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Reaction of soybean cultivars to *Meloidogyne incognita* race 3

Reação de cultivares de soja a *Meloidogyne incognita* raça 3

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Abstract

Soybean crops are attacked by several pathogens. Among these, there are nematodes of the *Meloidogyne* genus, which are responsible for yield reduction. Among several control methods against these pathogens, one consists of using resistant cultivars. The objective of this study was to evaluate the reaction of 27 soybean cultivars to the nematode *Meloidogyne incognita* race 3. Two experiments were conducted under greenhouse conditions, in a completely randomized design, with 27 treatments and six replications. Plants were kept in plastic cups with capacity for 400 mL of sterilized substrate (soil + sand) and were artificially inoculated with a suspension of 2000 eggs and J2 *of M. incognita* race 3/plant. Evaluations were performed sixty days after inoculation (DAI), in order to determine nematode population density in the roots and reproduction factor (RF). Through experiment results, four soybean cultivars were selected, of which three were resistant (BRSGO Paraíso, NA 7255 RR and NS 7490 RR) and one was susceptible (BRSGO Santa Cruz). The cultivars aforementioned were inoculated with four different *M. incognita* Race 3 inoculum concentrations. Thus, an experiment was conducted in a completely randomized design, in a 4 x 4 factorial, with 12 replications. Evaluations were performed at 10 DAI, when the number of J2 and J3/root system was observed. At 45 DAI, nematode population density in the roots was evaluated. NS 7476, NS 7490 RR, NA 8015 RR, NA 7620 RR, BRSGO Graciosa, P98Y70, CD 237 RR, P98Y51 and UFU Milionária cultivars showed resistant behavior, as they were similar to the resistance standards used. NA 7255 RR and NS7490 RR cultivars behaved as *M. incognita* race 3-resistant with the use of the inoculum concentration of 4000 eggs/plant, which seems to be the ideal concentration to evaluate the reaction of cultivars to this nematode. Evaluations of *M. incognita* race 3 penetration in the roots were not a good parameter to evaluate cultivar behavior.

Additional keywords: genetic resistance; inoculum concentration; root-knot nematode; root penetration.

Resumo

A cultura da soja é atacada por vários patógenos e entre esses estão os nematoides do gênero *Meloidogyne,* responsáveis pela redução de produtividade. Entre os vários métodos de controle desses patógenos, tem-se o uso de cultivares resistentes. O objetivo deste estudo foi avaliar a reação de 27 cultivares de soja ao nematoide *Meloidogyne incognita* raça 3. Dois experimentos foram conduzidos em condições de casa de vegetação, em delineamento inteiramente casualizado, com 27 tratamentos e seis repetições. As plantas foram mantidas em copos plásticos com capacidade para 400 mL de substrato (solo + areia) esterilizados e foram inoculadas artificialmente com uma suspensão de 2000 ovos e J2 de *M. incognita* raça 3/planta. As avaliações foram realizadas sessenta dias após a inoculação (DAI), determinando-se a densidade populacional do nematoide nas raízes e o fator de reprodução (FR). A partir dos resultados destes experimentos foram selecionadas quatro cultivares de soja, sendo três resistentes (BRSGO Paraíso, NA 7255 RR e NS 7490 RR), uma suscetível (BRSGO Santa Cruz), e foram inoculadas com quatro diferentes concentrações de inóculo de *M. incognita* raça 3. Assim, foi conduzido um experimento em delineamento inteiramente casualizado, em esquema fatorial 4 x 4, com 12 repetições. Avaliações foram realizadas aos 10 DAI observando-se o número de J2 e J3/sistema radicular. Aos 45 DAI avaliou-se a densidade populacional do nematoide nas raízes. As cultivares NS 7476, NS 7490 RR, NA 8015 RR, NA 7620 RR, BRSGO Graciosa, P98Y70, CD 237 RR, P98Y51 e UFU Milionária apresentaram comportamento de cultivares resistentes por se assemelharem aos padrões de resistência utilizados. As cultivares NA 7255 RR e NS7490 RR se comportaram como resistentes a *M. incognita* raça 3 com o uso da concentração de inóculo de 4000 ovos/planta, que parece ser a concentração ideal para avaliação da reação de cultivares a este nematoide. Avaliações da penetração de *M. incognita* raça 3 nas raízes não se constituíram em um bom parâmetro para avaliar o comportamento das cultivares.

Palavras-chave adicionais: concentração de inóculo; nematoide de galhas; penetração nas raízes; resistência genética.

Introduction

Soybean (*Glycine max* (L.) Merrill) worldwide production in the 2013/14 season was of 281,715,000 tons, of which Brazil was responsible for 85,000,000 tons in an area of 29,200,000 ha. Thus, Brazil ranks second in world production, only behind the United States of America, which produced 88,599,000 tons. China is the main soybean-consuming country, with a total demand of 78,930,000 tons and production of only 12,500,000 tons. Therefore, China needs to import about 66,430,000 soybean tons, of which 24,618,000 tons were imported from Brazil until July 2013 (AGRIANUAL, 2014). According to Costa (2005), soybean is the most significant crop of Brazil's agribusiness due to its great ability to produce protein and oil, which can generate different products for human and animal consumption. Through soybean crushing, bran and crude oil are obtained, as well as derivatives, such as degummed oil, margarines and fats.

