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Weed reaction to parasitism by the guava root-knot nematode, *Meloidogyne enterolobii*

Reação de plantas daninhas ao parasitismo pelo nematoide das galhas da goiabeira, *Meloidogyne enterolobii*

MOTA, Erika Araújo[1]; SOUZA JUNIOR, Francisco Jorge Carlos[2]; SANTOS, Carmem Dolores Gonzaga[3]

[1] 0000-0001-6799-4028; Federal University of Ceará (*Universidade Federal do Ceará*), Fortaleza, Ceará (CE), Brazil. Email: erikaaraujom@yahoo.com.br;

[2] 0000-0003-2086-9857; Federal Institute of Education, Science and Technology of Ceará (*Instituto Federal de Educação, Ciência e Tecnologia do Ceará*). Limoeiro do Norte, Ceará (CE), Brazil. Email: jorge.souza@ifce.edu.br

[2] 0000-0002-3364-7623; Federal University of Ceará (*Universidade Federal do Ceará*), Fortaleza, Ceará (CE), Brazil. Email: carmelo.ufc@gmail.com.

A B S T R A C T

The nematode *Meloidogyne enterolobii* (Yang & Eisenback) is one of the main pathogens associated with the guava tree (*Psidium guajava* L.) in northeastern Brazil. Considering the frequency with which weeds are found in fruit production areas and their potential as phytonematode hosts, the aim of this study was to investigate the reaction of weeds to the parasitism of *M. enterolobii*. The species evaluated for nematode infection were *Acanthospermum hispidum* D.C., *Ageratum conyzoides* L., *Alternanthera tenella* Colla., *Amaranthus deflexus* L., *A. spinosus* L., *A. viridis* L., *Bidens pilosa* L., Clitoria ternatea L., Datura stramonium L., Macroptilium lathyroides (L.) Urb., Merremia aegyptia (L.) Urb., *Solanum americanum* Mill., *S. paniculatum* L. and *Spigelia anthelmia* L. Of these, only six were classified as susceptible to the pathogen with factor breeding > 1.0: *S. paniculatum, S. americanum, B. pilosa, A. conyzoides, A. tenella,* and *M. aegyptia*, four species were resistant: *S. anthelmia, M. lathyroides, C. ternatea*, and *D. stramonium* and four species behaved as immune to the nematode: *A. hispidum, A. deflexus, A. spinosus, and A. viridis*. From the results obtained, it was found that weed species susceptible to *M. enterolobii* may pose a threat to the cultivation of guava, serving as a source of inoculum. Control measures in infested orchards should consider the hostability of these plants to the nematode in order to recommend their careful removal from the cultivated area.

R E S U M O

O nematoide *Meloidogyne enterolobii* (Yang & Eisenback) é um dos principais patógenos associado à cultura da goiabeira (*Psidium guajava* L.) na região nordeste do Brasil. Considerando a frequência com que as plantas daninhas são encontradas nas áreas de produção de frutíferas e de seu potencial como hospedeiras de fitonematoides, o objetivo deste estudo foi investigar a reação de plantas invasoras ao parasitismo de *M. enterolobii*. As espécies avaliadas quanto à infecção pelo nematoide foram *Acanthospermum hispidum* D.C., *Ageratum conyzoides* L., *Alternanthera tenella* Colla., *Amaranthus deflexus* L., *A. spinosus* L., *A. viridis* L., *Bidens pilosa* L., *Clitoria ternatea* L., *Datura stramonium* L., *Macroptilium lathyroides* (L.) Urb., *Merremia aegyptia* (L.) Urb., *Solanum americanum* Mill., *S. paniculatum* L. e *Spigelia anthelmia* L. Destas, apenas seis foram classificadas como suscetíveis ao patógeno com fator de reprodução > 1,0: *S. paniculatum, S. americanum, B. pilosa, A. conyzoides, A. tenella* e *M. aegyptia,* quatro espécies foram resistentes: *S. anthelmia, M. lathyroides, C. ternatea* e *D. stramonium* e quatro espécies comportaram-se como imunes ao nematoide: *A. hispidum, A. deflexus, A. spinosus* e, *A. viridis*. A partir dos resultados obtidos, verificou-se que as espécies de plantas daninhas suscetíveis a *M. enterolobii* podem constituir-se ameaça ao cultivo de goiabeira servindo como fonte de inóculo. Medidas de controle em pomares infestados devem considerar a hospedabilidade dessas plantas ao nematoide para a recomendação de sua criteriosa remoção da área cultivada.

