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Antagonistic potential and histopathology of *Meloidogyne javanica* on *Macrotyloma axillare* cv. Java

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Abstract

Root-knot nematodes are obligate parasites, so changes at their feeding sites can limit their development. Alterations to feeding sites is one of the main actions taken by antagonistic plants. The aim of this study was to assess the response and histopathology of interactions between *Meloidogyne javanica* and the roots of *Macrotyloma axillare* cv. Java. The penetration and development of the nematode was assessed from 8 to 30 days after inoculation (DAI) with 3000 eggs + second-stage juveniles (J2) of *M. javanica*. The reproduction factor (RF) was assessed at 60 DAI, with two inoculation levels, 700 and 1000 eggs + J2, and the changes in the development of nematodes at the third (J3) and fourth juvenile (J4) stages was delay, despite the presence of J2 inside the roots, and no adult females were found in the *M. axillare* cv. Java roots. RF was 0.31 and 0.39 for *M. axillare* cv. Java and 3.40 and 4.52 for soybean at inoculation levels of 700 and 1000 eggs + J2, respectively. The feed cells in *M. axillare* cv. Java could not effectively nourish the nematode, which led to deformed females 30 DAI. The feed cells and nematode development, however, were normal in soybean. *M. axillare* cv. Java was resistant to *M. javanica* and had an antagonistic potential, because it did not prevent the nematode from penetrating the roots but had a negative effect on *M. javanica* due to the inefficiency of the feeding site.

Keywords: Antagonistic; Crop Rotation; Leguminous; Resistance; Root-knot nematode.

Introduction

The genus *Meloidogyne*, often known as root-knot nematodes, contains the main species of plant parasitic nematodes due to its wide geographical distribution and broad range of hosts (Jones et al., 2013). More than 100 *Meloidogyne* species parasitize various plants worldwide. Economic losses are estimated at US\$ 157 billion globally (Abad et al., 2008), with crop losses of about 30-50% in banana, tobacco, cassava, soybean and other crops (Fourie et al., 2001; Coyne et al., 2006). *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood are among the main species that affect the soybean (Castro et al., 2003).

Parasitism by root-knot nematodesis complex and involves the formation of feeding sites characterized by hypertrophic and multinucleated cells (Williamson; Gleason, 2003; Abad et al., 2009). The nematodes absorb the nutrients they need for their development from these cells. Any interference at the feeding site can impair the development of the nematode and lead to its death (Williamson; Hussey, 1996; Faria et al., 2003).

Plants resistant to the nematode do not necessarily inhibit it, but the nematode can disrupt the formation of nourishing cells

that become less nourishing (Moritz et al., 2008a). In addition to the formation of ineffective feeding sites, these plants may have other natural defensive responses to pathogenic attacks, such as hypersensitive reactions (HRs) and the sudden and programmed death of infected tissues and tissues surrounding the feeding site (Watanabe; Lam, 2004).

The genetic improvement of plants has focused on nematode resistance. Some antagonistic plants, such as *Crotalaria* species, are examples of the formation and maintenance of the feeding sites. The efficiency of such species in controlling these nematodes has been broadly demonstrated (Inomoto et al., 2006; Ohara et al., 2012; Danahap; Wangang, 2015). Histopathological analyses applied to *Crotalaria spectabilis* Roth and *C. juncea* L. parasitized by *M. javanica* indicated the formation of fewer hypertrophic giant cells, which have few nuclei and lack vacuoles. These effects lowered the efficiency of nematode feeding, and HRs were induced around the nourishing cells in both *Crotalaria* species (Silva et al., 1990).

Antagonistic activity is known for some species, but other species have been rarely studied. *Macrotyloma axillare* (E.

Mey.) Verdc. cv. Java, which belongs to the Family Fabaceae, is a cross between two other cultivars: Archer and Guata. This cultivar has been used in recovery programs focused on degraded soils and revegetation due to its ability to protect soil as a cover crop (Silva et al., 2007). Previous studies have found that this plant has the potential to control *M. javanica* because of its possible antagonistic effect; the nematode can penetrate the roots but has limited reproduction (Miamoto et al., 2016).

Macrotyloma axillare cv. Java has received little attention but may be an option for crop rotation tocontrol nematodes. The aim of this study was to assess the penetration, development and reproduction of *M. javanica* in the roots of *M. axillare* cv. Java and to conduct a histopathological study of the infection caused by the nematode.

