

Behavior of 'IPR 100' and 'Apoatã IAC 2258' coffee cultivars under different infestation levels of *Meloidogyne paranaensis* inoculum

Elder Andreazi*¹, Gustavo Hiroshi Sera², Ricardo Tadeu de Faria¹, Tumoru Sera², Inês Cristina de Bastista Fonseca¹, Andressa Cristina Zamboni Machado², Luciana Harumi Shigueoka¹, Filipe Gimenez Carvalho¹, Fernando César Carducci²

¹Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid, Km 380, Cx. Postal 10.011, CEP 86.057-970, Londrina-PR-Brazil

²Instituto Agronômico do Paraná (IAPAR), Rodovia Celso Garcia Cid, Km 375, Três Marcos, CEP 86047-902, Londrina-PR-Brazil

*Corresponding author: elderfsp@gmail.com

Abstract

This study aimed to evaluate the behavior of the cultivars 'IPR 100' and 'Apoatã IAC 2258' under different infestation levels of *Meloidogyne paranaensis* inoculum. The experiment was conducted in a randomized block design and 3 × 6 factorial arrangement (three cultivars and six inoculum levels). The following inoculum levels were used: 0; 500; 1,500; 3,000; 5,000; and 8,000 eggs plant⁻¹. The root fresh weight and number of eggs were evaluated at 110 days after inoculation. The number of eggs and second-stage juveniles per gram of root (nematodes g⁻¹), reproduction factor (RF), reduction in reproduction factor (RRF), and host susceptibility index (HSI) were determined. The RF, RRF, and HSI were used to classify the resistance levels of the cultivars. The results showed significantly lower levels of nematodes g⁻¹ in IPR 100 and 'Apoatã' compared with that of the 'Catuaí' as check cultivar. The 'IPR 100' and 'Apoatã IAC 2258' cultivars exhibited resistance to *M. paranaensis* regardless of the inoculum level used. Based on the nematodes g⁻¹, RF, RRF, and HSI, the 'IPR 100' cultivar was more resistant to *M. paranaensis* than 'Apoatã IAC 2258.' Under the experimental conditions adopted here, the initial populations of 1,500 and 3,000 eggs plant⁻¹ are the most suitable for testing resistance to *M. paranaensis*. However, 3,000 eggs plant⁻¹ was the most efficient population because it can be used to classify the cultivars into different levels of resistance.

Keywords: breeding; *Coffea*; cultivars; resistance; root-knot nematode.

Abbreviations: 'Apoatã'_'Apoatã IAC 2258'; 'Catuaí'_'Catuaí Vermelho IAC 99'; HR_highly resistant; HS_highly susceptible; HSI_host susceptibility index; IAPAR_Instituto Agronômico do Paraná; IP_initial populations; MR_moderately resistant; MS_moderately susceptible; nematodes g⁻¹_eggs and second stage juveniles per gram of root; R_resistant; RF_reproduction factor; RRF_reduction in reproduction factor; S_susceptible.

Introduction

Brazil is responsible for more than 1/3 of the worldwide production and export of coffee, which makes Brazil the largest producer and exporter worldwide (ICO, 2014). With increased coffee consumption in recent decades and phytosanitary problems affecting the crop, production is no longer sufficient to meet the demand. To increase the supply, new technologies must be developed to increase yield and control pests and diseases.

Plant-parasitic nematodes are currently one of the most important causes of reduced yield (Campos and Villain, 2005) because they are difficult to control. There are also a limited number of resistant cultivars available. Once nematodes established at a coffee site, it is almost impossible to eradicate them (Gonçalves and Silvarolla, 2007).

