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Scientific Article

Initial inoculum density, evaluation time, and reproduction of *Meloidogyne enterolobii* in 'Paluma' guava plants

Densidade inicial de inóculo, época de avaliação e reprodução de *Meloidogyne enterolobii* em goiabeira 'Paluma'

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ABSTRACT – Guava decline is a complex disease caused by the interaction between *Meloidogyne enterolobii* and *Neocosmospora falciformis* (Syn.: *Fusarium solani*). Thus, selecting *M. enterolobii*-resistant genotypes within the genus *Psidium* is essential for controlling this disease, and developing a resistant cultivar of *Psidium guajava* could significantly impact this issue. Thus, the objective of this study was to assess the response of the guava plants of the cultivar Paluma to different densities of *M. enterolobii* inoculum. Guava seedlings were inoculated with 500, 1,000, 5,000, 10,000, and 20,000 eggs + second-stage juveniles (J2) of *M. enterolobii* per plant. Root and shoot fresh weights, shoot dry weight, root length, plant height, and stem base diameter were evaluated at 70 and 135 days after inoculation (DAI). Total number of *M. enterolobii* eggs + J2 in the root system and nematode reproduction factor nematode were assessed. Nematode multiplication in roots was not proportional to increases in initial inoculum density; thus, the best plant responses to nematode multiplication in the evaluated cultivar were found for the lowest tested densities. The reproduction factor decreased as the inoculum density was increased, at both evaluations (70 and 135 DAI).

Keywords: *Psidium guajava*. Root-knot nematode. Reproduction factor.

RESUMO – O declínio-da-goiabeira é uma doença complexa, causada por *Meloidogyne enterolobii* em interação com *Neocosmospora falciformis* (sin. *Fusarium solani*). Por causa disso, a seleção de genótipos resistentes a *M. enterolobii* dentro do gênero *Psidium* é muito importante para o controle da doença. A detecção desta resistência em *Psidium guajava* seria, ainda, de maior impacto. Assim, este trabalho teve por objetivo avaliar a reação de *P. guajava* 'Paluma' a diferentes densidades de inóculo de *M. enterolobii*. Mudas de goiabeira foram inoculadas com 500, 1000, 5000, 10000 e 20000 ovos + juvenis de segundo estádio (J2) de *M. enterolobii*/planta. Aos 70 e aos 135 dias após a inoculação (DAI), foram avaliadas as massas de matérias frescas de raiz, de parte aérea e de matéria seca de parte aérea, bem como o comprimento de raiz, a altura da planta e o diâmetro do colo. Os números totais de ovos + J2 de *M. enterolobii* nos sistemas radiculares e os fatores de reprodução foram as variáveis nematológicas determinadas. A multiplicação do nematoide nas raízes não foi proporcional ao aumento da densidade de inóculo inicial e, por isso, nas menores densidades, encontraram-se as melhores respostas de multiplicação do nematoide na cultivar avaliada. Observou-se, então, que, conforme se aumentou a densidade de inóculo, obtiveram-se menores fatores de reprodução médios nas duas avaliações (70 e 135 DAI).

Palavras-chave: *Psidium guajava*. Nematoide-das-galhas. Fator de reprodução.

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INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical climate species; India and Brazil are the largest producing countries of white-fleshed and red-fleshed guava, respectively (ASHOKKUMAR et al., 2021). São Paulo, Pernambuco, and Rio de Janeiro are the main guava producing states in Brazil (IBGE, 2024). Guava is rich in vitamin C and phenolic compounds responsible for antimutagenic and antioxidant activities (FRANZON et al., 2009; ZAHIN; AHMAD; AQIL, 2017). These attributes, along with low production costs, make this fruit important for agriculture in several tropical and subtropical countries (RAI; JAISWAL; JAISWAL, 2009; RODRÍGUEZ et al., 2010).

However, several diseases can occur in guava orchards, and root parasitism by the nematode *Meloidogyne enterolobii* Yang & Eisenback (Syn.: *M. mayaguensis* Rammah & Hirschmann), combined with the fungus *Neocosmospora falciformis* (L. Lombard and Crous, 2015, Syn.: *Fusarium solani*), is the main phytosanitary problem for guava production (GOMES et al., 2011; BINDHU et al., 2022). Symptoms include leaf bronzing, stem and branch discoloration, changes in fruit shape and maturation, yellowing and falling of leaves, and plant death (CARNEIRO et al., 2001; CASTRO et al., 2017). Root galls, necrosis of large roots, and fewer secondary roots exacerbate symptoms of

nutritional deficiency (GOMES et al., 2014; BELLÉ et al., 2019; VELOSO; CÂMARA; SOUZA, 2021).