Among nematode genera capable of parasitizing soybeans throughout the world, Ferraz (2001) and Embrapa (2011) highlight *Meloidogyne* sp. (Goeldi, 1887), *Rotylenchulus reniformis* (Linford & Oliveira, 1940), *Pratylenchus brachyurus* (Godfrey, 1929) and *Heterodera glycines* (Ichinohe, 1952). In Brazil, gall-inducing species are the main cause of crop damage and, for at least 50 years, have been a constant concern for Brazilian soybean farmers, consisting of an obstacle for food production. Gall-inducing species cause high direct losses due to production reduction, and high indirect losses due to limiting agricultural use in infested areas. In addition, these species also have wide geographic distribution (Moura, 1996; Ferraz, 2001). In the *Meloidogyne* genus, more than 80 species are described, among which the main soybean-infesting species, according to Ferraz, (2001) and Miranda et al., (2011), are *M. javanica* (Treub, 1885) and *M. incognita* (Kofoid & White, 1919; Chitwood, 1949).

Inclusion of root-knot nematode-resistant or tolerant cultivars in crop rotation or no-till systems is a valuable tool against Root-Knot Nematode (RKN) problems in Brazilian soybean crops (Ferraz, 2001). Genetic resistance is the most affordable and effective method of controlling diseases. However, in most of the cases, genetic resistance either does not exist or exists at a low level, or resistance is broken due to pathogen genetic variability (Yorinori & Kiihl, 2001). Therefore, research for resistance against root-knot nematodes is constant. Field and laboratory tests, as well as advanced molecular biology tests have been often conducted (Ferraz, 2001).

In order to use genetic resistance as a control measure, genotype reaction against nematode attacks must be known. According to Silva (2001), the first report of soybean resistance against root-knot nematodes in Brazil was made in 1952. Currently, there are several resistant soybean cultivars (Embrapa, 2011), although there are soybean cultivars that had their reactions to root-knot nematodes still not known. Thus, research to identify these genotypes for their use in RKN control is required.

According to Mendes & Rodriguez (2000), soybean genotype evaluation to identify resistance sources against RKN has resulted in conflicting information, depending on the criteria used. The authors aforementioned classified soybean cultivars RS-6 (Guassupi), RS- (Jacuí), UFV-15 (Uberlândia) and UFV/ITM-1 as hyper susceptible to *M. incognita* race 3 by using Canto-Sáenz criteria (Sasser et al., 1984). However, if Oostenbrink (1966) criteria were used, cultivars would be classified as resistant, because they had a reproduction factor lower than one (RF < 1.0). Dias et al. (2010), through the use of Canto-Sáenz criteria (Sassser et al., 1984), classified soybean cultivars M-Soy 8001, BRS Baliza RR and BRS Eva as tolerant to *M. ethiopica* (Whitehead, 1968). However, the cultivars aforementioned had RF greater than one (RF > 1.0), and would be classified as susceptible according to the Oostenbrink criteria (1966).

According to Moura (1997), resistant plants may show galls in the absence of nematode reproduction, while susceptible plants may not form galls, what hampers the use of this parameter in resistance evaluations. Penetration by the same amount of J2 that occur in non-resistant roots may occur even in resistant plants. However, in resistant genotypes, J2 can undergo different processes within the plant, returning to the soil or dying soon afterwards. In addition, when J2 begin their development in resistant plants, there is developmental impairment and their life cycle may not be concluded (Moura et al., 1993). Nematode life cycle interruption in resistant genotypes may occur due to resistance mechanism, as verified by Carneiro et al. (2005), who noticed high impairment in *M. incognita* J2 development when they were inoculated in resistant cotton genotypes. The J2 did not manage to establish and maintain giant cells, reducing nematode reproduction.

Difficulties to evaluate genotypes may occur due to nematode population density variations, which affect differently the soybean root system. This can be observed in Rocha et al. (2008), who evaluated *M. javanica* inoculum levels, ranging from 0 to 300 eggs, and observed that the highest inoculum concentration occurred with the highest number of females within the roots, not affecting root system weigh, although inoculum levels used in that study were considered low. Asmus & Ferraz (2001), while evaluating inoculum levels ranging from 0 to 97200 eggs, observed that leaf area index, total shoot dry matter, fresh root matter and grain yield were reduced due to nematode concentration increase.

The objective of this study was to evaluate the reaction of 27 soybean cultivars inoculated with *M. incognita* race 3, also evaluating the penetration and development of *M. incognita* race 3 in four soybean cultivars submitted to different inoculum concentrations.

Material and methods

Three experiments were carried out under greenhouse conditions, located in the geographical coordinates 16º35'47 "South and 49º16'47" West, 728 m elevation. Two experiments evaluated the reaction of 27 soybean cultivars to *Meloidogyne incognita* race 3, and one experiment evaluated the penetration and development of *M. incognita* race 3 in four soybean cultivars inoculated with four different inoculum concentrations. The first two experiments were conducted in a completely randomized design, with 27 treatments and six replications. The third experiment was conducted in a completely randomized design, in a 4 x 4 factorial, with twelve replications.

