Antagonistic Effects of Java against Plant Parasitic Nematodes

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Abstract

One of the main phytonematode control alternatives is the use of antagonistic plants in crop rotation or succession systems. Although java legume shows antagonist potential to control *Meloidogyne javanica*, its reaction to other nematodes was not investigated so far. Thus, the aim of the current study is to assess the penetration and reproduction factor (RF) of *M. incognita*, *Rotylenchulus reniformis* and *Pratylenchus brachyurus* in java, using soybean as control. Plants individually inoculated with 1000 specimens from each nematode were assessed 5, 10, 15, 20, 25 and 30 days after inoculation (DAI) for nematode penetration and development. The RF was assessed under two inoculum levels of each nematode (1000 and 700 specimens) 60 days after inoculation, for *M. incognita* and *R. reniformis*; and 80 days after inoculation, for *P. brachyurus*. *Meloidogyne incognita* and *R. reniformis* showed lower penetration and reproduction in java (RF < 1), whereas *P. brachyurus* showed higher penetration and RF > 1. Thus, the java legume can be considered resistant to *M. incognita* and *R. reniformis*, as well as susceptible to *P. brachyurus*.

Keywords: *Macrotylloma axilare*, *Meloidogyne inconita*, *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, antagonism

1. Introduction

The cultivation of antagonistic plants through crop rotation or succession in order to control nematodes is cited as one of the most efficient practices, since it presents several positive results (Pudasaini et al., 2006; Oka, 2010; Santana et al., 2012a). Antagonist plants can be defined as those holding substances able to inhibit or affect the action of another living organism (Chitwood, 2002). Root exudates produced by these plants may have an allelopathic or even nematicidal effect; they also present mechanisms able to inhibit nematode development by interfering in their feeding process or interrupting their life cycle (Marla et al., 2008; Nyczepir & Thomas, 2009; Andrade et al., 2010).

One of the main nematode control issues lies on the inadequate crop management, which worsens the problems they cause, specially in soybean crops (*Glycine max* (L.) Merril) because of mixed populations found in cultivated areas (Guzmán, 2011), and in Brazil the most common species are *Meloidogyne javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *Pratylenchus brachyurus* (Godfrey) Filipjev and Sch. Stekhoven, *Heterodera glycines* (Ichinohe), and *Rotylenchulus reniformis* (Linford & Oliveira).

Some legumes may be used to control these nematode species and can act in different ways to control these parasites, including production of secondary metabolites with nematicidal effect in their roots and shoots. As exemple, there is the monocrotaline, which is a pyrrolizidine alkaloid (Colegate et al., 2012), produced by *Crotalaria* spp., able to cause egg and juvenile mortality or to change nematode reproduction (Wang et al., 2002; Marla et al., 2008; Leandro & Asmus, 2015) and L-Dopa produced by mucuna (*Mucuna* spp. and *Stizolobium* spp.), which reduces the final nematode populations, increases mortality, and decreases femea *Meloidogyne* spp. formation (Barbosa et al., 1999). In addition, plants work as traps, since they attract juvenile nematodes and compromise their development after penetration (Curto et al., 2015; Miamoto et al., 2016). Antagonist plants may also be resistant to a particular pathogen, thus generating an incompatible reaction, called hypersensitivity reaction (HR), in which the programmed death of infected plant tissues happens as natural defense to pathogen

attacks (Watanabe & Lam, 2004). The HR may help restricting nematode infection or reproduction, since they are obligate parasites that feed on cellular compounds (Gabriel & Rolfe, 1990; Williamson & Kumar, 2006; Mur et al., 2008; Melillo et al., 2014).

Some legume species such as *Macrotyloma axillare* (E. Mey.) Verdc. cv. Java—or simply java—remain poorly studied. The aforementioned species, which was introduced in Brazil in 2004 (ABRASEM, 2006), is native to Indonesia and derives from the crossing between two *M. axillare* cultivars, namely: Archer and Guatá. It is a forage legume of perennial cycle, and does not demand high soil fertility in order to develop (Paiva et al., 2008). According to Miamoto et al. (2016), java plants may show antagonist potential, since *M. javanica* penetration per root gram was high, although the RF (reproduction factor) was low. In other words, this plant is highly attractive to *M. javanica*, sometimes even more attractive than soybean plants; however, it does not allow the nematode to complete its life cycle.