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Introduction

Weeds compete directly with plant species cultivated by growth factors such as water, light, space and nutrients, or indirectly, due to allelopathic action, through the release of chemical substances that may be toxic, stimulating or harmful to the development of other plant species (Soares et al., 2010; Vasconcelos et al., 2012). The losses caused by competition for nutrients reduce the productivity of crops that can vary between 45% and 95% according to the species of invaders present in the area, with the level of infestation, the edaphoclimatic conditions, which may result in economic unviability and, in extreme cases, in total loss of production (Araujo-Junior et al., 2015; Vilà & Hulme, 2017). The presence of weeds in agricultural areas is thus one of the main biotic factors that interfere in the cultivation of plants of agronomic interest in tropical regions and the predominance of weed species in the area may still interfere negatively, since the use of herbicides may be used, which would be harmful to the environment (Bester et al., 2020; Kozlowski et al., 2002; Schaffner et al., 2020).

In addition to spatial and nutritional competition in the soil, weed plants can act as alternative hostesses of several phytopathogens of economic importance, such as fungi, bacteria, viruses and nematodes (Sales Júnior et al., 2019). In the literature there are reports of several associations between weeds and phytoparasitic nematodes, particularly with the nematode of galls, *Meloidogyne Goeldi* (Ponte, 1968; Manso et al., 1994; Roese & Oliveira, 2004). This nematode affects the root system compromising the absorption of water and nutrients, interfering, in general in the growth of plants, reducing their production. Gall nematode has worldwide distribution and there are more than 100 identified species of the pathogen associated with numerous crops, thus constituting one of the major problems for agricultural production (Álvarez-Ortega et al., 2019; Jones et al., 2013; Przybylska & Obrępalska-Stęplowska, 2020).

The species *M. enterolobii* Yang & Eisenback was first reported in Brazil parasitizing guava (*Psidium guajava* L.) in the northeast region (Carneiro et al., 2001) and, since then, has increased its importance due to the losses caused in the crop, and may in some cases cause a 100% reduction in production (Barbosa, 2001). In general, guava cultivars parasitized by *M. enterolobii* present symptoms of galls with root rot, leaves with tanning, yellowing, maple burning and fall, generalized decline with the consequent reduction in the number and size of fruits, sometimes occurring the early death of the plant (Reis et al., 2011). Species *M. enterolobii* is polyphagous and parasite several crops of economic importance in Brazil such as pineapple (*Ananas comosus* L. Merril.), acerola (*Malpighia emarginata* D.C.), banana (*Musa spp.*), sweet potatoes (*Ipomoea batatas* L. Lam), eggplant (*Solanum melongena* L.), sunflower (*Helianthus annuus* L.), papaya (*Carica papaya* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* Thunb. Matsum & Nakai) and soybeans (*Glycine max* L. Merrill), among others, with wide geographic distribution (Almeida et al., 2011; Bitencourt &

Silva, 2010; Carneiro et al., 2001; Silva et al., 2016; Silva & Krasuski, 2012; Luquini et al., 2019; Rosa et al., 2015).

In view of the frequency with which weeds occur in fields and orchards competing for nutrients with cultivated plants and considering their importance as potential phytomes hostesses, the objective of this work was to investigate the reaction of invasive plant species to the parasitism of *M. enterolobii*.

Materials and Methods

The experimental trials with weeds and *M. enterolobii* were carried out in the greenhouse and in the Phytopathology Laboratory of the Federal University of Ceará (*Universidade Federal do Ceará - UFC*). Nematode inoculum was obtained from infected guava roots from orchards in the county of Acaraú, CE. The identification of the nematode species was performed using the technique of electrophoresis of isoenzymes in polyacrylamide gel described by Carneiro and Almeida (2001). Subsequently, the inoculum was multiplied in plants of coleus, *Coleus scutelarioides* (L.) Benth., good plant species host of nematode (Silva; Santos, 2012). The extraction of eggs of *M. enterolobii* for inoculation of the plants in the assays was carried out according to the methodology of Coolen and D'Herde (1972).

14 weed species were evaluated: *Acanthospermum hispidum* D.C., *Ageratum conyzoides* L., *Alternanthera tenella* Colla., *Amaranthus deflexus* L., *A. spinosus* L., *A. viridis* L., *Bidens pilosa* L., *Clitoria ternatea* L., *Datura stramonium* L., *Macroptilium lathyroides* (L.) Urb., *Merremia aegyptia* (L.) Urb., *Solanum americanum* Mill., *S. paniculatum* L. e *Spigelia anthelmia* L. Weed seeds, properly identified and used in the study, were collected at the Experimental Farm Lavoura Seca (Fazenda Experimental Lavoura Seca) of UFC, located in Quixadá, CE. The seeds of each species were soed in pots of 2 L containing soil substrate and manure (2:1), previously autoced. After germination, the seedlings were transplanted to 0.5 L pots, remaining one plant per pot, with eight replicates of each species. After 24 hours of transplantation, the weeds were individually inoculated with a suspension containing eggs and second stage juveniles (J2) of *M. enterolobii*. The inoculum consisting of 5,000 eggs/J2 was distributed in three holes made near the root of the plants. As a positive control of *M. enterolobii* inoculum, coleus seedlings were used. All inoculated plants were kept in a greenhouse during the experiment with a temperature ranging from 29 ± 3°C. The design used was completely randomized, with 15 treatments (14 weed species and control), with 8 replications each.

