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Seed treatment and pre-inoculation of soybean: effect of storage period and agrochemicals on the physiological quality of seed and yield

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Industrial seed treatment of soybean [*Glycine max* (L.) Merr.] has increased over the last years. New technologies have been developed to allow the inoculation procedure, which is traditionally done at the day of sowing, to be performed with the industrial treatment days or even weeks before sowing. Since little is known about the compatibility of agrochemicals and pre-inoculation, the objective of this study was to determine whether the storage period and the combination of fungicides and insecticides could negatively affect the physiological quality and yield of soybean seeds that were pre-inoculated. Soybean seeds received fourteen seed treatments that consisted of different fungicides and insecticides and were pre-inoculated with *Bradyrhizobium elkanii.* The seeds were treated and stored for 51 days until it was sown in the field. Every 17 days the physiological quality of the seed was assessed. The results shown that pre-inoculation did not affect the physiological quality of seeds. However, some combinations of agrochemicals, as well as storage period reduced seed vigor and seed germination, while increased abnormal seedlings. The findings of this study indicated that some combinations of fungicides and insecticides can have adverse effect on the physiological quality of seed that is stored for up to 51 days before sowing, but none of them jeopardized the nodulation and soybean yield under field conditions.

Key words: Biological nitrogen fixation, *Bradyrhizobium*, compatibility, fungicide, *Glycine max*, HiCoat, industrial seed treatment, insecticide, polymer.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most important agricultural crops grown around the world.

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Numerous pathogens (Henning, 2005) and pests (Hoffman-Campo et al., 2000) can cause yield losses by jeopardizing seed germination and initial establishment of plants under field conditions. Hence, seed treatment has been considered as a cost-effective tool that can be used to manage pests and diseases that occur soon after sowing (Goulart, 1998; Pereira et al., 2010a).

Industrial seed treatment of soybean is a practice that has gained more prominence over the last years (França-Neto et al., 2015). Immediately after processing, seed producer companies treat, bag and then store the seeds until sowing (Conceição et al., 2016; Brzezinski et al., 2017). Since growers do not need to treat the seeds on-farm, the sowing process is optimized (Brzezinski et al., 2015), which permits to sow the crop during the most ideal period to achieve high yields (Meotti et al., 2012). In addition, there is no need for the growers to have specific machines to perform the seed treatment on-farm (Brzezinski et al., 2017), which reduces the exposure of workers to toxic products (Abrasem, 2017).

Nevertheless, industrial seed treatments still face many challenges that need to be addressed regarding the storage period of the seed and the compatibility of agrochemicals with inoculants. Previous studies demonstrated that seed treatment with fungicides and insecticides (Pereira et al., 2010a; Dan et al., 2012; Ferreira et al., 2016; Camilo et al., 2017) as well as the period of storage (Krohn and Malavasi, 2004; Dan et al., 2010; Piccinin et al., 2013) can have negative effects on physiological quality of soybean seed. As a result, seed with low vigor can decrease seedling emergence (Khaliliaqdam et al., 2012) and reduce yield (Scheeren et al., 2010), which could seriously lead to unfeasibility of industrial seed treatments.

Inoculation of nitrogen-fixing bacteria of the *Bradyrhizobium* genus is a very important component of soybean production systems (Hungria et al., 2006; Salvagiotti et al., 2008); therefore, it needs to be considered by the companies that offer industrial seed treatment to the growers. Inoculation been performed traditionally at the day of sowing; however, due to the reduction of efficiency of the sowing process; pre-inoculation has been assessed as an alternative to the conventional inoculation (Zilli et al., 2010; Silva Junior et al., 2016; Anghinoni et al., 2017). Pre-inoculation is a practice that consists of inoculating soybean seeds days or even weeks before sowing (Anghinoni et al., 2017); therefore, growers do not need to worry about rushing and inoculating the seeds at the day of sowing.

Fungicides and insecticides applied to the seeds can be harmful to *Bradyrhizobium* spp. (Campo et al., 2009; Campo et al., 2010; Pereira et al., 2010b; Zilli et al., 2010; Costa et al., 2013; Gomes et al., 2017), and this is a challenge that needs to be addressed. Thus, new technologies have been developed with the aim of enabling pre-inoculation on soybean seeds by adding polymers to the inoculant (Fernandes Júnior et al., 2009; Silva Júnior et al., 2012), which allows better survival of the bacteria without negatively affect nodulation and crop yield (Pereira et al., 2010a).