Results

Penetration of M. javanica in roots of M. axillare cv. Java and soybean

The interaction between the factors 'time' and 'plant' was significant based on the number of J2 in the roots. Fewer J2 were found in the *M. axillare* cv. Java roots than the soybean roots 10, 12 and 14 DAI, but soybean and *M. axillare* cv. Java had similar numbers of J2 at the other times (Table 1). The factor 'time', however, was not significant for either plant.

'Plant' and 'time' did not interact significantly for the number of third-stage juveniles (J3), but both factors was individually significant. Fewer J3 were in the *M. axillare* cv. Java roots (average 1.31) than the soybean roots (7.39), whereas the means were lower for the evaluation of the factor 'time' 8 DAI, followed by the values recorded 10, 12, 14 and 16 DAI, when they were compared to the other evaluations (data not shown).

The interaction between the factors was significant for the number of fourth-stage juveniles (J4), except for 8 and 16 DAI when the numbers of juveniles in *M. axillare* cv. Java and soybean were similar. The number of J4 was higher in soybean roots at all times (Table 1). The evaluation time applied to each plant indicated that soybean had the lowest value 8 DAI, followed by the values assessed 10 and 16 DAI, when they were compared to the other times. The number of J4 in *M. axillare* cv. Java, however, did not differ significantly at the different DAI; the mean number of J4 varied from 0.0 to 0.6 (Table 1).

The first evaluation, conducted 8 DAI, identified J3 and J4in the soybean roots, whereas J3 and J4 in *M. axillare* cv. Java were identified 10 and 14 DAI, respectively (Table 1).

Females in the soybean roots were first observed 14 DAI, but no adult females were observed in *M. axillare* cv. Java until the end of the evaluations (Table 1). The interactions were significant, with differences between the plants from 20 to 30 DAI, and the number of females was always higher in the soybean roots. The evaluation time applied to soybean therefore indicated higher mean female numbers during this evaluation period, and the mean number of females in *M. axillare* cv. Java did not differ significantly during the entire evaluation period.

Finally, the total number of nematodes indicated that the means were higher in soybean at all times evaluated, except

for 8 and 16 DAI when the means in different plants did not differ significantly from each other (Table 1). The mean factor 'time'was lowest 8 and 16 DAI in soybean and at the first four evaluations, 8, 10, 12 and 14 DAI, in *M. axillare* cv. Java.

The number of J2 per gram of root was higher in *M. axillare* cv. Java roots at all times evaluated, except for 12 and 14 DAI (Table 2). 'Time' was a significant factor for *M. axillare* cv. Java, which had the highest means at 8 DAI, followed by 16 and 18 DAI. The number of J3 per gram ofroot was higher in soybean 10, 12 and 14 DAI, the same as 28 DAI in *M. axillare* cv. Java, but did not differ significantly at the other times. The factor 'time' did not differ significantly in soybean, and the means were highest in *M. axillare* cv. Java at 28 DAI (Table 2).

Only the factor 'plant' significantly affected the number of J4 per gram of root, and the soybean mean (1.38) was higher than for *M. axillare* cv. Java (0.43) (data not shown). The number of females per gram of root was highest in soybean roots after 20 DAI. 'Time' was only significant for soybean, which had highest means at the same evaluation time (Table 2).

The total number of nematodes per gram of root was highest in *M. axillare* cv. Java 8 DAI, followed by 16, 18 and 28 DAI but did not differ significantly for soybean (Table 2).

Reproduction factor of M. javanica in roots of M. axillare cv. Java and soybean

'Time' and 'plant' did not significantly interact for the total number of nematodes, nematodes per gram of root (NG) or the reproduction factor (RF), but each factor individually differed significantly (Table 3). The factors 'inoculum level' and 'plant' significantly affected the total number of nematodes (TN), and TN was higher for the inoculation with 1000 nematodes than the inoculation with 700 nematodes; TN was lower for *M. axillare* cv. Java than soybean (Table 3).

RF and NG differed significantly between the between plants and inoculum levels, and were lower in *M. axillare* cv. Java than soybean (Table 3). RF was 0.31 and 0.39 in *M. axillare* cv. Java and 3.40 and 5.52 in soybean inoculated with 700 and 1000 nematodes, respectively (Table 3).

