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# **General Entomology**

# Treatment of bean seeds (*Phaseolus vulgaris* L.) with systemic insecticides for the management of *Cerotoma arcuata* (Olivier) (Coleoptera: Chrysomelidae)

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**Abstract.** Bean (*Phaseolus vulgaris* L.) cultivation has great agricultural and food importance, but this crop production is generally affected by severe infestations of *Cerotoma arcuata* (Olivier) (Coleoptera: Chrysomelidae) in the northern region of Brazil. An alternative for leaf beetle control is the use of physiological insecticides acting in the initial phase of plant growth, through pre-sowing seed treatment. In this sense, this study aimed to evaluate the effects of systemic insecticides, based on thiamethoxam, imidacloprid and fipronil, on the germination performance of bean seeds, and to verify the efficacy of these insecticides on the control of *C. arcuata*. The research was carried out in two stages. Initially, under laboratory conditions, the influence of the insecticides on the germinating power of seeds was evaluated through the following parameters: germination, emergence, electrical conductivity and dry mass of the aerial part. In the field, the efficacy of the insecticides on the plant stand, number of insects per plant and level of foliar damage at 21 and 28 days of planting were evaluated. The insecticides investigated did not affect the germinating power of seeds when compared to the control. Regarding field results, there was a lower incidence of *C. arcuata* and less leaf damage to plants from seeds treated with systemic insecticides, showing greater crop protection in the period studied.

Keywords: Bean leaf beetle; Control; Defoliation; Germination; Neonicotinoid.

ean (*Phaseolus vulgaris* L.) cultivation has great agricultural and food importance in several regions (HIOLANDA et al. 2018), being Brazil one of the largest producers and consumers of beans in the world (CONAB 2020). Among the phytosanitary problems associated with this cultivation it is worth mentioning the occurrence of *Cerotoma arcuata* (Olivier) (Coleoptera: Chrysomelidae) in the northern region of Brazil (ALECIO et al. 2010; FAZOLIN & ESTRELA 2004; FAZOLIN et al. 2007), where this insect pest disperses thus promoting severe infestations.

Adults of *C. arcuata* attack the bean leaves and other legumes while their larvae feed on roots and also on nodules, where nitrogen fixation (BNF) occurs, causing a delay in the bean plant development and a decrease in crop production (TEIXEIRA & FRANCO 2007; BOIÇA JUNIOR *et al.* 2014). The control of *C. arcuata* in bean cultivation has been done mainly through the use of residual insecticide (FAZOLIN *et al.* 2007), which is applied four times/crop cycle, mainly due to the short duration of action on the target insects. However, the continuous and indiscriminate use of insecticides can result the evolution of resistant pest populations, in addition to causing problems for man and the environment (PIMENTEL *et al.* 2012; SOUSA *et al.* 2012).

The use of high-quality seeds is a key factor for the formation of a uniform stand, enabling the maximization of the action of other inputs and production factors used in plantation (Costa *et al.* 2017). Thus, seed treatment with insecticides is a practice that has been increasingly adopted as an alternative for plant protection during the establishment phase in the

field, not only aiming at plant protection, but also to improve the performance of the initial development of plants, or during their vegetative cycle, resulting in greater use of their productive potential (CASTRO *et al.* 2008; LEMES *et al.* 2015).

It is worth noting that seed treatment practice with systemic insecticides makes it possible to reduce the number of foliar applications, which often need to be initiated soon after seedling emergence (MAIENFISCH *et al.* 2001). After sowing, the compounds are released from the seeds and, due to their low vapor pressure and water solubility, they are slowly absorbed by the roots (GOULSON 2013). Seed treatment is considered one of the most efficient methods for insecticides use (ALBAJES *et al.* 2003). However, this technology generally does not protect plants during the entire growing season. For seed treatment to be successful it needs to be based on product information, regarding its effects on the germination power of the seeds, its spectrum, duration of action, toxicology and compatibility with other products (BARROS *et al.* 2001; VIEIRA *et al.* 2003).

