# Thiamethoxam treated bean seeds performance during storage

César Iván Suárez Castellanos<sup>1</sup>, Andreia da Silva Almeida<sup>1</sup>, Carolina Terra Borges<sup>1</sup>, Cristiane Deuner<sup>1</sup>, Geri Eduardo Meneghello<sup>1</sup>

<sup>1</sup> Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Avenida Eliseu Maciel, s/n, Campus Capão do Leão, CEP 96050-500, Capão do Leão-RS, Brasil. E-mail: cesarivansuarez@gmail.com; andreiasalmeida@yahoo.com.br; carol\_tborges@hotmail.com; cdeuner@yahoo.com.br; gmeneghello@gmail.com

## ABSTRACT

After sowing, seeds are exposed to the action of soil pests and adverse environmental conditions that can reduce the expression of their physiological potential. A solution to these problems is the seed treatment with insecticides to protect the seeds and seedlings against the action of pests and to promote plant growth. The aim of the experiment was to evaluate the influence of thiamethoxam combined with storage on the physiological performance of bean seeds, IAPAR Siriri cultivar. Seeds were treated with Crusier 350 FS<sup>®</sup> in doses of 0, 100, 200, 300, 400 and 500 mL 100 kg<sup>-1</sup> of seeds, and for evaluation of treatment effect, seeds were submitted to germination and vigor tests (accelerated aging, cold test and emergence in seedbed) at 0, 3, 6, 9 and 12 months after treatment in a completely randomized 6 x 5 factorial design with four replications. Treatment of bean seeds with thiamethoxam doses between 200 and 300 mL 100 kg<sup>-1</sup> of seeds allows germination and vigor expression as well as storage potential under controlled conditions, decreasing the rate of germination loss over time.

Key words: germination; Phaseolus vulgaris L.; vigor

# Desempenho de sementes de feijão tratadas com thiamethoxam durante o armazenamento

#### RESUMO

Após a semeadura, as sementes são expostas à ação de pragas do solo e condições ambientais adversas que podem reduzir a expressão do seu potencial fisiológico. Uma solução para esses problemas é o tratamento de sementes com inseticidas, cuja finalidade é proteger as mesmas e as plântulas oriundas contra a ação de pragas e promover o crescimento das plantas. O objetivo do experimento foi avaliar a influência do thiamethoxam combinado com o armazenamento sobre o desempenho fisiológico de sementes de feijão, cv. IAPAR Siriri. As sementes foram tratadas com Crusier 350 FS<sup>®</sup> nas doses de 0, 100, 200, 300, 400 e 500 mL 100 kg<sup>-1</sup> de sementes, sendo que para avaliação do efeito dos tratamentos as sementes foram submetidas a testes de germinação e vigor (envelhecimento acelerado, teste de frio e emergência em canteiro) aos 0, 3, 6, 9 e 12 meses após o tratamento, em delineamento experimental inteiramente casualizado em esquema fatorial 6 x 5 com quatro repetições. O tratamento de sementes de feijão cv. IAPAR Siriri com doses de thiamethoxam entre 200 e 300 mL 100 kg<sup>-1</sup> de sementes viabiliza a expressão da germinação e do vigor, bem como seu potencial de armazenamento em condições controladas, diminuindo a taxa de perda de germinação através do tempo.

Palavras-chave: germinação; Phaseolus vulgaris L.; vigor

## Introduction

Bean (*Phaseolus vulgaris* L.) is a species of the plant family Fabaceae that has great agricultural importance, especially in Brazil, where national production in the three harvests of 2015/2016 reached 2,593.1 million tons of grains (Conab, 2016). The success of crops depends on environmental factors, agronomic management, crop density, plant distribution in the area, cultural and phytosanitary treatment, quality and health of the seeds used, among others.

Rufino et al. (2010) reported that despite having high physiological quality, when seed are planted, they may suffer the action of soil insects, thereby reducing the number of plants per unit area, which directly affects the crop yield. According to Dan et al. (2012a), the action of soil pests damage seeds and seedlings resulting in productivity losses. This indicates that seed treatment with preventive insecticides is a good alternative to ensure proper plant population. Furthermore, plants are also exposed to various stresses and adverse conditions in the field, which may eventually decrease the expression of their yield potential (Almeida et al., 2009).

