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# Physiological potential and initial development of soybean plants as a function of seed treatment<sup>1</sup>

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## ABSTRACT

Seed treatment is a process that helps to control insects and soil-borne pathogens, besides allowing a given crop to reach its maximum production potential and protect its seedlings. This study aimed to evaluate the physiological performance of seeds and the initial development of soybean, as a function of seed treatment. The study was based on a completely randomized design, with five treatments [imidacloprid + thiodicarb (75 + 225 g a.i. 100 kg<sup>-1</sup> of seeds); chlorantraniliprole (62.5 g a.i. 100 kg<sup>-1</sup> of seeds); cyantraniliprole (72 g a.i. 100 kg<sup>-1</sup> of seeds), fipronil + thiophanate-methyl + pyraclostrobin (5 + 45 + 50 g a.i. 100 kg<sup>-1</sup> of seeds); and control (seeds without treatment)]. Initially, the physiological quality of the seeds was evaluated by determining the first germination count, final germination and accelerated aging, with four replications. After that, the effect of the seed treatment on the soybean plant development was evaluated by analyzing the leaf area, number of leaves, shoot height, root and shoot dry mass and fresh mass of root nodules, with ten replications. The seed treatment with fipronil + pyraclostrobin + thiophanate-methyl allows an increased germination when the seeds are subjected to the accelerated aging test, besides an increment in the shoot height and leaf area of the plants.

**KEYWORDS:** *Glycine max*, germination, seed accelerated aging.

## RESUMO

Potencial fisiológico e desenvolvimento inicial de plantas de soja em função do tratamento de sementes

O tratamento de sementes é um processo que auxilia no controle de insetos e patógenos iniciais, além de possibilitar à cultura atingir seu potencial máximo de produção e promover proteção à plântula. Objetivou-se avaliar o desempenho fisiológico de sementes e o desenvolvimento inicial de plantas de soja, em função do tratamento de sementes. O experimento foi conduzido em delineamento inteiramente casualizado, com cinco tratamentos [imidacloprid + thiodicarb (75 + 225 g i.a. 100 kg<sup>-1</sup> de sementes); chlorantraniliprole (62,5 g i.a. 100 kg<sup>-1</sup> de sementes); cyantraniliprole (72 g i.a. 100 kg<sup>-1</sup> de sementes); fipronil + thiophanate-methyl + pyraclostrobin (5 + 45 + 50 g i.a. 100 kg<sup>-1</sup> de sementes); e testemunha (sementes sem tratamento)]. Inicialmente, foi avaliada a qualidade fisiológica das sementes, por meio de primeira contagem de germinação, germinação final e envelhecimento acelerado, com quatro repetições. Posteriormente, foi avaliado o efeito do tratamento de sementes no desenvolvimento inicial das plantas de soja, por meio de análise da área foliar, número de folhas, comprimento da parte aérea, massa seca de raiz e parte aérea e massa fresca de nódulos, em dez repetições. O tratamento com fipronil + pyraclostrobin + thiophanate-methyl proporciona maior germinação em sementes submetidas ao teste de envelhecimento acelerado, além de incremento no comprimento da parte aérea e área foliar das plantas.

**PALAVRAS-CHAVE:** *Glycine max*, germinação, envelhecimento acelerado de semente.

## INTRODUCTION

The combination of expanded soybean production and intensive farming in the same area has favored the occurrence of pests and diseases that compromise the crops stand and initial development (Lauxen et al. 2016).

To avoid damage to seeds and seedlings, and ensure an adequate plant population, seed treatment with insecticides and fungicides is the most commonly adopted practice (Ferreira et al. 2016, Brzezinski et al. 2017, Castellanos et al. 2017). This practice has a relatively low cost and lower environmental impact due to the lower amount of active ingredients per

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applied area relatively to the total application area (Cunha et al. 2015). Thus, the number of registered products available for this purpose increases every year (Tavares et al. 2014, Cunha et al. 2015).

