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Compatibility among fungicide treatments on soybean seeds through film coating and inoculation with *Bradyrhizobium* strains

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ABSTRACT. This research evaluated the development of soybean plants grown in a greenhouse, from seeds that were treated with fungicide, film coated and inoculated with *Bradyrhizobium*. Seeds from the soybean cultivar 'Vencedora' were submitted to treatments disposed in a factorial 5 x 3 x 2 design. The seeds were treated with Derosal Plus® with polymer; Tegran® with polymer; Derosal Plus® without polymer, Tegran® without polymer; and without fungicide and without polymer. After the chemical treatment, the seeds were inoculated with peat or liquid inoculants, and without inoculant. The chemical treatments were used either previous to or after the six months of storage. Seed inoculation was performed with CPAC15 and BR29 strains, immediately before sowing in Leonard pots (two seedlings per pot). The plants at R_2 stage were evaluated through the number and dry matter weight of nodules, the shoot dry matter weight, and the chlorophyll content. The results indicated that the treatments with Derosal Plus or Tegran-associated or not with the polymer, and independent of the application time-did not affect nodule establishment and development, or nitrogen fixation when the seeds were inoculated. Liquid and peat inoculants applied to the seeds were equally efficient in the formation of nodules in soybean plants; however, the liquid inoculant promoted higher shoot dry matter weight.

Key words: *Glycine max*, polymer, Tegran®, Derosal Plus®, *Bradyrhizobium*.

RESUMO. Compatibilidade entre tratamento fungicida de sementes de soja via peliculização e inoculação com estirpes de *Bradyrhizobium*. Esta pesquisa foi conduzida com o objetivo de avaliar o desenvolvimento em casa-de-vegetação da cultura da soja, proveniente de sementes submetidas ao tratamento fungicida, peliculização e inoculação com *Bradyrhizobium*. Sementes da cultivar Vencedora foram submetidas a tratamentos dispostos em fatorial 5 x 3 x 2, sendo: sem fungicida e sem polímero, Derosal Plus® com polímero, Tegran® com polímero, Derosal Plus® sem polímero e Tegran® sem polímero; inoculante líquido, turfoso ou não-inoculadas; antes ou após seis meses de armazenamento. A inoculação das sementes foi realizada com as estirpes CPAC15 e BR29, imediatamente antes da semeadura em vasos tipo Leonard, onde se manteve duas plântulas por vaso. As plantas, em estágio R_2, foram avaliadas por meio de: número e matéria seca de nódulos, matéria seca da parte aérea e teor de clorofila. Conclui-se que o tratamento das sementes de soja com os fungicidas Derosal Plus® ou Tegran® associados ou não a polímeros, e independentemente da época de aplicação, não afeta a formação dos nódulos quando as sementes são inoculadas. Inoculantes líquido e turfoso aplicados às sementes são igualmente eficientes na formação de nódulos em plantas de soja, no entanto, o inoculante líquido promove maior matéria seca da parte aérea.

Palavras-chave: *Glycine max*, polímeros, Tegran®, Derosal Plus®, *Bradyrhizobium*.

Introduction

Soybean yield has increased with the acquisition of appropriate technologies by producers, the advance of research and the obtaining of new and more productive varieties that are less susceptible to adverse conditions (LINZMEYER JÚNIOR et al., 2008).

Associated with this yield increase is the reduction of costs in the soybean production, which has made the Brazilian soybean more competitive in the international market. Using the symbiosis between the plant and the *Bradyrhizobium* genus, decreases the cost of soybean production due to the lower amount of nitrogen fertilizers required

(ALVES et al., 2003; HUNGRIA et al., 2005; SENEVIRATNE et al., 2000).

In order to supply the necessary nitrogen to the soybean crop, symbiotic bacteria require favorable conditions to establish themselves. The strain used the dosage of inoculants, the fungicides used to treat the seeds and the environmental conditions are the main factors that interfere on the biological nitrogen fixation (BNF) (ALBINO; CAMPO, 2001; SCHOLLES; VARGAS, 2004; VIEIRA NETO et al., 2008). Additionally fungicides may present different toxicity levels, reducing the number of inoculant cells. This may cause a reduction in the number of nodules, thereby affecting the BNF (ANDRÉS et al., 1998; ANNAPURNA, 2005; BIKROL et al., 2005).

