Joint use of fungicides, insecticides and inoculants in the treatment of soybean seeds[1]

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ABSTRACT

The interference of the joint application of pesticides with seed inoculation on the survival of *Bradyrhizobium* has been reported in the last years. So, the objective of this study was to evaluate the joint use of fungicides, insecticides and inoculant in the treatment of soybean seeds on various parameters of *Bradyrhizobium* nodulation in soybean as well as on crop productivity parameters. The experiment was conducted during the 2013/2014 crop in the experimental field of the Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso – Campo Novo do Parecis Campus. The seeds of TMG 133 RR variety were sown in pots. It was used a randomized block design in a 4 x 4 + 1 factorial, four fungicides (1: fludioxonil + metalaxyl-M, 2: carboxine + thiram, 3: difeconazole and 4: carbendazim + thiram), four insecticides (1: fipronil 250 SC, 2: thiamethoxam, 3: imidacloprid + thiodicarpe and 4: imodacloprid 600 FC) and an inoculant (SEMIA 5079 and SEMIA 5080), common to all treatments, with three replications. The experiment was not repeated. The joint application of fungicide and insecticide with inoculant does not affect nodulation, foliar N content and vegetative growth of the plants as well as the masses of grains per plant and 100-grain mass. The use of the carbendazim + thiram mixed with fipronil and carbendazim + thiram mixed with imidacloprid provides less number of pods per plant and grains per plant, reflecting in reductions in the production of soybean grains. In this way, the fungicide carbendazim + thiram, regardless of the combined applied insecticide, is the most harmful to *Bradyrhizobium* spp.

Key words: biological nitrogen fixation; *Bradyrhizobium* ssp; *Glycine max* L. Merrill; grain yield; nodulation.

RESUMO

Uso conjunto de fungicidas, inseticidas e inoculante no tratamento de sementes de soja

Nos últimos anos têm surgido relatos sobre a interferência da aplicação de defensivos agrícolas em conjunto com a inoculação das sementes de soja na sobrevivência dos *Bradyrhizobium*. Dessa forma, o presente trabalho teve por objetivo avaliar o efeito do uso conjunto de fungicidas, inseticidas e inoculante no tratamento de sementes de soja sobre vários características da nodulação de *Bradyrhizobium* na soja e, também, em parâmetros de produtividade da cultura. O experimento foi realizado durante a safra 2013/2014 no campo experimental do Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso – Campus Campo Novo do Parecis. A semeadura foi realizada em vasos, com a variedade TMG 133 RR. Adotou-se o delineamento em blocos casualizados, em esquema fatorial 4 x 4 + 1, sendo quatro fungicidas (1: fludioxonil + metalaxil-M, 2: carboxina + thiram, 3: difeconazol e 4: carbendazim + thiram), quatro inseticidas (1: fipronil 250 SC, 2: thiamethoxam, 3: imidacloprido + tiodicarpe e 4: imodacloprido 600 FC) e um inoculante (SEMIA 5079 e SEMIA 5080), comum a todos os tratamentos, com três repetições. O experimento não foi repetido. A aplicação conjunta de fungicida e inseticida com inoculante não prejudica a nodulação, o teor de N foliar e crescimento vegetativo das plantas, assim como as massas de grãos por planta e de 100 grãos. O uso das misturas carbendazim +

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thiram com fipronil e carbendazim + thiram com imidacloprido proporciona menor número de vagens por planta e de grãos por planta, refletindo em reduções na produção de grãos de soja. Desta forma, o fungicida carbendazim + thiram, independentemente do inseticida aplicado combinado, é o mais prejudicial aos *Bradyrhizobium* spp.

Palavras-chaves: *Bradyrhizobium* ssp; fixação biológica de nitrogênio; *Glycine max* L. Merrill; nodulação; produtividade de grãos.

INTRODUCTION

Soybean, (*Glycine max* L. Merrill) is a dicotyledonous plant of the Fabaceae family, grown in most regions in the world due to the practicality of cultivation, the various purposes of its products and derivatives, and because of its wide socioeconomic importance (Neves, 2011).