The advancement of industrial seed treatments depends on the identification of products that could be combined with pre-inoculation without compromising the symbiotic relationship of soybean with *Bradyrhizobium.* Hence, the objectives of this study were to investigate whether the storage period and the combination of fungicides and insecticides can negatively affect the physiological quality, nodulation and yield of soybean seeds that were pre-inoculated with *B. elkanii*.

MATERIALS AND METHODS

The study was carried out in two stages: i) evaluation of the physiological quality of soybean seeds in laboratory, and ii) evaluation of nodulation and soybean yield under field conditions.

Seed treatments with agrochemicals

All fungicides and insecticides used in this study were commercial products recommended for seed treatment (Table 1). The dose used was the one in the label recommended by the manufacturer. The products were mixed according to their respective doses, and water was added to bring the volume to 5 ml. The mixture was applied on 1.5 kg of seed of the soybean cultivar 'Nidera 7310' that was previously placed into a plastic bag, following by agitation to uniformly distribute the products on the seeds. The bags were maintained on a laboratory bench for one hour for the seeds to dry and then pre-inoculation was performed. A non-treated check without application of fungicide and insecticide was added to the experiment.

Pre-inoculation procedure

Pre-inoculation was performed with commercial products manufactured and commercialized by BASF S/A using the HiCoat® technology, which consists of 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and SEMIA 5019 at 5 x 10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and SEMIA 5019 at 5 x 10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water. A volume of 9.5 ml kg^{-1} was applied on the seeds, which were maintained on a laboratory bench to dry for an hour.

Physiological quality of seed

The experiment was carried out under controlled conditions in the Laboratory of Field Crops at the Universidade Estadual do Centro-Oeste (UNICENTRO) in Guarapuava, Paraná, Brazil. The experiment design was a completely randomized with six replications. The treatments consisted of a factorial arrangement of four periods (0, 17, 34 and 51 days after treatment) and fourteen seed treatments (Table 1). After treatment with the agrochemicals and pre-inoculation, seeds were stored in paper bags and maintained at room temperature. The physiological quality of seed was evaluated over time with samples from the same paper bag. Fifty soybean seeds were arranged in a "germitest" paper previously soaked in distilled water for a period of 24 h, and another sheet was used to cover the seeds. The paper sheets were

Table 1. Treatments used in this study to evaluate the effect of fungicides and insecticides associated to pre-inoculation of *Bradyrhizobium elkanii* using the HiCoat® technology in soybean seeds.

Treatment	Active ingredient	Type of active ingredient[a]	Trade name	Dose of commercial product (ml kg^{-1})[b]
1	Non-treated and non-inoculated control	-	-	-
2	Pre-inoculation (PI)[c]	-	-	-
3	PI + Thiamethoxan	I	Cruiser®	2
4	PI + Carbendazim + Thiram + Thiamethoxan	F + F + I	Derosal® + Cruiser®	2 + 2
5	PI + Carbendazim + Thiram + Imidacloprid	F + F + I	Derosal® + Gaucho®	2 + 2
6	PI + Carbendazim + Thiram + Fipronil	F + F + I	Derosal® + Standak®	2 + 2
7	PI + Imidacloprid	I	Gaucho®	2
8	PI + Fludioxonil + Metalaxyl-M	F + F	Metaxyl-M®	1
9	PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarb	F + F + I + I	Metalaxyl-M® + CropStar®	1 + 2
10	PI + Fludioxonil + Metalaxyl-M + Thiamethoxan	F + F + I	Metalaxyl-M® + Cruiser®	1 + 2
11	PI + Fludioxonil + Metalaxyl-M + Imidacloprid	F + F + I	Metalaxyl® + Gaucho®	1 + 2
12	PI + Fludioxonil + Metalaxyl-M + Fipronil + Thiophanate-methyl + Pyraclostrobin	F + F + I + F + F	Metalaxyl-M® +Standak® Top	1 + 2
13	PI + Fipronil	I	Standak®	2
14	PI + Fipronil + Thiophanate-methyl + Pyraclostrobin	I + F + F	Standak® Top	2

