinifolium, Cybistax antisyphilitica, Dalbergia miscolobium, Jacaranda mimosifolia, Echinolaena inflexa, Elionurus muticus, Eriochrysis filiformis, Eriotheca pubescens, Eugenia tomentosa,

Calophyllum brasiliense, Hymenaea stilbocarpa, Inga fagifolia, Inga heterophylla, Kielmeyera coriaceae, Kielmeyera speciosa, Myracrodruon urundeuva, Paspalum plicatulum, Physocalymma scaberrimum, Stryphnodendron adstringens, Handroanthus caraíba, Handroanthus chrysotrichus, Handroanthus impetiginosus, Handroanthus serratifolius, Pseudobombax tomentosum and Terminalia tomentosum.

Table 3 – Averages of Reproduction Factor (RF), Egg Mass Index (EMI), Gall Index (GI) and Number of Eggs and J2 per Root Grass (NEJ2 / GR) in plant species behaved as hosts or potential hosts after four months from inoculation with 5,000 eggs and eventual J2 of *Meloidogyne* spp.

Species	Experiment 1				Experiment 2			
	EMI	GI	NEJ2/GR	RF	EMI	GI	NEJ2/GR	RF
Meloidogyne javanica								
Andropogon bicornis	1,2	0	857,6	7,21	1,4	0	120,9	0,67
Esenbeckia leiocarpa	1	0,4	55,72	0,23	0,8	0,2	177,4	0,73
Triplaris gardneriana	2,6	2,2	156,4	2,93	0	0	0	0
Solanum lycopersicum	5	5	1740	5,02	5	5	988	4,3
Meloidogyne morocciensis								
Copaifera langsdorffii	0,6	0,2	0,87	0,26	0,6	0,6	0,88	0,004
Triplaris gardneriana	3	1,8	159,7	1,79	0,8	1	4,84	0,05
Solanum lycopersicum	5	5	10677	33,59	5	5	7032	10,59
Meloidogyne incognita								
Andropogon bicornis	1,6	0	543,4	1,58	2,8	0	95,59	0,42
Copaifera langsdorffii	1,2	1,8	1772	3,15	0	0	18,05	0,05
Guibourtia hymenifolia	1,4	1,6	291	0,64	0	0	0	0
Triplaris gardneriana	2,6	3,8	365,2	5,97	0	0	5,58	0,08
Solanum lycopersicum	5	5	24760	76,86	5	5	947,2	6,95

Source: Authors (2019)

In where: RF = Reproduction Factor; EMI = Egg mass index; GI = Gall index; NEJ2/GR = Number of eggs and J2 per gram of root.

Plant species that behaved as potential hosts were *Copaifera langsdorffii* for *Meloidogyne morocciensis* (FR = 1.15 and FR = 3.20), *Esenbeckia leiocarpa* for *Meloidogyne javanica* (FR = 1.1) and *Guibourtia hymenifolia* for *Meloidogyne incognita* (FR = 2.26). *Andropogon bicornis* reacted as host to *Meloidogyne javanica* and *Meloidogyne incognita*;

Copaifera langsdorffii as host of *Meloidogyne incognita* and *Triplaris gardneriana* as host of *Meloidogyne javanica*, *Meloidogyne incognita* and *Meloidogyne morocciensis*.

For most inoculated plant species, the average of reproduction factors produced by *Meloidogyne* spp. was low, which supports the hypothesis of genetic resistance of the plant species to the nematodes. The non-uniform reaction of replicates, classified as hosts and potential hosts to *Meloidogyne* spp., supports the hypothesis of intraspecific genetic variability in the cerrado plants tested in this work. This hypothesis gains strength when analyzing individual reproduction factors and not on average. As an example, we have *Triplaris gardneriana* inoculated with *Meloidogyne javanica* in experiment 1. The five replicates presented RFs that varied from 0.5 to 11.36, obtaining an average RF = 2.93. *Andropogon bicornis* also inoculated with *Meloidogyne javanica* showed an average of RF = 7.21 and a variation of RF from 0 to 35. For *Meloidogyne morocciensis*, there is *Triplaris gardneriana* with RF ranging from 0.50 to 2.65 and an average of RF = 1.79. *Meloidogyne incognita* inoculated on *Andropogon bicornis* produced RFs ranging from 0 to 5.0 and RF average = 1.58; *Copaifera langsdorffii* with RF ranging from 0 to 11.80 and RF average of 3.15, as well as *Triplaris gardneriana* with RF ranging from 0.20 to 7.77 and RF average of 5.97.