Different methodologies are used to classify plant resistance to root-knot nematodes. However, the lack of methodological standards for initial inoculum density is one of the challenges in characterizing genotype resistance, mainly in perennial crops, including guava (BURLA et al., 2010).

Several factors affect nematode reproduction, including environmental conditions, inoculum density, and plant exposure time to the nematode (WUYTS et al., 2007). Resistance is often evaluated using high initial inoculum populations. Studies evaluating resistance in *Psidium* spp. have been conducted using inoculation of 10,000 or 20,000 *M. enterolobii* eggs (CARNEIRO et al., 2007; MARTINS et al., 2013). However, high initial nematode populations can cause competition among individuals for feeding sites in the host, leading to reproduction factors below 1.0, characterizing resistance even in susceptible plants (GRECO; DI VITO, 2009; STARR; MERCER, 2009).

Oliveira et al. (2019) evaluated the response of *Psidium* spp. under three densities of *M. enterolobii* inoculum and found reductions in reproduction factor when using the highest tested densities. These findings reinforce the importance of evaluating the response of *Psidium* spp. to different densities of *M. enterolobii* inoculum, as already pointed out in evaluations conducted at 135 and 180 days after inoculation (BURLA et al., 2010). Similarly, the reproduction factor of *M. incognita* (Kofoid & White) Chitwood in cucumber (*Cucumis sativus* L.) decreased under inoculum densities higher than 2,000 eggs (KAYANI; MUKHTAR; HUSSAIN, 2018). Coffee plants (*Coffea arabica* L.) inoculated with 3,500, 5,000, and 6,500 eggs of *M. paranaensis* (Carneiro, Carneiro, Abrantes, Santos & Almeida) were evaluated at 120 and 180 days after inoculation, and the results showed that high inoculum levels can reduce nematode multiplication (HOLDERBAUM et al., 2021).

Thus, the objective of this study was to assess the response of a *P. guajava* cultivar Paluma to increasing densities of *M. enterolobii* inoculum at 70 and 135 days after inoculation.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse (mean temperature of 28 °C) and in the Laboratory of Nematology of the Brazilian Agricultural Research Corporation (Embrapa Semiarid), in Petrolina, PE, Brazil (09°04'14.0"S and 40°19'03"W) from August to November 2021.

Guava seeds of the cultivar Paluma were sown in expanded polystyrene trays with a sterilized substrate; at 38 days after germination, the seedlings were transplanted to 3-liter plastic bags containing autoclaved soil (121 °C for one hour) and vermiculite (2:1 v v^{-1}).

The *Meloidogyne enterolobii* inoculum was prepared using a pure population maintained on tomato plants (*Solanum lycopersicum* L. cultivar Rutgers) grown in autoclaved soil, following the methodology proposed by Hussey and Barker (1973) modified by Boneti and Ferraz (1981). The suspension was calibrated in counting chambers under an optical microscope for the application of 500, 1,000, 2,000, 5,000, 10,000, and 20,000 eggs + second-stage juveniles (J2) per plant, according to the treatments. Inoculation was performed 15 days after transplanting by applying suspension with eggs and J2 into three holes of 2 cm deep equidistant 3 cm from the plant's stem base.

The plants were grown in soil fertilized with a 10-24-12 N-P-K formulation at 30 and 90 days after inoculation and irrigated daily according to water demand.

Root and shoot fresh weights (g) and shoot dry weight (g) were measured on a digital scale at 70 and 135 days after inoculation. Shoot dry weight was determined by drying plant tissues in a forced air circulation oven at 65 °C for 3 days. Root length (cm) and plant height (cm) were measured using a tape measure, and stem base diameter (cm) was measured using a digital caliper.

Root fresh weight was determined by separating the root systems from the aerial parts of the plants and washing them under running water; excess water was removed with paper towels. The roots were then processed, following the methodology proposed by Boneti and Ferraz (1981). The total numbers of *M. enterolobii* eggs + J2 in the root systems were determined in a counting chamber using an inverted microscope (Nikon® TS100).

The reproduction factor (RF) was determined by the equation $RF = Pf / Pi$, where Pf is final population and Pi is the initial population (Pi = 500; 1,000; 2,000; 5,000; 10,000; and 20,000 eggs + J2). Plants with RF < 1 were considered resistant, while those with RF > 1 were considered susceptible (OOSTENBRINK, 1966).

The experiment was conducted in a completely randomized design with six replications, using a 2×6 factorial arrangement consisted of two evaluation times (70 and 135 after inoculation) and six inoculum densities (500; 1,000; 2,000; 5,000; 10,000; and 20,000 eggs + J2).