Soybean Cultivar Reactions to *M. incognita* race 3

Twenty-three soybean cultivars, without information regarding resistance to *Meloidogyne* sp. (Embrapa, 2011), plus two cultivars known to be resistant and two susceptible were tested. Cultivars used as resistant standards were BRSGO 8860 RR and BRSGO Paraíso and, as susceptible standards, BRSGO Raíssa and BRSGO Santa Cruz (Embrapa, 2011).

Four seeds were placed in plastic cups with 400 mL capacity on November 17, 2011 (first experiment) and December 20, 2011 (second experiment). The substrate consisted of a 1:1 mixture of soil and sand, previously sterilized by autoclaving. After thirteen days, each plot was thinned, leaving two plants/pot. Immediately after thinning, inoculation was conducted by depositing inoculum suspension containing 2,000 eggs and J2 of *M. incognita* race 3 in each pot.

The inoculum was obtained from a *M. incognita* race 3 population kept on tomato 'Santa Cruz' under greenhouse conditions. During the experiment conduction period, plants were watered daily, in order to keep the proper soil moisture level for plant growth. Maximum and minimum temperatures in the greenhouse were taken daily.

Sixty days after inoculation (DAI), nematode extractions and quantifications were performed, as well as reproduction factor (RF) determination. Nematode extraction was performed according to Coolen & D'Herde (1972). After extraction, nematodes were stored and preserved in Golden X solution, according to Hooper (1970). Counting was done under optical microscope (100x magnification), with the aid of a Peters counting slide.

The reproduction factor (RF) was obtained by dividing the final population (Fp) by the initial population (Ip), according to Oostenbrink (1966). Moura & Régis (1987) criteria was used to identify the behavior of each cultivar. In this classification, the cultivar with the highest RF is used as the susceptible standard. Then, this cultivar is compared with each of the others e and the reduction percentage of the RF is calculated. Each cultivar is rated as highly susceptible (0 to 25% RF reduction), susceptible (26 to 50% RF reduction), low resistance (51 to 75% RF reduction), moderately resistant (76 to 95% RF reduction), resistant (96 to 99% RF reduction) or highly resistant (100% RF reduction).

Data were transformed to $\sqrt{x}$ and analysis of variance was conducted for the first and second experiments individually. Afterwards, the Hartley test (Ramalho et al., 2000) was conducted to verify variance homogeneity between experiments. When there was homogeneity, joint analysis of the experiments was performed. As significant interaction between the experiments was observed they were analyzed separately. Means were compared by the Scott-Knott's test at 5% probability.

M. incognita race 3 penetration and development in soybean cultivars submitted to different inoculum concentrations.

In order to carry out this experiment, four soybean cultivars were selected, two of which were known as resistant (BRSGO Paraíso) and susceptible (BRSGO Santa Cruz), according to Embrapa (2011). The two other cultivars (NA 7255 RR and NS 7490 RR) were chosen for their lack of information on the reaction to *M. incognita* race 3 and were rated as moderately resistant in previous experiments.

The seeds were sown in 400 mL plastic cups containing previously autoclaved substrate (1:1 mixture of soil and sand) on April 10, 2012. After thirteen days of sowing, thinning was performed remaining plant/pot. Then, plants were inoculated with *M. incognita* race 3 at concentrations of 500, 1,000, 2,000 and 4,000 eggs+ J2/plant).

At ten DAI, six replications of each treatment were removed, in order to evaluate nematode penetration. First, fresh root matter was determined. Afterwards, root staining was conducted by the technique described by Byrd et al. (1983), using acid fuchsin.

At 45 DAI, the six remaining replications of each treatment were removed. After plant fresh root matter was determined, nematodes were extracted according to Coolen & D'Herde (1972), through which the amount of eggs and J2 by root system was obtained (final population - Fp). RF was determined by the ratio between final population (Fp) and initial population (Ip), according to Oostenbrink (1966), and cultivar classification was conducted using the criteria of Moura & Régis (1987).

After quantification, data were transformed into $\sqrt{x + 0.5}$, in order to proceed analyzes of variance. When significant differences were observed among cultivars, Tukey's test at 5% probability was applied for mean comparison. When there were differences between inoculum concentrations, regression analysis was performed. Analyzes were performed using the SISVAR statistical application (Ferreira, 2011).

Results and discussions

Reaction of Soybean Cultivars to *M. incognita* Race 3

Data joint analysis showed significant interaction (P < 0.05) between experiments, indicating that they should be analyzed separately. Through population densities, it was observed in both experiments that four cultivar groups were formed (Table 1). In the first experiment, the cultivars that had the highest values were TMG 1288 RR, NA 7337 RR and AN 8943, which had higher values than the other cultivars, including BRSGO Raíssa and BRSGO Santa Cruz, used as susceptible standard. In the second experiment, the NA 7337 RR cultivar had the highest population density (P < 0.05), and AN 8843 and TMG 1288 RR equaled the BRSGO Santa Cruz susceptible standard. Although the four formed groups did not exactly consist of the same cultivars, there was a coincidence for most of them. Thus, consistency was observed in the most susceptible group, in which, in addition to the two cultivars used as standard, TMG 1288 RR, NA 7337 RR and AN 8843 were also present. These three cultivars had RF <1.0 in the first experiment, but were rated as low resistance, susceptible and highly susceptible, respectively, according to the criteria by Moura & Régis (1987). In the second experiment, the same cultivars showed RF close to 1.0 and were rated as susceptible, highly susceptible and susceptible, respectively. Therefore, it is possible to state the susceptibility of these three cultivars to *M. incognita* race 3 based on our results.