Seed treatment with products such as carbofuran (Freitas et al., 2001) and aldicarb (Reddy et al., 1990) have shown that some insecticides, besides acting as protectors, can interfere with the physiological and morphological plant behavior. Thus, the used of these insecticides has dual purpose, namely, protecting seeds and plants against the pests and promoting plant growth (Horii et al., 2007), either under normal or stressful situations.

For example, Castellanos et al. (2015) found that treatment of bean seeds with thiamethoxam increased plants chlorophyll content, and Adams et al. (2014) found that treatment of soybean seeds with thiamethoxam mixed with fungicides or plant growth regulators decreased seed vigor loss during storage.

Thiamethoxam is one of the insecticides used for seed treatment. This is a systemic neonicotinoid product that, besides its insecticide function, has bioactivator properties. This means that thiamethoxam is an organic substance that modifies plant growth, has the potential to interfere with DNA transcription, gene expression and affect membrane proteins, metabolic enzymes and mineral nutrition (Castro & Pereira, 2008).

Thiamethoxam acts on the expression of genes that synthesize and activate enzymes related to plant growth, increasing the production of plant hormone precursor amino acids (Castro, 2006). Furthermore, Cataneo (2008) reported that this insecticide increases the expression of seed vigor, dry matter accumulation, photosynthetic rate and root depth.

The positive effect of thiamethoxam seed treatment on initial seed performance has been observed in cotton (Lauxen et al., 2010), rice (Almeida et al., 2011), black oats (Almeida et al., 2012) and soybean (Dan et al., 2012b). However, information on the effect of that insecticide on seeds when they are stored after treatment is still missing. For these reasons, the objective of this study was to evaluate the physiological performance of thiamethoxam treated bean seeds under storage for 12-months.

## **Material and Methods**

The experiment was conducted in the Teaching Seed Analysis Laboratory of the Graduate Program on Seed Science and Technology, Federal University of Pelotas, located in the municipal district of Capão do Leão - RS. Bean seeds, IAPAR Siriri cultivar, were treated with the Cruiser 350 FS<sup>®</sup> insecticide which has thiamethoxam as active ingredient. The product was applied at increasing doses, corresponding to six treatments: 0 (control), 100, 200, 300, 400 and 500 mL 100 kg<sup>-1</sup> of seeds. Each dose was equivalent to application of 0, 35, 70, 105, 140 and 175 g i.a. 100 kg<sup>-1</sup> of seeds.

The spray volume used was 0.6 L 100 kg<sup>-1</sup> of seeds for all treatments, with the solution supplemented with distilled water for each treatment. The volume was applied with a 1 mL micropipette at the bottom of a plastic bag and then dispersed to cover <sup>3</sup>/<sub>4</sub> of the bag. Then, the seeds were added and the bag was manually agitated until a homogeneous coverage of the product was obtained in the portion of treated seeds. After this procedure, the seeds were removed and spread on plastic trays, where they remained for 24 hours to dry. Afterwards they were packed in paper bags and stored in a cold chamber with relative humidity of 60% at 16°C.

Seed quality was evaluated right after treatment and every three months until the 12th month of storage through germination tests, accelerated aging, cold test and emergence in seedbed. There were a total of five evaluations (0, 3rd, 6th, 9th and 12th month).

Germination test: the test was performed in Germitest paper rolls moistened with distilled water at a ratio of 2.5 times of their dry weight. Two hundred seeds were used per replicate, divided into four rolls with 50 seeds each, which were transferred to a germination chamber and maintained at constant temperature of 25°C. Normal seedling counts were carried out at five and nine days according to Seed Analysis Rules - RAS (Brasil, 2009) and results were expressed in percentages.

Accelerated aging test: the test was conducted through the gerbox method; a single seed layer was put on a suspended metal screen in a box containing 40 ml of distilled water. The gerbox were closed and transferred to a *Biological Oxygen Demand* (B.O.D.) camera at the temperature of 41°C for 48 hours (Marcos Filho, 2005). After this period, seeds were taken from the gerbox and germinated according to the methodology described above for the germination test. The evaluation was performed on the fifth day after sowing. Normal seedlings were counted and results were expressed in percentages.