The main coffee-parasitic nematodes in Brazil are *Meloidogyne incognita* and *Meloidogyne paranaensis*, which exhibit aggressive behavior that prevents the establishment of plantations, and *M. exigua*, which is important because of its widespread geographical distribution (Gonçalves and Silvarolla, 2007). The *M. paranaensis* was reported for the first time in Paraná State, Brazil (Carneiro et al., 1996) and

has rapidly spread throughout the arable regions of the states of Paraná, São Paulo (Carneiro and Almeida, 2000; Gonçalves and Silvarolla, 2001; Oliveira et al., 2001; Carneiro et al., 2005), and Minas Gerais (Castro et al., 2003, Castro and Campos, 2004, Castro et al., 2008). There are also reports of its occurrence in the states of Goiás (Silva et al., 2009) and Espírito Santo (Barros et al., 2011).

The use of resistant cultivars has proven to be the most efficient control measure at infested sites. However, the sources of resistance in *Coffea arabica*, which is the most commercially important coffee species, is scarce, especially for resistance to *M. paranaensis* and *M. incognita* (Gonçalves and Silvarolla, 2007). The 'Apoatã IAC 2258' rootstock of the species *C. canephora* exhibited resistance to the nematodes *M. exigua* (Salgado et al. 2005), *M. incognita*, and *M. paranaensis* (Sera et al., 2006; Fonseca et al., 2008). Recently, the ungrafted 'IPR 100' cultivar of *C. arabica* was released on the market, which is resistant to *M. paranaensis* (Sera et al., 2007; Ito et al., 2008; Sera et al., 2009) and *M. incognita* race 1 (Kanayama et al., 2009) and race 2 (Ito et al., 2008). However, the effectiveness of resistance in 'IPR

100' and 'Apoatã IAC 2258' cultivars at higher inoculum levels is not known.

In the nematode \times host plant interaction with high initial nematode populations, roots of the host plant may be severely damaged by pathogen attack. Thus, there is substantial competition between individuals for feeding sites on the host, which maintains the reproduction factor at levels below 1.0. Such behavior characterizes the resistance reaction, even in plants susceptible to the nematode (Greco and Di Vito, 2009). Thus, the reaction of resistant cultivars at different inoculum levels is an important factor because at high nematode population densities, the roots of resistant plants that are intolerant to the pathogen or have hypersensitive resistance reactions can express cell death linked to the resistance mechanism. Such behavior was previously observed for the coffee \times *M. exigua* relationship (Anthony et al., 2005), although these plants are compromised by parasitism, and unreliable results have been produced in coffee breeding (Greco and Di Vito, 2009).

Therefore, this study aimed to evaluate the behavior of the 'IPR 100' and 'Apoatã IAC 2258' cultivars at different levels of *M. paranaensis* inoculum in a greenhouse.

Results

Cultivar \times inoculum level interaction

The interaction between cultivars and *M. paranaensis* inoculum levels was significant for the value of nematodes g^{-1} root (nematodes per gram of roots). A lower nematodes g^{-1} root value was observed for *M. paranaensis* in the 'IPR 100' and 'Apoatã' cultivars, and the value was significantly different from that of 'Catuaí' at all of the inoculum levels (Table 1). The graphical representation showed a more significant increase in the regression curve approaching $P_i = 3,000$ eggs for the three cultivars. At this level, there was a trend towards stabilization for 'Apoatã.' For 'Catuaí,' the curve showed a trend towards stabilization starting at $P_i = 3,000$, and a reduction in nematodes g^{-1} root starting at $P_i = 5,000$. In 'IPR 100,' the stabilization appeared to occur close to $P_i = 8,000$; however, the increase in the curve was drastically reduced starting at $P_i = 3,000$ (Fig 1).

Classifying the resistance level of the cultivars

Based on the RF, the 'IPR100' and 'Apoatã' cultivars behaved as R (resistant) and 'Catuaí' cultivar behaved as S (susceptible) at all population levels used. Overall, the RF was lower with increasing inoculum levels, which was expected because of the increased competition for feeding sites and possible destruction of the root system, although this effect did not occur evenly in 'Apoatã' (Table 2).

