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Reaction of potential guava rootstocks to *Meloidogyne enterolobii*

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ABSTRACT

Root-knot nematode *Meloidogyne enterolobii* is the main phytosanitary problem of guava cultivation in Brazil. Among the strategies to manage the problem, the best prospects are in identifying or developing cultivars or rootstocks that are resistant to this nematode. To identify plants with potential as rootstocks for guava, the reaction of araçá (wild guava) to *M. enterolobii* was assessed in a greenhouse experiment. Seven araçá species were evaluated (*Eugenia stipitata*, *Psidium acutangulum*, *P. cattleyanum* 'yellow', *P. friedrichsthalianum*, *P. guajava* var. *minor*, *P. guineense*, and *Psidium* sp.). The plants were inoculated with a suspension of 3,000 eggs of *M. enterolobii*, using eggplant as control treatment. The parameters fresh root mass, number of eggs and second stage juveniles (J2) per root system, the reproduction factor ($RF = Pf/Pi$), and araçá reaction were determined during the experiment. RF of the araçá species *E. stipitata*, *P. cattleyanum* 'yellow', and *P. friedrichsthalianum* was less than one ($RF < 1$), therefore resistant to *M. enterolobii*. The araçá trees had good root system development and the susceptible plants showed many root galls, high number of eggs and J2, and *Fusarium solani* and *Rhizoctonia solani* root rot. The araçá species, *P. cattleyanum* 'yellow', *P. friedrichsthalianum*, and *E. stipitata* are resistant to *M. enterolobii* and can be tested as potential guava rootstocks.

Keywords: *Psidium guajava*; araçá species; root-knot nematode; genetic resistance.

RESUMO

Reação de possíveis porta-enxertos de goiabeira a *Meloidogyne enterolobii*

O nematoide das galhas, *Meloidogyne enterolobii*, é o principal problema fitossanitário para o cultivo da goiabeira. Entre as estratégias para o manejo deste nematoide, as melhores perspectivas estão na identificação ou no desenvolvimento de cultivares ou porta-enxertos resistentes. Buscando elucidar quais plantas poderão ser testadas como porta-enxertos de goiabeira, um experimento foi conduzido em casa de vegetação com o objetivo de avaliar a reação de araçazeiros frente a *M. enterolobii*. Sete espécies de araçazeiros foram avaliadas (*Eugenia stipitata*, *Psidium acutangulum*, *P. cattleyanum* 'amarelo', *P. friedrichsthalianum*, *P. guajava* var. *minor*, *P. guineense* e *Psidium* sp.), as quais foram inoculadas com uma suspensão de 3.000 ovos de *M. enterolobii*, sendo utilizadas plantas de berinjela como tratamento controle. Durante as avaliações, foram determinados a massa fresca de raízes, o número de ovos e juvenis de segundo estágio (J2) por sistema radicular, o fator de reprodução [$FR = Pf/Pi$] e a reação dos araçazeiros. Os araçazeiros, *E. stipitata*, *P. cattleyanum* 'amarelo' e *P. friedrichsthalianum* tiveram fator de reprodução menor que um ($FR < 1$), sendo, portanto, resistentes a *M. enterolobii*. Nesses araçazeiros houve bom desenvolvimento do sistema radicular e nas plantas suscetíveis ($FR > 1$) foram observadas muitas galhas, elevado número de ovos e J2 e necroses nas raízes, com a presença de *Fusarium solani* e *Rhizoctonia solani*. Os araçazeiros, *P. cattleyanum* 'amarelo', *P. friedrichsthalianum* e *E. stipitata* são resistentes ao *M. enterolobii* e podem ser testados como possíveis porta-enxertos de goiabeira.

Palavras-chave: *Psidium guajava*; araçazeiros; nematoide das galhas; resistência genética.

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INTRODUCTION

In the late 1980s, severe damage to guava (*Psidium guajava*) caused by root-knot nematodes was diagnosed and attributed to *Meloidogyne incognita* [(Kofoid & White) Chitwood] (Moura & Moura, 1989). Later, these damages were related to *Meloidogyne enterolobii* (Yang & Eisenback), and, in Brazil, the first recorded parasitism occurred in irrigated orchards of Bahia and Pernambuco (Carneiro *et al.*, 2001).