Table 1 - Population density (PD), reproduction factor (RF), % of reduction compared to the susceptible standard and classification (C) of soybean cultivars inoculated with *M. incognita* race 3.

Cultivar	(1)Experiment 1				(1)Experiment 2			
	PD* (eggs and J2/10g root)	RF*	% inhibition	(2)C	PD* (eggs and J2/10g root)	RF*	% inhibition	(2)C
BRSGO 8860 RR (R)3	82 a	0.003 a	100	HR	566 a	0.06 a	96	R
BRSGO Paraíso (R)3	102 a	0.008 a	99	R	399 a	0.06 a	96	R
NS 7476	148 a	0.01 a	99	R	817 a	0.10 a	93	MR
NS 7490 RR	216 a	0.01 a	99	R	997 a	0.11 a	93	MR
NA 8015 RR	227 a	0.01 a	99	R	1397 a	0.08 a	95	MR
NA 7255 RR	251 a	0.02 a	98	R	1535 a	0.23 a	85	MR
NA 7620 RR	379 a	0.02 a	98	R	478 a	0.05 a	97	R
BRSGO Graciosa	380 a	0.02 a	98	R	1272 a	0.09 a	94	MR
UFU Xavante	437 a	0.03 a	97	R	4232 b	0.67 b	55	PR
UFU Carajás	440 a	0.03 a	97	R	1047 a	0.19 a	87	MR
BRS 206	470 a	0.02 a	98	R	1339 a	0.16 a	89	MR
P98Y70	489 a	0.03 a	97	R	1022 a	0.11 a	93	MR
UFUS Riqueza	520 a	0.03 a	97	R	3497 b	0.49 b	67	PR
TMG 1179 RR	666 b	0.02 a	98	R	1336 a	0.17 a	89	MR
CD 237 RR	691 b	0.04 a	96	R	1439 a	0.12 a	92	MR
UFU Guarani	850 b	0.08 a	91	MR	1873 a	0.34 a	77	MR
TMG 1181 RR	955 b	0.10 a	89	MR	6261 c	0.67 b	55	PR
P98Y51	990 b	0.05 a	95	MR	1187 a	0.09 a	94	MR
UFUS Impacta	1218 b	0.11 a	88	MR	4802 b	0.67 b	55	PR
A 7002	1303 b	0.07 a	93	MR	4851 b	0.65 b	56	PR
UFU Milionária	1448 b	0.06 a	94	MR	1390 a	0.15 a	90	MR
AN 8500	2105 c	0.19 b	80	MR	8184 c	0.85 b	43	S
BRSGO Raíssa (S)3	2413 c	0.28 b	70	PR	4689 b	0.64 b	57	PR
BRSGO Santa Cruz (S)3	3340 c	0.28 b	70	PR	9063 c	1.25 c	16	HS
TMG 1288 RR	4984 d	0.45 b	52	PR	6045 c	1.03 c	31	S
NA 7337 RR	5907 d	0.48 c	49	S	11167 d	1.49 c	Standard	HS
AN 8843	7638 d	0.94 d	Standard	HS	6869 c	0.99 c	34	S
Mean	1431	0.12			3250	0.42		
CV (%)	43.52	64.44			30.43	37.95		

(1)- Means followed by the same letter in the columns do not differ (Scott & Knott's, 5% probability)
(2)- Classification proposed by Moura & Régis (1987). Where HS = Highly susceptible, S = Susceptible, PR = Poorly resistant. MR = Moderately resistant, R = Resistant, HR = Highly resistant.
3- Classification described by Embrapa (2011), where R = Resistant, S = Susceptible.
* - Values transformed into $\sqrt{x}$ for statistical analysis.

Although good nematode development was observed in plant roots, with population densities ranging from 82 to 7,638 eggs and J2/10 g of roots in the first experiment, and from 399 to 11,167 eggs and J2/ 10 g of roots in the second experiment, very low RF was observed, especially in the first experiment. Low RF values were also observed by Mendes & Rodriguez (2000), who evaluated 13 soybean cultivars inoculated with *M. incognita* races 1, 2, 3 and 4 and observed that almost all cultivars had RF < 1. This hampers cultivar behavior evaluation in relation to *M. incognita*, and could lead to erroneous classification, since different resistant and susceptible standard cultivars were used. Thus, it is considered that, in the present study, cultivar classification according to the criteria by Moura & Régis (1987) is the most adequate.

When using the classification by Moura & Régis (1987), it was observed that BRSGO Santa Cruz cultivar, which was the susceptibility standard in the first experiment, showed different behavior in the second experiment. The AN 8843 cultivar, although having RF < 1.0, was considered the susceptible standard for the first experiment, while the cultivar NA 7337 RR was the susceptible standard for the second experiment, both being classified as highly susceptible.