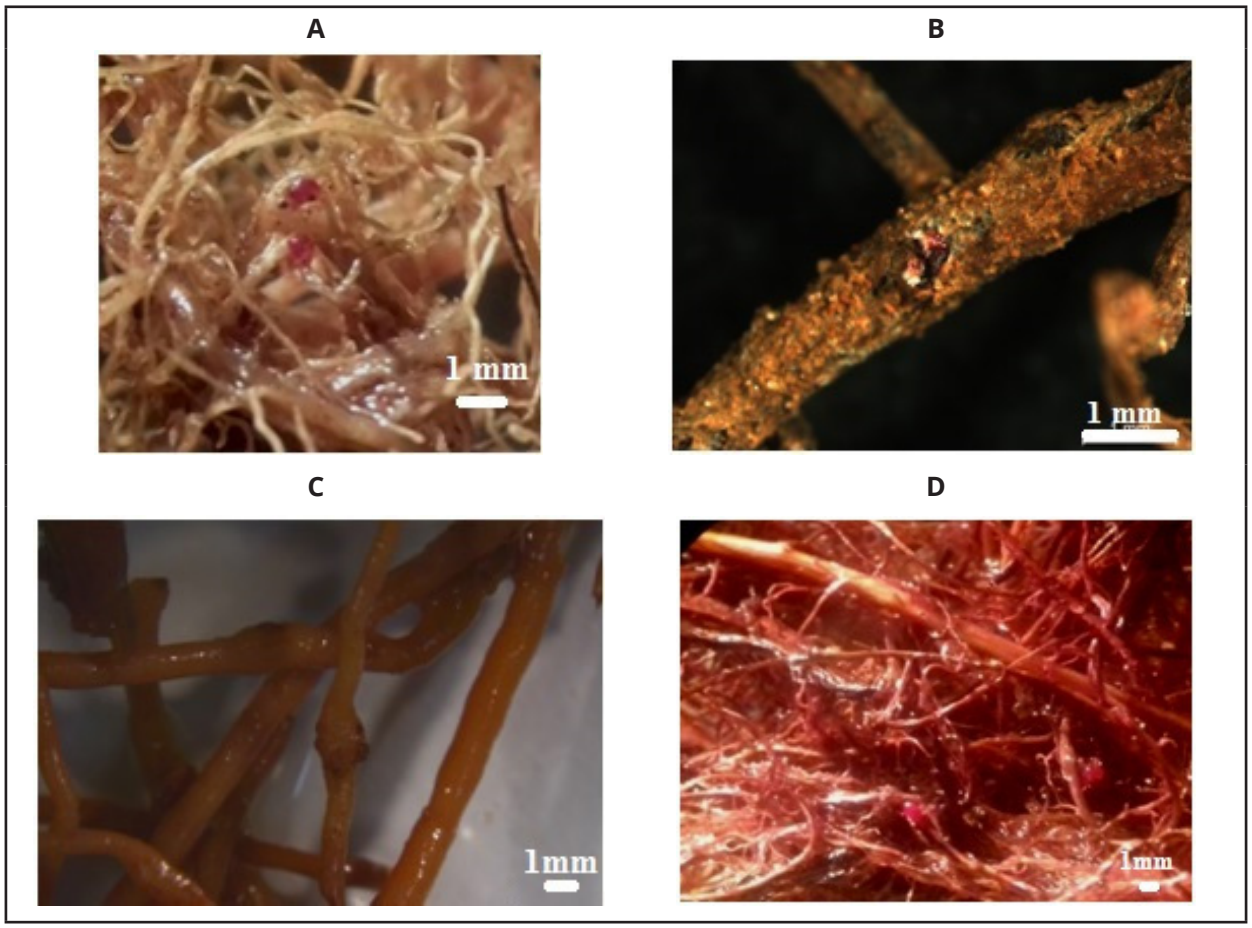
Of the plant species studied, *Triplaris gardneriana* was exclusive in allowing the reproduction of *Meloidogyne javanica*, *Meloidogyne incognita* and *Meloidogyne morocciensis* at mean rates of RF> 1.0. *Andropogon bicornis* is host of *Meloidogyne javanica* and *Meloidogyne incognita*, and *Copaifera langsdorffii* is host of *Meloidogyne incognita*. *Meloidogyne paranaensis*, a nematode species not yet detected in native cerrado, did not show reproductive rates that demonstrated population increase or even maintenance of the population in the inoculated plants. *Meloidogyne incognita* was the nematode species with the highest number of host plants with an average of RF> 1.0 for three hosts, followed by *Meloidogyne javanica* with two hosts and *Meloidogyne morocciensis* with one host plant.