Histopathology of the interaction between M. javanica and M. axillare cv. Java

The histopathological study conducted at 10 DAI assessed the formation of feeding sitesin both plants. Granulating nourishing cells with up to five nuclei formed in soybean (Figure 1A). The cells were unorganized and hypertrophic in the *M. axillare* cv. Java roots (Figure 1B).

The soybean samples at 15 DAI indicated the presence of nematodes and the normal formation of feeding sites and multinucleated giant cells (Figure 1C). The Java samples also indicated the presence of nematodes but no feeding sites, only a slight cellular disorganization (Figure 1D). Adult females and feeding sites had fully developed by 30 DAI, with characteristic cellular modifications at the feeding sites, including multinucleated giant cells, thick walls, dense cytoplasm and small vacuoles (Figure 1E), indicating normal nematode development. Some females in *M. axillare* cv. Java were deformed (Figure 1F), probably due to the incomplete

	Evaluation Time																							
Trat.	8		10	1	2	1	4	:		1	.8		20		22		24		26		28		30	
	J2																							
Soybean	6.7	aA	10.2	aA	12.0	aA	10.5	aA	6.2	aA	5.2	aA	11.5	aA	5.5	aA	5.5	aA	10.2	aA	9.2	aA	6.7	aA
Java	5.7	aA	5.0	bA	3.7	bA	2.2	bA	9.2	aA	11.0	aA	6.0	aA	5.7	aA	8.2	aA	6.7	aA	6.0	aA	7.5	aA
	CV (%)) 27.34																						
Trat.							J4																	
Soybean	0.5	aC	7.5	aA	14.7	aB	6.7	aA	5.7	aB	14.2	aA	17.7	aA	7.0	aA	9.7	aA	8.5	aA	11.5	aA	10.0	aA
Java	0.0	aA	0.0	bA	0.0	bA	0.2	bA	0.7	aA	0.2	bA	0.0	bA	0.2	bA	0.5	bA	0.2	bA	0.7	bA	0.7	bA
	CV (%)) 46.01																						
Trat.	Femal	es																						
Soybean	0.0	aB	0.0	aB	0.0	aB	0.5	aB	0.0	aB	1.5	aB	5.5	aA	3.0	aA	3.7	aA	3.0	aA	7.2	aA	4.2	aA
Java	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	bA										
	CV (%)	CV (%) 61.31																						
Trat.	Total	Number																						
Soybean	8.7	aB	26.2	aA	34.7	aA	24.0	aA	15.5	aB	28.7	aA	42.2	aA	25.5	aA	26.7	aA	28.7	aA	36.7	aA	33.2	aA
Java	5.7	aB	5.2	bB	4.0	bB	2.5	bB	10.7	aA	12.7	bA	8.5	bA	7.5	bA	10.5	bA	9.0	bA	10.2	bA	10.0	bA
	CV (%)) 19.13																						

Table 1. Number of second (J2) and fourth (J4) stage juveniles, total number of *Meloidogyne javanica* females in the root system of *Macrotymola axillare* cv. Java and soybean cv. Pintado assessed between the 8 and 30 day after inoculation (DAI) with 3000 nematode eggs + J2 of *M. javanica*.

For each phase of development of the nematode and the evaluation period, means followed by the same lowercase letter in the column and upper case in the line do not differ by the Scott-Knott's and Bonferroni T test at 5% probability. Trat. = treatment. CV = coefficient of variation.

Table 2. Number of second (J2) and third (J3) stage juveniles and the total number of females in *Meloidogyne javanica* per gram of *Macrotymola axillare* cv. Java and soybean cv. Pintado roots assessed between the 8 and 30 day after inoculation (DAI) with 3000 nematode eggs + J2 of *M. javanica*.