BRSGO 8860 RR and BRSGO Paraíso cultivars, which were used as resistant standards, were the ones with the lowest population densities (Table 1) in the first experiment. In the second experiment, NA 7620 RR along with the two cultivars aforementioned, showed lower population densities. In addition, BRSGO 8860 RR and BRSGO Paraíso were the cultivars with the lowest RF in the first experiment (0.003 and 0.008, respectively). In the second experiment, these two cultivars had the second lowest RF, which was only higher than that of the NA 7620 RR, although they were statistically equal (Table 1). Therefore, the resistance behavior described for these two cultivars was confirmed.

In the first experiment the cultivars BRSGO Raíssa, BRSGO Santa Cruz and TMG 1288 RR were rated as of low resistance (Table 1), as they reduced the RF between 51% and 75% compared to the susceptible standard (AN 8843). Most of cultivars (14 cultivars) were rated as resistant, and BRSGO 8860 RR was rated as highly resistant. It is noted that, in the first experiment, there were higher reproduction factor inhibition percentages compared to the second experiment.

In the second experiment, the BRSGO Santa Cruz cultivar was rated as highly susceptible by the Moura & Régis (1987) criteria, followed by cultivars AN 8500, AN 8843 and TMG 1288 RR, which were rated as susceptible. In this experiment, 13 cultivars were rated as moderately resistant, representing the largest group (Table 1). NA 7620 RR, BRSGO 8860 RR and BRSGO Paraíso cultivars, that had lower RF and higher reduction of the RF in relation to the susceptible standard, were rated as resistant cultivars (Table 1).

The cultivar BRSGO Raíssa, which, according to EMBRAPA (2011), is susceptible to *M. incognita* race 3, behaved as of low resistance in both experiments (Table 1). BRSGO Santa Cruz had the same behavior as BRSGO Raíssa in the first experiment, when they registered a RF equal to 0.28 and were rated as of low resistance, contradicting its description of being susceptible (Embrapa, 2011). The cultivars UFU Xavante and UFUS Riqueza also showed contradictory behavior in the two experiments, and were rated as resistant in the first experiment and as of low resistance in the second experiment.

Mendes and Rodriguez (2000) affirm that contradictory results found among different ratings in the literature may be due to the adoption of different methodologies to classify cultivars reactions. An example may be observed in the study of Dias et al. (2010) in which the reaction of soybean cultivars inoculated with *M. enterolobii* Yang & Eisenback (1983) were rated according to Canto-Sáenz classification (Sasser et al., 1984). Thus, the cultivar BRSGO Paraíso was rated as tolerant, even though it had a reproduction factor of 6.5. According to Oostenbrink criteria (1966), BRSGO Paraíso would be a susceptible cultivar because it showed RF > 1. Differences between classification results in studies by different authors may also be due to the nematode isolate aggressiveness variation. Tihohod & Ferraz (1986), when analyzing two *M. javanica* isolates inoculated in soybean cultivars observed that cultivars inoculated with the isolate from Itamarati farm, state of Mato Grosso, Brazil, produced four to five times more galls and egg masses compared with plants inoculated with Capinópolis isolate from estate of Minas Gerais. According to Dall'Agnol et al. (1984), in studies carried out in a naturally infested field, gall indexes do not have the same meaning for evaluations made in different locations or years because soil infestations not the same. Thus, uneven spatial nematode distribution could lead to escapes (Asmus & Andrade, 1996). Due to the factors aforementioned, which interfere with cultivar classification and generate conflicting results, Mendes and Rodriguez (2000) reported that the selection of soybean genotypes resistant to gall-forming nematodes, in particular to *M. incognita* species, has been problematic.

Based on the results of the two experiments (Table 1), it is possible to highlight a cultivar group that had resistant or moderately resistant behavior and presented RF inhibition percentage above 90%. They were the cultivars NS 7476, NS 7490 RR, NA 8015 RR, NA 7620 RR, BRSGO Graciosa, P98Y70, CD 237 RR, P98Y51 and UFU Milionário, in addition to the two resistant standards used. These were considered the best cultivars, among those tested, for use in areas infested with *M. incognita* race 3.

M. incognita race 3 penetration and development in soybean cultivars submitted to different inoculum concentrations

In the evaluation conducted at ten days after inoculation, second (J2) and third-stage (J3) juveniles were observed inside the roots, which were quantified separately. Statistical analysis revealed significant interaction (P < 0.05) between cultivars and inoculum concentrations for these two variables.

In the comparison between cultivars, it was observed that the BRSGO Paraíso, which was used as resistant standard, did not confirm its resistant behavior (Table 2). At the concentration of 500 eggs per plant, the four soybean cultivars showed the same behavior. From the concentration of 1,000 eggs/plant, cultivars differed, and BRSGO Santa Cruz cultivar, which was used as the susceptible standard, was the one with the lowest J2 and J3 mean number in the roots. This cultivar only showed higher J2 and J3 numbers in the roots compared to the other cultivars when plants were submitted to the inoculum concentration of 2,000 eggs/plant. Even so, it has been statistically equal to BRSGO Paraíso.

Table 2 - Second (J2) and third-stage (J3) juveniles per root system of four soybean cultivars under different *M. incognita* race 3 inoculum concentrations ten days after inoculation.