The obtained data were transformed into log(x+1), analyzed, and subjected to ANOVA and regression analysis using the programs Genes and Sisvar (CRUZ, 2013; FERREIRA, 2019).

RESULTS AND DISCUSSION

According to the analysis of variance, the interaction between the evaluated factors (inoculum density and evaluation time) had no significant effect on the assessed vegetative growth variables. Significant results were found for total number of eggs + J2 and reproduction factor of *Meloidogyne enterolobii* (Table 1).

Table 1. Analysis of variance for root fresh weight (RFW; g), root length (RL; cm), stem base diameter (SD; cm), plant height (PH; cm), shoot fresh weight (SFW; g) and shoot dry weight (SDW; g) of 'Paluma' guava plants, and total number of eggs + J2 (NEJ) and reproduction factor (RF) of *Meloidogyne enterolobii*.

Source of variation	DF	Mean squares			
		RFW	RL	SD	PH
Time (T)	1	0.5134^{ns}	0.0015^{ns}	0.0084*	49.37*
Density (D)	5	0.0852^{ns}	0.0130^{ns}	0.0030^{ns}	0.0059^{ns}
T × D	5	0.0620^{ns}	0.0036^{ns}	0.0034^{ns}	0.0066^{ns}
Error	60	0.0937	0.0004	0.0016	0.0081
Total	71				
Overall mean		1.5516	1.5550	0.1378	0.9525
CV (%)		19.7	4.2	29.3	9.4
		SFW	SDW	NEJ	RF
Time (T)	1	0.0647^{ns}	0.0004^{ns}	0.1941^{ns}	0.1940^{ns}
Density (D)	5	0.1379^{ns}	0.1252^{ns}	0.3252^{ns}	6.7793*
T × D	5	0.0523^{ns}	0.0585^{ns}	0.4238*	0.4238**
Error	60	0.0776	0.0909	0.1744	0.1744
Total	71				
Overall mean		1.6868	1.2287	5.2838	1.7838
CV (%)		16.5	24.5	7.9	23.4

**, * = significant at 1% and 5% probability levels, respectively, by the F-test. ^{ns} = not significant. Data transformed into log(x+1).

The interaction between the factors was significant at 1% and 5%, respectively, for reproduction factor and total number of eggs + J2. This result highlights the importance of considering the initial inoculum density used and the evaluation time of experiments, mainly those searching for *M. enterolobii*-resistant genotypes. The evaluation timing of the experiment is essential, mainly for evaluating the root systems under adequate conditions.

The means found for total number of eggs + J2 were similar for the tested inoculum densities at both evaluations (Table 2). However, the results found at 70 and 135 days after inoculation (DAI) differed for the initial densities of 2,000 and 5,000 eggs + J2 per plant.

Table 2. Means for total number of eggs + J2 (NEJ2) and reproduction factor (RF) of *Meloidogyne enterolobii* at 70 and 135 days after inoculation of inoculation of 'Paluma' guava plants with different initial inoculum densities.

Initial inoculum density (eggs + J2 plant^{-1})	Days after inoculation			
	70	135	70	135
	NEJ2^{ns}	NEJ2^{ns}	RF	RF
500	175160a	638093a	350.3a	1,276.2a
1,000	253533a	364057a	253.5a	394.4a
2,000	229973b	788697a	114.9b	394.3a
5,000	351513a	154080b	70.3a	30.8b
10,000	202113a	200107a	20.2a	20.0a
20,000	162267a	158373a	8.1a	7.9a

*Original data. Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test at a 5% probability level. ^{ns} = not significant.

The reproduction factor (RF) depends on the total number of eggs. The highest mean RF found at 135 DAI was 1,276.2 for the initial inoculum density of 500 eggs + J2. However, the RF decreased as the inoculum density was increased, decreasing to 7.9 for the highest density (20,000 eggs + J2 per plant). Thus, high initial nematode populations can cause competition among individuals for feeding sites in roots of host plants. This results in RFs below 1.0 at the end of experiments, erroneously characterizing the plant's response as resistance, even for nematode-susceptible plants, confirming results found in other studies (GRECO; DI VITO, 2009; STARR; MERCER, 2009).

The effect of the interaction between the factors was significant for RF (Table 1), showing that the selection of *P. guajava* genotypes for resistance to *M. enterolobii* can be affected by evaluation time and initial inoculum densities, as reported by Burla et al. (2010).

A significant difference between RF means was found in the first (70 DAI) and second (135 DAI) evaluation times for the inoculum densities of 2,000 and 5,000 eggs + J2 per plant. The RF at 135 DAI was approximately three-fold higher than that found at 70 DAI for the density of 2,000 eggs + J2 per plant. However, there was no significant difference between RFs found for the initial densities of 10,000 and 20,000 eggs + J2 at any of the evaluation times; RF decreased at 70 and 135 DAI, reaching 8.1 and 7.9, respectively.