The RRF value showed that the 'IPR 100' and 'Apoatã' cultivars behaved as HR at all inoculum levels (Table 2). In 'IPR 100,' except for at 500 eggs, which showed 10% plants behaving as MS, 100% of the plants were classified between HR (highly resistance) and MR (mild resistance) at all of the other levels. Similarly, in 'Apoatã IAC 2258,' only 10% of plants were HS at 3,000 eggs, whereas the remainder of the plants were classified between HR and MR at the other studied levels. Most of the plants of both cultivars were classified as HR (Table 3).

Using the HSI, the 'IPR 100' cultivar was HR at the 1,500-8,000 egg level and resistant at the 500 egg level, whereas 'Apoatã' was only HR at the 1500 egg level and R at the other levels (Table 4). All of the plants of the 'IPR 100' cultivar (100%) exhibited HR and MR at all levels studied. 'Apoatã' had 10% S plants at the 3,000 egg level, whereas

the remainder were classified between HR and MR at the other levels (Table 5).

A small rate of segregation for susceptibility was expected in 'Apoatã' because it is a cross-pollinated species (Gonçalves and Silvarola, 2007). The presence of 10% moderately susceptible plants at the 500 eggs level in 'IPR 100' based on RRF, can be explained by the cross-fertilization rate (5 to 10%) that naturally occurs in *C. arabica* because the seeds were obtained by open pollination.

Effect of inoculum level on the cultivars

Higher RF values were obtained at egg densities of 500, 1,500, and 3,000, demonstrating the resistance of 'IPR 100' and Apoatã to *M. paranaensis* (Table 2). In the inoculations with high initial populations, increased competition for feeding sites can occur among nematodes (Greco and Di Vito, 2009), which may have caused the reduced RF at the 5,000 and 8,000 eggs $plant^{-1}$ levels and could explain why the RF in the susceptible control did not increase proportionally with increasing inoculum level. Limited variation occurred for the nematodes root g^{-1} values in the resistant 'IPR 100' and 'Apoatã' cultivars between the 3,000 and 5,000 egg levels, and this pattern was also observed in the 'Catuaí' cultivar, which presented a slight increase at the 5,000 egg level (9,766.8) compared with the 3,000 egg level (8,345.1). Compared with the initial population of 8,000, the difference was considerable, with approximately 1.7-fold higher values relative to the 5,000 egg level for the three genotypes (Table 1, Fig 1). In addition, the lowest RFs were found at the 8,000 egg level (except for 'Apoatã'), primarily in the susceptible control. Initial *M. paranaensis* populations of approximately 500 and 1,500 eggs were sufficient for classifying the plants between resistant and susceptible; however, more expressive nematodes g^{-1} values were found starting at 3,000 eggs $plant^{-1}$ (Table 1, Fig 1).

Discussion

One of the characteristics of *M. paranaensis* is its remarkable ability to destroy the root system of susceptible coffee plants (Gonçalves and Silvarolla, 2007; Silva et al., 2009). Thus, plant resistance may be difficult to classify when only the nematode reproduction rate, RF and RRF are considered. Additionally, competition for feeding sites increases at high population densities, which can be further exacerbated by the destruction of the root system, which also occurs in resistant cultivars. A previous study suggested that coffee plant resistance to certain pathogens such as *M. exigua* occurs after the nematode penetrates and propagates via a hypersensitive reaction conferred by a major resistance gene, which causes extensive cell destruction at the nematode feeding site and throughout the entire root system (Anthony et al., 2005). The HSI was used here to minimize possible interference in classifying resistance caused by differences among the root volume of the evaluated cultivars (Gonçalves and Ferraz, 1987, with modifications) because it is an index that considers the number of nematodes root g^{-1} when classifying the resistance levels. The comparison between the two indices (RRF and HSI) shows altered mean resistance reaction between 'Apoatã' and 'IPR 100.' According to the RRF, both cultivars were HR at all inoculum levels, whereas the HSI showed that the 'IPR 100' was more resistant than 'Apoatã' at the 3,000, 5,000, and 8,000 levels. The R reaction observed in both 'IPR 100' and 'Apoatã' at the 500 level (HSI) may be related to the low number of nematodes g^{-1} observed in 'Catuaí,' indicating that this initial population

Table 1. Mean number of eggs and second-stage juveniles of *Meloidogyne paranaensis* per gram of roots (nematodes root g⁻¹) of coffee plants at different inoculum levels.