Cultivar	Inoculum concentration				
	500	1000	2000	4000	Média
			(1)J2		
BRSGO Santa Cruz	(2)17.00 a	0.33 a	146.46 c	78.66 a	60.54
BRSGO Paraíso	29.33 a	26.83 b	100.83 bc	147.00 b	76.00
NA 7255 RR	9.16 a	34.33 b	59.33 ab	67.33 a	42.54
NS 7490 RR	9.33 a	18.16 b	31.50 a	68.33 a	31.83
Média	16.20	19.91	84.45	90.33	
CV (%)			26.13		
			(1)J3		
BRSGO Santa Cruz	(2)2.83 a	0.17 a	60.16 b	33.50 b	24.17
BRSGO Paraíso	8.17 a	5.50 ab	39.33 b	24.83 ab	19.46
NA 7255 RR	1.17 a	8.50 b	19.33 a	16.00 a	11.25
NS 7490 RR	2.17 a	4.33 ab	8.00 a	15.00 a	7.37
Mean	3.58	4.62	31.71	22.33	
CV (%)			33.78		

(1)- Data were transformed into $\sqrt{x + 0.5}$ for statistical analysis
(2)- Means followed by the same letter in the columns do not differ from each other (Tukey, 5% probability)

The cultivars NA 7255 RR and NS 7490 RR, for which there was no resistance record in the literature, were generally the ones that showed the lowest *M. incognita* race 3 penetration in the roots compared to the standards used, mainly in the highest inoculum concentrations. These two cultivars, although showing an increasing number of J2 and J3 in the roots as the inoculum concentration was increased (Figures 1B and 1D), still had lower values than those shown by BRSGO Santa Cruz and BRSGO Paraíso.

Cultivar BRSGO Santa Cruz increased J2 penetration and J3 presence up to inoculum concentrations of 2770 and 3640 eggs/plant, respectively. From these concentrations, nematode penetration in the roots began to decrease (Figure 1A). The same occurred with the J3 number found in the roots of cultivar BRSGO Paraíso, which begin to decrease from the concentration of inoculum of 2,821 eggs/plant (Figure 1C). It is possible that, from these inoculum concentrations, competition for feeding sites among nematodes restricted their penetration and development. According to Asmus & Ferraz (2001), the population, at low initial levels, grows exponentially for a short period of time, but due to competition for food, growth rates start to reduce. This fact was observed by Windham & Barker (1986), who analyzed the damage potential of *M. incognita* in soybean cultivars and noticed sequential decrease in the final population when initial populations were of 1,250, 5,000 and 20,000 eggs.

On the other hand, J2 penetration of into BRSGO Paraíso roots grew linearly as inoculum concentrations increased (Figure 1C). Moritz et al. (2008) observed that, on the eighth day after inoculation, most of nematodes that had penetrated in the roots of susceptible and resistant soybean cultivars were still at the J2 developmental stage, remaining parallel to the root central cylinder. This may indicate that resistance mechanisms of the resistant cultivars were not slowing nematode overall development yet. Moura et al. (1993) observed that in *M. incognita*-resistant soybean cultivars, plant root cells became disorganized and necrotic 10 days after nematode inoculation, and feeding sites formation did not occur. Thus, the resistance mechanism performance was observed, reducing development for J3 stage.