Thus, given the potential of java plants to control *M. javanica*, and due to the scarcity of studies focused on applying it in the management of other nematodes composing mixed populations in soybean crops, the aim of the current study was to assess *M. incognita*, *R. reniformis* and *P. brachyurus* penetration and reproduction in java plants.

2. Materials and Methods

The current experiment followed a completely randomized design and was conducted in a greenhouse at the State University of Maringá, located at latitude 23°47′28.4″ S, longitude 53°15′24.0″ W, and altitude 379 m. Seedlings from java and soybean cultivar CD 206 (control) were germinated in polystyrene trays containing Bioplant® commercial substrate in order to assess nematode penetration in the experiment conducted with *M. incognita*. Similarly, seedlings from cultivar Pintado were used to assess nematode penetration in the experiment conducted with *R. reniformis* and *P. brachyurus*. Fifteen (15) days after germination, the aforementioned seedlings were transplanted into pots containing 700 mL soil (typical Dystrophic Red Latosol) sterilized through autoclaving at 120 °C for 2 hours.

Two days after transplantation, they were inoculated with an initial *M. incognita* population (IP) comprising 700 eggs and eventual second-stage juveniles (J2), or with 700 *R. reniformis* or *P. brachyurus* specimens, in four holes open in the soil surrounding each plant. Pure *M. incognita*, *R. reniformis* and *P. brachyurus* populations were previously multiplied in tomato (cultivar Santa Clara), soybean (cultivar Pintado) and maize plants (cultivar Al Bandeirantes), respectively. They were extracted from the root system, according to the methodology by Hussey and Barker (1973), adapted by Boneti and Ferraz (1981) to be applied to *M. incognita* and *R. reniformis*, as well as by Coolen and D'Herde (1972) to *P. brachyurus*.

Four plants from each treatment were collected every five days, from the 5th to the 30th day after inoculation (DAI), for evaluation purposes. The root system of these plants was carefully separated from the shoot, washed and deposited on paper towel to remove the water excess. Next, the fresh root mass was set and the roots were, subsequently, stained with acid fuchsin, according to the method described by Byrd Junior et al. (1983), for further evaluation.

Temporary slides containing root fragments were mounted to assess the entire root volume. *Meloidogyne incognita* development stages J2, J3, J4 (second-, third- and fourth-stage juveniles, respectively) and adult females; *R. reniformis* immature and adult females; as well as the total number of *P. brachyurus* nematodes penetrating the roots, were analyzed. The analyses were performed in a light microscope, at 100x magnification.

In addition to nematode penetration, java susceptibility to nematodes was tested at two inoculum levels. The same procedure adopted for seedling production, as well as the same cultivars, were applied to each nematode. Seedlings were transplanted to pots containing 700 mL autoclaved soil, 15 days after germination, as it was previously described. Two days after transplantation, java and soybean plants were inoculated with 700 or 1000 *M. incognita* eggs and occasional J2, or with 700 or 1000 *R. reniformis* or *P. brachyurus* specimens as previously described; the experiment adopted seven repetitions for each nematode species and for each inoculum level.

Assessments were conducted 60 (*M. incognita* and *R. reniformis*) and 80 days after inoculation (*P. brachyurus*). Plants were collected and their shoot was discarded. The root system of each plant was collected, carefully washed, and placed on absorbent paper to remove the water excess. Roots were weighed and subjected to the extraction processes mentioned above. Next, the total number of each nematode in the roots was assessed; such number was divided by the root mass in order to find nematodes/root gram. In addition, the reproductive factor (RF) was calculated; RF = Final population/Initial population (Oostenbrink, 1966). Furthermore, 100 cm³ soil samples were collected and subjected to *R. reniformis* and *P. brachyurus* nematode extraction, according to the

methodology by Jenkins (1964). In these cases, the total nematode population was calculated through the sum of total nematodes in the roots and in the soil. The analyses were conducted in Peters' slide, under optical microscope at 100× magnification.

Data were subjected to analysis of variance and compared, for each nematode and parameter, through Bonferroni T test, at 5% probability level, in the Sisvar statistical software (Ferreira, 2014).