	Evaluation Time																							
Trat.	8		10		12	12			16		18		20		22	22		24		5	28		30	
	J2.g ⁻¹																							
Soybean	3.3	bA	4.0	bA	3.6	aA	3.1	aA	1.5	bA	1.4	bA	3.2	bA	1.7	bA	1.2	bA	2.4	bA	1.7	bA	1.1	bA
Java	44.2	aA	18.2	аB	12.9	аB	6.2	аB	27.7	aA	24.7	aA	12.6	aB	15.1	аB	17.6	аB	10.4	aB	19.6	aA	13.4	aB
	CV (%) 37.18																							
Trat.	J3.g ⁻¹				_																			
Soybean	0.7	aA	3.0	аа	2.2	aA	1.8	aA	1.0	aA	2.0	aA	2.1	aA	3.2	aA	1.7	aA	1.6	aA	1.8	bA	2.1	aA
Java	0.0	aC	0.7	bC	0.6	bC	0.0	bC	2.6	aB	3.7	aB	5.3	aB	3.7	аB	3.9	аB	3.6	aB	11.5	aA	3.2	aB
	CV (%) 37.77																						
Trat.	Females.g ⁻¹																							
Soybean	0.0	aB	0.0	аB	0.0	аB	0.1	аB	0.0	аB	0.4	aB	1.5	aA	0.9	aA	0.7	aA	0.6	aA	1.5	aA	0.7	aA
Java	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	aA	0.0	bA										
	CV (%)) 30.37																						
Trat.	Total	Numbe	r.g ⁻¹																					
Soybean	4.3	bA	9.6	aA	10.3	aA	7.1	aA	4.1	bA	7.9	bA	12.1	aA	8.2	aA	5.9	bA	6.8	aA	7.4	bA	5.8	bA
Java	44.2	aA	18.9	аB	13.5	аB	6.9	аB	32.6	aA	29.1	aA	18.0	aB	19.5	аB	22.2	аB	14.6	aB	33.6	aA	18.1	aB
	CV (%) 29.02																						

For each phase of development of the nematode and the evaluation period. means followed by the same lowercase letter in the column and upper case in the line do not differ by the Scott-Knott's and Bonferroni T test at 5% probability. Trat. = treatment. CV = coefficient of variation.



Fig 1. Effect of nematodes on roots of *Macrotyloma axillare*. **A**: Feeding sites (multinucleated giant cells) formed through the parasitism of *Meloidogyne javanica* in soybean at 10 DAI. **B**: Feeding site (giant cells without apparent nuclei) formed by the parasitism of *M. javanica* in *Macrotymola axillare* cv. Java at 10 DAI. **C**: Feeding site (multinucleated giant cells) formed by the parasitism of *M. javanica* in soybean at 15 DAI. **D**: *M. javanica* parasitizing *M. axillare* cv. Java roots at 15 DAI, cell disorganization and malformed feeding site. **E**: *M. javanica* female parasitizing soybean roots at 30 DAI, six granulating and multinucleated cells. **F**: Deformed *M. javanica* female in *M. axillare* cv. Java root at 30 DAI. * = giant cells, N = nematode, x = cell disorganization.

Table 3.	Total number o	f nematod	es (NT),	number of r	nemato	odes per gra	m of r	oot (N	NG) and r	eprodu	ction f	factor	[.] (FR)	of M	eloida	ogyne
javanica	in Macrotyloma	a axillare c	v. Java a	and soybean	roots,	inoculated	with ir	nitial _I	populatio	n (IP) d	of 700	and	1000	eggs	+ J2 (of <i>M</i> .
javanica																

		1	NT		_		_	NG			_				
Treat.	In	nocul	umlevel				Inc	oculumlevel			Ino				
	700		1000		?		700	1000	?		700	1000	?		
Soybean	3401		4524		3963.1	а	634	863	748.6	а	3.40	4.52	3.96	а	
Java	318		392		355.3	b	93	134	114.1	b	0.31	0.39	0.35	b	
?	1860.1	В	2458.3	Α			-	-	-		-	-	-		
	CV (%) 14.16							17.56		CV (%) 11.09					

For each inoculum level means followed by the same letter in the column do not differ by the Bonferroni T test at 5% probability. Trat. = treatment. CV = coefficient of variation.

formation of the feeding sites or to reduced hypersensitivity, although we found no evidence of this mechanism in any of the slide fragments.

Discussion

M. javanica efficiently penetrated the *M. axillare* cv. Java roots; the number of J2 penetrating this plant was similar to the number of J2 in the soybean roots. The number of nematodes per gram of root was higher in *M. axillare* cv. Java than soybean. J3 and J4, however, appeared later; no females were found in *M. axillare* cv. Java. We thus hypothesized that *M. axillare* cv. Java was antagonistic to *M. javanica*. Antagonist plants are characterized by their mode of action, because they have a negative effect on nematode development in the root system (Chitwood, 2002). Their mode of action can change depending on the species; these changes can be characterized by repelling the nematode due to the production of allelopathic compounds or by allowing penetration, but they inhibit the life cycle of the nematode because of the resistance mechanisms (Chitwood, 2002; Nyczepir; Thomas, 2009).