At 45 days after inoculation, the 120 plants were removed and the roots cut and taken to the laboratory for counting the number of galls (NG), number of egg mass (NMO), number of eggs (NO) and calculation of egg mass index (IMO) and reproduction factor (FR). The NG and NMO count was performed under stereoscopic microscope and the IMO was performed according to the Taylor and Sasser scale (1978) that applied notes ranging from 0 to 5, according to the amount of galls or egg masses: 0 = absence of galls; 1 = from 1 to 2 galls; 2 = from 3 to 10 galls; 3 = from 11 to 30 galls; 4= from 31 to 100; 5 = above 100 galls.

The FR was calculated by the quotient between the final and initial egg population for each treatment (FR = Pf/Pi), where: Pf = final population and Pi = initial population. The FR value was used to perform the classification proposed by Oostenbrink (1966), where species are classified as: immune (I) if FR = 0, resistant (R) if FR < 1 and susceptible (S) when FR > 1.

Statistical analyses were performed with the Assistat program (Silva & Azevedo, 2006). The means of the treatments were submitted to variance analysis (ANOVA) and compared by the Tukey test ($p \leq 0.05$).

Results and Discussion

The variables NG, IG, NMO, IMO, NO and FR of the parasitism of *M. enterolobii* inoculated in the 14 weeds are presented in Chart 1.

Chart 1.

Average number of galls (NG), gall index (IG), average number of egg masses (NMO), egg mass index (IMO), average number of eggs (NO), reproduction factor (FR) in weed species inoculated with *Meloidogyne enterolobii*.

Plant species	NG	IG	NMO	IMO	NO	FR	Reaction*
Solanum paniculatum	400.5 a	5	96.5 b	4	46,800 a	9.4	S
Solanum americanum	327.9 b	5	104.3 b	5	22,195 b	4.4	S
Bidens pilosa	306.6 b	5	155 a	5	13,716 d	2.7	S
Ageratum conyzoides	160.5 c	5	46 c	4	11,070 d	2.2	S
Alternanthera tenella	483.5 a	5	6.4 d	2	5,965 e	1.2	S
Merremia aegyptia	276.6 b	5	18.4 d	3	5,940 e	1.2	S
Spigelia anthelmia	132.5 c	5	2.3 d	2	150 f	0.03	R
Macroptilium lathyroides	7.3 d	2	2.9 d	2	103 f	0.02	R
Clitoria ternatea	65.4 d	4	2.0 d	1	95 f	0.02	R
Datura stramonium	30.2 d	3	0.6 d	0.5	12 f	0.01	R
Acanthospermum hispidum	10.2 d	2	0 d	0	0 f	0	I
Amaranthus deflexus	0	0	0 d	0	0 f	0	I
Amaranthus spinosus	0	0	0 d	0	0 f	0	I
Amaranthus viridis	0	0	0 d	0	0 f	0	I
Control (coleus)	284.4 b	5	61.3 c	4	31,200 c	6.2	S
CV	37.06	20.45	51.58	19.50	24.11	19.15	-

Source: own authorship.

*Note: Averages followed by the same letter in the column do not differ statistically from each other by the Tukey test at 5% probability. *Reaction according to Oostenbrink (1966): immune (I; FR = 0), resistant (R; FR < 1) and susceptible (S; FR ≥ 1).*

The average number of galls observed in the root system of weeds ranged from 7.3 to 483.5, except for the species *A. derflexus*, *A. spinosus* and *A. viridis*, which did not present any gall. The average number of egg masses ranged from 0.6 to 155, and the presence of egg mass was not observed in *A. derflexus*, *A. hispidum*, *A. spinosus* and *A. viridis*. Regarding the average number of eggs in the roots, there was a variation of 100 to 46,800 eggs/root, except for the four species mentioned above that had no egg mass in the roots (Chart 1). In the species *A. conyzoides*, *A. tenella*, *B. pilosa*, *M. aegyptia*, *S. americanum* and *S. paniculatum*, FR ranging from 1.2 to 9.4 was observed, and the same were classified as susceptible to nematode. In *C. ternatea*, *D. stramonium*, *M. lathyroides* and *S. anthelmia*, although the gall index varied from 2 to 5, the FR obtained was very low (0.01 to 0.03), and the plants were considered very resistant. Finally, in the species *A. hispidum*, *A. deflexus*, *A. spinosus* and *A. viridis*, the FR was zero, indicating that they did not allow the multiplication of the pathogen, even though in *A. hispidum* the gall index was higher than 2 (Chart 1).