3. Results

Meloidogyne incognita did not show statistical difference in the number of J2, as well as in the total number of nematodes penetrating java roots, in comparison to soybean roots, at the 5th and 10th DAI. The number of nematodes per root gram was higher in the java root at the 10th DAI (Table 1). According to the assessments conducted in the 15th, 20th, 25th and 30th DAI, the number of J2, total nematode and nematode per root gram were larger in soybean plants, except at the 20th DAI, when there was no difference between treatments in the number of J2 and nematodes per root gram (Table 1).

Third-stage *M. incognita* juvenile (J3) formation started at the 15th DAI, both in soybean and java plants; the number of J3 in soybean roots was larger in all the herein assessed periods. The J4 formation started at the 15th DAI in soybean plants and at the 20th DAI in java plants (Figure 1). The largest number of J4 nematodes in soybean roots was recorded at the 20th and 25th DAI; however, this parameter did not show difference between treatments at the 30th DAI. The first adult females were recorded in soybean plants at the 20th DAI, whereas java plants presented female formation in the roots at the 25th DAI. The largest number of adult females was recorded in soybean plants at the 25th DAI (Figure 1); however, there was no difference between treatments at the 30th DAI (Table 1).

Table 1. Number of second- (J2), third- (J3) and fourth-stage (J4) female *Meloidogyne incognita* juveniles, total number of nematodes (TN) and nematodes per root gram (NRG) in the root system of java and soybean cultivar CD 206, assessed between the 5th and the 30th day after inoculation (DAI) with 700 nematode eggs and eventual juveniles

Treatment	J2	J3	J4	Female	TN	NRG
5 DAI						
Java	8.75^{ns}	-	-	-	8.75^{ns}	18.61 b
Soybean	3.50	-	-	-	3.50	1.95 a
MSD	6.0	-	-	-	6.0	5.90
10 DAI						
Java	3.50^{ns}	-	-	-	3.50^{ns}	5.36^{ns}
Soybean	8.25	-	-	-	8.25	3.18
MSD	6.79	-	-	-	6.79	4.50
15 DAI						
Java	9.50 a	0.25 a	0.0^{ns}	-	9.75 a	5.49 a
Soybean	29.25 b	4.75 b	0.50	-	34.50 b	41.21 b
MSD	12.28	4.0	0.91	-	9.75	10.49
20 DAI						
Java	12.75^{ns}	3.00 a	0.25 a	0.00^{ns}	16.00 a	9.61^{ns}
Soybean	12.25	20.25 b	14.75 b	1.00	48.25 b	10.40
MSD	6.16	8.55	7.17	1.29	12.14	7.33
25 DAI						
Java	3.50 a	2.75 a	1.25 a	0.25 a	7.75 a	7.31 a
Soybean	23.75 b	27.25 b	23.25 b	2.00 b	76.25 b	26.58 b
MSD	17.20	14.37	11.90	1.52	37.35	3.20
30 DAI						
Java	3.50 a	1.50 a	1.50^{ns}	0.50^{ns}	7.00 a	2.26 a
Soybean	99.75 b	26.00 b	3.00	3.00	131.75 b	32.88 b
MSD	20.05	20.72	4.00	3.04	41.42	6.25

Note. Means followed by the same letter did not differ from each other in the Bonferroni t-test, at 5% probability. ns = not significant. MSD: minimum significant difference.

There was no significant difference in the number of immature female *R. reniformis* nematodes in the assessments conducted at the 5th, 10th, 15th and 20th DAI; this number was larger in soybean plants at the 25th and 30th DAI, only (Table 2). Adult female formation was recorded in both cultures after the 15th DAI (Figure 2); this parameter did not show significant difference from the 15th DAI on (Table 2). There was also no difference between treatments in the total number of nematodes, according to the assessments conducted up to the 20th DAI. However, this variable presented higher values in soybean roots from the 25th DAI (Table 2). Java plants showed larger number of nematodes per root gram in the assessments conducted at the 5th, 10th, 15th and 20th DAI; however, this parameter presented higher values in soybean plants at the 30th DAI (Table 2).