Soybean grains present high protein content (about 6.5% N), which makes nitrogen the most required element by the crop, which needs 80 kg ha^{-1} of nitrogen (N) for every 1000 kg of produced grains. Nitrogen can be supplied to the soybean crop by the use of nitrogen fertilizers and by the biological nitrogen fixation process (BNF) (Hungria *et al*., 2001). However, soybean cropping has been carried out without the application of mineral nitrogen fertilizer inasmuch as the biological nitrogen fixation process (BNF) can effectively replace it. In this case, the nitrogen supply is from 60 to 250 kg ha^{-1}, which can increase crop productivity, especially in nitrogen-poor soils, with a reduction in the production costs and in the negative effects of the excess of nitrate in the springs (Hungria *et al.*, 2007; Marks, 2008).

Biological nitrogen fixation is performed by the symbiotic association of diazotrophic microorganisms with the roots of plants. The process begins when seeds are germinating or later, when the roots of the seedlings exude molecules that chemically attract *Bradyrhizobium japonicum*, stimulating their growth in the rhizosphere and triggering the expression of several genes, both of the bacterium and the host plant. The bacteria then penetrate the soybean roots and cause the growth of some specific plant cells, forming the nodules where they will be hosted. Hence, nitrogen fixation occurs by the rapid incorporation of ammonia synthesized with hydrogen ions (H^+), abundant in bacterial cells, which are transformed into ammonium ions (NH_4^+), which will then be distributed to the host plant and be incorporated into different forms of organic N such as ureides, amino acids and amides (Hungria *et al.*, 2001).

Nodulation may be influenced by biotic factors such as synergism or antagonism with other representatives of soil biota or among the rhizobia themselves, by abiotic factors such as soil pH, nutrient availability, moisture, temperature, soil physical structure, and by other cultural practices such as the treatment of seeds with fungicides and insecticides. These factors are responsible for reducing nodulation and consequently crop productivity (Bizarro, 2004).

Seed treatment with fungicides and insecticides is responsible for the reduction in the number of viable bacterial cells in the seeds, caused by the direct contact of the active ingredient as well as by the contact of the solvent of the phytosanitary product with the bacterium and by the action in the formation of the nodules (Campo & Hungria, 1999).

The treatment of soybean seeds with fungicides may promote a reduction by 20% in the number of bacterial cells in the seeds, two hours after the treatment and inoculation, as reported in experiments testing the fungicides benomyl + captan, benomyl + thiram, benomyl + tolylfluanid, carbendazim + captan, carbendazim + thiram, difeconazole + thiram, thiabendazole + thiram and thiabendazole + tolyfluanid and inoculation of strains SEMIA 5019 and SEMIA 5079 (Campo & Hungria, 1999). The less toxic fungicides were thiabendazole + tolylfluanid, thiabendazole + thiram and thiabendazole + captan. However, when they were kept in contact with the bacterium for 24 hours, the number of cells was reduced by 60%.

Due to this possible incompatibility, the objective of this work was to evaluate the effects of the joint use of fungicides, insecticides and inoculants in the treatment of soybean seeds on *Bradyrhizobium* populations and on the development of the crop.

MATERIAL AND METHODS

The experiment was carried out from December/2013 to April/2014, in pots that were firstly placed in a greenhouse and after the formation of the second trifoliate leaf, the pots were transferred to open environment in the experimental area of the Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso - Campo Novo do Parecis Campus (latitude 13º40’31"S, longitude 57º53’31" W, altitude of 572 meters), in order to guarantee natural environmental conditions. The climate of the region is tropical hot and humid (Aw), according to the precepts of Koppen. Figure 1 shows the average rainfall and temperatures that occurred during the experimental period. Their average values were: 29.8; 23.6 and 20.3 °C for maximum, average and minimum temperatures, respectively, as well as a precipitation height of 1512 mm.