Beyond plant protection, some of these products can provide positive results on plant productivity and development, reducing the loss of seed vigor due to storage (Carvalho et al. 2011, Adam et al. 2014). As an example, at 45 days after the plant emergence, there is a positive effect on the initial soybean growth by applying abamectin, imidacloprid + thiodicarb, thiamethoxam and fipronil + thiophanate-methyl + pyraclostrobin. On the other hand, Dan et al. (2012), who studied soybean seeds treated with thiamethoxam, fipronil and imidacloprid, observed that the accumulation of fresh mass is not affected by the treatments, if evaluated during the initial plant development.

One of the factors influencing the initial soybean development is the interaction between phytosanitary products used for seed treatment and nitrogen-fixing bacteria belonging to the *Bradyrhizobium* genus (Pereira et al. 2010). However, studies regarding this factor are scarce. Thus, considering the importance of the phytosanitary seed treatment against insects and pathogens and the relevance of these products in maintaining the crop yield with an adequate plant stand, this study aimed to evaluate the physiological performance and initial soybean plant development, as a function of seed treatment.

## MATERIAL AND METHODS

The study was conducted in a greenhouse at the Universidade Estadual Paulista, in Botucatu, São Paulo state, Brazil, from February to April 2018. The soybean seed BMX Potência RR cultivar was used, and the experimental design for the physiological seed testing was completely randomized, with five treatments and four replications. For determining the leaf area, number of leaves, shoot height, root and shoot dry mass and fresh mass of root nodules, the experimental design was completely randomized, with ten replications. Each replication was represented by one pot with two plants. The treatments were as it follows: imidacloprid + thiodicarb (Cropstar®), which consisted of 75 + 225 g a.i. 100 kg<sup>-1</sup> of seeds, respectively; chlorantraniliprole (Dermacor®) at 62.5 g a.i. 100 kg<sup>-1</sup> of seeds; cyantraniliprole (Fortenza 600 FS®) at 72 g a.i. 100 kg<sup>-1</sup> of seeds;

fipronil + pyraclostrobin + thiophanate-methyl (Standak Top®) at 50 + 5 + 45 g a.i. 100 kg<sup>-1</sup> of seeds; and control (seeds without treatment).

For each treatment, water and the respective phytosanitary product in the correct dosage to 0.5 kg of seeds were added to 1.0 L plastic bags, to attain a final volume of 600 mL 100 kg<sup>-1</sup> of seeds. Seeds were subsequently shaken vigorously until fully covered by the products (Cunha et al. 2015).

To evaluate the effect of the seed treatment on its physiological quality after the applied phytosanitary product had dried, the following tests were performed: seed germination: using 50 seeds per replication on germitest paper previously moistened with water at 2.5 times the mass of the dry paper, germination was conducted in a controlled environment at 25 °C (Brasil 2009), with results expressed in percentage of normal seedlings; first germination count: conducted at five days after the beginning of the germination test, on paper (germitest), by counting the normal seedlings, with results shown as percentage (Brasil 2009); accelerated aging: a gerbox containing 40 mL of distilled water was used, in which 200 seeds for each treatment were distributed in a single layer, covering the entire surface of a metal screen above the water level. After that, the gerbox containing the seeds was covered and placed in a BOD incubator at 41 °C, for 48 h (Marcos Filho 1999). After this period, the seeds underwent the germination test described previously. Data were shown in percentage.

To evaluate the initial development, an experiment was conducted in a greenhouse on February 21 (2018), using 50 plastic pots with capacity of 0.7 L filled with substrate (Tropstrato®) comprising pine bark, peat, vermiculite, single superphosphate and potassium nitrate. Upon sowing, three seeds were placed in each pot, in which two plants were maintained after thinning, at 10 days after sowing (DAS). To determine the initial growth, the soybean plants were cut at the substrate level at 35 DAS. The variables analyzed were: leaf area (cm<sup>2</sup>), measured with a LI-3100 Area Meter (LI-COR Inc. Lincoln, NE, EUA), using leaves detached from the petioles; number of leaves, taking the mean of two plants; shoot height (cm), measured with a millimeter ruler from the substrate surface to the last leaf in development; shoot and root dry mass (g), using a kiln at 65 °C, for 72 h; and fresh mass of root nodules (g), in which the nodules were manually removed from the roots with the aid of a 3-mm mesh sieve

and running water. After draining the water excess, the fresh mass of the nodules was determined with a precision scale ( $d = 0.0001$  g) and, to obtain the mean value of each pot, the values were divided by two.