However, under certain environmental conditions, the treatment of soybean seeds with fungicides is essential to ensure an appropriate stand, to control important pathogens transmitted by seeds, and to reduce the chances of introducing pathogens in areas free of diseases. Furthermore, fungicide treatment helps maintain seed quality during storage periods (CARDOSO et al., 2004).

The use of fungicides in association with polymers used in film coating provides soybean seeds with severaleneficial agronomic traits. Among these benefits is an improved retention of fungicides (SAMPAIO; SAMPAIO, 1998; SILVEIRA, 1998), and insecticides and fungicides, among other products, that may act where they are really needed. The toxicity of such chemicals on nitrogen-fixing bacteria may be reduced. The use of polymers gives seeds additional protection against pathogens. In addition, polymers increase the safety during handling, due to the known toxic effect of fungicides and insecticides to humans (ROBANI, 1994).

The present study assessed the performance of soybean plants grown under greenhouse conditions, from seeds submitted to fungicide treatment, film coating and inoculation with *Bradyrhizobium* strains.

Material and methods

Soybean seeds of "Vencedora" cultivar were treated with either the thiabendazole + thiram (Tegran®) or carbendazin + thiram (Derosal Plus®) fungicides. They were treated at commercial doses of 200 mL 100 kg^{-1} seed (17 + 0.7 g a.i. kg^{-1} of seeds and 0.3 + 0.7 g a.i. kg^{-1} of seeds, respectively) associated or not with film coating (200 mL polymers 100 kg^{-1} seed). The control did not receive any treatment. The treatments were undertaken in two phases: at the moment of sowing, and six months before sowing (anticipated treatments). After product application, the seeds were stored in multiple layer packing, under conventional storage conditions.

Treatments were arranged in a completely randomized 5 x 3 x 2 factorial design. Each had three repetitions. The chlorophyll content and nodule numbers were transformed to $\sqrt{(X+0.5)}$ and then submitted to ANOVA and Tukey tests.

Inoculation was performed at the time of sowing using commercial liquid or peat inoculant (Stoller®). These contained the *Bradyrhizobium* strains SEMIA 5079 (CPAC15 - *B. japonicum*) and SEMIA 5019 (BR29 - *B. elkanii*). The liquid inoculants had a minimum concentration of 4 x 10^9 of viable cells $gram^{-1}$, at a dose of 220 mL 50 kg^{-1} seed. The peat inoculants used 220 g 50 kg^{-1} of seeds pre-moistened with 300 mL of sucrose solution at 10%, as commercially recommended.

The seeds were sown in Leonard jars having washed-sand + vermiculite (1:1) as a substrate. The nutritive solution was composed by 10 mL of K_2HPO_4 (2%); 10 mL of $MgSO_4$ $7H_2O$ (2%) + NaCl (2%); 30 mL of $CaHPO_4$ (10%); 10 mL of $FeCl_3$. $6H_2O$ (1.4%); 1 mL of micronutrient solution (2.86 g of H_3BO_3, 2.03 g of $MnSO_4$ $4H_2O$, 0.22 g of $ZnSO_4$ $7H_2O$, 0.08 g of $CuSO_4$ $5H_2O$ and 0.09 g of $NaMoO_4$ H_2O per liter). The volume was completed to four liters, and the pH was adjusted to 6.7 (VINCENT, 1970). The jars, substratum (0.7 L), and nutrient solution (0.7 L) were sterilized in an autoclave for 60 minutes, at 120°C.

Four seeds were sown in each jar and after emergency of seedlings, only two plants were left. The anticipated treatment seeds (which were treated six months before sowing) were not disinfected, since disinfection by moisture can rupture the tegument. The jars were kept in a greenhouse and the volume of nutritious solution was added when necessary.

At full flowering (R_2), the plants were evaluated for the following characteristics: nodule number and dry matter weight per plant (dried at 60°C in a forced air oven, up to constant weight); shoot dry matter weight (dried at 60°C in a forced air oven, up to constant weight) and chlorophyll content (determined from leaves localized at the superior and medium part of the plant, using a SPADI chlorophyllometer).

