ANTAGONIST EFFECT OF *ARACHIS PINTOI* ON *MELOIDOGYNE PARANAENSIS* AND *M. INCOGNITA*

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Summary. The antagonist effect of *Arachis pintoi* to *Meloidogyne paranaensis* and *M. incognita* races 1, 2, 3, and 4 was examined in two glasshouse experiments. In the first, seedlings of four *A. pintoi* accessions and "Rutgers" tomato were planted in pots containing a sterilized substrate infested with 10,000 nematode eggs and/or juveniles. In the second, finely chopped *A. pintoi* tissue was incorporated in the substrate of the pots used in the first experiment, which were then re-infested with the remaining nematode population. Examination of *A. pintoi* root stained in B Floxine revealed that no penetration by *M. incognita* and *M. paranaensis* juveniles had occurred and hence there was no gall or egg mass formation. On the other hand, tomato favored the increase of the nematode population, mainly of the *M. paranaensis* and *M. incognita* race 2, which showed the largest population means densities. Acessions 1 and 4 of *A. pintoi* displayed the largest and the smallest root weight, respectively. The roots of the tomato plant sharing the pots with *A. pintoi* accession 4 had larger galls and more egg masses than those sharing pots with accession 1. The incorporation of the non-infested *Arachis* tissues significantly reduced the number of galls and egg masses in the tomato plant roots. In general, the *A. pintoi* accessions had an antagonist effect on the nematodes, suggesting that they could be used as an intercalated crop or cover crop to reduce *M. paranaensis* and *M. incognita* populations.

Among the root-knot nematodes, the most frequently found species in Brazil is *Meloidogyne incognita* which has been described in four races (Taylor and Sasser, 1978). This characteristic often results in a serious problem for crop selection in annual or sequential rotation systems, which are promising options for nematode control in infested areas. Alternative control methods, such as the use of organic fertilizers incorporated into the soil, which stimulate the proliferation of microbial agents (Badra *et al.*, 1979), and the incorporation of parts of plants species that have antagonist properties (Sing and Sitaramaiah, 1967; Gonzaga and Ferraz, 1994a) are reported to be efficient.

Gonzaga and Ferraz (1994b) observed that various plants are potentially valuable species to control *M. incognita* races 3 and *M. javanica*. Mucuna, crotalaria, black oat, peanut and castorbean have also been used in culture rotation systems and in areas infested with root gall nematodes (Carneiro and Carneiro, 1982). *Arachis* cultivars resistant to *M. incognita* species could be important for agricultural management practices used to control nematodes. In fact, it has been shown that *A. pintoi* cultivar Amarillo performs well on different insects, diseases and nematodes (Cook and Franklin, 1988; Cook and Loch, 1993; Cook *et al*, 1994).

Araya and Cheves (1997) studied the control of *Meloidogyne* species in the banana cultivation with *A. pintoi* e *Geophilla macropoda* but failed to achieve conclusive results. Jonathan *et al.* (1999) concluded that species of *Meloidogyne* and *Rotylenchulus reniformis* did not reproduce in *A. pintoi* roots and that this cultivation, intercalated with the banana, minimized the losses caused by nematodes. Good results were also obtained by Domínguez-Valenzuela *et al.* (1990) and Marbán-Mendoza *et al.* (1992b) on tomato and by Vallejos and La Cruz (1992) on coffee but not by Herrera and Marbán-Mendoza (1999) in microplots.

The present study investigated the performance of four different *A. pintoi* accessions for the control of *M. paranaensis* and *M. incognita* races and their possible use an intercalated crop or cover crop.

MATERIAL AND METHODS

Two experiments were carried out in a glasshouse at the Paraná Agronomic Institute – IAPAR, Londrina, PR, Brazil, from September 1999 to April 2000. A randomized block design with ten replications was used in the first experiment, where the treatments consisted of seedlings of four *A. pintoi* Krapovickas *et* Gregory accessions and of "Rutgers" tomato (*Lycopersicon esculentum* Mill.) planted into plastic pots containing 500 g of previously sterilized substrate (with methyl bromide at 250 ml/m^3) made of forest soil, manure and washed sand in the proportion 1:1:1 (v/v/v) infested with *M. paranaensis* Carneiro *et al.* and *M. incognita* (Kofoid *et* White) Chitw. races 1, 2 3 and 4. Seedlings of *A. pintoi* were transplanted to the pots at the fourth to the sixth permanent leaf stage.