It was used a complete randomized block experimental design with 4 x 4 + 1 factorial scheme, with four fungicides (fungicide 1: fludioxonil + metalaxyl-M; fungicide 2: carboxin + thiram; fungicide 3: difeconazole and fungicide 4: carbendazim + thiram), four insecticides (insecticide 1: fipronil 250 SC, insecticide 2: thiamethoxam; insecticide 3: imidacloprid + thiodicarpe and insecticide 4: imodacloprid 600 FC), all with inoculant, and an additional treatment only with inoculant. Symbiosis nod® soybean, containing the strains SEMIA 5079 and SEMIA 5080 (*B. japonicum*), with guaranteed concentration of $7.2x10^9$ viable cells mL^{-1} were used. The experimental plot consisted of two pots, each containing two soybean plants with three replicates per treatment.

The pots presented 15 cm of upper radius, 10 cm of lower radius and 21 cm of height (10.45 dm^3) and were filled with soil collected from an area not used for cultivation for three consecutive crops, classified as Typic dystrophic Red Latosol with clayey texture, according to the Brazilian Soil Classification System (Embrapa, 2013). Its granulometry in the 0 -0.20 m depth is 506 g kg^{-1} clay, 134 g kg^{-1} silt and 360 g kg^{-1} sand, and the initial chemical characterization, performed according to Raij *et al*. (2001) showed the following values: pH ($CaCl_2$) = 5.7; O.M. = 26 g dm^{-3}; P (resin) = 5.9 mg dm^{-3}; K, Ca, Mg and H + Al = 1.5; 32; 11 and 40 $mmol_c dm^{-3}$, respectively, with V = 54.8%.

Sowing fertilization was performed based on the recommendations of Embrapa, 2011, considering soil analysis and expected yield of 3000 kg ha^{-1}. Based on the recommended doses per hectare, doses proportional to the volume of soil contained in the vessels were calculated, applying 283.5 mg of P_2O_5 (simple superphosphate with 18% P_2O_5, 16% Ca and 8% S), 252.0 mg Ca and 126.0 mg S in addition to 157.5 mg K_20 (potassium chloride with 60% K_20). The fertilizers were diluted in water, homogenized and distributed in each pot. Topdressing fertilization (V4, fourth trifoliate leaf) was calculated in the same manner and according to the crop needs (Embrapa, 2011). It was applied 157.5 mg of K_2O and 1.91 mg of Mn (manganese sulphate with 30% Mn and 17% S) and 1.08 mg S were diluted in water, homogenized and distributed to each pot.

For each treatment, 0.25 kg of soybean seeds of TMG 133RR variety at a cycle ranging from 122 to 129 days were used for the region of Parecis in the State of Mato Grosso.

The following doses and active principles were used according to the need of each experimental unit to form the different fungicide/insecticide combinations: 0.5 mL carbendazim + thiram (150 + 350 g L^{-1}); 0.25 mL fludioxonil (25 + 10 g L^{-1}); 0.085 mL difeconazole (250 g L^{-1}); 0.75 mL carboxin + thiram (200 + 200 g L^{-1}) and 0.5 mL fipronil (250 g L^{-1}); 0.75 mL thiamethoxam (350 g L^{-1}); 1.75 mL imidacloprid + thiodicarpe (150 + 450 g L^{-1}) and 0.5 mL imidacloprid (600 g L^{-1}) -1), diluted in distilled water for the homogenization of the volume. The amount of each product was calculated by their respective commercial