The nodule number and chlorophyll content were transformed to $\sqrt{(X+0.5)}$ and submitted to ANOVA. The data were analyzed by the SISVAR package, version 4.0. The averages between the chemical treatments and the inoculants were compared by the Tukey test, at a level of 5% of probability.

Results and discussion

The ANOVA analyses evidenced significant interactions in fungicides x inoculants x storage for nodule number, dry matter weight and chlorophyll content. Interactions inoculants x fungicides and storage of seeds for shoot dry matter weight were also observed.

The results of the nodule numbers per plant did not present significant differences between applied fungicides, either before to or after seed storage, when the seeds were inoculated (Table 1). Similar results were observed by Revellin et al. (1993), who observed that the fungicides Quinolate Pro® (carbendazim and copper oxide), Vitavax 200 FF® (carboxin and thiram) and Monceren® (pencycuron) had little or no effect on *B. japonicum* survival, and on soybean nodulation and production. Bueno et al. (2003) also did not find significant effects of these fungicides on the nodulation of soybean cv. 'Embrapa 48', grown in pots with soil and inoculated with the same strains.

Nevertheless, the action on *B. japonicum* by the fungicides thiran (ANDRÉS et al., 1998); Germipro UFB® (carbendazin and iprodione); Apron 35J (metalaxyl); and Tachigaren® (hymexazol) (REVELLIN et al., 1993) can reduce the bacteria survival and soybean nodulation.

For seeds that were not inoculated, low nodule formation was observed. This is probably related to the low number of viable cells of *Bradyrhizobium* because, in these conditions, the seeds had only natural contamination by nitrogen-fixing bacteria. Despite the larger variation in the nodule numbers to the random contamination, significant harmful effects of the treatments were not observed.

The dry matter of nodules was significantly higher when the seeds were inoculated with peat or liquid inoculants.

Dry matter of nodules from plants originating from inoculated seeds with liquid or peat inoculants did not show significant differences among the chemical treatments used before or after the storage.

The nodule number and dry matter weight observed in the different treatments corroborate the results obtained by Peres et al. (1993), which are between 15 to 30 nodules or 100 to 200 mg of dry mater of nodules during flowering time.

The results of shoot dry matter weight showed no effects from the chemical factor treatment when the seeds were inoculated with peat or not inoculated (Table 2). However, for the seeds inoculated with liquid, shoot dry matter weight showed higher values for the treatments with Tegran® and polymer when compared with plants from seeds treated with Derosal Plus® with or without polymer, and Tegran® without polymer. However, none of these treatments differed from the treatments without fungicide and without polymer, indicating the absence of toxic effects from the fungicides and polymer applied.

Plants stemming from seeds treated with chemical products before storage presented a significant decrease in shoot dry matter weight (1.69 g) when compared with the seeds treated after storage (2.00 g). This was probably due to the harmful effects of the permanence of the fungicides with the seeds during storage (KROHN; MALAVASI, 2004), which negatively affected plant development.

The results of leaf chlorophyll content did not show significant differences among the treatments independently of the fungicide treatment, the time of application and the type of used inoculant. This shows that these products do not affect the bacteria used in the inoculation or fixation of nitrogen (Table 3).

Peat or liquid inoculants did not show significant differences, but they showed, in general, a higher content of leaf chlorophyll compared to the leaves of the plants originating from not inoculated seeds.

Table 1. Nodule number and dry matter weight (g) in soybean plants originating from seeds treated or not with fungicide, before or after the storage period, associated or not to polymers, and inoculated or not with *Bradyrhizobium* strains.