The nematodes were multiplied on "Rutgers" tomato plants and the eggs produced were extracted following the Hussey and Barker method, modified by Bonetti and Ferraz (1981). The suspension was adjusted to a mean concentration of 1,000 eggs and juveniles/ml. In-

oculation of each pot was carried out at the 15th day after transplanting seedling by applying 10 ml of the suspension (containing approximately 10,000 nematode eggs and/or juveniles) in two holes 1 to 2 cm deep near the seedling base.

Fifteen days after infestation, the number of juveniles penetrating the roots was assessed in two plants from each treatment. The canopy was discarded and the root system was washed in running water and then stained as described by Byrd *et al.* (1983). The number of juveniles present in 100 ml of soil was evaluated on the 120th day according to methodology of Jenkins (1964) and the number of galls and eggs masses per root gram were counted. At this last assessment, the canopy corresponding to each plant was cut and kept for use in the second experiment. To sample the plant root systems, the contents of each pot were placed on a flat surface and a 100 ml sample of the substrate was taken and the sample was carefully washed and weighed. A 1 g root sample was collected and immersed in a 0.015% B Floxine solution for 15 minutes for egg mass staining.

In the second experiment, the *A. pintoi* plant root system was finely chopped and immediately incorporated in to the substrate of the pots used in the first experiment, which were then infested by the remaining nematode populations. The canopy was also finely chopped, placed on the substrate surface for ten days and then incorporated. Simultaneously, a "Rutgers" tomato plant was planted into each of the pots. A completely randomized design with treatments in a factorial four x two x five scheme (*A. pintoi* accessions x incorporated plant tissue/non-incorporated plant tissue x nematode populations) and five replications was used.

The root weight and number of galls and egg masses per root system of the tomato plants were assessed 45 days after planting. The root system was removed from the pots, washed in running water and immersed in a B Floxine solution.

The data from both experiments were transformed to $\sqrt{(x+0.05)}$ and submitted to analysis of variance, mean comparison analysis and interpretation. The Tukey test at the 5% level of probability was used for mean comparisons.

RESULTS AND DISCUSSION

Penetration of *M. paranaensis* or *M. incognita* juveniles in the first experiment was not observed in the stained roots of the four *A. pintoi* accessions. The root exudates of this legume may have inhibited juvenile hatching and penetration, as reported by Peacock (1959), Tarjan (1960) and Miller and Ahrens (1969). Our results suggest further studies to determine the mechanism involved in the process that prevents pathogen penetration.

The analysis of variance of the root weight showed that there was positive interaction among the *A. pintoi* accessions and the *Meloidogyne* species. The lowest weight was observed for the tomato plant roots, which differed statistically from the others, regardless of the nematode species or race. Nematode species effects were detected only in the tomato plants. There were no significant differences for *M. incognita* races 1, 2, 3 and 4, and it was not possible to statistically differentiate between *M. paranaensis* and *M. incognita* race 2 for the tomato plant roots (Table I). *A. pintoi* prevented gall and egg mass formation in its roots, regardless of the nematode species and/or race or of the accession used. These results comply with the ones obtained by Marbán-Mendoza *et al.* (1992b), who verified that there was no gall formation in *A. pintoi* roots exposed to *M. incognita* during the experimental period.