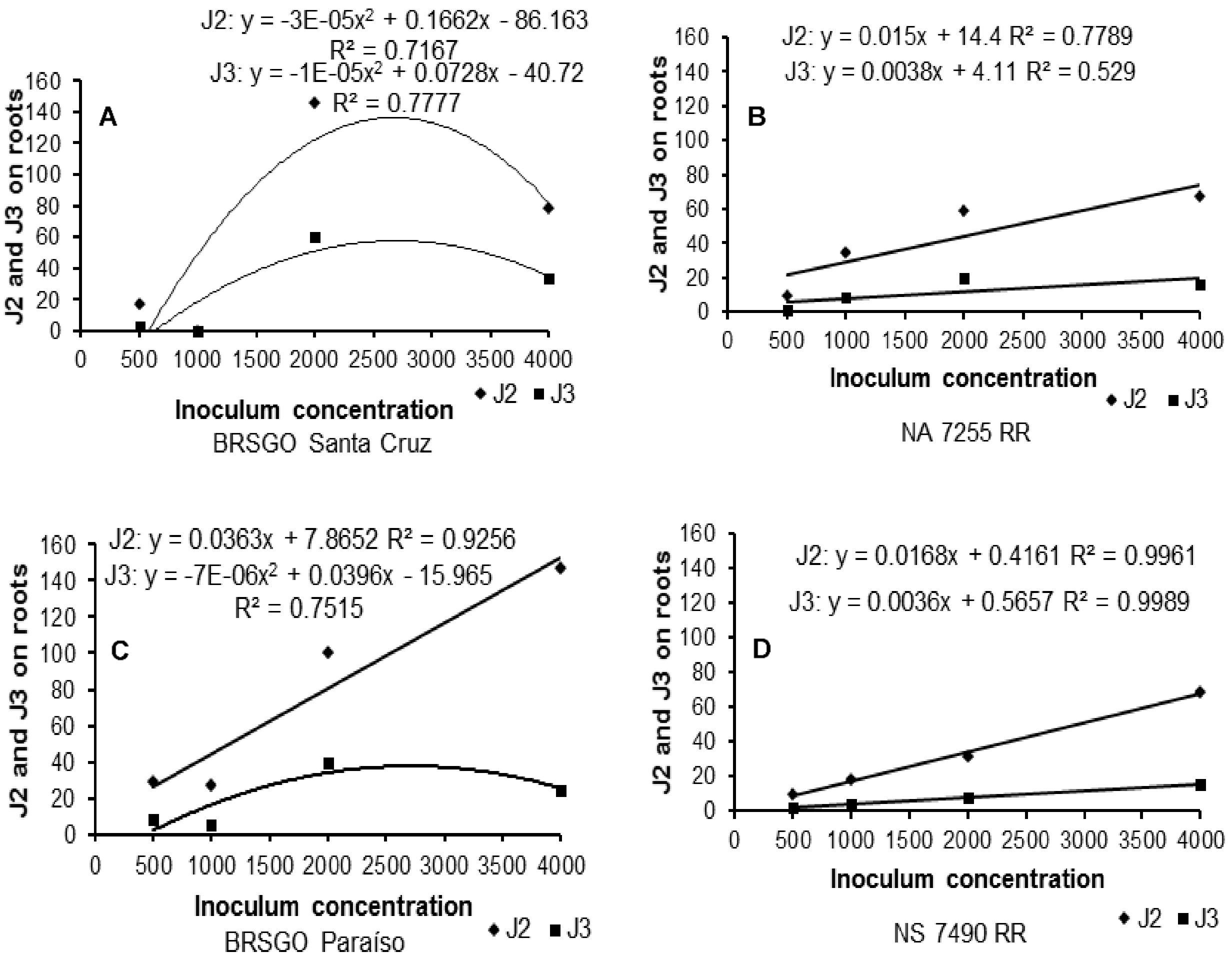

Figure 1 - Second (J2) and third-stage (J3) juveniles on *M. incognita*, race 3, in soybean cultivar roots ten days after inoculation, under different inoculum concentrations.

The highest J2 amount per root system observed at 10 days in the resistant cultivar BRSGO Paraíso at concentrations of 1,000 and 4,000 eggs and J2, compared to the susceptible cultivar BRSGO Santa Cruz, contradicts the results found by Moura et al. (1993). When these authors evaluated *M. incognita* penetration at four DAI, it was observed that susceptible cultivars had larger numbers of nematodes than the resistant cultivars. Herman et al. (1991) observed that increase in the J2 amount in roots of resistant cultivars occurred after the 16th day of inoculation. The authors related this fact to the migration rate of nematodes to the substrate after five days of inoculation. According to Carneiro et al. (2005), some resistance mechanisms act to prevent the nematode development cycle without preventing their penetration into the plant tissue. According to Faria et al. (2003), one of the resistance mechanisms is phytoalexin accumulation in resistant hosts, which coincides with the hypersensitivity reaction, functioning as nematostatic phytoalexins, drastically affecting nematode function and preventing its development. However, this resistance mechanism did not appear in the present study because the nematode was able to penetrate and develop in the roots.

The nematode final population in the roots was evaluated at 45 days after inoculation, considering that the nematode life cycle would have been completed at this time. According to Ferraz & Mendes (1992) and Moura (1996) the *Meloidogyne* life cycle is generally completed in 25 days at temperatures around 28 °C. In the greenhouse where the experiment was installed ambient temperatures ranged from 22ºC to 35ºC. In this evaluation (eggs and J2/10 g of root) there was a significant interaction between "cultivars" and "inoculum concentrations" ($P < 0.05$). Susceptible cultivar BRSGO Santa Cruz only differed from the resistant cultivar BRSGO Paraíso at the highest inoculum concentration (Table 3). In general, this is the concentration used for experiments with this nematode. At this same inoculum concentration NA 7255 RR and NS 7490 RR had a similar behavior to that of BRSGO Paraíso, although they showed lower population densities even when submitted to the inoculum concentration of 2000 eggs/plant.

Tabela 3 - *Meloidogyne incognita* race 3 final population density in the roots of four soybean cultivars submitted to different inoculum concentrations, 45 days after inoculation.

Cultivar	(1)Inoculum concentration				
	(2)500	(2)1000	(2)2000	(2)4000	Média
BRSGO Santa Cruz	477.83 a	446.11 b	935.16 bc	1619.33 b	869.62
BRSGO Paraíso	144.66 a	181.66 ab	1319.50 c	381.50 a	506.83
NA 7255 RR	1884.66 b	143.66 ab	310.16 a	623.66 a	740.54
NS 7490 RR	136.83 a	73.66 a	437.16 a	598.00 a	311.41
Mean	661.00	211.29	750.50	805.00	
CV (%)			36.63		

(1)- Data were transformed into √ (x + 0.5) for statistical analysis
(2)- Means followed by the same letter in the columns do not differ from each other (Tukey, 5% probability)

M. incognita race 3 final populations in the roots of cultivars BRSGO Santa Cruz (Figure 2A) and NS 7490 RR (Figure 2B) showed linear growth as inoculum concentrations per plant were increased. However, values observed for NS 7490 RR were much lower and often lower than those observed in the resistant cultivar BRSGO Paraíso (Figure 2C).