3.3 Symptoms on the roots of plants inoculated w*ith Meloidog*yne spp.

The symptoms produced varied according to the inoculated plant species (Figure 2). Of the host-plant species, *Andropogon bicornis* did not show gall formation and egg masses very evident on the root system (Figure 2A). *Copaifera langsdorffii* showed cracks with egg masses on the roots, but without evident galls (Figure 2B). *Triplaris gardneriana* presented evidente galls of different sizes and egg masses (Figure 2D).

Of the potential hosts, *Esenbeckia leiocarpa* presented egg masses only in newer lateral roots, without gall formation (Figure 2C). *Guibourtia hymenifolia* presented necrosis and small and barely visible galls on lateral roots, with egg mass formation.

Figure 2 – Symptoms induced by *Meloidogyne* spp. on the roots of host-plants and potential host-plants from Cerrado: A - *Andropogon bicornis* (arrow indicating egg mass); B - *Copaifera langsdorffii* (arrow indicating crack); C - *Esenbeckia leiocarpa* (arrow indicating swelling with egg mass); D - *Triplaris gardneriana* (arrow indicating gall with egg mass)



Source: Authors (2019)

The results obtained in this study revealed new hosts for the tested *Meloidogyne* species. In the case of a sedentary endoparasite nematode, its survival is related to parasitism and reproduction in plants.

Andropogon bicornis L., a species popularly known as 'capim-rabo-de-burro', allowed the reproduction of *Meloidogyne incognita* and *Meloidogyne javanica* in its root system, having been classified as host of these nematodes and, being this, the first report in literature. Previously, Café Filho and Huang (1988) reported *Pratylenchus brachyurus* in association with *Andropogon bicornis* roots in the cerrado.

In addition to *Andropogon bicornis*, other grasses were tested in this study for *Meloidogyne* spp. such as *Paspalum pilosum*, *Paspalum plicatulum*, *Eriochrysis filiformis*, *Elionurus muticus* and *Echinolaena inflexa*. These grasses did not allow the reproduction of *Meloidogyne* spp., having been classified as non-hosts.

The nematodes *Meloidogyne javanica* and *Meloidogyne incognita* were reported by Silva *et al*. (2013) in a cerrado soil sample collected in the Clean Field phytophysiognomy, an area with a predominance of grasses, among which are the species tested in this study. Considering that *Meloidogyne javanica* and *Meloidogyne incognita* need host-plants for their survival, it is likely to infer that, in the phytophysiognomies of Cerrado where *Andropogon bicornis* occurs, both nematodes will infect and reproduce in this host.

Triplaris gardneriana is a plant species adapted to wetlands and commonly known as 'pau-de-formiga' or 'Pajeú', being indicated for reforestation of riparian forests (LACERDA; BARBOSA; BARBOSA, 2007). In the cerrado, it is found in the phytophysiognomies 'Cerradão' and gallery forests (IMAÑA-ENCINAS; MACEDO; PAULA, 2007). This study demonstrated that this species is a new host for *Meloidogyne javanica*, *Meloidogyne incognita* and *Meloidogyne morocciensis*, having no report in the literature of nematodes associated with this plant species. *Meloidogyne incognita* and *Meloidogyne javanica* were previously reported by Silva *et al*. (2013) from soil collected in gallery forest, being an indication that *Triplaris gardneriana* may host these nematodes

in this type of vegetation. Another plant species present in gallery forests is *Copaifera langsdorffii* (Leguminosae, Caesalpinoideae). This tree has demonstrated the potential for the maintenance and reproduction of *Meloidogyne incognita* populations in the cerrado.