Significant interactions among nematode species and races was observed in the analysis of the number of juveniles in 100 ml of substrate (Table II). *A. pintoi* may have had an antagonist effect with suppressive action on the development of the nematode populations. A decrease in the number of second stage juveniles was detected in the substrate where *A. pintoi* developed com-

Table I. Mean root weight (g) of four *Arachis pintoi* accessions and tomato plants cultivated in substrate infested with *Meloidogyne paranaensis* and *M. incognita* races 1, 2, 3 and 4. [1]

Meloidogyne sp.	Accession 1	Accession 2	Accession 3	Accession 4	Tomato
M. paranaensis	23.1 Aa	22.8 Aa	24.9 Aa	24.5 Aa	5.7 Bb
M. incognita race 1	21.7 Aa	24.0 Aa	23.8 Aa	23.2 Aa	11.0 Ba
M. incognita race 2	23.6 Aa	23.6 Aa	23.4 Aa	23.8 Aa	7.1 Bab
M. incognita race 3	21.2 Aa	22.3 Aa	23.7 Aa	24.3 Aa	10.8 Ba
M. incognita race 4	22.6 Aa	23.1 Aa	22.7 Aa	23.4 Aa	11.0 Ba
CV 15.7%					

[1] Means followed by the same upper case letter, in the same column and the same lower case letter in the same row, did not differ at the 5% level of probability by the Tukey test.

pared with those where the tomato plant was grown. In general, the highest number of juveniles occurred with *M. paranaensis* and *M. incognita* race 2. These results indicate that the *A. pintoi* accessions were not hosts for the *M. incognita* species and/or races and may function as an efficient means of control since they produced an increased decline in the juvenile populations.

Concerning the second experiment, the data showed the following significant interactions for root weight and egg mass number per tomato plant root system variables: a) plant material incorporation x *Arachis* accessions; b) incorporation x species and/or *Meloidogyne* race; and c) accession x species and/or race. For root galling, the significant interactions were: a) incorporation x accession; and, b) incorporation x species and/or race (Tables III and IV).

The incorporation of *A. pintoi* significantly reduced the tomato root weight in all accessions, but the smallest one was associated with the incorporation of tissues of accession 4 (Table III). This suggests that the plant material had a toxic effect in the soil. Domínguez-Valenzuela and La Cruz (1990) obtained identical results using *A. pintoi* as green cover in a palm tree cultivation, observed smaller plant development. Root systems of tomato plants cultivated in the substrate where the canopy of *A. pintoi* was incorporated showed significant reduction in gall and egg mass formation, regardless of the accession used.

The results of the incorporation x species and/or nematode race interaction are shown in Table IV. The influence of the incorporation of *A. pintoi* plant parts on the tomato plant root weight for all the species and/or races were assessed. The lowest root weight was observed in the tomato plants cultivated in substrate infested with *M. incognita* race 2 and incorporated with *A. pintoi* parts. The influence of incorporation was also

Table II. Mean number of juveniles of *M. paranaensis* and *M. incognita* races 1, 2, 3 and 4 in 100 ml substrate in which *A. pintoi* accessions and tomato plants were incorporated. *[1]

Meloidogyne sp.	Accession1	Accession 2	Accession 3	Accession 4	Tomato
M. paranaensis	15.3 Ba	15.7 Ba	16.4 Ba	17.4 Ba	861.9 Aa
M. incognita race 1	3.8 Bc	3.7 Bc	4.6 Bbc	4.3 Bb	574.4 Ac
M. incognita race 2	10.9 Bab	10.3 Bab	8.6 Bb	12.2 Ba	752.5 Ab
M. incognita race 3	4.7 Bc	5.9 Bbc	3.5 Bc	5.1 Bb	472.5 Ad
M. incognita race 4	5.7 Bbc	7.1 Bbc	3.7 Bc	5.4 Bb	409.4 Ae
CV (%) 10.8					

* Data was transformed in $\sqrt{(x+0.5)}$ for the statistical analysis.
[1] Means followed by the same upper case letter in the same column and the same lower case letter on the same row did not differ at the 5% level of probability by the Tukey test.