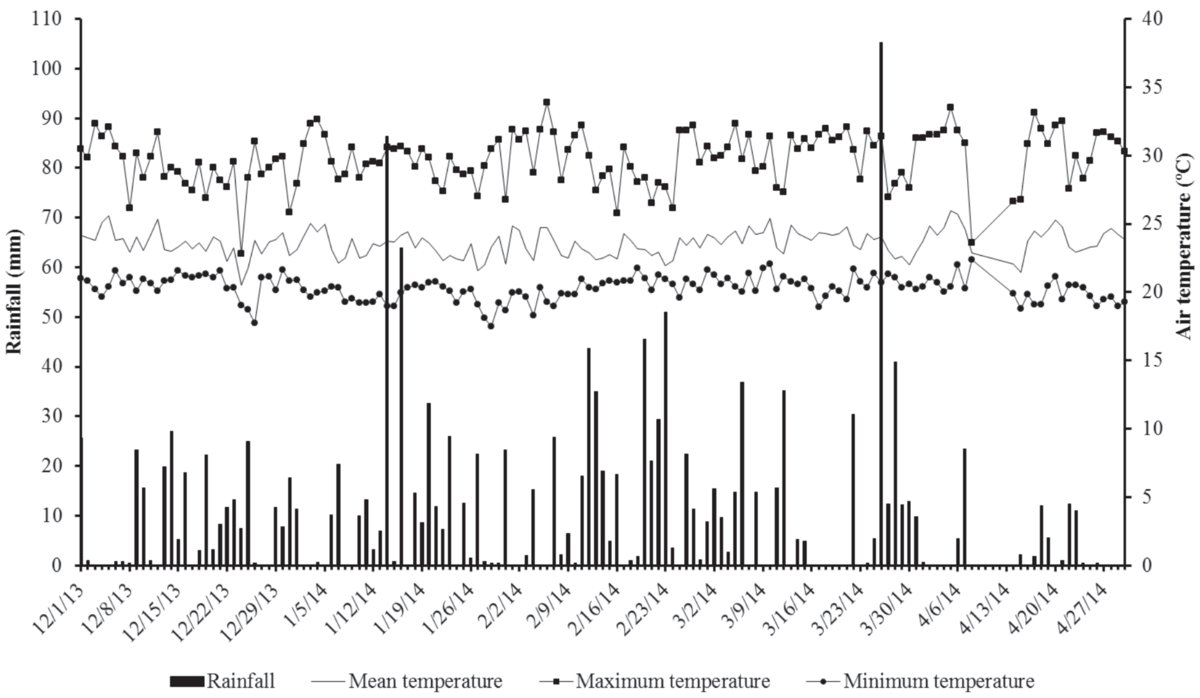

Figure 1: Rainfall and average temperatures in the experimental area in Campo Novo do Parecis, State of Mato Grosso from December/ 2013 to April/ 2014.

recommendations. The seeds were placed in plastic bags to receive the treatment by means of slow stirring. After drying the seeds, inoculation was carried out with 0.5 mL of Simbiose nod® soybean and immediate sowing, on December 20, with five seeds per pot. At ten days after emergence of the seedlings (DAE), thinning was performed, leaving two plants per pot. The plants were transferred to the open environment at 30 DAE to provide similar field conditions.

Control of insects *Diabrotica speciosa* (Germar), *Bemisia tabaci* (Genn.), *Elasmopalpus lignosellus* (Zeller) and *Helicoverpa armigera* (Hübner) was carried out by applying a commercial dose of 0.13 mL fipronil, 0.13 mL imidacloprid and 0.6 mL of thiamethoxam + Lambda-cyhalothrin. A commercial dose of 0.2 mL of Azoxystrobin + Ciproconazole and 0.2 mL of Trifloxystrobin + Prothioconazole was used for the control of target spot diseases (*Corynespora cassiicola* - Berk. & Curt.), Asian soybean rust (*Phakopsora pachyrhizi* - Sidow & Sidow) and anthracnose (*Colletotrichum dematium* var. Truncata – Schw). Applications with insecticides and fungicides started at 10 and 15 DAE, respectively, and repeated every 7 days, until the R6 stage (final grain filling).

The evaluations were carried out in two steps. In the first, when the plants were at R1 stage (first flower emitted at any node on the main stem). The total collection, collection of the aerial part and roots of the two plants of one of the pots of each repetition was carried out. In the second step, the same procedure was performed with the plants at R7 (physiological maturation).