Cultivar	Inoculum levels (eggs plant ⁻¹)*					
	0	500	1,500	3,000	5,000	8,000
'IPR 100'	0.0 a	15.1 a	26.1 a	47.6 a	46.6 a	86.9 a
'Apoatã'	0.0 a	28.2 a	36.0 a	148.5 a	147.9 a	244.5 a
'Catuai'	0.0 a	1041.2 b	4297.6 b	8345.1 b	9766.8 b	15257.7 b
CV(%)	=	38.95				

*Means followed by the same letter in the columns do not differ according to Tukey's test (p≤0.01). Data are log(x+1) transformed.

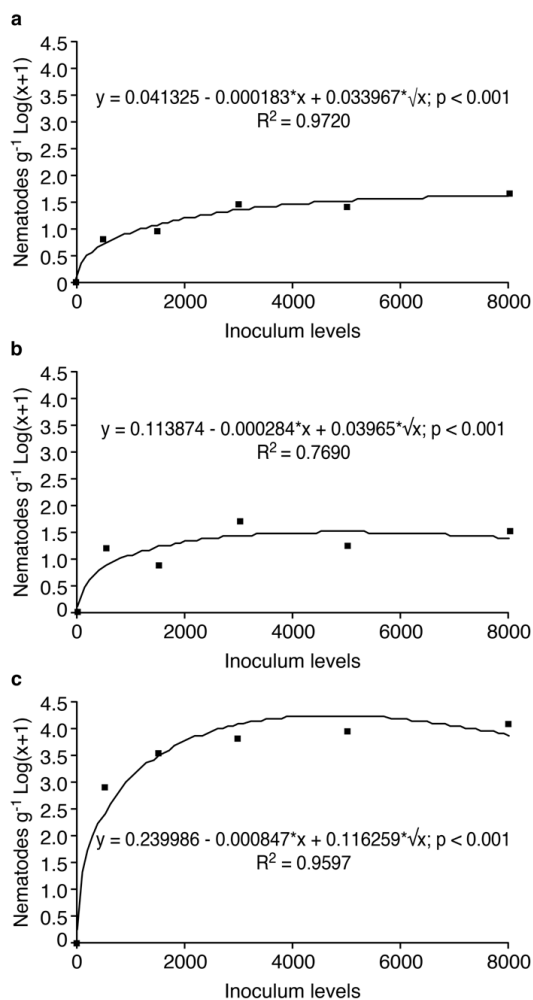


Fig 1. Number of eggs and second-stage juveniles of nematodes per gram of roots of coffee plants (nematodes root g⁻¹) at different *Meloidogyne paranaensis* inoculation levels. a = 'IPR 100'; b = 'Apoatã IAC 2258'; and c = 'Catuai Vermelho IAC 99'

Table 2. Reproduction factor (RF), reduction in reproduction factor (RRF), and resistance reaction (rr) of coffee cultivars subjected to different *Meloidogyne paranaensis* inoculum levels (N).