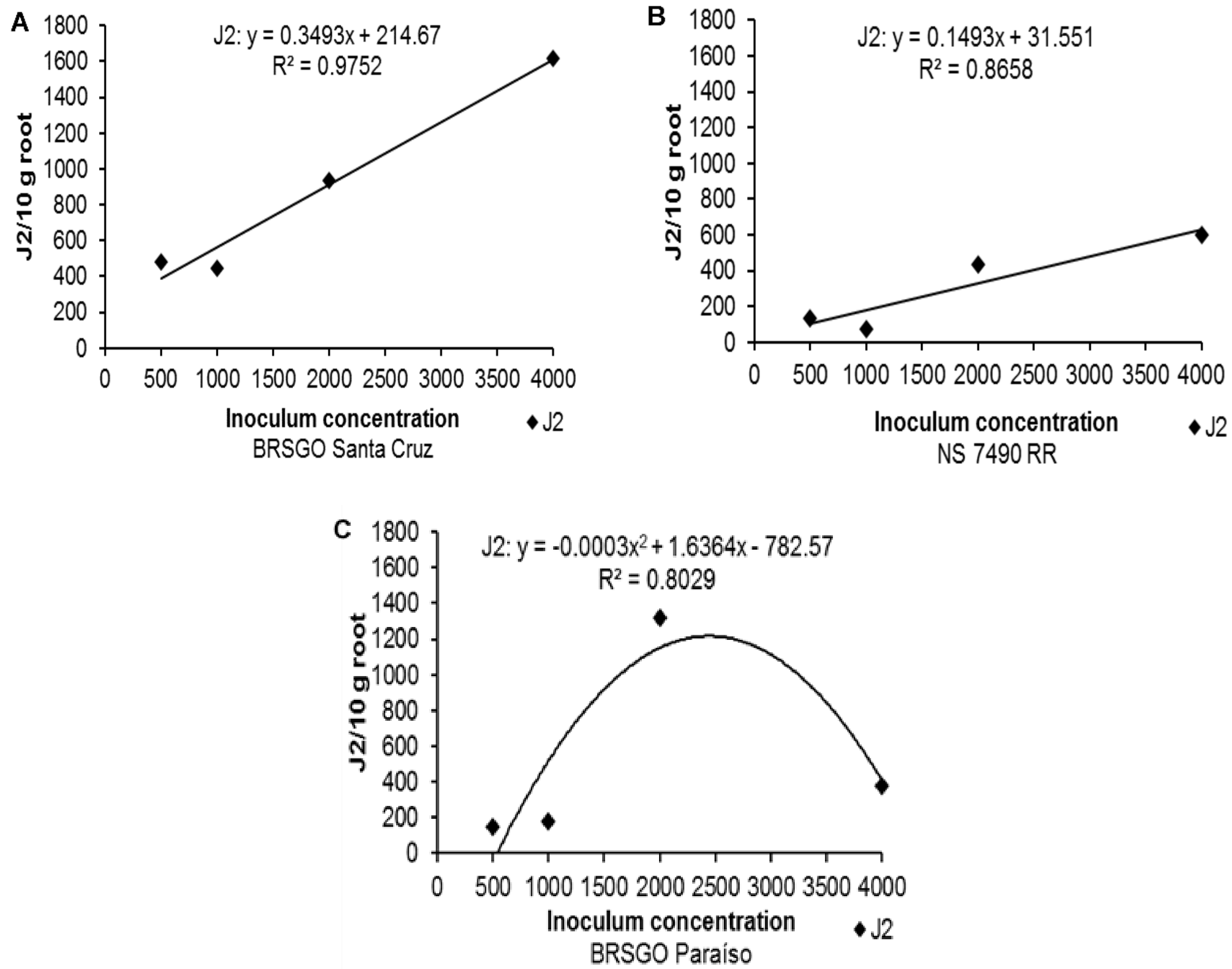

Figure 2 - *Meloidogyne incognita* race 3 final population in the roots of soybean cultivars submitted to different *M. incognita* race 3 inoculum concentrations, evaluated at 45 days after inoculation.

Cultivar BRSGO Paraíso had a final population increase up to the concentration of 2,727 eggs. After this inoculum concentration, nematode development restriction occurs, indicating that resistance is effective when plants are submitted to inoculum concentrations above 2,700 eggs/plant (Figure 2C).

With the results obtained in this study, it is concluded that the cultivars NA 7255 RR and NS

7490 RR are resistant to *M. incognita* race 3, although resistance is only apparent with inoculum concentrations of 4,000 eggs/plant. It is also concluded that evaluation of nematode penetration in roots is not a good parameter to evaluate cultivar reaction and to discriminate them as resistant or susceptible.

Conclusion

The cultivars NS 7476, NS 7490 RR, NA 8015 RR, NA 7620 RR, BRSGO Graciosa, P98Y70, UFUS Riqueza, CD 237 RR, P98Y51 and UFU Milionária showed resistant behavior, as they were similar to the resistance standards used. NA 7337 RR, AN 8843 and TMG 1288 RR were considered susceptible to *M. incognita*, race 3.

The cultivars NA 7255 RR and NS 7490 RR were resistant to *M. incognita* when submitted to inoculum concentrations above 1,000 eggs/plant.

The concentration of 4,000 eggs/plant is ideal to evaluate cultivar reaction to *M. incongita*.

Nematode penetration evaluation in roots is not a good parameter to discriminate the resistance of cultivars.

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