Time	Chemical	*Bradyrhizobium*			*Bradyrhizobium*		
		Without	Peat	Liquid	Without	Peat	Liquid
		Number of nodules			Nodule dry matter (g)		
Before	Without fungicide, without polymer	6 b B	58 a A	69 a A	0.018 a B	0.163 a A	0.194 ab A
	Derosal Plus®, with polymer	16 ab B	58 a A	65 a A	0.047 a B	0.152 a AB	0.229 ab A
	Tegran®, with polymer	25 ab B	49 a AB	85 a A	0.029 a C	0.200 a B	0.310 a A
	Derosal Plus®, without polymer	44 a A	56 a A	67 a A	0.089 a B	0.214 a A	0.155 b AB
	Tegran®, without polymer	3 b B	61 a A	69 a A	0.004 a B	0.140 a A	0.230 ab A
After	Without fungicide, without polymer	30 abc B	65 a AB	80 a A	0.025 b B	0.200 a A	0.274 a A
	Derosal Plus®, with polymer	75 a A	51 a A	68 a A	0.235 a A	0.268 a A	0.233 a A
	Tegran®, with polymer	6 c B	59 a A	77 a A	0.034 b C	0.170 a B	0.290 a A
	Derosal Plus®, without polymer	16 cb B	69 a A	93 a A	0.055 b C	0.204 a B	0.335 a A
	Tegran®, without polymer	37 ab A	57 a A	76 a A	0.090 b B	0.178 a AB	0.273 a A

Means followed by the same capital letter in the line and lowercase in the column, for each time of seed evaluation do not differ statistically amongst themselves by Tukey's test at 5% probability.

Table 2. Shoot dry matter weight (g) of soybean plants originating from fungicide-treated seeds, associated or not to polymers, and inoculated or not with *Bradyrhizobium* strains.

Chemical	*Bradyrhizobium*		
	Without	Peat	Liquid
Without fungicide, without polymer	1.16 a B	1.68 a B	2.35 ab A
Derosal Plus®, with polymer	1.52 a B	1.99 a AB	2.17 b A
Tegran®, with polymer	0.87 a C	1.91 a B	2.99 a A
Derosal Plus®, without polymer	1.46 a B	2.22 a A	2.25 b A
Tegran®, without polymer	1.19 a B	1.68 a AB	2.22 b A

Means followed by the same capital letter in the line and lowercase in the column for each time of seed evaluation do not differ statistically amongst themselves by Tukey's test at 5% probability.

Table 3. Leaf chlorophyll content (chlorophyllometer SPADI) in soybean plants originating from inoculated or non-inoculated seeds, treated or not with fungicide, associated or not to polymers, before to or after the storage period.

Time	Chemical	*Bradyrhizobium*		
		Without	Peat	Liquid
Before	Without fungicide, without polymer	44 b B	303 a A	361 a A
	Derosal Plus®, with polymer	143 ab B	324 a A	303 a A
	Tegran®, with polymer	160 ab B	301 a AB	358 a A
	Derosal Plus®, without polymer	210 a A	307 a A	368 a A
	Tegran®, without polymer	133 ab B	269 a AB	357 a A
After	Without fungicide, without polymer	50 c B	324 a A	322 a A
	Derosal Plus®, with polymer	317 a A	348 a A	337 a A
	Tegran®, with polymer	48 c B	346 a A	358 a A
	Derosal Plus®, without polymer	137 bc B	373 a A	344 a A
	Tegran®, without polymer	181 ab B	365 a A	350 a A

Means followed by the same capital letter in the line and lowercase in the column for each time of seed evaluation do not differ statistically amongst themselves by Tukey's test at 5% probability.

Conclusion

The treatment of soybean seeds with the fungicides Derosal Plus® or Tegran®, associated or not with polymer, and independently of the application time, did not affect the establishment and development of nodules and the nitrogen fixation when the seeds were inoculated with *Bradyrhizobium* strains SEMIA 5019 (BR29) and SEMIA 5079 (CPAC15). Liquid and peat inoculants applied to the seeds were equally efficient in the formation of nodules in soybean plants; however, liquid inoculants promoted higher values of shoot dry matter weight.

References

ALBINO, U. B.; CAMPO, R. J. Efeito de fontes e doses de molibdênio na sobrevivência do *Bradyrhizobium* e na fixação biológica de nitrogênio em soja. **Pesquisa Agropecuária Brasileira**, v. 36, n. 3, p. 527-534, 2001.

ALVES, B. J. R.; BODDEY, R. M.; URQUIAGA, S. The success of BNF in soybean in Brazil. **Plant and Soil**, v. 252, n. 1, p. 1-9, 2003.

ANDRÉS, J. A.; CORREA, N. S.; ROSAS, S. B. Survival and symbiotic properties of *Bradyrhizobium japonicum* in the presence of thiram: isolation of fungicide resistant strains. **Biology and Fertility of Soils**, v. 26, n. 2, p. 141-145, 1998.