The antagonistic potential of this culture to control *M. javanica* was recently reported. Nematode penetration in the root system was higher in *M. axillare* cv. Java than soybean, but RF was lower and similar to that for the *Crotalaria* species. This result indicated that the nematode life cycle was negatively affected by the culture (Miamoto et al., 2016). Miamoto et al. (2018) also reported that the plants had an antagonistic potential against *M. incognita*.

The response of *M. axillare* cv. Java to *M. javanica* was similar to the responses reported for other pathosystems involving antagonistic plants, such as *Cajanus cajan* L. (Mill) cv. Fava Larga × *Heterodera glycines* Ichinohe (Valle et al., 1997) and *C. cajan*cv. IAPAR 43 × *M. javanica* (Miamoto et al., 2016). Nematodes penetrated the roots of these plants but did not complete their life cycle. The result was similar for *C. spectabilis* and *Meloidogyne* spp.; the plants attracted the nematodes to the root system but did not allow the nematodes to efficiently reproduce and developto the next stage (Curto et al., 2015; Miamoto et al., 2016).

Plant antagonism against nematodes can also occur due to the production of toxic substances, as in *Tagetes* spp., whose antagonistic effect has been attributed to the production of α -terthienyl. In addition to inhibiting nematode development after root penetration, α -terthienyl directly affects egg hatching and the juveniles in the soil; it thus impairs infectionby the pathogen (Debprasad et al., 2000; Hussain et al., 2011; Ferreira et al., 2013). Similarly, the production of substances by *C. cajan* toxic to the nematode can inactivate and kill *M. exigua* Goeldi juveniles (Amaral et al., 2002). The chemical composition of extracts of *M. axillare* cv. Java should thus be studied in the future.

The reaction of a resistant plant can be similar to an antagonist, as observed in studies about the genotype of cotton species resistant to *M. incognita*, in which most of the nematodes penetrating the roots did not pass the J2 stage, because they did not establish efficient feeding sites (Carneiro et al., 2005). A study of the resistance of soybean cultivars CD 214 RR and CD 203 susceptible and resistant, respectively, to *M. paranaensis* was due to nematode penetration and initial development, with J3 formation unaffected but with the

number of females affected (Moritz et al., 2008b). This result was similar to that for *M. axillare* cv. Java in our study, in which no adult females developed, despite the initial penetration and development.

The histopathological examination found that the feeding site was inefficient for nourishing the nematodes in the interaction between *M. Javanica* and *M. axillare* cv. Java, because the females were atrophic. These results were similar to those for white *Avena sativa* L. cv. IPR Afrodite infected with *M. incognita*, which had underdeveloped parasites at 18 DAI (Marini et al., 2016). Marini et al. (2016) also reported the formation of small and malformed giant cells, indicating that the nematode could induce the formation of the feeding sites but could not establish complete differentiation, thereby influencing its own development.

Dysfunctional feeding sitesmay be associated with HRs, which kill cells adjacent to the infection site (Moffett et al., 2002). This reaction has been observed in the pathosystems of *M. exigua* and coffee trees (Anthony et al., 2005) and of *M. incognita* and cotton (Motta et al., 2012), demonstrating that incompatible interactions between nematodes and plants can cause localized death in tissues adjacent to the nematode feeding site. Such plant defensive reactions may not limit parasite invasion or the induction of feeding sites but may limit nematode growth and development (Williamson; Gleason, 2003).

Our results indicated a lack of necrosis, suggesting HRs, but the stimulus for the formation and maintenance of giant cells differed between M. axillare cv. Java and soybean. This result corroborates other studies, including the interaction between the cotton genotypes resistant to *M. incognita*, which developed HRs but formed small feeding sites with small cells, apparent extensive cell disorganization and no thickening of the secondary membrane wall (Carneiro et al., 2005). Similarly, the pathosystem for the coffee cv. Apoatã IAC 2258 and M. exigua demonstrated that the lack of resistance was not only due to HR but also to a set of defensive responses constituted, or induced, after the infection by the nematode. These responses led to the malformation of the feeding site, J2 emigration and the inhibition of nematode development and reproduction. Thus, resistant plants have mechanisms that prevent the establishment of feeding sites, because fewer nourishing cells form. These cells are smaller and consequently can less efficiently feed the nematode, impairing its development (Moritz et al., 2008b). The resistant plants and some antagonistic species therefore did not prevent nematode penetration but compromised its development. The nematodes could die before completing their life cycle.