[a]Type of product: I = insecticide; F = fungicide.
[b]All products were mixed and distilled water was used to bring the volume to 5 ml that was applied to one kilogram of seed.
[c]Pre-inoculation was performed with the HiCoat® Technology: a mixture of 300 g of peat inoculant (Adhere HC®, *Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water.

rolled and placed in an incubator at 25°C in the dark. Each sheet was considered as a replication. At the fifth day, seed vigor was evaluated according to Brasil (2009). The rolled papers were returned to the incubator for three more days and then germination, abnormal seedlings and non-germinated seeds were determined according to Brasil (2009). All data were expressed as percentage (%) of the total seeds placed on each paper sheet.

Nodulation and soybean yield

The study was carried out under field conditions during the cropping season 2009/2010 at Santa Cruz Farm in Guarapuava, Paraná, Brazil. The field is located in a region with humid subtropical climate (Cfb) and at approximately 1,100 m of altitude. The soil of the experimental area is classified as a Brown Latosol (Embrapa, 2013).

The experimental design was a randomized complete block with four replications and 16 treatments. Soybean seeds that were treated, pre-inoculated and stored for 51 days were used in this experiment. In addition to the treatments used for evaluation of physiological quality of seed, one treatment with application of nitrogen fertilizer and one treatment with standard inoculation at the day of sowing were added as controls. The experimental plot was composed of four rows spaced 0.40 m apart with 5.5 m length. The useful area for evaluation corresponded to the two central rows without 0.50 m of each extremity for 3.60 m².

Desiccation of triticale (X *Triticosecale* Wittimack) was performed with glyphosate herbicide (720 g ha^{-1} a.i.) 30 days before sowing, which was performed on 15 Dec 2009. Fertilization was performed at the day of sowing of the triticale crop with 14 kg ha^{-1} of N, 34 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O. The soybean crop was not fertilized. This protocol follows the standard procedure done by the farmer. Management of weeds, pests and diseases was performed when needed following the appropriate recommendation.

The number of plants was counted at V2 growth stage to determine the density of plants. Nodulation was evaluated at full flowering (R2) stage. Three soybean plants were selected from each plot, and the root system was collected by placing a cylindrical metal device with 10 cm diameter and 10 cm height. The soil was washed off the roots with tap water, and the nodules were collected with a metal screen and counted. The nodules were placed in paper bags that were maintained in a drying oven at 65ºC for 72 h. Dry mass of the nodules was then determined in mg per plant.

The plants in the two central rows of the experimental plot were manually harvested and threshed. The soybean seeds were weighted and seed moisture was measured.

Table 2. Mean square values for the effects of seed treatment and storage period on the physiological quality of soybean seeds.

Effect	Degrees of freedom	Mean square values			
		Seed vigor[a]	Seed germination[a]	Abnormal seedlings[a]	Non-germinated seeds[a]
Seed treatment (ST)	13	2.65 **	1.96 **	7.12 **	0.49 **
Storage period (SP)	3	6.27 **	1.15 **	4.94 **	0.41 ns
ST x SP	39	0.43 **	0.33 **	1.19 **	0.16 ns
Error	280	0.15	0.11	0.47	0.16
Mean		8.85	9.11	4.15	1.23
Coefficient of variation (%)		4.42	3.59	16.52	32.08

[a]Variables in percentage were previously transformed to √x+1 for statistical analysis. [ns] non-significant and ** significant at 1% probability level.

Soybean yield was determined in kg ha^{-1} at 13% moisture. A sample was taken and used to determine the thousand-grain mass by counting and weighing 300 grains.

Data analysis

The variables expressed as a percentage were transformed to √(x + 1). All data were submitted to analysis of variance. The means of seed vigor, seed germination, abnormal seedlings and non-germinated seeds were compared by Scott Knott's test at 5% probability level, whereas the means of nodulation and soybean yield were compared by Tukey's test a 5% probability level. All the analyses were performed using the statistical program Sisvar 5.6 (Ferreira, 2000).