The statistical analysis was performed with the Sisvar software (Ferreira 2011). The results were submitted to analysis of variance using the F-test, and the mean values were compared by the Tukey test ( $p \leq 0.05$ ). The data obtained from the initial plant development were transformed according to  $(x + 1)^{0.5}$ .

## RESULTS AND DISCUSSION

The treatments were not effective throughout the entire evaluation of the germination period, indicating that the evaluated phytosanitary products did not affect germination (Table 1). Other authors also did not find any difference among treatments for seed germination when evaluating the effect of insecticides on seed treatments in soybean plants (Dan et al. 2012, Cunha et al. 2015, Santos et al. 2018). Soybean seeds treated with thiamethoxam, fipronil and imidacloprid did not present any difference for germination when compared with the control treatment (Dan et al. 2012). Similarly, Santos et al. (2018) found that it was possible to store soybean seeds treated with fipronil + pyraclostrobin + thiophanate-methyl for 150 days in a cold chamber without germination loss.

For the accelerated aging test, fipronil + pyraclostrobin + thiophanate-methyl showed a greater germination rate (69 %), followed by the control (49 %) and imidacloprid + thiodicarb (44.5 %), which did not differ among themselves. Similar results were found by Ferreira et al. (2016), which verified that fipronil + pyraclostrobin + thiophanate-methyl provided a higher percentage of normal seedlings, in relation to other treatments involving seeds treated

and stored for two months. Active ingredients may affect the seedling development under adverse conditions, immediately after the treatment or after storage (Antonello et al. 2009). Therefore, the care with which seeds are treated with chlorantraniliprole and cyantraniliprole should be higher, thus avoiding exposing the seeds to adverse conditions or storage for long periods.

In relation to the parameters leaf area and shoot height, the statistical analysis showed a difference, when compared to the control treatment (Table 2), which showed the lowest values for these variables ( $97.27 \text{ cm}^2$  and  $15.70 \text{ cm}$ , respectively). The treatment with fipronil + pyraclostrobin + thiophanate-methyl presented the highest results, with increments of 22.4 % and 15.48 % for leaf area and shoot height, respectively, when compared to the control. The other treatments did not differ from the control.

These results showed that phytosanitary products may promote alterations in the initial development of soybean plants. Some molecules may promote physiological and biochemical effects that can modify the plant growth, morphology and productivity (Macedo & Castro 2011). As an example, the treatment of soybean seeds with the insecticides thiamethoxam, imidacloprid, aldicarb and a biostimulant led to the formation of thinner roots, which characterized a tonic effect (Castro et al. 2008).

On the other hand, the insecticides carbofuran, acephate and imidacloprid + thiodicarb reduced the height of soybean plants, in relation to the control; however, those who received the treatment with thiametoxam, fipronil and imidacloprid showed no differences for this variable (Dan et al. 2012). The effect of seeds treated with fungicides and insecticides on the soybean plant development was evaluated by Cunha et al. (2015). The authors observed that, at 30 days after the emergence, there

Table 1. First germination count (FGC), final germination (FG) and germination of soybean seeds (BMX Potência RR cultivar) submitted to accelerated aging (AA), as a function of seed treatment.

Active ingredient	FGC (%)	FG (%)	AA (%)
Imidacloprid + thiodicarb	78	84	45 b
Chlorantraniliprole	75	80	37 c
Cyantraniliprole	74	78	24 d
Fipronil + pyraclostrobin + thiophanate-methyl	76	85	69 a
Control	78	81	50 b
$F_{\text{calc}}$	0.89 <sup>ns</sup>	1.82 <sup>ns</sup>	163.01 <sup>**</sup>
CV (%)	5.00	4.98	5.90

Means followed by the same letter (column) do not differ among them by the Tukey test ( $p \leq 0.05$ ). \*\* Significant at 1 % of probability; <sup>ns</sup> not significant.

Table 2. Shoot height (SH), number of leaves (NL) and leaf area (LA) of soybean plants (BMX Potência RR cultivar) treated with phytosanitary products.