Table 2. Number of immature *Rotylenchulus reniformis* females, adult females, total nematode (TN) and nematode per root gram (NRG) in the root system of java and soybean cultivar Pintado, assessed between the 5th and the 30th day after inoculation (DAI) with 700 specimens

Treatment	Immature Females	Adult Females	TN	NRG
5 DAI				
Java	5.00^{ns}	-	5.00^{ns}	13.22 b
Soybean	7.25	-	7.25	4.61 a
MSD	13.08	-	13.08	6.84
10 DAI				
Java	10.00^{ns}	-	10.00^{ns}	8.12 b
Soybean	18.25	-	18.25	4.39 a
MSD	13.01	-	13.01	3.16
15 DAI				
Java	16.50^{ns}	0.25^{ns}	16.75^{ns}	4.30 b
Soybean	22.00	1.75	23.75	2.47 a
MSD	14.02	2.05	13.18	1.55
20 DAI				
Java	11.75^{ns}	0.00^{ns}	11.75^{ns}	3.41 b
Soybean	17.25	1.00	18.25	2.29 a
MSD	15.50	1.29	14.37	1.00
25 DAI				
Java	12.25 a	0.25^{ns}	12.50 a	2.15^{ns}
Soybean	19.00 b	1.00	20.00 b	1.98
MSD	4.75	2.38	4.94	1.43
30 DAI				
Java	11.00 a	0.25^{ns}	11.25 a	1.31 a
Soybean	19.50 b	1.25	20.75 b	2.89 b
MSD	5.88	1.29	6.56	0.19

Note. Means followed by the same letter did not differ from each other in the Bonferroni t-test, at 5% probability. ns = not significant. MSD: minimum significant difference.

No statistical difference was observed in the total number of *P. brachyurus* penetrating java and soybean roots, according to assessments conducted at the 5th, 10th, 15th and 20th DAI (Table 3, Figure 3). However, the number of nematodes per root gram was larger in java than in soybean roots, according to assessments conducted at the 5th, 10th and 15th DAI (Table 3). Nonetheless, assessments performed at the 25th and 30th DAI showed that the total number of nematodes was larger in soybean roots, although the variable 'nematode per root gram' did not show difference between treatments (Table 3).

Table 3. Total number of *Pratylenchus brachyurus* nematodes and nematodes per root gram (NRG) in the root system of java and soybean cultivar Pintado, assessed between the 5th and the 30th day after inoculation (DAI) with 700 specimens

Treatment	Total number	NRG
5 DAI		
Java	102.75^{ns}	176.57b
Soybean	62.25	95.72a
DMS	55.19	76.56
10 DAI		
Java	39.50^{ns}	95.20b
Soybean	26.50	32.75a
DMS	29.25	29.85
15 DAI		
Java	66.25^{ns}	122.40b
Soybean	130.50	57.65a
DMS	120.21	33.37
20 DAI		
Java	106.75^{ns}	131.70^{ns}
Soybean	222.75	85.75
DMS	137.93	63.47
25 DAI		
Java	72.75a	92.70^{ns}
Soybean	276.50b	76.07
DMS	134.37	84.52
30 DAI		
Java	39.75a	34.55^{ns}
Soybean	140.25b	40.35
DMS	83.47	24.97

Note. Means followed by the same letter did not differ from each other in the Bonferroni t-test, at 5% probability. ns = not significant. MSD: minimum significant difference.
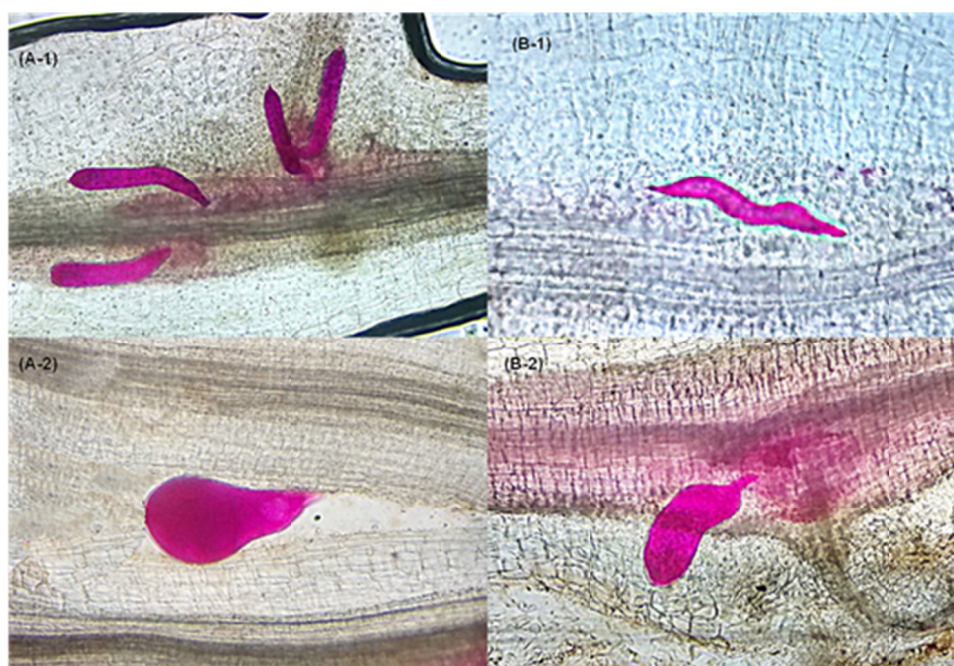