RESULTS AND DISCUSSION

Physiological quality of seed

Seed vigor, seed germination and abnormal seedlings were significantly affected by the seed treatment, the storage period and the interaction, whereas the percentage of non-germinated seeds was significantly influenced by the seed treatments (Table 2).

Vigor is one of the main characteristics regarding to the physiological quality of seed that need to be considered for an appropriate establishment of a crop in the field (Scheeren et al., 2010). Storage for 51 days did not significantly affect seed vigor for the non-inoculated and non-treated control as well as for the pre-inoculation with HiCoat® technology (Table 3). This indicates that the use of inoculant with polymer did not jeopardize the physiological quality of seed similarly to what was reported by other authors (Conceição et al., 2016).

Application of the insecticide imidacloprid associated with the fungicides fludioxonil + metalaxyl-M and with carbendazim + thiram had the lowest seed vigor (Table 3) and lowest seed germination (Table 4) compared to the other treatments previous to the storage as well as 51 days after storage of the seeds. These effects were associated with increase in the percentage of abnormal seedlings (Table 5), but did not affect the percentage of non-germinated seeds (Table 6). Application of imidacloprid without association with any of the fungicides caused significant reduction of seed vigor after 51 days of storage (Table 3). Seed treatment with carbendazim + thiram + thiamethoxan, fludioxonil + metalaxyl-M + imidacloprid + thiodicarbe, fludioxonil + metalaxyl-M + thiamethoxan also reduced seed vigor compared to the control after 51 days of storage of the seeds (Table 3). Previous studies did not observe a negative effect of application of imidacloprid only (Castro et al., 2008; Dan et al., 2013, 2012), however, the association of imidacloprid + thiodicarb reduced vigor and germination of soybean seeds (Dan et al., 2012; Bortoletto et al., 2017). Sometimes when products are combined, there can be modification in some characteristics of the mixture that become more toxic to the seeds compared to the product alone. Moreover, the effect of the toxicity might not only be due to the active ingredients but also due to some component added in the commercial product to serve as a vehicle to the active ingredient (Kintschev et al., 2014).

Several studies had shown that the application of the insecticide thiamethoxan negatively affect the physiological quality of seed (Castro et al., 2008; Piccinin et al., 2013). In this study, no significant difference regarding to seed vigor was observed from the non-inoculated and non-treated control when only thiamethoxan was applied to the pre-inoculated seeds even after 51 days of storage (Table 3). However, there was reduction in seed germination after 51 days of storage when thiamethoxan was applied isolated as well as associated with fludioxonil + metalaxyl-M (Table 4). These results are contrary to what was observed by other authors (Barros et al., 2001; Dan et al., 2012; Dan et al., 2013; Ferreira et al., 2016; Bortoletto et al., 2017), where no negative effects of thiamethoxan on physiological quality of seed occurred. The differences among studies could be due to the storage conditions, dose applied and

Table 3. Vigor (%)[a,b] of soybean seeds treated with different associations of fungicides and insecticides, pre-inoculated[c] with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

Seed treatment	Storage period (days) after seed treatment			
	0	17	34	51
Non-inoculated and non-treated control	84.0^{aA}	84.0^{aA}	86.0^{aA}	79.0^{aA}
Pre-inoculation (PI)	87.0^{aA}	85.7^{aA}	84.0^{aA}	79.0^{aA}
PI + Thiamethoxan	85.3^{aA}	77.3^{bA}	82.0^{aA}	72.7^{aB}
PI + Carbendazim + Thiram + Thiamethoxan	77.7^{bA}	83.0^{aA}	73.3^{bA}	66.7^{bB}
PI + Carbendazim + Thiram + Imidacloprid	69.0^{cB}	73.0^{bA}	76.3^{bA}	62.7^{bB}
PI + Carbendazim + Thiram + Fipronil	91.3^{aA}	76.0^{bB}	75.0^{bB}	80.3^{aB}
PI + Imidacloprid	85.3^{aA}	66.7^{bC}	76.3^{bB}	65.7^{bC}
PI + Fludioxonil + Metalaxyl-M	84.3^{aA}	82.7^{aA}	83.0^{aA}	73.3^{aB}
PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe	79.7^{bA}	78.3^{aA}	79.0^{aA}	62.7^{bB}
PI + Fludioxonil + Metalaxyl-M + Thiamethoxan	81.3^{bA}	82.0^{aA}	85.7^{aA}	69.7^{aB}
PI + Fludioxonil + Metalaxyl-M + Imidacloprid	57.0^{dB}	72.3^{bA}	63.0^{cB}	62.0^{bB}
PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin	86.7^{aA}	83.0^{aA}	84.7^{aA}	75.0^{aB}
PI + Fipronil	88.7^{aA}	72.7^{bA}	83.7^{aA}	71.0^{aB}
PI + Fipronil + Tiophanate-methyl + Pyraclostrobin	90.3^{aA}	83.7^{aA}	82.3^{aA}	76.0^{aB}