ANNAPURNA, K. *Bradyrhizobium japonicum*: survival and nodulation of soybean as influenced by fungicide treatment. **Indian Journal of Microbiology**, v. 45, n. 4, p. 305-307, 2005.

BIKROL, A.; SAXENA, N.; SINGH, K. Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicides seed treatment. **African Journal of Biotechnology**, v. 4, n. 7, p. 667-671, 2005.

BUENO, C. J.; MEYER, M. C.; SOUZA, N. L. Efeito de fungicidas na sobrevivência de *Bradyrhizobium japonicum* (Semia 5019 e Semia 5079) e na nodulação da soja. **Acta Scientiarum. Agronomy**, v. 25, n. 1, p. 231-235, 2003.

CARDOSO, P. C.; BAUDET, L.; PESKE, S. T.; LUCCA FILHO, O. A. Armazenamento em sistema a frio de sementes de soja tratadas com fungicida. **Revista Brasileira de Sementes**, v. 26, n. 1, p. 15-23, 2004.

HUNGRIA, M.; FRANCHINI, J. C.; CAMPO, R. J.; GRAHAM, P. H. The importance of nitrogen fixation to soybean cropping in South America. In: WERNER, D.; NEWTON, W. E. (Ed.). **Nitrogen fixation in agriculture**: forestry ecology and environment. Dordrecht: Kluwer Academic Publishers, 2005. p. 25-42.

KROHN, N. G.; MALAVASI, M. M. Qualidade fisiológica de sementes de soja tratadas com fungicidas durante e após o armazenamento. **Revista Brasileira de Sementes**, v. 26, n. 2, p. 91-97, 2004.

LINZMEYER JUNIOR, R.; GUIMARÃES, V. F.; SANTOS, D. E.; BENCKE, M. H. Influência de retardante vegetal e densidades de plantas sobre o crescimento, acamamento e produtividade da soja. **Acta Scientiarum. Agronomy**, v. 30, n. 3, p. 373-379, 2008.

PERES, J. R. R.; MENDES, I. C.; SUHET, A. R.; VARGAS, M. A. T. Eficiência e competitividade de estirpes de rizóbio para soja em solos de cerrado. **Revista Brasileira de Ciência do Solo**, v. 17, n. 2, p. 357-363, 1993.

REVELLIN, C.; LETERME, P.; CATROUX, G. Effect of some fungicide seed treatments on the survival of *Bradyrhizobium japonicum* and on the nodulation and yield of soybean [*Glycine max* (L.) Merr.]. **Biology and Fertility of Soils**, v. 16, n. 3, p. p. 211-214, 1993.

ROBANI, H. Film coating horticultural seed. **Hort Technology**, v. 4, n. 2, p. 104-105, 1994.

SAMPAIO, N. V.; SAMPAIO, T. G. Sementes: com as cores da eficiência. **A granja do ano**, v. 54, n. 12, p. 16-18, 1998.

SCHOLLES, D.; VARGAS, L. K. Viabilidade da inoculação de soja com estirpes de *Bradyrhizobium* em solo inundado. **Revista Brasileira de Ciência do Solo**, v. 28, n. 6, p. 973-979, 2004.

SENEVIRATNE, G.; VAN HOLM, L. H. J.; EKANAYAKE, E. M. H. G. S. Agronomic benefits of rhizobial inoculant use over nitrogen fertilizer application in tropical soybean. **Field Crops Research**, v. 68, n. 3, p. 199-203, 2000.

SILVEIRA S. Recobertura como medida para proteção da semente. **Seed News**, v. 5, n. 4, p. 34-35, 1998.

VIEIRA NETO, S. A.; PIRES, F. R.; MENEZES, C. C. E.; MENEZES, J. F. S.; SILVA, A. G.; SILVA, G. P.; ASSIS, R. L. Formas de aplicação de inoculante e seus efeitos sobre a nodulação da soja. **Revista Brasileira de Ciência do Solo**, v. 32, n. 2, p. 861-870, 2008.

VINCENT, J. M. **A manual for practical study of the root-nodule bacteria**. Oxford: Scientific Publications, 1970.

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