Figure 1. *Meloidogyne incognita* in soybean and java roots A-1: J3/J4 of *M. incognita* in soybean plant at the 15th DAI; A-2: *M. incognita* adult female in soybean plant at the 30th DAI; B-1: J3 of *M. incognita* in java plant at the 15th DAI; B-2: *M. incognita* immature female in java plant at the 30th DAI
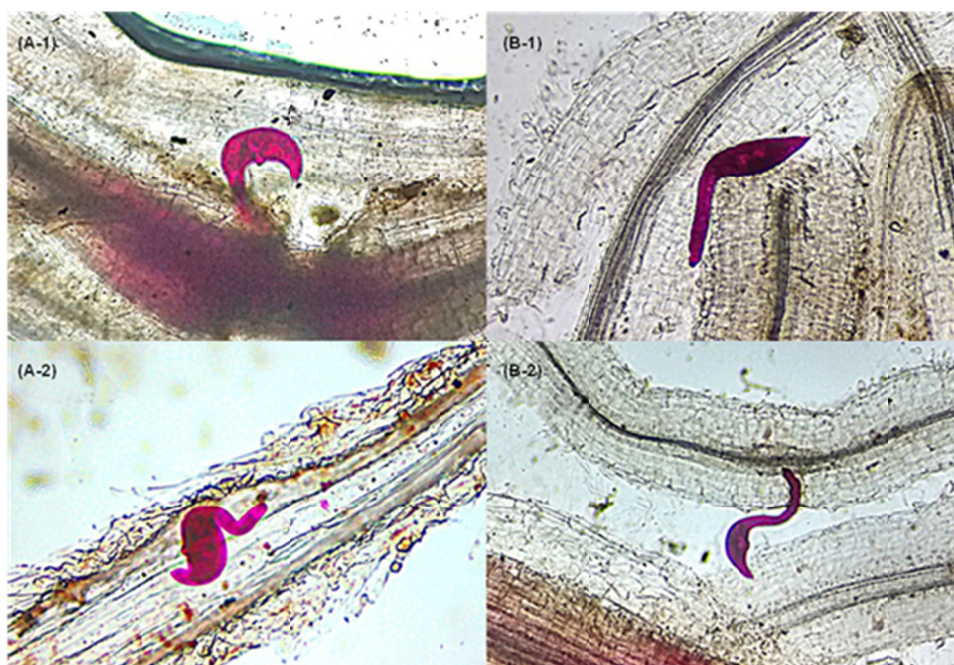
Figure 2. *Rotylenchulus reniformis* in soybean and java roots. A-1: *R. reniformis* adult female in soybean plant at the 15th DAI; A-2: *R. reniformis* adult female in soybean plant at the 30th DAI; B-1: *R. reniformis* immature female in java plant at the 15th DAI; B-2: *R. reniformis* immature female in java plant at the 30th DAI
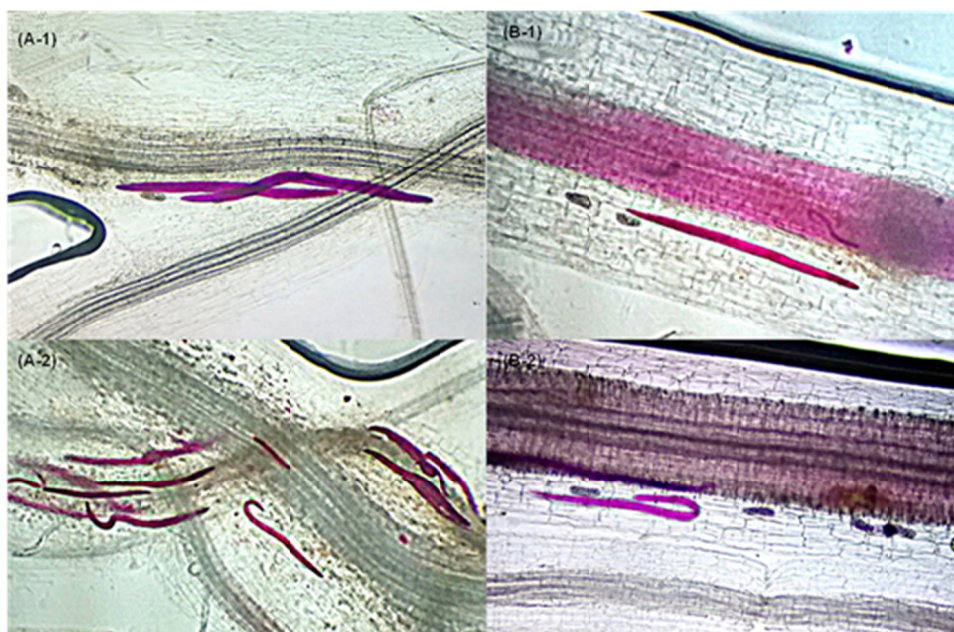