[a]Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. [b]Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c]Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water.

Table 4. Germination (%)[a,b] of soybean seeds treated with different associations of fungicides and insecticides, pre-inoculated[c] with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

Seed treatment	Storage period (days) after seed treatment			
	0	17	34	51
Non-inoculated and non-treated control	84.0^{bA}	89.0^{aA}	88.7^{aA}	83.0^{bA}
Pre-inoculation (PI)	88.3^{aA}	87.0^{aA}	85.7^{aA}	88.0^{aA}
PI + Thiamethoxan	85.3^{bA}	81.3^{bB}	87.0^{aA}	78.0^{cB}
PI + Carbendazim + Thiram + Thiamethoxan	78.3^{bB}	85.0^{aA}	83.7^{bA}	76.3^{cB}
PI + Carbendazim + Thiram + Imidacloprid	69.0^{cB}	79.7^{bA}	80.7^{bA}	73.0^{cB}
PI + Carbendazim + Thiram + Fipronil	91.3^{aA}	79.0^{bB}	82.7^{bB}	89.7^{aA}
PI + Imidacloprid	85.3^{bA}	75.3^{bB}	79.3^{bB}	73.7^{cB}
PI + Fludioxonil + Metalaxyl-M	85.0^{bA}	86.3^{aA}	86.7^{aA}	81.7^{bA}
PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe	80.3^{bA}	83.0^{bA}	81.3^{bA}	79.3^{bA}
PI + Fludioxonil + Metalaxyl-M + Thiamethoxan	83.3^{bA}	87.7^{aA}	86.7^{aA}	76.0^{cB}
PI + Fludioxonil + Metalaxyl-M + Imidacloprid	60.3^{dC}	79.0^{bA}	68.3^{cB}	69.0^{cB}
PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin	87.3^{aA}	86.3^{aA}	89.7^{aA}	82.0^{bA}
PI + Fipronil	88.7^{aA}	81.0^{bB}	86.3^{aA}	78.0^{cB}
PI + Fipronil + Tiophanate-methyl + Pyraclostrobin	90.3^{aA}	87.3^{aA}	86.0^{aA}	80.0^{bB}

[a]Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. [b]Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c]Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water.

application procedure. This demonstrates the importance of performing multiple studies under different conditions to better assess the effect of agrochemicals in the physiological quality of seed.

Previous to the storage, germination was higher than the non-treated and non-inoculated control when soybean

Table 5. Abnormal seedlings (%)[a,b] of soybean from seeds treated with different associations of fungicides and insecticides, pre-inoculated[c] with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

Seed treatment	Storage period (days) after seed treatment			
	0	17	34	51
Non-inoculated and non-treated control	15.7 c A	11.0 b A	11.3 c A	15.6 b A
Pre-inoculation (PI)	11.7 d A	13.0 b A	13.3 c A	12.0 b A
PI + Thiamethoxan	14.0 d A	17.0 a A	12.0 c A	19.7 b A
PI + Carbendazim + Thiram + Thiamethoxan	21.0 c A	14.7 b B	16.0 b B	23.0 a A
PI + Carbendazim + Thiram + Imidacloprid	30.7 b A	20.0 a B	18.7 b B	26.7 a A
PI + Carbendazim + Thiram + Fipronil	8.7 d B	20.0 a A	16.3 b A	10.3 c B
PI + Imidacloprid	13.7 d B	22.3 a A	18.3 b B	25.3 a A
PI + Fludioxonil + Metalaxyl-M	15.0 c A	13.3 b A	12.3 c A	17.3 b A
PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe	19.7 c A	16.0 a A	18.3 b A	20.0 b A
PI + Fludioxonil + Metalaxyl-M + Thiamethoxan	16.0 c B	12.3 b B	13.3 c B	23.7 a A
PI + Fludioxonil + Metalaxyl-M + Imidacloprid	39.0 a A	20.7 a B	31.7 a A	29.7 a A
PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin	12.3 d A	12.3 b A	10.0 c A	17.7
PI + Fipronil	11.3 d B	18.0 a A	12.3 c B	19.7 b A
PI + Fipronil + Tiophanate-methyl + Pyraclostrobin	9.3 d B	11.0 b B	13.3 c B	19.0 b A