Variables analyzed at R1:

- **Number of viable nodules (NVN):** The nodules were detached from the roots and cut in the middle to count the number of reddish-colored nodules, indicative of viability (Hungria *et al*., 1997). The results were expressed in number per plant.
- **Dry mater of the viable nodules (DMVN):** The viable nodules were packed in paper bags and dried in an oven at a temperature of 50 °C until reaching a constant mass, weighed on a precision scale (0.001g) and the results expressed in g per plant;
- **Relationship between dry matter of nodules and number of viable nodules (DMPN):** It was obtained by dividing the dry mass by the total number of viable nodules, transforming it into a 100-nodule dry mass;
- **Fresh and dry mass of the aerial part (FMAP; DMAP) and roots (RFM; RDM):** The aerial part and the roots of the plants were separated, washed and dried in an oven at 50 °C until reaching a constant mass, weighed on a precision scale (0.001g) and the results expressed in g per plant;
- **Foliar nitrogen content (FNC):** The third trifoliate leaf was removed from the four plants corresponding to each treatment. After washing in distilled water and drying in an oven at 50 °C until constant mass, the trifoliate leaves were ground, and the nitrogen content was determined by sulfuric solution digestion, distillation and titration by Kjeldahl (Embrapa, 2009a). The results were expressed as total nitrogen content in g kg^{-1} dry mass.

Variables analyzed at R7:

- **100-grain mass (HGM):** it was determined on the basis of a sample of 100 grains by adjusting the moisture content to 13% (wet basis moisture, w%), whose mass was measured on a precision scale (0.001g) and expressed in g;
- **Number of pods per plant (NPP) and number of grains per pod (NGP):** They were determined by the count in the two plants of each plot to make the final average, after the manual thresh of the pods;
- **Number of grains per plant (NGP):** It was determined by counting the grains of the two plants of each plot, achieving the final average;
- **Soybean grain production (PRO):** It was determined by the average mass obtained in the two plants of each plot by adjusting the moisture content to 13% (w%), and expressed in g per plant.

The data were submitted to analysis of variance (F test) using ASSISTAT statistical software (Silva & Azevedo, 2002), and the means of each of the 16 possible combinations were compared among each other and with the additional treatment (control) by the Dunett's test at $p < 0.05$.

RESULTS AND DISCUSSION

The number of viable nodules, dry matter of viable nodules and the ratio between dry mass by the number of viable nodules did not present statistical difference between treatments (Table 1), showing that the joint application of fungicide, insecticide and inoculant did not have influence in the treatment of seeds, on the nodulation process.

These results are in accordance with the data obtained by Bueno *et al*. (2003), who observed that the fungicides carboxin + thiram, difenoconazole, fludioxonil + metalaxyl-m and carbendazim + thiram, also used in this study, as well as the other fungicides, benomyl, carbendazim, thiabendazole + thiram, methyl thiophanate, captan, thiram, tolylfluanid and metalaxyl, applied mixed with inoculant containing SEMIA 5019 and SEMIA 5079 strains in seed treatment did not cause a significant reduction of nodulation. Similarly, Bacchi *et al*. (2004) found no harmful effects on the number of viable nodules and dry matter of viable nodules when thiram, tolylfluanid, difeconazole, carbendazin, carbendazin + thiram, thiabendazole + tolylfluanid, methyl thiophanate, carboxyn + thiram, thiabendazole + thiram and fludioxonil + metalaxyl were used combined with inoculant containing the same bacterial strains.

The non-significant reduction of nodulation in soybean plants, or the production of nodules in amounts greater than the additional treatment with only inoculant may be related to the action of the fungicides on soil phytopathogens and on antagonistic pathogens of the bacteria, besides the non-interference of the fungicides at the pH of the soil solution, providing an adequate environment for the development of bacteria (Bueno *et al*., 2003). However, it may occur detrimental effects such as those reported by Pereira *et al*. (2010a), who registered a reduction by 51.6% in nodule numbers, in soybean seeds inoculated with SEMIA 5019 and SEMIA 5079 strains and treated with carbendazin + thiram and thiabendazole + thiram in relation to untreated inoculated seeds, perhaps due to influence of storage.

When evaluating the survival of *Bradyrhizobium japonicum* (SEMIA 5079 and SEMIA 5080) and the effects on soybean nodulation and productivity, Costa *et al*. (2013) also observed a reduction of 24.7% in the number of nodules when fludioxonil + mefenoxam was used and a reduction of 48.2% when using carbendazim + thiram, as well as a decrease between 26.5 and 49.4% in the mass of nodules when the fungicides carbendazim + thiram and fludioxonil + mefenoxam were applied.