Figure 3. *Pratylenchus brachyurus* in soybean and java roots. A-1: *P. brachyurus* in soybean plant at the 15th DAI; A-2: *P. brachyurus* in soybean plant at the 30th DAI; B-1: *P. brachyurus* in java plant at the 15th DAI; B-2: *P. brachyurus* in java plant at the 30th DAI

With respect to the reproduction of the three nematode species studied, in plants inoculated with 700 specimens, the total number of nematodes and the number of nematodes per root gram were larger in soybean roots, used as control (Table 4). Java plants were susceptible to *P. brachyurus* (RF = 1.87), although they showed RF lower than that recorded in soybean plants (RF = 4.21). On the other hand, the herein assessed legume showed RF equal to 0.51 for *M. incognita*, as well as equal to 0.92 for *R. reniformis* (Table 4).

Results recorded in plants inoculated with 1000 specimens were similar to the previously observed ones, *i.e.*, variables "total nematodes" and "nematodes per root gram" presented the lowest values in java roots (Table 4). In addition, java plants confirmed susceptibility to *P. brachyurus* (RF = 2.12), as well as resistance to *M. incognita* (RF = 0.61) and to *R. reniformis* (RF = 0.91) (Table 4).

Table 4. Total nematodes (TN), nematodes per root gram (NRG) and reproduction factor (RF) of *Meloidogyne incognita*, *Rotylenchulus reniformis* and *Pratylenchus brachyurus*, in java and soybean roots inoculated with initial population (IP) comprising 700 and 1000 specimens

IP = 700			
Treatment	TN	NRG	RF
M. incognita			
Java	358 a	85 a	0.51 a
Soybean	3101 b	367 b	4.43 b
CV%	40.83	31.55	40.84
R. reniformis			
Java	650 a	25 a	0.92 a
Soybean	1772 b	84 b	2.53 b
CV%	17.30	37.81	17.30
P. brachyurus			
Java	1870 a	363 a	1.87 a
Soybean	4217 b	731 b	4.21 b
CV%	29.72	31.11	29.72
IP = 1000			
Treatment	TN	NRG	RF
M. incognita			
Java	614 a	183 a	0.61a
Soybean	3997 b	908 b	3.99 b
CV%	18.20	77.80	3.99
R. reniformis			
Java	907 a	32 a	0.91 a
Soybean	2318 b	150 b	2.37 b
CV%	20.88	27.69	20.88
P. brachyurus			
Java	2120 a	603 a	2.12 a
Soybean	6055 b	1104 b	6.05 b
CV%	44.67	36.14	44.67

Note. Means followed by the same letter did not differ from each other in the Bonferroni t-test, at 5% probability. CV = Coefficient of variation.