Cold test: the test was performed in Germitest paper rolls moistened with distilled water at a ratio of 2.5 times of their dry weight. Two hundred seeds per replication were distributed in four rolls with 50 seeds each. After seeding, the rolls were wrapped in plastic bags and kept in a B.O.D. at 10°C for seven days. After this period, the rolls were removed from the bags and transferred to a germinator at 25°C, where they remained for five days. In the fifth day, the counting of normal seedlings was carried out and results are presented in percentages (Barros et al., 1999). Emergence test: the test was performed in seedbeds containing soil as substrate, seeded with four replications of 50 seeds per treatment in 1m-length lines, to a depth of about three centimeters and 0.1 m of spacing. Irrigation of the seedbeds was performed daily at the morning, with the final percentage of seedlings computed 21 days after sowing.

The experiment was conducted in a completely randomized 6 x 5 factorial design, corresponding to six bioactivator doses and five storage times after application of the product, with 4 repetitions. Analysis of variance was performed using the statistical software R (R Core Team, 2014) and in the case of significant differences, polynomial regressions and response surfaces were performed with p < 0.05.

# **Results and Discussion**

Significant interactions between thiamethoxam doses and storage time were found in germination and accelerated aging tests, while significant main effects of the two factors were found in the cold test and seedbed emergence, but without interaction between factors (Table 1).

Figure 1A shows the surface data on the percentage of germination in function of the interaction between thiamethoxam doses and storage times. The adjusted model is a second degree polynomial in which the maximum response point was 95% of germination at 2.8 months of storage at a thiamethoxam dose of 235 mL 100 kg<sup>-1</sup> of seeds.

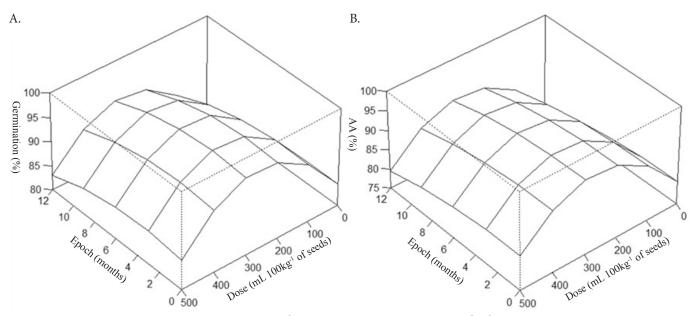
Seeds had stable germination until the third month of storage, and after this, germination was reduced regardless the thiamethoxam dose used (Figure 1A). We can also see a greater germination percentage of seeds treated with thiamethoxam doses between 200 and 300 mL 100 kg<sup>-1</sup> of seeds, while doses below or above this range led to germination percentages similar to the control, what was observed in all evaluation periods. Almeida et al. (2011) observed a similar response in rice seeds and attributed this phenomenon to a probable phytotoxic effect of thiamethoxam in high concentrations.

Positive results with the use of thiamethoxam were observed by Clavijo (2008) in rice seeds, Almeida et al. (2009) in carrot seeds, and Lauxen et al. (2010) in cotton seeds. According to Serciloto (2002), these positive effects of the product on seed germination happen because the external application of some bioactivators stimulates the biosynthesis of hydrolytic enzymes involved in the metabolic processes of germination.

The results of the accelerated aging test had a similar behavior to the germination, where best performance in all evaluation periods occurred with doses between 200 and 300 mL 100 kg<sup>-1</sup> of seeds. Furthermore, germination after seed aging gradually dropped with storage time, regardless the thiamethoxam doses applied. This behavior, represented by the surface response in Figure 1B, follows a second degree polynomial with a maximum response point of 93% in the first

Table 1. Mean square of germination (G), accelerated aging (AA), cold test (CT) and seedbed emergence (SE) according to treatment of bean seeds, IAPAR Siriri cultivar, with various doses of thiamethoxam and storage for 12 months. Capão do Leão - RS. 2015.