Table III. Root weight and gall and egg mass formation in root systems of tomato plants grown in a substrate with finely chopped tissue of four *A. pintoi* accessions incorporated. *[1]

Tomato		Accession 1	Accession 2	Accession 3	Accession 4
Root weight	Incorporate	14.0 Ab	13.6 Ab	13.0 Bb	12.6 Bb
(g)	Not Incorporate	17.5 Aa	17.0 ABa	17.3 Aa	16.4 Ba
Galls	Incorporate	1.5 Ab	1.4 Ab	1.6 Ab	2.1 Ab
(root system)	Not Incorporate	12.8 Aa	15.3 Aa	11.7 Ba	15.8 Aa
Egg masses	Incorporate	0.6 Ab	0.7 Ab	1.0 Ab	1.3 Ab
(root system)	Not Incorporate	10.0 Ba	12.0 Aa	9.0 Ba	12.6 Aa
CV (%) root weight 4.6					
CV (%) galls 18.6					
CV (%) egg masses 19.0					

* Number of galls and egg masses data were transformed to $\sqrt{(x+0.5)}$ for statistical analysis.
[1] Means followed by the same upper case letter in the same column and the same lower case letter in the same row did not differ at the 5% level of probability by the Tukey test.

Table IV. Effect of finely chopped *A. pintoi* tissue incorporation in substrate infested with *M. paranaensis* and *M. incognita* races on root weight and gall and egg mass formation in tomato roots. *[1]

Meloidogyne sp.	Root weight (g)		Galls (root system)		Egg masses (root system)	
	Incorporate	Not incorp.	Incorporate	Not incorp.	Incorporate	Not incorp.
M. paranaensis	13.8 Ba	18.0 Aa	4.1 Ba	27.0 Aa	2.7 Ba	20.8 Aa
M. incognita race 1	13.4 Ba	16.2 Ac	0.7 Bc	7.1 Ad	0.0 Bb	5.1 Ad
M. incognita race 2	12.7 Bb	17.1 Ab	1.9 Bb	16.9 Ab	1.6 Ba	14.0 Ab
M. incognita race 3	13.5 Ba	16.9 Ab	0.6 Bc	8.1 Acd	0.1 Bb	6.3 Acd
M. incognita race 4	13.3 Ba	17.0 Ab	0.7 Bc	10.2 Ac	0.2 Bb	8.2 Ac
CV (%)	4.6		18.6		19.0	

* Number of galls and egg masses data were transformed to $\sqrt{(x+0.5)}$ for statistical analysis.
[1] Means followed by the same upper case letter in the same column and the same lower case letter in the same row did not differ at the 5% level of probability by the Tukey test.

Table V. Effect of finely chopped *A. pintoi* tissue incorporation in substrate infested with *M. paranaensis* and *M. incognita* races on tomato root weigh (g). *[1]

Meloidogyne sp.	Accession 1	Accession 2	Accession 3	Accession 4
M. paranaensis	16.6 Aa	15.7 Ba	15.5 Ba	15.7 Ba
M. incognita race1	14.4 Bb	15.4 Aa	15.3 Aa	14.3 Bbc
M. incognita race 2	16.1 Aa	15.1 Ba	14.8 Ba	13.5 Cc
M. incognita race 3	16.0 Aa	15.2 Ba	14.9 Ba	14.6 Bb
M. incognita race 4	15.8 Aa	15.3 Aa	15.1 ABa	14.3 Bbc
CV (%) root weight 4.6				
CV (%) galls 18.6				
CV (%) egg masses 19.0				

* Data was transformed to $\sqrt{(x+0.5)}$ for statistical analysis.
[1] Means followed by the same upper case letter in the same column and the same lower case letter in the same line did not differ at the 5% level of probability by the Tukey test.