However, the insecticides thiamethoxan and imidacloprid were not harmful to nodulation, when applied in the treatment of seeds combined with inoculant containing the strains SEMIA5019, SEMIA 5079 and SEMIA 5080 (Lamas *et al*., 2004; Franco *et al*., 2008). However, Pereira *et al*. (2010b) evidenced a significant reduction in the mass of nodule when the seeds were treated with imidacloprid and carbosulfan insecticide combined with inoculant containing BR96 strain. So, it was characterized that the interference of these insecticides in soybean nodulation depended on the strain used in the inoculation.

Table 1: Means for the number of viable nodules, dry matter of the viable nodules, ratio between dry matter mass and number of viable nodules, foliar nitrogen content, fresh mass of the aerial part and dry matter of the aerial part according to the joint use of fungicide, insecticide and inoculant in the treatment of soybean seed

	Number of viable nodules					**Dry matter of viable nodules**				
	I1[++]	**I2**	**I3**	**I4**	**Mean**	**I1**	**I2**	**I3**	**I4**	**Mean**
F1[+]	222	344	245	176	249	0.60	0.90	0.89	0.55	0.73
F2	219	152	227	187	197	0.89	0.39	0.65	0.58	0.63
F3	290	234	222	307	263	0.78	0.74	0.80	0.89	0.80
F4	211	201	206	213	208	0.67	0.47	0.74	0.67	0.64
Mean	236	233	228	221	229	0.73	0.63	0.77	0.67	0.70
Additional			*223*					*0.72*		
CV%			37.75					30.36		
Ratio between dry matter mass and number of viable nodules						Foliar nitrogen content (%)				
F1	0.23	0.26	0.36	0.33	0.30	42.9	47.2	39.2	44.9	43.6
F2	0.43	0.26	0.28	0.31	0.32	43.9	45.8	43.1	45.3	44.5
F3	0.27	0.30	0.33	0.29	0.30	40.3	44.4	39.8	41.7	41.6
F4	0.31	0.25	0.35	0.31	0.31	44.8	44.3	44.2	41.1	43.6
Mean	0.31	0.27	0.33	0.31	0.31	43.0	45.5	41.6	43.2	43.7
Additional			*0.32*					*49.19*		
CV%			12.13					11.38		
Fresh mass of the aerial part (g)						Dry mass of the aerial part (g)				
F1	52.7	63.1	52.5	41.9	52.5	11.4	13.3	11.7	9.2	11.4
F2	61.5	52.5	45.7	48.8	52.1	13.0	10.8	9.2	10.5	10.9
F3	44.0	50.1	65.3	61.1	55.1	8.7	10.6	13.1	12.9	11.3
F4	48.6	38.8	60.3	43.7	47.8	11.0	8.5	11.8	9.3	10.1
Mean	51.7	51.1	55.9	48.9	53.3	11.0	10.8	11.4	10.5	11.2
Additional			*75.7*					*15.3*		
CV%			31.6					31.5		

[+] fungicide 1: fludioxonil + metalaxyl-M; fungicide 2: carboxin + thiram; fungicide 3: difeconazole and fungicide 4: carbendazim + thiram; [++] insecticide 1: fipronil 250 SC; insecticide 2: thiamethoxam, insecticide 3: imidacloprid + thiodicarpe and insecticide 4: imodacloprid 600 FC

Foliar nitrogen content (FNC) was not affected by fungicide and insecticide applications (Table 1). The total nitrogen content in the leaf dry mass of most of the plants from the treated seeds, as well as those in which the seeds were only inoculated, was 45 g kg^{-1} on average, a sufficient amount for the good development of the soybean crop (Embrapa, 2009b). These results indicate the high symbiotic efficiency of the *Bradyrhizobium* bacteria strains contained in the inoculants, resulting in higher FNC in the soybean plants, as already observed by Sediyama (2012).

Fresh mass of the aerial part, root fresh mass, dry mass of the aerial part and root dry mass of the plants were not affected by the combined use of fungicide, insecticide and inoculant (Tables 1 and 2).