[a]Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. [b]Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c]Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water.

Table 6. Non-germinated seeds (%)[a,b] of soybean treated with different associations of fungicides and insecticides, pre-inoculated[c] with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

Seed treatment	Storage period (days) after seed treatment			
	0	17	34	51
Non-inoculated and non-treated control	0.3 aA	0.0 bA	0.0 aA	0.7 aA
Pre-inoculation (PI)	0.0 aA	0.0 bA	1.0 aA	0.0 aA
PI + Thiamethoxan	0.7 aA	1.7 bA	1.0 aA	2.3 aA
PI + Carbendazim + Thiram +Thiamethoxan	0.7 aA	0.3 bA	0.3 aA	0.7 aA
PI + Carbendazim + Thiram + Imidacloprid	0.3 aA	0.3 bA	0.7 aA	0.3 aA
PI + Carbendazim + Thiram + Fipronil	0.0 aA	1.0 aA	1.0 aA	0.0 aA
PI + Imidacloprid	1.0 aA	2.3 aA	2.3 aA	1.0 aA
PI + Fludioxonil + Metalaxyl-M	0.0 aA	0.3 bA	1.0 aA	1.0 aA
PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe	0.0 aA	1.0 aA	0.3 aA	0.7 aA
PI + Fludioxonil + Metalaxyl-M + Thiamethoxan	0.7 aA	0.0 bA	0.0 aA	0.3 aA
PI + Fludioxonil + Metalaxyl-M + midacloprid	0.7 aA	0.3 bA	0.0 aA	1.3 aA
PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methy + Pyraclostrobin	0.3 aA	1.3 aA	0.3 aA	0.3 aA
PI + Fipronil	0.0 bA	1.0 aA	1.3 aA	2.3 aA
PI + Fipronil + Tiophanate-methyl + Pyraclostrobin	0.3 aA	1.7 aA	0.7 aA	1.0 aA

[a]Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. [b]Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c]Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water.

seeds were inoculated and treated with fipronil, carbendazim + thiram + fipronil, fludioxonil + metalaxyl-M

Table 7. Mean square values for the effects of seed treatments on yield and nodulation of soybean.

Factor of variation	Degrees of freedom	Mean square				
		Yield (kg ha^{-1})	Thousand grain mass (g)	Number of plants per meter	Number of nodules per plant	Mass of nodules per plant (mg)
Block	3	120232.81 *	199.24**	0.8293^{ns}	190.47^{ns}	2830.68^{ns}
Treatment	15	50574.46^{ns}	30.84**	0.9059^{ns}	278.95 *	9283.66 **
Error	45	43112.09	15.25	0.4944	129.74	2878.3
Mean		3182.42	146.4	14.16	59.52	264.89
Coefficient of variation (%)		6.52	2.67	4.97	19.14	20.25

[a]Variables in percentage were previously transformed to √x+1 for statistical analysis. ^{ns} non-significant, * significant at 5% and ** at 1% probability level.

+ fipronil + tiophanate-methyl + praclostrobin, and fipronil + tiophanate-methyl + pyraclostrobin (Table 4). It was already reported in a previous study that seed treatment with fungicide (carboxin + thiram) increased the percentage of germinated seeds compared to the non-treated control (Brand et al., 2009). These results are contrary to what was observed by Ferreira et al. (2016) who reported that fipronil + tiophanate-methyl + pyraclostrobin had negative effect on physiological quality of seed by reducing germination whereas it increased the percentage of abnormal seedlings. It is not very clear how a fungicide could improve the physiological quality of seed, but one hypothesis is that the fungicide can provided a better control of fungi that infected seeds and this could reflect in the physiological quality during the storage period (Brand et al., 2009).