Neonicotinoids and fipronil stand out among the systemic insecticides used for seed treatment (KocH *et al.* 2005; VAZQUEZ *et al.* 2014; COSTA *et al.* 2017). Systemic insecticides are applied to crops by different means, from foliar spraying to seed treatments and soil applications. The popularity of these insecticides is largely due to their high toxicity to invertebrates, the ease and flexibility of application, their long persistence and their systemic nature. However, research on the use of these insecticides for the control of *C. arcuata* in bean cultivation remains limited in the scientific literature, as

well as their physiological effects on the germination phase of the seeds. Taking into consideration the information presented, this study aimed to evaluate the effects of systemic insecticides, based on thiamethoxam, imidacloprid and fipronil, on the germination performance of bean seeds and to verify their efficacy on the control of *C. arcuata*.

## **MATERIAL AND METHODS**

The research consisted of two stages, the first conducted at the Seed Laboratory of the Universidade Federal do Acre (UFAC) and the second in the experimental area located in Catuaba Experimental Farm (10°4'36 "S; 67°37'0 "W), also belonging to the UFAC. Common bean seeds of Pérola cultivar were used.

The laboratory experiment was conducted from January to April 2012. The seeds were initially separated into five 200 g lots. These seeds were treated with the following products: T1 (thiamethoxam), T2 (imidacloprid), T3 (fipronil), T4 (fipronil + pyraclostrobin + methyl thiophanate), and the control consisted of seeds without insecticide treatment. After seed treatment, the first lot was sown and analyzed, then the other four lots were sown and analyzed after storage periods of 15, 30, 45 and 60 days.

Seeds were wet treated by diluting the insecticide in distilled water, at a dosage of 200 g commercial product (cp)/100 kg seeds for all the treatments, as indicated on the package insert. Only distilled water was used in the control treatment. The homogenization of the slurry with the seeds was carried out in plastic bags of 2 kg capacity. The solution was shaken for 2 min in order to homogenize and cover perfectly and uniformly the surface of the seeds with the insecticide. After this procedure, the effects of the insecticides were evaluated through the following analyses: germination, emergence, electrical conductivity and dry mass of the aerial part.

The germination test was performed according to the Rules for Seed Analysis (BRASIL 2009), using four samples of 50 bean seeds for each of the four repetitions. The substrate used was germitest paper, moistened with distilled water at a proportion of 2.5 times the weight of the paper. The seeds were arranged on two sheets of germination paper and then covered with another sheet of the same paper, and then wrapped, forming rolls. The rolls were placed in a vertical position inside a germinator and kept at a temperature of 25  $\pm$  1 °C. The final count was made after nine days, considering the normal seedlings, and the data were expressed as a germination rate.

For seedling emergence in sand, the experiments were performed from sowing seeds in polystyrene seedling trays with 200 cells, divided into four repetitions of 50 seeds. Initially the sand used as substrate was autoclaved at a temperature of 120 °C for 60 min to avoid possible contamination of the seed lot. Emerging seedlings were counted daily from the beginning (five days after installation) of the emergence to the moment (nine days after installation) of numerical stabilization of counts. The results were expressed as % of normal seedlings emerged in stabilization (BRASIL 2009).

Electrical conductivity was evaluated through three repetitions of 50 seeds each, weighed and packed in disposable plastic cups with a capacity of 180 mL, adding 75 mL of distilled water. Then the cups were kept in a BOD incubator at 25 °C. The reading was taken after 24 h of soaking, using an electrical conductivity meter (VIEIRA *et al.* 2001; FREITAS *et al.* 2016).

Dry mass of the aerial part was analyzed after stabilizing the emergence of the seedlings, which were initially washed and

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cut by separating the root and aerial part of 25 seedlings from each repetition. Later, the seedlings were stored in paper bags, weighed and then placed in an oven at 60 °C until the mass stabilization, in order to be weighed again and determine the aerial dry matter mass.

Data obtained were submitted to the arcsine square root transformation to follow the assumptions of the analysis of variance, and statistical analysis performed through the Sisvar Software (FERREIRA 2011). When significant, the germinal characteristics data, referring to the seeds treated with each insecticide, were submitted to regression adjustments in relation to the storage period, using the SigmaPlot software, version 14 (Systat Software, Inc., San Jose, CA, USA). Significant means were compared by Tukey's test at 5% probability. The experiment was performed in a completely randomized design (CRD) in a factorial scheme (5 treatments x 5 storage periods), with four repetitions.