Similarly, the application of fludioxonil + mefenoxam, carbendazim + thiram, carboxin, carboxin + thiram, fludioxonil, carbendazim, and thiram fungicides in the treatment of seeds combined with inoculation of *Bradyrhizobium* (SEMIA 5079 and SEMIA 5080) did not affect the production of dry mass of the aerial part when compared to treatment only with inoculant (Costa *et al*., 2013). However, when the seeds were treated with thiabendazole + thiaram, captan, carboxin + thiram, tolylfluanid and fludioxonil + metalaxyl-M via peliculization

Table 2: Means for root fresh mass, root dry mass, number of pods per plant, number of grains per pod, 100-grain mass and soybean production according to the joint use of fungicide, insecticide and inoculant in the soybean seed treatment

	Root fresh mass (g)					**Root dry mass (g)**				
	I1	**I2**	**I3**	**I4**	**Mean**	**I1**	**I2**	**I3**	**I4**	**Mean**
F1	19.7	22.4	20.2	22.0	21.1	2.9	3.6	3.1	2.9	3.1
F2	22.8	17.0*	24.2	19.9	21.0	3.5	2.4	3.0	2.6	2.9
F3	23.2	19.2	19.5	25.1	21.8	3.2	2.8	2.6	3.5	3.0
F4	19.4	16.2*	21.1	18.7	18.8	2.8	2.3	2.9	2.8	2.7
Mean	21.3	18.7	21.2	21.4	21.0	3.1	2.8	2.9	3.0	3.0
Additional			*27.1*					*3.7*		
CV%			16.8					21.3		
	Number of pods per plant					Number of grains per pod				
F1	35.2 A	33.8	31.8*	32.7*	33.4 A	2.4*	2.4	2.3*	2.3*	2.3 B
F2	36.8 A	29.5*	35.0	34.7	34.0 A	2.4*	2.3*	2.4	2.4*	2.3 B
F3	33.0 A	36.8	35.2	34.5	34.9 A	2.3*	2.4*	2.4	2.3*	2.3 B
F4	28.0* B	36.2	40.8	26.7*	32.9 B	2.4	2.4	2.4	2.3*	2.4 A
Mean	33.3	34.1	35.7	32.1	34.4	2.4 a	2.4 a	2.4 a	2.3 b	2.4
Additional			*44.5 A*					*2.5 aA*		
CV%			13.5					1.9		
	Number of grains per plant					100-mass grain (g)				
F1	83 A	81	73*	76* A	78	13.1	13.2	13.3	13.4	13.3
F2	87 A	68*	83	82 A	80	18.9	13.5	13.6	13.2	14.8
F3	77 A*	87	84	79* A	82	13.1	13.2	13.2	13.2	13.2
F4	67* B	88	99	62* B	79	13.3	13.0	13.1	13.1	13.2
Mean	79	81	85	75	82	14.6	13.2	13.4	13.2	13.6
Additional			*110 A*					*13.4*		
CV%			14.7					17.2		
	Soybean grain production (g per plant)									
F1	10.9 aB	10.7 a	9.8 a	10.3 a	10.4					
F2	15.9 aA	9.2* b	11.2 b	10.8 b	11.8					
F3	10.1* aB	11.4 a	11.1 a	10.5 a	10.8					
F4	8.9 bB	11.4 a	13.3 a	8.1* b	10.4					
Mean	11.5	10.7	11.3	9.9	11.1					
Additional			*14.7 A*							
CV%			20.0							

Means followed by the same lowercase letters in the row and the same uppercase letter in the column are not different from each other by the Dunett's test at 5%; + Fungicide 1: fludioxonil + metalaxyl-M, fungicide 2: carboxin + thiram, fungicide 3: difeconazole and fungicide 4: carbendazim + thiram; ++ insecticide 1: fipronil 250 SC, insecticide 2: thiamethoxam, insecticide 3: imidacloprid + thiodicarpe and insecticide 4: imodacloprid 600 FC; * Value statistically different from the additional treatment and inoculated with *Bradyrhizobium* strains (SEMIA 5019 and SEMIA 5079), a significant reduction was found in the dry mass of the aerial part of soybean plants (Pereira *et al*., 2009), as well as when the seeds were treated with the insecticides carbosulfan, imidacloprid, clothianidin and inoculated with bacteria of the genus *Bradyrhizobium*, strains BR86 and BR96 (Pereira *et al*. 2010b), ratifying the observations of this study.