M. enterolobii is the main limiting factor for guava cultivation and the typical symptoms of the disease are tanning, yellowing, and fall of leaves, leading to plant death (Carneiro *et al.*, 2001). The infected roots present numerous galls, generalized necrosis, and few radicles, which aggravate the symptoms of nutritional deficiency (Almeida *et al.*, 2011). In addition, infection by *M. enterolobii* in guava is generally associated with soil fungi (Gomes *et al.*, 2013), which interact synergistically resulting in greater damage to crops, as reported for common bean (*Phaseolus vulgaris*) and passion fruit (*Passiflora edulis* f. *flavicarpa*) (Fischer *et al.*, 2010; Al-Hazmi & Al-Nadary, 2015).

An estimate of direct damages in guava orchards associated with *M. enterolobii* infection was carried out in the states of Bahia, Ceará, Pernambuco, Rio Grande do Norte, and Rio de Janeiro and, reported a loss of more than US \$ 60 million, without accounting for impacts on other sectors of the crop production chain. In addition to the decapitalization of small-farmers, as guava orchards are usually cultivated by family farmers, rural labor unemployment is added and negatively affects the regional economy (Pereira *et al.*, 2009).

After its identification and the damage caused to guava, *M. enterolobii* gained worldwide importance, and its polyphagia was detected in many plants of economic interest, such as fruit (*Carica papaya* and *Malpighia emarginata*) and vegetables (*Citrullus lanatus*, *Lactuca sativa*, *Solanum lycopersicum*, *Solanum melongena*, among others) (Rosa *et al.*, 2015; Silva *et al.*, 2016).

Among the strategies for managing *M. enterolobii* in guava, the use of organic compost (avian litter and cattle manure) applied to the crown projection is more feasible in moderately infested orchards (Gomes *et al.*, 2010). The use of antagonistic plants and/or unfavorable hosts is also an alternative for managing nematodes, since they reduce the populations and are used as green manures (*Avena* spp., *Urochloa* spp., *Crotalaria* spp., *Mucuna* spp., *Pennisetum glaucum*, and *Raphanus sativus* var. *oleiferus*) or for grain production (*Arachis hypogaea*) (Cunha *et al.*, 2015; Rosa *et al.*, 2015; Silva & Santos, 2017).

However, the best prospects for the management of *M. enterolobii* in guava orchards are in the identification or development of resistant cultivars or rootstocks. Some

studies evaluated the reaction of araçá and guava trees to *M. enterolobii* and found sources of resistance (Almeida *et al.*, 2009; Freitas *et al.*, 2014; Biazatti *et al.*, 2016). However, most of the evaluated species are susceptible to *M. enterolobii* and results diverge for some araçá species as in *Psidium cattleianum* and *P. friedrichsthalianum* (Almeida *et al.*, 2009; Freitas *et al.*, 2014).

Thus, the objective of this study was to evaluate the reaction of araçá species to *M. enterolobii* and to diagnose the possible presence of fungi in the roots, seeking to identify which genotypes could be tested as rootstock for guava.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse in the municipality of Jaboticabal, São Paulo State, Brazil (21°14'33"S; 48°17'02"W; 563 m altitude), from August 21, 2013 to July 10, 2014.

A completely randomized design was used, with eight treatments (aráçá trees), one control (eggplant), and ten replications. The following wild araçá species were evaluated: yellow-aracá (*Psidium cattleianum*), araçazeiro-boi (*Eugenia stipitata*), araçazeiro-do-campo (*P. guineense*), araçazeiro-mirim (*Psidium* sp.), araçazeiro-pera (*P. acutangulum*), goiabeira-mirim (*P. guajava* var. *minor*), and two genotypes of Costa Rican guava (*P. friedrichsthalianum*).

Seeds were planted in perforated polyethylene trays (43 x 28 x 11 cm) filled with expanded vermiculite of medium texture, and kept under a 50% shade screen. The seedlings, approximately 5 cm in height, were transplanted into 3.8 L polyethylene vessels filled with a mixture (1:2) of soil and sand, which was autoclaved at 120 °C for 2 h. After transplanting, the seedlings were kept in the greenhouse free of nematodes for approximately four months to achieve better growth rate and development, mainly the root system, until inoculation.

The inoculum was obtained from roots of 'Paluma' guava, of a commercial plantation of approximately 12 years, in Vista Alegre do Alto, São Paulo State. A subpopulation of *M. enterolobii* was identified based on the morphological characters of the perineal pattern (Taylor & Netscher, 1974), the labial region morphology in males (Eisenback & Hirschmann, 1981), and the esterase isoenzymatic phenotype (Esbenshade & Triantaphyllou, 1990).