Table VI. Effect of finely chopped *A. pintoi* tissue incorporation in substrate infested with *M. paranaensis* and *M. incognita* races, on gall and egg mass formation in tomato roots. *[1]

Meloidogyne sp	Galls				Egg masses			
Accession.	1	2	3	4	1	2	3	4
M. paranaensis	12.5 Ba	15.5 ABa	14.9 ABa	19.4 Aa	9.2 Ba	11.1 Ba	12.0 ABa	14.7 Aa
M. incognita race1	3.5 Ab	3.7 Ac	3.9 Ac	4.5 Ac	2.2 Ab	2.5 Ac	2.5 Ac	3.0 Ac
M. incognita race 2	10.4 Aa	10.0 Ab	8.2 Ab	9.2 Ab	7.8 Aa	8.1 Aab	6.8 Ab	8.6 Ab
M. incognita race 3	3.6 Ab	5.9 Ac	2.8 Ac	5.3 Abc	2.7 ABb	4.7 Ac	1.5 Bc	3.8 Ac
M. incognita race 4	5.7 Ab	6.5 Ac	3.5 Ac	6.2 Abc	4.5 ABb	5.4 Abc	2.2 Bc	4.7 ABc
CV (%)	18.6				19.0			

* Data was transformed to $\sqrt{(x+0.5)}$ for statistical analysis.
[1] Means followed by the same upper case letter in the same column and the same lower case letter in the same line, did not differ at the 5% level of probability by the Tukey test.

observed on the number of galls and egg masses formed. The lowest gall number and egg mass means were observed with the incorporation of *A. pintoi* in substrate infested with *M. incognita* races 1, 3 and 4, which were statistically different from those infested with *M. incognita* race 2 and *M. paranaensis*. These results agree with reports by Sing and Sitaramaiah (1967) showing that the nematode activity can be affected by the addition of plant material, regardless of the mechanism or activity exercised.

The highest root weight values were observed in the substrate incorporated with *A. pintoi* accession 1 materials and infested with *M. paranaensis* and *M. incognita* races 2, 3 and 4 (Tables V and VI). The lowest root weight values were obtained with the incorporation of *A. pintoi* accession 1 in substrate infested with *M. incognita* race 1 and with the incorporation of *A. pintoi* accession 4 in substrates infested with *M. incognita* races 1, 2, 3 and 4. The largest gall number for accession 1 was observed in the substrate infested with *M. paranaensis* and *M. incognita* race 2. In the *A. pintoi* accession 2, 3 and 4, the greatest means were observed for *M. paranaensis*. Similar results were observed for the number of egg masses, where the highest means were also obtained in the substrate infested with *M. paranaensis* and *M. incognita* race 2. The highest egg mass means for *M. paranaensis* were found in the *A. pintoi* accession 2, 3 and 4.

The pronounced toxic effects on the nematodes, due to the incorporation of *A. pintoi* plant parts in the soil observed in the present study, was probably caused by the large concentration of plant material in the small pot soil volume. According to Badra *et al.* (1979), incorporation of plant material in the soil stimulates the microbiota and/or increases its activity; along with the compounds released during the decomposition of plant remains which has a toxic effect on the nematodes. This condition may have occurred after *A. pintoi* incorporation in the soil for this study since a sharp reduction in nematode numbers was observed at all stages that were assessed.

Another factor that may have contributed to the control of nematode activity by the incorporation of *A. pintoi* plant material was the condition in which the experiment was conducted. There was no egg mass formation in the treatment where sterilized soil was used, probably because the environment was unfavorable to phytonematodes. This result differs from that of Gonzaga and Ferraz (1994a), who did not observe effective nematode control with the incorporation of mucuna green matter in sterilized soil due to the eradication of antagonist microorganisms present in the soil; but this confirms the nematostatic action of the *A. pintoi* plant material used in the present study, since nematode reproduction was inhibited.

The incorporation of *A. pintoi* tissues significantly reduced the number of galls and egg masses in the tomato plant roots. Similar results were obtained by Marbán-Mendoza *et al.* (1992a) who obtained significant reductions in the numbers of galls formed by *M. incognita* in tomato plants seedling cultivated along with *A. pintoi*. They considered the presence of soluble lecitines released by radicular exudates to be mainly responsible for the nematicide properties found in this leguminous vegetable. The *A. pintoi* accessions showed, in general, an antagonist effect on the nematodes, which suggests that they could be used as intercalated culture or cover crop to reduce the *M. paranaensis* and *M. incognita* populations.

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