In relation to the anticipation of the treatment of soybean seeds with fungicide carbendazim + thiram, cellular protector and inoculant containing *Bradyrhizobium japonicum* cells (SEMIA 5079 and SEMIA 5080) on seed nodulation and plant growth, it was verified that root dry mass was positively influenced when the seeds were treated with fungicide and negatively when the seeds received inoculants and were immediately sown (Sediyama, 2012). However, application of the thiamethoxam insecticide combined with the inoculant (SEMIA 5079 and SEMIA 5080) did not promote a reduction in the dry matter of the aerial part and root dry matter in a study carried out by Franco *et al*. (2008).

The treatments affected the number of pods per plant (NVP) and number of grains per plant (NGP) (Table 2). A higher negative interference was observed in the combined use of carbendazim + thiram with fipronil, with a reduction of 37% in the number of pods per plant and a reduction of 39% in the number of grains per plant as well as in the combined use of carbendazim + thiram with imidacloprid, with a decrease of 40% in the number of pods per plant and 44% in the number of grains per plant, compared to plants of only inoculated seeds, that is, those from the additional treatment. For the other treatments, a reduction in the number of pods per plant between 8 and 33% was found and in the number of grains per plant, this reduction was of 10 to 33%, in relation to the only inoculant treatment.

As for the number of grains per pod (NGP) and 100-grain mass grains (MCG), no interference of the combinations of fungicide and insecticide occurred (Table 2), except between the factorial x control (additional) interaction for number of grains per pod and number of grains per plant. The productive components of the soybean crop are composed of the number of plants per area, number of pods per plant, number of grains per pod and mass of grains. The number of grains per pod shows less variation in different growing situations due to the uniformity of genetic improvement in the development of plants with a production capacity of two grains per pod, on average. However, there is a variability among the cultivars that allows the formation of pods with one, two, three and, less frequently, four grains (Mundstock & Tomas, 2005).

In addition, plants from seeds treated with carbendazim + thiram combined with polymer had significantly lower number of pods per plant than those with no fungicide treatment (Pereira *et al*., 2010a), as well as plants inoculated with *Bradyrhizobium japonicumm* (SEMIA 5079 and SEMIA 5080) and treated with carbendazim + thiram fungicide, produced lower number of pods per plant and MGP than those with no fungicide (Sediyama, 2012).

Hence, the lowest yields (PRO) (Table 2) were achieved from seedlings treated with the carbendazim + thiram mixed with imidacloprid, with a decrease of 45%, carbendazim + thiram mixed with fipronil with a reduction of 39%, carboxin + Thiram mixed with thiamethoxam with a reduction of 38%, difeconazole with fipronil, 31%, carboxin + thiram with imidacloprid 600 Fc, 27%, fludioxonil + mephenoxam with fipronil, 26% and carboxin + thiram with imidacloprid + thiodicarpe 23%, when compared to plants of only inoculated seeds, that is, from the additional treatment.

CONCLUSIONS

The joint application of fungicide and insecticide with inoculant does not affect nodulation, foliar N content and vegetative growth of the plants as well as the grain mass per plant and the 100-grain mass.

The use of carbendazim + thiram mixed with fipronil and carbendazim + thiram combined with imidacloprid provide smaller number of pods per plant and of grains per plant, reflecting in reductions in the production of soybean grains. Consequently, the fungicide carbendazim + thiram, regardless of the combined applied insecticide, is the most detrimental to *Bradyrhizobium* spp.

The combination of fungicides fludioxonil + metalaxyl-M, carboxin + thiram and difeconazole with the insecticides fipronil 250 SC and thiamethoxam, when associated with the inoculant *B. japonicum* (strains SEMIA 5079 and SEMIA 5080), cause small reductions in the productive characteristics of the plants when compared to the combinations carbendazim + thiram and imidacloprid + thiodicarpe and imodacloprid 600 FC, and should be preferred.s

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