We prepared a *M. enterolobii* egg suspension from infected roots using 0.5% aqueous solution of sodium hypochlorite (Hussey & Barker, 1973), on January 8, 2014. Then, the araçá seedlings were inoculated with a suspension of 10 mL, containing 3,000 eggs. The suspension was pipetted into six holes in the substrate around the collar of the plants. To assess the viability of

the inoculum, 'Comprida Roxa' eggplant plants were used as susceptibility pattern (control treatment).

The plants were irrigated according to need and fertilized according to production guidelines for guava seedlings (Franco & Prado, 2006). About six months after inoculation (183 days), the root system was removed, washed in running water, the excess water removed with paper towel, and the fresh mass determined. The roots were processed according to Hussey & Barker (1973) and, after extractions, the number of eggs and J2 of *M. enterolobii* in the root systems was estimated using a Peters' counting slide under light microscope. Araçá and eggplant root samples were sent to the Phytopathology Laboratory of the School of Agricultural and Veterinary Studies of the Universidade Estadual Paulista (FCAV/UNESP) to determine the presence of fungi. The identification, was performed through the morphological characteristics of fungi growth by direct observation in optical microscope.

The reproduction factor (RF) was determined by the equation $[RF = Pf/Pi]$ where: Pf = final population and Pi = initial population (Pi = 3,000 eggs). The araçá plants with $RF < 1$ were considered resistant and those with $RF > 1$ were considered susceptible (Oostenbrink, 1966). For the purpose of analysis, the number of eggs and J2 were transformed into $\log(x+5)$, submitted to analysis of variance by the F test, and the means were compared by the Tukey's test at 1% of probability ($p < 0.01$).

RESULTS AND DISCUSSION

M. enterolobii did not multiply in the species yellow-aracá (*Psidium cattleianum*) and Costa Rican guava (*P. friedrichsthalianum*), which were classified as resistant, with $RF < 1$ (Table 1), corroborating results in the literature (Freitas *et al.*, 2014; Biazatti *et al.*, 2016). Freitas *et al.* (2014) observed that the roots of yellow-aracá (*Psidium cattleianum*) and Costa Rican guava (*P. friedrichsthalianum*) were infected by *M. enterolobii* and the J2 induced formation of feeding sites, but, about 30 days after inoculation, the giant cells deteriorated and most of the specimens of *M. enterolobii* did not reach maturity.

The resistance of yellow-aracá (*Psidium cattleianum*) and Costa Rican guava (*P. friedrichsthalianum*) may result from a mechanism called post-infection or delayed resistance, in which the nematodes are able to penetrate the roots but do not develop (Freitas *et al.*, 2014). In plants possessing post-infection resistance, cell death around the area of infection occurs and prevents the formation and development of the feeding site, giving resistance to the plants. This type of resistance mechanism to gall nematodes was also reported for genotypes of cowpea

(*Vigna unguiculata*) and rice (*Oryza glaberrima*) (Das *et al.*, 2008; Cabasan *et al.*, 2014).

E. stipitata allowed a little multiplication of *M. enterolobii*, however, it was also classified as resistant ($RF < 1$) (Table 1). This result corroborates with the results of Almeida *et al.* (2009) who evaluated roots of the plants and obtained a reproduction factor of 0.09 after five months of inoculation. Similar behavior was found in the *Prunus* spp. \times *M. incognita* pathosystem (Khallouk *et al.*, 2011). Khallouk *et al.* (2011) observed that J2 were not able to dissolve the medial lamella between the root cells of *Prunus* spp., because Casparian strips reinforce the walls of the endoderm.

Due to the adaptability to different soil and climatic conditions, it is possible that *E. stipitata* possesses some mechanism of resistance similar to that observed in *Prunus* spp. (Khallouk *et al.*, 2011). In this case, the energy expenditure is high for the J2 to locate and establish the feeding sites, leading to starvation of juveniles due to lack of nutrients. Thus, the development to adult stage is inhibited, reducing the chances of reproduction, which may help explain the low reproductive factor observed in *E. stipitata*.

The species *P. acutangulum*, *P. guajava* var. *minor*, *P. guineense*, and *Psidium* sp. allowed nematode multiplication and were classified as susceptible ($RF > 1$), as well as with the 'Comprida Roxa' eggplant, which confirmed the viability of the inoculum (Table 1). The fungi *Fusarium solani* and *Rhizoctonia solani* were also identified in these species (araçá and eggplant) along with the galls, which are often associated with the decline of the guava tree (Gomes *et al.*, 2013).