The 'ipê' species such as *Handroanthus impetiginosus*, *Handroanthus serratifolius* and *Handroanthus caraiba* were classified as non-hosts for the nematodes tested in this study. The data obtained by Silva *et al*. (2013), corroborate with those found in this study where *Handroanthus impetiginosus* did not allow the reproduction of *Meloidogyne javanica* in its root system after artificial inoculation.

Despite the negative reaction of the 'ipê' species tested for *Meloidogyne javanic*a, Mendes and Cardoso (1978) reported *Meloidogyne javanic*a infecting *Handroanthus serrati*folius (sin. *Tabebuia serratifoli*a - yellow ipe). Later, Alonso et al. *(1999*) studied the virulence and aggressiveness of two isolates of *Meloidogyne javanica on Handroanthus serratifolius, bein*g one isolate from seedlings of yellow 'ipê' and the other from a different host. The authors reported that both isolates had the same reproductive rate on 'ipê' (FR = 2.49).

According to Alonso et al. (1999), *Handroanthus serratifolius* developed the following symptoms: stunting, giant cells in the protoxylem and protofloem, internal egg masses and extensive necrosis in the root cortex. In this study, it was not observed reduction in growth, but well-developed galls and necrosis, with few egg masses.

Natural infection of *Handroanthus impetiginosus* and *Handroanthus serratifolius* by *Meloidogyne incognita* was reported by Antes *et al*. (2012), in seedlings obtained from a commercial nursery in Western Paraná, Brazil. However, the same authors reported no infection in healthy seedlings of the same plant species when the natural population of *Meloidogyne incognita* was artificially inoculated, which suggests the occurrence of genetic variability in those plant species.

Handroanthus impetiginosus was also reported as host to *Meloidogyne arenaria* by Ferreira (1989). The same plant species was studied by Oliveira *et al*. (1995), who reported the damage caused by *Meloidogyne arenaria* and *Meloidogyne javanica* under different inoculum densities. Infected plants produced galls on the roots and a low reproductive rate of the nematode without plant-growth reduction for the different inoculum densities tested. According to Oliveira *et al*. (1995), the highest values of RF were RF = 0.61 for *Meloidogyne javanica* with inoculation of 9,000 eggs/J2 and RF = 2.2 for M. arenaria with inoculation of 3,000 eggs/J2.

Further investigation is necessary to comprove the status of *Esenbeckia leiocarpa*, *Guibourtia hymenifolia*, and *Copaifera langsdorffii*, suggested here as potential hosts of *Meloidogyne javanica*, *Meloidogyne incognita*, and *Meloidogyne morocciensis*, respectively.

The conflicting results reported in the literature and related to the reproductive rate of nematodes on plants might be caused by the intraspecific variability found in populations of plants and nematodes (ANTES *et al*., 2012) and by differences in the methodology used for running experiments such as inoculum density (OLIVEIRA *et al*., 1995) and its origin (ALONSO *et al*., 1999).

4 CONCLUSION

Triplaris gardneriana was classified as host of *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne morocciensis*; *Andropogon bicornis* as host of *Meloidogyne javanica* and *Meloidogyne incognita*, and *Copaifera langsdorffii* as host of *Meloidogyne incognita*. As potential host-plants were identified: *Esenbeckia leiocarpa* for *Meloidogyne javanica*; *Guibourtia hymenifolia* for *Meloidogyne incognita*, and *Copaifera langsdorffii* for *Meloidogyne morocciensis*. The remaining plant species were considered non-hosts for the three *Meloidogyne* isolates. There was no reproduction of *Meloidogyne paranaensis* on the plants used in this study, which were classified as non-hosts to this nematode. The symptoms observed on the plant roots after inoculation with *Meloidogyne* spp. were necrosis, cracks, galls, swelling and egg mass production.

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