Solanum paniculatum was the plant species that presented the highest reproduction factor (9.4), superior to the control coleus (6.2), indicating that it had high hostability to *M. enterolobii*. Behavior similar to that observed in *D. stramonium* and *A. hispidum* was reported for *Catharanthus roseus* infected with *M. paranaensis*. This species presented numerous galls in its roots, but nematode reproduction (FR = 0) did not occur, being considered by the authors as highly resistant (Mendonça et al., 2017).

The susceptibility of *A. spinosus*, *B. pilosa*, *S. americanum* to *M. enterolobii* was also reported by Kaur et al. (2007) and Bellé et al. (2019a) from experimental trials conducted with nematode in a greenhouse. However, the species *A. deflexus*, *A. spinosus* and *A. viridis* cited as susceptible by Bellé et al. (2019a) behaved as immune to *M. enterolobii* in this essay, being in disagreement with those authors. In surveys carried out in guava orchards, the species *M. aegyptia* and *S. paniculatum* were found with infection by *M. enterolobii* (Castro et al., 2019; Silva & Santos, 2017), confirming the results observed in this study.

The resistance of weeds *A. conyzoides*, *A. tenella* and *A. viridis* to *M. enterolobii* was previously reported by Pinheiro et al. (2019) in artificial inoculations similar to those performed in the present study. However, the results differed since *A. conyzoides* was classified as susceptible and all three species of the genus *Amaranthus* evaluated were immune to nematode (Chart 1). In a study carried out by Freire and Santos (2018), *S. anthelmia* and *D. stramonium* were classified as resistant to *M. enterolobii*, information confirmed in this evaluation.

The behavior of amaratáceas *A. derflexus*, *A. spinosus* and *A. viridis* and asterecea *Acanthospermum hispidum* observed in the assay suggests that these plants do not constitute an inoculum source for a guava orchard. The parasitism of *M. enterolobii* in *C. ternatea* and *A. hispidum* observed in this study is the first record of the susceptibility of these plant species to the pathogen.

The resistance of *M. lathyroides* to *M. enterolobii* observed in this study differs from the susceptibility to *M. incognita* observed by Brito et al. (2008).

The susceptibility of these weeds to other species of *Meloidogyne* have been reported by several authors in several regions of Brazil, such as *S. paniculatum* susceptible to *M. javanica* (Zem & Lordello, 1976), *S. americanum* to *M. incognita* and to *M. javanica* (Bellé et al., 2019b; Zem & Lordello, 1976; Mônaco et al., 2009), *B. pilosa* to species *M. hapla, M. incognita* and *M. javanica* (Bellé et al., 2019b; Ponte et al., 1977), *A. conyzoides* to species *M. arenaria*, *M. incognita* and *M. javanica* (Bellé et al., 2019b; Zem & Lordello, 1976), *A. tenella* to nematodes *M. incognita*, *M. javanica* and *M. paranaensis* (Bellé et al., 2019b; Mônaco et al., 2009), *S. anthelmia* to *M. javanica* (Ponte et al., 1977), *M. lathyroides* to *M. arenaria* and *M. incognita* (Brito et al., 2008; Ponte *et al.*, 1977), *C. ternatea* to *M. ethiopica* (Lima et al., 2009), *A. deflexus* to *M. exigua* and *M. paranaensis* (Lima et al., 1985; Mônaco et al., 2008), *A. spinosus* to species *M. graminicola* and *M. paranaensis* (Mônaco et al., 2008; Sperandio & Amaral, 1994; Roese & Oliveira, 2004) and *A. viridis* to *M. javanica* and *M. incognita* (Mônaco et al., 2009), which confirms the importance of investigating invasive plants as a source of nematode inoculum for crops. For invasive species *M. aegyptia* and *A. hispidum* no reports of infection were found by *Meloidogyne* spp. in the country.

Conclusions

Despite the high degree of polyphagism observed for *M. enterolobii*, of the 14 species evaluated only six of the weed species tested were susceptible to the nematode of guava galls. The species of the genus *Amaranthus* stood out for not presenting galls or egg mass, and zero FR indicating that they are not nematode hosts.

Weeds as alternative hosts can ensure the survival and multiplication of nematode in guava orchards. Thus, the control measures to be adopted in orchards infested with this parasite should take into account the hosting of these plants to the nematode to recommend the careful removal of these plant species from the cultivated area. The susceptibility of the species evaluated in this study corroborates to increase information on the parasitism of *Meloidogyne spp*. in weeds.

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