Along with xylem obstruction by gall-forming nematodes, the concomitant presence of soil fungi contributes to plant wilting in the hottest time of the day, even with adequate water. This shows that the synergistic effect of the pathosystem involving *M. enterolobii* and soil fungi, more frequently with the presence of *F. solani*, greatly aggravates plant damage, leading to the decline of guava trees (Gomes *et al.*, 2013).

In general, complex diseases involving nematodes occur because the penetration of these pathogens provides entry points for microorganisms, including fungi, limiting growth, yield and, in extreme cases, resulting in the death of plants. The severity of root rot in the presence of *M. enterolobii*, as observed in susceptible araçá trees, may be caused by nematode infection inducing anatomic and physiological changes and predisposing the roots to increased fungal infection (Porter & Powell, 1967). The interaction between nematodes and soil fungi causes significantly higher damage to cultivated plants (Fischer *et al.*, 2010; Gomes *et al.*, 2013; Al-Hazmi & Al-Nadary, 2015).

Table 1: Fresh mass of roots (RFM in grams), number of eggs, number of second-stage juveniles in the roots (J2), reproduction factor (RF), and reaction of araçá trees to *Meloidogyne enterolobii*

Treatment	RFM ^(x)	Egg ^(v; x)	J2 ^(v; x)	RF ^(x; w)	Reaction ^(y)
<i>Psidium cattleianum</i> 'yellow'	8.80d	0a	0a	0.00	R
<i>Psidium friedrichsthalianum</i> ^(z)	9.60c	0a	0a	0.00	R
<i>Psidium friedrichsthalianum</i> ^(z)	11.05b	0a	0a	0.00	R
<i>Eugenia stipitata</i>	10.95b	335b	175b	0.17	R
<i>Psidium acutangulum</i>	10.90b	83,630c	20,300c	34.64	S
<i>Psidium guajava</i> var. <i>minor</i>	17.50a	126,120cd	25,480c	50.53	S
<i>Psidium guineense</i>	10.05c	203,285cd	33,775d	79.02	S
<i>Psidium</i> sp.	16.95a	298,895d	44,875d	114.59	S
<i>Solanum melongena</i> (control)	11.15	276,480	74,880	117.12	S
Test F	26.94**	62.54**	75.19**		
CV (%)	15.28	10.07	2.92		

^(v) Original data; for analysis, the values were transformed into $\log(x+5)$. Means followed by different letters in the column are significantly different by the Tukey's test ($p < 0.01$). CV: coefficient of variation; **: significant at 1%.

^(x) Mean values of ten repetitions ($n=10$).

^(w) $RF = Pf/Pi$, where: RF: reproduction factor; Pf: final population; Pi: initial population.

^(y) Reaction of inoculated plants. $FR < 1$: resistant (R); $FR > 1$: susceptible (S).

^(z) Genotypes of *P. friedrichsthalianum*.

The resistant araçá trees showed good formation and distribution of roots, mainly of radicles, and absence of galls, except for *E. stipitata*, which had some galls. The araçá trees *P. guajava* var. *minor* and *Psidium* sp., susceptible to *M. enterolobii*, even with the highest root mass, showed the root system basically with supporting roots, few radicles, many galls and necrosis (Table 1). This relationship between the increase in root mass and the increase in the population of gall nematodes has been attributed to the combined effect of the growth of new roots in the nematode-infected sites and the high number of galls (Maleita *et al.*, 2012; Pereira *et al.*, 2016).

Secondary symptoms were observed in the canopy of the susceptible araçá trees (tanning, yellowing, and leaf fall), with greater intensity in *P. guajava* var. *minor* and *Psidium* sp. Secondary symptoms were caused by damage to the root system, mainly due to scarcity of radicles, which reduced the volume of soil explored. Because of the reduction in the volume of the root system, there was less absorption and transport of water and soil nutrients, which affected the development and growth of plants. Due to this chain of events, visual nutrient deficiency symptoms were observed in the leaves of araçá species susceptible to *M. enterolobii*, mainly for nitrogen, phosphorus, potassium and magnesium, which are similar to those described in guava trees (Almeida *et al.*, 2011).

CONCLUSIONS

M. enterolobii did not multiply on *P. cattleianum* 'yellow' and *P. friedrichsthalianum*.

E. stipitata allows a little multiplication of *M. enterolobii*.

The araçá species *P. cattleianum* 'yellow', *P. friedrichsthalianum*, and *E. stipitata* are resistant to *M. enterolobii* and can be tested as potential guava rootstocks.

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