After 51 days of storage, pre-inoculation with HiCoat® and application of carbendazim + thiram + fipronil presented higher seed germination (Table 4) and lower percentage of abnormal seedlings (Table 5) than the non-treated and non-inoculated control. The application of fipronil increased the percentage of non-germinated seeds after 51 days of storage, but none of the other treatments affected these variables (Table 5). Piccinin et al. (2013) also verified that the application of fipronil reduced the physiological quality of seeds after 180 days of storage, which, according to the authors, can be due to degenerative alterations in the metabolism of cells, disruption of cell membranes.

Seed germination with the application of fludioxonil + metalaxyl-M, fludioxonil + metalaxyl-M + imidacloprid + thiodicarbe, fludioxonil + metalaxyl-M + fipronil + tiophanate-methyl + praclostrobin, and fipronil + tiophanate-methyl + praclostrobin did not differ from the control, whereas the other treatments caused its reduction (Table 4). In previous studies, seed treatment with fipronil + tiophanate-methyl + pyraclostrobin associated with polymers were not harmful to seed germination (Camilo et al., 2017; Santos et al., 2018). Similarly, treatment with carbendazim + thiram + imidacloprid + thiodicarb + micronutrient + polymer + inoculant did not cause any physical and physiological damage to the soybean seeds (Segalin et al., 2013). This indicates that these products could be applied without compromising the physiological quality of soybean seeds.

The minimum value for seed germination according to Brazilian regulations is 80% (Mapa, 2013). In this study, not all treatments provided this level of germination. This is important to be considered by companies that intend to use industrial seed treatments, otherwise the seeds cannot be commercialized.

Nodulation and soybean yield

Soybean yield and number of plants per meter were not affected by the treatments, whereas thousand grain mass, number of nodules per plant and mass of nodules per plant were significantly influenced by the treatments (Table 7).

The application of nitrogen fertilizer significantly reduced the number and the mass of nodules per plant (Table 8) as already reported by other authors (Hungria et al., 2006; Salvagiotti et al., 2008; Anghinoni et al., 2017). The energy costs required for the biological nitrogen fixation process is usually very high (Taíz and Zeiger, 2004; Minchin and Witty, 2005), therefore, when large amounts of nitrogen are easily available in the soil, the plant supply its needs by absorbing the nutrient from the soil rather than establishing a symbiotic relationship. However, the addition of nitrogen fertilizer to the soybean crop in Brazil is usually non-profitable (Hungria et al., 2006). As a result, growers still prefer to rely on the inoculation to supply nitrogen to the plant.

Pre-inoculation did not affect the number, mass of nodules per plant, and yield when compared to the standard inoculation performed at the day of sowing (Table 8). This indicates that the use of peat inoculant + liquid inoculant + polymer maintains the viability of the nitrogen-fixing bacteria for a proper establishment of a symbiotic relationship. These results corroborate with previous reports of no adverse effect on yield due to the

Table 8. Soybean yield, thousand grain mass, initial number of plants per meter and nodulation of soybean for each seed treatment with fungicides and insecticides, pre-inoculation[c] with *Bradyrhizobium elkanii* and storage at room temperature for 51 days.