Sources of variation –	Mean Square				
	DF	Germination	Accelerated aging	Cold test	Emergence in seedbed
Seed storage	4	94.7208 *	93.6792 *	96.8125 *	71.9458 *
Thiamethoxam doses	5	493.3483 *	583.3483 *	583.84 *	599.02 *
Doses x storage	20	4.0108 *	4.0942 *	4.1775	4.0658
Residue	90	0.9417	1.0361	2.5333	2.4111
CV (%)		1.0789	1.1681	1.8401	1.712



 $G(\%) = 84.488 + 0.19 \text{ epoch} + 0.0911 \text{ dose} - 0.0002 \text{ dose}^2 + 0.0005 \text{ epoch} * \text{ dose} - 0.056 \text{ epoch}^2; R^2 = 0.9343; p < 0.0000 \text{ AA}(\%) = 80.078 + 0.069 \text{ epoch} + 0.102 \text{ dose} - 0.0002 \text{ dose}^2 + 0.0006 \text{ epoch} * \text{ dose} - 0.039 \text{ epoch}^2; R^2 = 0.9339; p < 0.0000 \text{ Figure 1. A. Germination percentage (G%); B. Normal seedling percentage in the accelerated aging test (AA%) of bean seeds, IAPAR Siriri cultivar, in function of the interaction between increasing thiamethoxam doses and storage time up to 12 months. Capão do Leão - RS. 2015.$ 

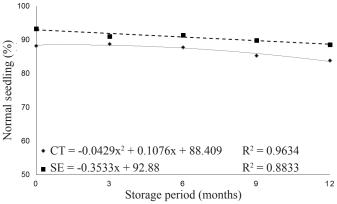
month of storage at a dose of 255 mL 100 kg<sup>-1</sup> of seeds. This improved performance observed in thiamethoxam treated seeds can be explained by the fact that the active agent be transported through the cells and influence the expression of proteins that help the plant to withstand environmental stressful conditions (Tavares et al., 2008).

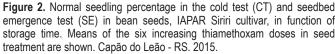
The analysis of seed vigor under storage through the cold and seedbed emergence tests showed that regardless the dose used, the percentage of normal seedlings decreased as the storage time increased (Figure 2). However, cold test data fitted a quadratic model where seed vigor was retained until six months of storage; after this time, the fall in the percentage of normal seedlings was obvious (Figure 2).

Seedbed emergence data fitted a negative linear model, in which the percentage of seedlings decreased at a rate of 0.3 percentage points per month of storage, losing around 5 percentage points over the 12-month period of the experiment (Figure 2). These results corroborate Borges et al. (2014) who observed that thiamethoxam treated rice seeds had reduced vigor over the storage period as assessed through cold and field emergence tests.

The analysis of vigor behavior of seeds through the cold and seedbed emergence tests, in function of the thiamethoxam dose (Figure 3) showed that both data fitted a quadratic model. In the cold test, the maximum point was 91%, and in the seedbed emergence test, this was 96% at thiamethoxam doses of 251 and 247 mL 100 kg<sup>-1</sup> of seeds, respectively. Similar results were obtained by Almeida et al. (2012) in black oat seeds, when they observed that seedling emergence was stimulated by thiamethoxam seed treatment. According to Nunes (2006), soybean seeds treated with thiamethoxam contain higher amino acid concentration and increased enzymatic activity and hormone synthesis resulting in decreased crop time and increased yield in the field because plants become more tolerant to stress.

The results show clearly that the use of the product brings benefits that translate into a better performance of seedlings, which in turn will have a direct impact at the stand of a crop, allowing faster and uniform establishment. Therefore, crops that have this feature also have significantly increased production potential.





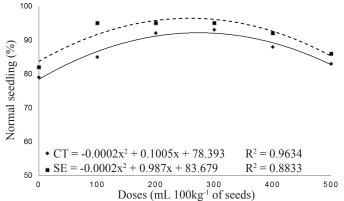


Figure 3. Normal seedling percentage in the cold (CT) and seedbed emergence (SE) tests in bean seeds, IAPAR Siriri cultivar, in function of the treatment with increasing thiamethoxam doses. Mean values of five storage times are shown. Capão do Leão - RS. 2015.

#### Conclusion

Treatment of bean seeds, IAPAR Siriri cultivar, at thiamethoxam doses between 200 and 300 mL 100 kg<sup>-1</sup> of seeds enables germination and vigor expression and the storage potential under controlled conditions, decreasing the rate of germination loss over time.

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