N	'IPR 100' ¹				'APOATÃ' ¹				'CATUAI' ¹			
	RF	rr ²	RRF	rr ³	RF	rr ²	RRF	rr ³	RF	rr ²	RRF	rr ³
0 ⁴	0.00		0.00	-	0.00	-	0.00	-	0.00		0.00	
500	0.51	R	96.8	HR	0.64	R	96.0	HR	15.83	S	0.00	HS
1,500	0.28	R	98.3	HR	0.24	R	98.5	HR	16.55	S	0.00	HS
3,000	0.23	R	98.5	HR	0.49	R	96.8	HR	15.01	S	0.00	HS
5,000	0.15	R	98.6	HR	0.26	R	97.4	HR	10.28	S	0.00	HS
8,000	0.15	R	98.5	HR	0.30	R	97.0	HR	9.95	S	0.00	HS

¹'Catuai' cultivar was used as the control to calculate the RRF.

²R = resistant; S = susceptible; based on RF (Oostenbrink, 1966).

³HR = highly resistant; based on RRF (Moura and Regis, 1987).

⁴At inoculum level (N) 0 used as control do not have resistance reaction (rr).

Table 3. Percentage of coffee plants that were highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible (HS) to *Meloidogyne paranaensis* at different inoculum levels (N) based on the reduction in reproduction factor (RRF).

N (eggs plant ⁻¹)	'IPR 100' ¹						'Apoatã IAC 2258' ¹					
	HR	R	MR	MS	S	HS	HR	R	MR	MS	S	HS
0												
500	50	30	10	10	0	0	60	10	30	0	0	0
1,500	80	10	10	0	0	0	90	0	10	0	0	0
3,000	100	0	0	0	0	0	80	0	10	0	0	10
5,000	90	0	10	0	0	0	70	30	0	0	0	0
8,000	100	0	0	0	0	0	80	10	10	0	0	0

¹'Catuai' Vermelho IAC 99' cultivar was used as the control to calculate the RRF.

Table 4. Reaction of coffee plants to *Meloidogyne paranaensis* at the mean value according to the host susceptibility index (HSI) at different inoculum levels (N).

N (eggs plant ⁻¹)	'IPR 100' ¹		'Apoatã IAC 2258' ¹	
	HSI	Reaction ²	HSI	Reaction ²
0 ³	0,0	-	0,0	-
500	1.5	R	2.7	R
1,500	0.6	HR	0.8	HR
3,000	0.6	HR	1.8	R
5,000	0.5	HR	1.5	R
8,000	0.6	HR	1.6	R

¹'Catuai' Vermelho IAC 99' cultivar was used as the susceptible control to calculate the HSI.

²R = resistant; and HR=highly resistant.

³ At inoculum level (N) 0 used as control do not have resistance reaction (rr).

Table 5. Percentage of coffee plants rated as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible (HS) to *Meloidogyne paranaensis* at different inoculum levels (N) according to the host susceptibility index (HSI).

N (eggs plant ⁻¹)	'IPR 100' ¹						'Apoatã IAC 2258' ¹					
	HR	R	MR	MS	S	HS	HR	R	MR	MS	S	HS
0												
500	40	50	10	0	0	0	30	50	20	0	0	0
1,500	60	40	0	0	0	0	60	40	0	0	0	0
3,000	80	20	0	0	0	0	60	30	0	0	10	0
5,000	90	10	0	0	0	0	70	30	0	0	0	0
8,000	80	20	0	0	0	0	50	50	0	0	0	0

¹'Catuai' Vermelho IAC 99' cultivar was used as the susceptible control to calculate the HSI.

could not occupy all of the feeding sites in the susceptible control. Because the resistance reaction of the HSI is based on the susceptible control, lower susceptibility levels in 'Catuai' reduce the resistance reaction of the 'IPR 100' and 'Apoatã' cultivars. This implies that at the initial population and under the conditions of this study, it was not possible to differentiate 'IPR 100' from 'Apoatã' because 'IPR 100' was classified at a lower resistance level.