Treatment	Yield (kgha^{-1})[a]	Thousandgrainmass(g)[a]	Numberofplantspermeter[a]	Numberofnodulesperplant[a]	Massofnodulesperplant(mg)[a]
Non-inoculatedandnon-treatedcontrol	3207[a]	147.8[ab]	13.66[a]	51.5[ab]	238.3[a]
StandardInoculation	3301[a]	146.3[ab]	14.66[a]	59.0[ab]	266.7[a]
NitrogenFertilization(200kgha^{-1}N)	3158[a]	139.0[b]	14.46[a]	33.0[b]	93.3[b]
Pre-inoculation(PI)	3315[a]	149.0[ab]	13.54[a]	57.8[ab]	268.3[a]
PI+Thiamethoxan	3056[a]	141.9[ab]	13.00[a]	55.5[ab]	267.5[a]
PI+Carbendazim+Thiram+Thiamethoxan	3235[a]	147.9[ab]	14.66[a]	59.0[ab]	265.8[a]
PI+Fludioxonil+Metalaxyl-M+Imidacloprid	3210[a]	146.7[ab]	14.50a	65.0[a]	270.7[a]
PI+Carbendazim+Thiram+Fipronil	3145[a]	146.1[ab]	14.58[a]	64.2[a]	287.5[a]
PI+Imidacloprid	3192[a]	146.7[ab]	13.92[a]	64.0[a]	293.3[a]
PI+Fludioxonil+Metalaxyl-M	2916[a]	146.3[ab]	14.63[a]	63.5[a]	271.7[a]
PI+Fludioxonil+Metalaxyl-M+Imidacloprid+Thiodicarbe	3276[a]	148.2[ab]	14.08[a]	65.7[a]	280.0[a]
PI+Fludioxonil+Metalaxyl-M+Thiamethoxan	3191[a]	147.4[ab]	14.33[a]	67.0[a]	293.3[a]
PI+Carbendazim+Thiram+Imidacloprid	3147[a]	148.7[ab]	14.04[a]	57.2[ab]	270.7[a]
PI+Fipronil+Tiophanate-methyl+Pyraclostrobin	3372[a]	149.7[a]	14.00[a]	60.2[ab]	277.5[a]
PI+Fipronil	3147[a]	143.4[ab]	13.96[a]	62.0[ab]	297.5[a]
PI+Fludioxonil+Metalaxyl-M+Fipronil+Tiophanate-methyl+Pyraclostrobin	3050[a]	147.1[ab]	14.46[a]	67.5[a]	295.8[a]
Mean	3182	146.4	14.20	59.5	264.9
CoefficientofVariation(%)	6.52	2.67	4.97	19.1	20.2

[a] Means followed by the same letter do not differ statistically from each other by Tukey's Test at 5% probability.
[b] Standard inoculation was performed at the day of sowing with the liquid inoculant Gelfix® (*B. elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}).

use of pre-inoculation with *Bradyrhizobium* spp. (Anghinoni et al., 2017; Machineski et al., 2018). All these results are contrary to what was found by Brzezinski et al. (2015) and Zilli et al. (2009), where the authors mentioned that treatment of soybean seeds with insecticide and fungicides before sowing hinders the establishment of soybean in the field and reduces nodulation of the plants. This is probably due to the toxic effect of some agrochemicals that reduces the survival of the nitrogen-fixing bacteria. However, the difference may be due to the absence of polymers in both studies and the fact that seeds were stored for 240 days before sowing in the study by Brzezinski et al. (2015). This corroborates with the study by Krohn and Malavasi (2004) who reported that seeds treated with agrochemicals and stored for more than four months led to lower emergence of seedlings in the field.

Seed treatments with fungicide and insecticides can be used in an integrated management program to control diseases and pests as a preventative tactic. According to a survey performed in Brazil, some advantages of the use of industrial seed treatments have been related to higher efficiency of the sowing process, economy of labor and time, lower risk of intoxication by growers, higher precision regarding to the dose used, more uniform coverage of the seeds, reduction of production costs, guarantee of acquisition of seeds with good quality, combat seed piracy, among others (França-Neto et al., 2015).

This finding indicates that although some seed treatments affected the physiological quality of seed, there was no significant effect on the establishment of the plants in the field and yield. Hence, the pre-inoculation of soybean seed using the HiCoat® technology could be performed on treated seeds and stored for up to 51 days without compromising yield. Some companies have added nematicides and micronutrients such as cobalt and molybdenum to the seed; therefore, additional studies are required to evaluate the association of these products with pre-inoculation. Likewise, further studies should perform the seed treatment with the machines used for industrial seed treatment in order to verify if a better coverage of the seed would have an impact in the physiological quality. Furthermore, evaluation of other soybean cultivars is necessary to verify whether different genotypes would have distinct effects regarding seeds treatments as well as the response to pre-inoculation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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