Materials and Methods

Experimental area and design

The experiments were conducted in a greenhouse and laboratory at the State University of Maringá $(23^{\circ}47'28.4''S, 53^{\circ}15'24.0''W; 379 m a.s.l.)$. The study had a completely randomized design for each of two experiments, based on a 2×12 factorial arrangement (2 plants and 12 evaluation times) for the penetration test and a 2×2 arrangement (2 plants and 2 inoculation levels) for the reproduction test.

Plant and soil materials

Seedlings of *M. axillare* cv. Java and the soybean cv. Pintado (control) were obtained by germination in polyethylene trays containing a commercial substrate for evaluating penetration. These seedlings were transplanted to 700-mL pots containing a 1:1 mixture of soil and sand15 d after germination. The mixture was autoclaved at 120 °C for 2 h.

Penetration of M. javanicainto the roots of M. axillare cv. Java and soybean

The seedlings were inoculated with an initial population of 3000 eggs + second-stage juveniles (J2) of *M. javanica* per plant two days after transplantation. The nematode suspension was deposited in four equidistant holes (approximately 2cm deep) in the soil around each plant. The nematodes used as inoculum were obtained from a pure population raised in tomato plants (cv. Santa Clara) under greenhouse conditions. They were extracted from the root system based on the methodology by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981).

Four plants of each treatment were harvested every two days from 8 to 30 days after inoculation (DAI) to assess nematode penetration and development. The root systems of these plants were carefully separated from the shoots, washed and deposited on paper towels to remove excess water. The roots were then weighed and stained with acid fuchsin (Byrd Junior et al., 1983). Temporary slides with root fragments from each plant were prepared and analyzed using a light microscope at a magnification of 100×. The number of nematodes penetrating the root systems was assessed, and the developmental stage was classified as juveniles in the second, third and fourth stages (J2, J3 and J4, respectively) or females.

Reproduction factor (RF) for M. javanicain theroots of M. axillare cv. Java and soybean

Nematode reproduction was also assessed when the plants were inoculated two days after transplantation with two initial inoculations (II) of 700 and 1000 eggs + J2 of *M. javanica*. Plants were maintained in the greenhouse for 60 days, and the root systems were then collected, washed and weighed. The nematodes were extracted as described above and quantified using Peters slides under a light microscope to evaluate the final population (FP). RF for the nematodes was then calculated following the method by Oostenbrink (1966), in which RF = PF/PI. Seven replicates were assessed in each treatment.

Statistical analysis

The recorded data were subjected to analyses of variance and transformed as $\sqrt{(x + 0.5)}$. The Shapiro Wilk test was used to evaluate normal distributions. The means of the factor 'plant' were compared using the Bonferroni T test, whereas 'time' was compared using the Scott-Knott test, both at 5% probability level. The tests were conducted using Sisvar software.

Histological analysis

M. axillare cv. Java and soybean seeds were sowed in 200-mL pots and inoculated with 1000 eggs + J2 of *M. javanica* 15 d after germination for evaluating the histopathology.

Four roots were carefully removed and washed for the evaluations conducted 10, 15 and 30 DAI. The roots were prepared for further fragments based on Pergard et al. (2005). Twenty root fragments (approximately 0.5 cm) were sectioned, and root areas with visible galls were selected and immersed in a fixer (2% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer at pH 7.00) under continuous shaking. The root samples were then dehydrated in 10, 30, 50, 70, 80, 90 and 100% ethanol, and the fragments were deposited in microtubes and impregnated with resin (Technovit 7100), based on the manufacturer's protocol. Fragments (1-2 µm) were sectioned with a Luptec MRP 2015 ultramicrotome and deposited in drops of distilled water on microscope slides over a heating plate to dry and fix the fragments. The samples were then stained with 0.5% toluidine blue and observed in a light microscope to visualize nematode development and the formation of feeding sites. The selected histopathological fragments were photographed for analyzingand comparing M. axillare cv. Java and soybean.

Conclusions

Macrotyloma axillare cv. Java was resistant to *M. javanica* and was potentially antagonistic. It did not prevent nematodes from penetrating the roots but had a negative effect on the life cycleby the formation of inefficient feeding sites for nourishing the nematode.

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