Active ingredient	SH (cm)	NL	LA (cm <sup>2</sup> )
Imidacloprid + thiodicarb	17.33 ab	11.30	116.49 ab
Chlorantraniliprole	16.46 ab	10.30	99.99 ab
Cyantraniliprole	16.99 ab	10.65	107.27 ab
Fipronil + pyraclostrobin + thiophanate-methyl	18.13 a	10.90	119.06 a
Control	15.70 b	11.00	97.27 b
F <sub>calc</sub>	4.78**	1.23 <sup>ns</sup>	3.55*
CV (%)	3.71	4.63	7.45

Means followed by the same letter (column) indicate no difference by the Tukey test ( $p \leq 0.05$ ). \*\* Significant at 1 % of probability; <sup>ns</sup> not significant. Data transformed according to  $(x + 1)^{0.5}$ .

were no differences among the treatments for plant height and stalk diameter. Tavares et al. (2007) did not observe any differences for the shoot height of soybean seedlings after the seed treatment with five doses of thiamethoxam.

The results showed that the shoot dry mass, root dry mass and fresh mass of nodules did not differ among the treatments (Table 3), corroborating those obtained by Dan et al. (2012), although this study found significant differences related to vigor, phytointoxication and soybean plant height. In addition, no significant differences were observed for shoot dry mass accumulation on soybean plants at 30 DAS. Tavares et al. (2014) tested fungicides and insecticides on seed treatment and observed that, for the BMX Potência RR cultivar, there were no differences for shoot dry mass.

Colman et al. (2012) tested the insecticides fipronil, fipronil + pyraclostrobin + thiophanate-methyl, thiamethoxam and imidacloprid + thiodicarb and concluded that these products do not provide any gains in the soybean shoot dry mass and plant height, if compared to the control.

In relation to the nodule formation on roots, the results from the treatments did not differ significantly

from the control; however, this study did not conduct any inoculations. Notably, the presence of bacteria in substrates is uncommon; however, as soybean plants are noduliferous and the material used was not sterilized, the presence of nitrogen-fixing microorganisms is one possible reason for the nodule formation on roots. Studies on the treatment of seeds with fungicides have shown that they can reduce the nodule formation on soybean roots (Revellin et al. 1993, Campo & Hungria 2000). Thus, new studies are necessary to evaluate the effect of seed treatment with fungicides combined with bacteria inoculation.

Silva et al. (2011) showed that the reduced soil-based nodule formation may be related to active and/or inert ingredients contained in products used for seed treatment or for pH alteration. It may also be influenced by nutrients, growth regulators or resistance inductors, which are products capable of increasing the pH and making spray mixtures more harmful to bacteria. Khudhur & Askar (2013) explained that the nodule mass on roots may decrease due to bacteria mortality, after being exposed to phytosanitary products.

Thus, it is known that the seed treatment is considered an efficient method for the use of

Table 3. Shoot dry mass (SDM), root dry mass (RDM) and fresh mass of nodules (FMN) of soybean (BMX Potência RR cultivar) treated with phytosanitary products.

Active ingredient	SDM (g)	RDM (g)	FMN (g)
Imidacloprid + thiodicarb	0.4655	0.4715	0.1891
Chlorantraniliprole	0.4141	0.4598	0.1728
Cyantraniliprole	0.4751	0.4739	0.1935
Fipronil + pyraclostrobin + thiophanate-methyl	0.4859	0.4856	0.1878
Control	0.4287	0.4004	0.1901
F <sub>calc</sub>	1.48 <sup>ns</sup>	2.07 <sup>ns</sup>	0.058 <sup>ns</sup>
CV (%)	2.77	2.54	4.34

<sup>ns</sup> Not significant. Data transformed according to  $(x + 1)^{0.5}$ .



phytosanitary products in the control of pest and diseases during the initial development of crops (Castro et al. 2008, Balardin et al. 2011). However, the relation among chemical substances present in the pesticides used may either damage or benefit soybean crops.

## CONCLUSIONS

1. For the BMX Potência RR soybean cultivar, the tested phytosanitary products (imidacloprid + thiodicarb, chlorantraniliprole, cyantraniliprole, fipronil + thiophanate-methyl + pyraclostrobin) do not influence the germination, dry mass accumulation and root nodule formation;
2. The treatment with fipronil + pyraclostrobin + thiophanate-methyl increases the germination of seeds submitted to the accelerated aging test, in addition to increment the plant height and leaf area.

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