4. Discussion

The java legume proved to be resistant to *M. incognita*, since although adult females were formed, the total number of nematodes and nematodes per root gram at the end of the cycle was smaller than that recorded in soybean plants. Consequently, the low RF showed that nematode multiplication was negatively affected. However, java did not prevent the initial penetration; it was attractive to the nematode, but reduced its reproduction. These results corroborate those recorded for *M. javanica*, in which the number of nematodes per java root gram was larger than that recorded in soybean plants. Nevertheless, java was resistant to *M. javanica* (FR = 0.12); although it is attractive to root-knot nematodes, it does not allow them to efficiently complete their life cycle (Miamoto et al., 2016). Nematodes belonging to genus Meloidogyne showed similar results in other pathosystems such as *C. spectabilis* Roth, *C. juncea* L. and *C. paulina* Scrank, which were attractive to *M. javanica* juveniles, allowed high penetration into the root system, but did not allow adult female formation up to the 45th DAI (Silva et al., 1989b).

Thus, it is hypothesized that the action of java on *M. incognita* may be related to antagonistic substances affecting nematode development. Furthermore, it is possible inferring that the mechanism making this plant resistant is post-infectional and does not inhibit nematode penetration. In other words, nematodes penetrate the roots and induce the feeding site, but somehow such sites are not efficient in nurturing them or are inactivated by some plant resistance gene (Williamson & Kumar, 2006). This type of reaction has been recorded in other studies conducted with *Cajanus cajan* (L.) Mill (pigeon pea) cv. Fava Larga—*H. glycines* (Valle et al., 1997) and *Tagetes* spp.—*M. incognita*, *M. javanica*, *P. brachyurus* and *R. reniformis* (Belcher & Hussey, 1977; Bakker et al., 1979; Huang, 1984; Siddiqui & Alam, 1987; Hussain et al., 2011). In the specific case of *Tagetes* spp., the antagonistic reaction results from alpha-tertienyl production, since this substance has direct effect on nematodes (Gommers & Bakker, 1988).

Another hypothesis for the post-infection reaction is the activation of hypersensitivity reaction (HR) (Dhandaydham et al., 2008). This phenomenon is elucidated by the presence of resistance genes in the host (H) and avirulence genes (Avr) in nematodes; the interaction between these genes triggers a cascade of plant defense responses (Fuller et al., 2008). The HR is initially triggered by the production of reactive oxygen species (ROS) (Mishra et al., 2012); it is followed by other molecular events such as the accumulation of salicylic acid, nitric oxide and different intracellular calcium levels (Muret et al., 2008). Such accumulation results in the localized and rapid death of cells composing the feeding site or of cells adjacent to it (Williamson, 1999), fact that is common in different associations between plants and nematodes (Branch et al., 2004; Anthony et al., 2005; Motta et al., 2012). This hypothesis is corroborated by histopathological analyses applied to *C. spectabilis* and *C. juncea* parasitized by *M. javanica*, in which the HR showed formation of giant cells, although in smaller number and size, with dense cytoplasm, few nuclei and, generally, without large vacuoles; such features may have made giant cells less efficient in the nematode nutrition process (Silva et al., 1990). The same type of reaction and histopathological features were recorded in tomatoes treated with resistance inducers against *M. incognita*, besides increased lignin in the nourishing cells (Melillo et al., 2014).

Java plants penetrated by *R. reniformis* showed RF < 1, which is the limit value for a plant to be considered resistant. Thus, it is necessary taking particular care at the time to plant this species in order to reduce the number of nematodes. It is possible saying that the java plant does not allow *R. reniformis* population increase, although it does not drastically reduce its population. Despite the scarcity of studies about *R. reniformis* antagonistic plants, there are reports about some species able to control it, namely: stylosanthes 'Campo Grande' (*Stylosanthes capitata* × *S. macrocephala* M. B. Ferreira & N. M. Sousa Costa), peanut (*Arachis hypogaea* L.) and velvet bean (*Mucuna deeringiana* (Bort.) Merr); which were able to reduce the final parasite population in the field (Gardiano et al., 2014). Some crotalaria species are also cited as resistant to *R. reniformis*, since they present RF < 1 (Silva et al., 1989c; Leandro & Asmus, 2015). Results reported for species *C. juncea* were similar to those recorded in the current study, *i.e.*, the nematode was able to penetrate the roots; however, low reproduction rates and plant interference in female development were recorded (Wang et al., 2001; Marla et al., 2008).