The altered behavior in susceptible coffee genotypes at considerably low levels of nematodes was also found for *M. exigua*, where the susceptible "Mundo Novo" cultivar plants were classified as tolerant at an inoculum level of 1,000 eggs plant⁻¹ but not at higher levels (2,000, 4,000, 8,000, and 12,000) (Gonçalves, 1998). In addition, despite the increased population size for all of the inoculum levels, the highest RF was reported for the initial population of 2,000 eggs plant⁻¹. The authors attributed this finding to an ideal ratio that occurs at this level (2,000) between the number of nematodes and quantity of roots available. Although these results are close to those obtained in our study, it is important to note that the plants in the aforementioned study were inoculated in 300-mL containers at the cotyledon stage and evaluated after 100 days. Additionally, *M. exigua* behaves less aggressively compared with *M. paranaensis*. Thus, different results related to the most efficient inoculum level are likely to have

occurred because of changes in the substrate type, container size, plant age, evaluation time after inoculation, and aggressiveness level of the nematode species.

In this study, the RF and nematodes root g⁻¹ values showed that the initial populations between 500 and 3,000 eggs were sufficient for differentiating between resistant and susceptible genotypes. These values could ensure a beneficial *M. paranaensis* multiplication without extensive destruction of the root system and excessive competition among nematodes. According to the RF, nematodes g⁻¹ (Table 1), and regression curve (Fig 1), the most suitable levels were between 1,500 and 3,000 eggs, and according to the RF, nematodes root g⁻¹, regression curve, and HSI, the most efficient inoculum level was 3,000 eggs because at this level, good nematode multiplication occurred and the resistant cultivars could be classified into different resistance levels.

Resistance to *M. paranaensis* in *C. arabica* is limited despite being found in certain cultivars and lines, such as 'Icatu Vermelho IAC 3888' (Gonçalves and Silvarolla, 2007), "Icatu" progeny (Matiello et al., 2010), wild *C. arabica* accessions from Ethiopia (Anthony et al., 2003; Boisseau et al., 2009), and 'IPR 106' progeny (Ito et al., 2008). Although resistance to *M. paranaensis* in 'IPR 100' has already been reported by other authors (Sera et al., 2007; Ito et al., 2008; Sera et al., 2009), the behavior of this cultivar

in initial populations higher than 3,000, 5,000, and 8,000 eggs plant⁻¹ had not yet been evaluated. In this study, the level of resistance of 'IPR 100' was similar to that of 'Apoatã', which is considered resistant to *M. paranaensis* (Sera et al., 2006; Fonseca et al., 2008). However, it is possible that the level of resistance of 'IPR 100' is higher than that of 'Apoatã' because when HSI was used, the 'IPR 100' cultivar exhibited less nematodes g⁻¹ at all inoculum levels, higher amounts of HR plants according to both indices (RRF and HSI), and increased resistant at the 3,000, 5,000, and 8,000 levels despite a lack of significant differences among cultivars.

Based on all of the parameters used in the resistance evaluations (nematodes g⁻¹, RF, RRF, and HSI), 'IPR 100' was more resistant to *M. paranaensis* than 'Apoatã IAC 2258.' 'Apoatã IAC 2258' resistance to *M. paranaensis* is derived from *C. canephora*, whereas resistance in 'IPR 100' it is most likely derived from *C. liberica* because this cultivar was derived from the cross between 'Catuai' and an Arabic coffee plant of the BA-10 series, which carries *C. liberica* genes (Sera et al., 2007; Ito et al., 2008; Sera et al., 2009). To date, resistance to *M. paranaensis* in *C. liberica* has not been tested. However, the major resistance gene of *C. canephora* is likely the same as that of *C. liberica*; thus, 'IPR 100' may have minor genes that increase its level of resistance compared with that of 'Apoatã'.

Materials and Methods

Plant cultivars and experimental setup

The following coffee cultivars were evaluated: 'IPR 100'; 'Apoatã' and 'Catuai', which was used as the susceptible control. The seedlings originated from open-pollinated seeds that were germinated in sand and transplanted at the cotyledon stage into 700-mL plastic pots containing a soil and sand mixture (1:1 ratio) as substrate, which was previously sterilized in a greenhouse at 100°C for three hours with moisture at field capacity. Fertilization was performed according to the recommendations for farming coffee seedlings, and pH was corrected according to the results of the soil chemical analysis.