Although a susceptibility response was confirmed by RFs higher than 1.0 (OOSTENBRINK, 1966), the nematode multiplication in the roots was not proportional to increases in initial inoculum density. Oliveira et al. (2019) evaluated the response of accessions of *Psidium* spp. to different densities of *M. enterolobii* inoculum and found similar results; the highest nematode multiplication was found for the lowest initial inoculum density tested; the highest RFs varied from 17 to 592 for the density of 600, and from 1.0 to 239 for the density of 1,600 eggs + J2 per plant; and the RF dropped to 3.0 when using an inoculum density of 2,000 eggs + J2 per plant.

Inoculum density and evaluation time are essential factors as they can affect the results regarding total number of eggs and RF, which are the most important nematode variables evaluated in experiments focused on identifying genotypes resistant to *Meloidogyne* species. The high significance of inoculum density as a source of variation found in the present study denotes the importance of this variable for determining resistance of accessions of *Psidium* spp. to *M. enterolobii*.

Martins et al. (2013) evaluated an initial population of 20,000 eggs, divided into two inoculations of 10,000 eggs per plant with a 30-day interval, and found five accessions of *Psidium* spp. resistant and eight susceptible to *M. enterolobii*. Carneiro et al. (2007) found resistance in *P. friedrichsthalianium* (A. Berg) Nied. and *P. cattleianum* Sabine when evaluating an initial population of 10,000 eggs of *M. enterolobii* per plant. Similar results were found by Burla et al. (2010), who found significant differences in plant response to the nematode. Thus, evaluating the individual response of plants to the nematode is an important step due to the high frequency of false-positive results caused by the lack of infection sites in plants with developing root systems. The number of false-positive may be significantly higher at inoculum densities exceeding 10,000 eggs per plant.

The data of total number of eggs and RF fitted a polynomial regression model and were significant at 70 and 135 DAI (Table 3).

Table 3. Regression analysis for total number of eggs + J2 (NEJ2) and reproduction factor (RF) of *Meloidogyne enterolobii* at 70 and 135 days after inoculation of 'Paluma' guava plants with different initial inoculum densities.

Source of variation	Equation	CV (%)	F-test	R^2 (%)
	70 days after inoculation			
NEJ2	$y = 1E\text{-}12x^3\text{-}3E\text{-}08x^2+0.0002x+5.0301$	6.43	1.421^{*}	75.09
RF	$y=6E\text{-}09x^2\text{-}0.0002x+2.463$	22.3	16.834^{**}	95.16
	135 days after inoculation			
NEJ2	$y=4E\text{-}09x^2\text{-}0.0001x+5.6971$	8.36	2.942^{*}	77.74
RF	$y=1E\text{-}08x^2\text{-}0.0003x+2.9084$	24.30	23.521^{**}	95.70

CV = coefficient of variation. ** and * = significant at 1% and 5% probability levels by the F-test, respectively. ^{ns} = not significant. Data transformed into log(x+1).

According to the regression analysis, the R^2 value found for RF exceeded 95% at both evaluation times, explaining the correlation between inoculum density and final nematode population. The means found for RF were lower at initial densities of 10,000 and 20,000 eggs + J2 but greater at lower initial inoculum densities at both evaluation times.

Kayani, Mukhtar and Hussain (2018) compared the response of cucumber cultivars, susceptible and resistant to *M. incognita*, under different inoculum densities and found reductions in RF as the inoculum density was increased, with inverse correlations shown in regression equations. Débia et al. (2020) evaluated carrot plants (*Daucus carota* L.) and found reductions in the number of nematodes per root system as the inoculum density was increased. Similarly, Andreazzi

et al. (2015) evaluated population densities of *M. paranaensis* in different coffee genotypes and reported that population densities between 500 and 3,000 eggs per plant were sufficient to distinguish resistant from susceptible genotypes based on the results of RF and total number of eggs + J2 at 110 DAI.

The findings of the present study showed a reduction in RF at both evaluation times as the initial inoculum density was increased. Thus, as indicated in previous studies (BURLA et al., 2010; OLIVEIRA et al., 2019), evaluating plant resistance under different inoculum densities, including low densities, is essential to prevent the discard of promising guava genotypes by breeding programs due misinterpretation of resistance responses to *M. enterolobii*.

CONCLUSION

The multiplication of *M. enterolobii* in roots of *P. guajava* plants of the cultivar Paluma was not proportional to increases in initial inoculum density. The reproduction factor decreased as the initial inoculum density was increased, at both evaluation times (70 and 135 days after inoculation).

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