Results showed that java was more attractive to *P. brachyurus* than soybean; however, java somehow reduced the reproductive rate of this nematode at the 80th DAI, since the RF was lower than that of the control, although it was higher than one. Antagonist plants negatively affect nematode population in different ways; one of them lies on attracting the parasite and allowing its penetration, but hindering its development and reproduction; these plants are also considered unfavorable hosts (Ferraz & Valle, 1997). However, the term 'unfavorable host' or 'bad host' often refers to plants presenting lower RF, although close to 1.0, *i.e.*, plants considered resistant (Wang et al., 2002). Thus, researchers consider some botanic species moderate hosts, since they present RF higher than 1.0, although significantly lower than that recorded in the control (Inomoto et al., 2006; Machado et al., 2007; Borges et al., 2010). These studies corroborate the current results, since java plants presented RF values 1.87 and 2.12 when they were inoculated with 700 and 1000 specimens, respectively, and were considered susceptible to the nematode (Oostenbrink, 1966); however, java RF values were significantly lower than those recorded in soybean plants (4.21 and 6.05) inoculated with the respective inoculum levels. Similar reproduction factor was recorded in *C. cajan* cv. Fava Larga (RF = 1.57) inoculated with *P. brachyurus* (Inomoto et al., 2006). However, this cultivar was assessed against two *P. brachyurus* isolates from different regions—Pb20 and Pb24—and presented RF 0.78 and 4.28, respectively, whereas the respective values for soybean cv. Pintado were 1.60 and 6.15 (Machado et al., 2007).

Plants with potential to control other nematode species may respond differently to *P. brachyurus* or even to distinct populations of this species, as it was observed in *C. cajan* cv. 'IAPAR 43', which enabled Meloidogyne ethiopica Whitehead and R. reniformis control (Lima et al., 2009; Gardiano et al., 2012). Similar result was

recorded for *C. juncea*, which allowed reducing *M. javanica* populations (Inomoto et al., 2008; Miamoto et al., 2016), although it was not efficient in controlling *P. brachyurus* (Braz et al., 2016). It is worth highlighting that *C. juncea* recorded different RFs for different *P. brachyurus* populations; populations from Goiás State presented RF = 2.16 (Braz et al., 2016), those from Rio de Janeiro State recorded RF = 1.13, and populations from Mato Grosso State recorded 4.27 (Machado et al., 2007). Thus, it is common for antagonist plants to present varying reactions when they are exposed to a specific nematode genus, species or population; at first, such variation may appear to be more prominent in nematodes with migratory feeding habit.

It is worth pinpointing that plants whose RF is higher than 1, even when this value is lower than that of the susceptible plant, should be carefully used or analyzed in the field, since assessments under experimental conditions are often carried out between the 60th and 90th days after inoculation. However, there are some examples such as the black velvet (*Stizolobium aterrimum* Piper and Tracy), in which *P. zeae* Graham showed RF = 1.76 at the 60th DAI. This value was lower than that recorded for maize, which was used as control (RF = 36.40); however, *P. zeae* showed RF = 16.69 at the 110th DAI, when it did not statistically differ from maize, which showed RF = 20.40 (Santana et al., 2012a).

It is essential conducting further studies to help better understanding the mode of action of antagonistic plants on migratory nematodes, since their behavior overall differs from that observed in the sedentary ones. In this case, *C. spectabilis* may be used as an example, although it reduces *P. brachyurus* reproduction (Santana et al., 2012b; Rosa et al., 2013; Debiasi et al., 2016). Such activity is attributed to the low attractiveness of the plant or even to its higher resistance to *P. brachyurus* and *P. zeae* penetration (Silva et al. 1989a; Santana et al. 2012a). Differently, crotalaria plants are attractive to sedentary nematodes; however, they present giant cell dysfunction, fact that makes them less efficient in feeding the parasite (Silva et al., 1990).

Java plants do not prevent *M. javanica* and *R. reniformis* penetration; however, it is considered resistant to both nematodes, since it shows reproduction factor lower than 1. Thus, it is necessary conducting additional studies with R. reniformis, since the herein recorded reproductive factor was very close to 1. Java is susceptible to *P. brachyurus*, although it presents reproduction factor lower than that recorded for soybean plants.

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