The experiment was conducted in a greenhouse at the headquarters of the IAPAR in Londrina, Paraná State, Brazil, between August 14 and November 26, 2012. The mean maximum and minimum temperatures during the experimental period were 34.6°C and 20.1°C, respectively.

Collection, quantification, and inoculation of *Meloidogyne paranaensis*

The inoculum was extracted from pure populations that were confirmed via electrophoresis and multiplied in 'Santa Clara' tomato cultivar plants and 'Catuai' cultivar coffee plants under greenhouse conditions. The eggs were extracted according to Boneti and Ferraz (1981) and adjusted in the suspension for 1,000 eggs mL⁻¹ by counting in a Peters chamber under an optical microscope. Inoculation was performed when the plants began exhibiting three pairs of well-developed leaves. The eggs were placed in three holes with a depth of approximately 1 cm, which were excavated around each plant. The following inoculum levels or IP of *M. paranaensis* were evaluated: 0; 500; 1,500; 3,000; 5,000; and 8,000 eggs plant⁻¹.

Evaluation method

The evaluations were performed at 110 days after inoculation. The roots were washed carefully under running water and weighed, and the eggs were then extracted from the root system according to Boneti and Ferraz (1981). After extraction, the number of eggs was quantified by counting in a Peters chamber. The number of nematodes root g⁻¹ was determined from the data on the weight of the root fresh and number of eggs and second-stage juveniles.

The RF was estimated as the difference between the final and initial nematode populations, where RF ≤ 1 = resistant and RF > 1 = susceptible (Oostenbrink, 1966). The RF and nematodes g⁻¹ data helped determine the best initial populations for evaluating resistance to *M. paranaensis*.

To classify the resistance levels of the cultivars the RRF and HSI were used. RRF was calculated based on the RF data using the following formula: RRF = [(RF of the susceptible control - RF of the treatment) ÷ RF of the susceptible control] × 100 (Moura and Regis, 1987). Based on the RRF, the genotypes were classified according to the scale of Moura and Regis (1987) with modifications, where 0 to 25% = HS; 25.1 to 50% = S; 50.1 to 75% = MS; 75.1 to 90% = MR; 90.1 to 95% = R; and 95.1 to 100% = HR.

The HSI was calculated using the formula HSI = (nematodes g⁻¹ of the treatment ÷ nematodes g⁻¹ of the susceptible control) × 100 (Gonçalves and Ferraz, 1987, with modifications). The HSI values were used to classify the resistance levels of the coffee plants according to the criteria by Fassuliotis (1985) with modifications as follows: 0 to 1% = HR; 1.1 to 10% = R; 10.1 to 25% = MR; 25.1 to 50% = MS; 50.1 to 75% = S; and 75.1 to 100% = HS.

Statistical analysis

The experiment was conducted in a randomized block design with a 3 × 6 factorial arrangement (three cultivars and six inoculum levels), with 10 replicates of one plant each. The nematodes g⁻¹ data were log(x+1) transformed and tested for normality (Shapiro-Wilk) and homogeneity of the variances (Hartley). Next, an analysis of variance and Tukey's test were performed, and the means were compared at 1% probability. The effect of the inoculum levels was analyzed by constructing orthogonal polynomials.

Conclusions

Coffea arabica cv. IPR 100 and *C. canephora* cv. Apoatã IAC 2258 were resistant to *Meloidogyne paranaensis*. 'IPR 100' presented higher resistance level to *M. paranaensis* when compared with 'Apoatã IAC 2258'. The initial populations of 1,500 and 3,000 eggs plant⁻¹ are the most suitable for testing resistance to *M. paranaensis*. However, 3,000 eggs plant⁻¹ was the most efficient population because it can be used to classify the cultivars into different levels of resistance.

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