The second stage of the research was carried out between the months of March and June 2012, consisting of a common bean plantation at Catuaba Experimental Farm, belonging to the Universidade Federal do Acre, to verify the effectiveness of insecticides in field conditions. The experimental area of  $45 \times 30$  m was hoed for soil preparation and elimination of weeds. The planting was done with the help of a manual seed planter. Each experimental plot had dimensions of  $4 \times 8$  m, with spacing between rows of 0.5 m and the working area consisting of the three main rows excluding 1 m of edge.

The plant stand was analyzed at 21 days after planting, using a wood square frame of 1 m<sup>2</sup>, which was randomly thrown three times within the working area of each plot. Then, the plants were counted within the square frame in the working area, the average number of plants was calculated and the final result expressed in ha<sup>-1</sup>. To determine the incidence of *C. arcuata*, five plants were chosen at random within the plot area and the number of insects on their leaves was counted. The evaluations were carried out 21 and 28 days after planting. For leaf damage level, five plants were randomly chosen inside the working area of the plot, being assigned a rating scale from 1 to 5, where 1 consisted of a plant without leaf damage and 5 for a totally affected plant. This evaluation occurred at 21 and 28 days after planting.

The experiment was conducted in a randomized block design (RBD), with five treatments (control and insecticides) and four repetitions (blocks). The results were verified through SHAPIRO & WILK (1965) for normality of errors and Hartley F-maximum test for homogeneity of variances. Later they were submitted to analysis of variance and then compared by the Tukey's test at a level of 5% significance.

### **RESULTS AND DISCUSSION**

For germination, no significant interaction was found between insecticide factors and storage period of treated seeds (F<sub>16.75</sub>=1.21, P=0.28). But there was significant interaction between these two factors for the emergence of seedlings in sand (F<sub>16,75</sub>=1.82, *P*=0.04). The three-parameter sigmoid model (y=a/1+exp(-(x-b/c))) was the best fit for seedling emergence data in relation to the seed storage period, for control and for T3 (R<sup>2</sup>=0.99, P≥0.01), and a four-parameter sigmoid model (y= a+b/(1+exp(-(x-c)/d))) was the best fit for T1 and T2 ( $R^2$ =0.99,  $P \ge 0.03$ ), and therefore the models used (Figure 1). None of the regression analysis models fitted significantly for T4 emergence data (P>0.05). Overall, the emergence reduced with the increasing storage period of seeds in all treatments (Figure 1). Seeds treated with the insecticides T1 (thiamethoxam) and T2 (imidacloprid) showed lower emergence rates in the 45-day storage period (P<0.05, Table 1).



**Figure 1.** Emergence of seedlings sown with seeds treated with systemic insecticides and stored for 60 days. The symbols represent the averages of the repetitions and the lines represent the tendency of the emergence in relation to the storage period. Control: no insecticide, T1: thiamethoxam, T2: imidacloprid, T3: fipronil, T4: fipronil + pyraclostrobin + methyl thiophanate.

Veriebles	Treatments	Storage period (days)				<b>C</b> \ (0()	
Variables		0	15	30	45	60	CV (%)
	Control	99.00 a	99.00 a	98.38 a	97.13 a	90.75 a	
	T1	99.13 a	98.88 a	98.38 a	93.13 b	92.00 a	1.80
Emergence (%)	T2	98.25 a	98.13 a	98.00 a	93.88 b	93.25 a	
	T3	99.38 a	99.13 a	97.50 a	96.50 ab	93.00 a	
	Τ4	99.25 a	96.75 a	96.88 a	96.50 ab	90.25 a	
Electrical conductivity (µS cm⁻¹ g⁻¹)	Control	1.23 b	1.27 c	1.30 b	1.22 c	1.17 c	
	T1	1.50 a	1.40 b	1.51 a	1.47 b	1.54 a	
	T2	1.27 b	1.35 bc	1.28 b	1.38 c	1.32 b	3.92
	Т3	1.57 a	1.57 a	1.51 a	1.61 a	1.60 a	
	T4	1.11 c	1.14 d	1.18 c	1.12 c	1.13 c	
Dry mass of the aerial part (g)	Control	3.53 ab	3.71 a	3.41 a	2.93 b	2.54 a	
	T1	4.05 ab	3.85 a	3.86 a	3.29 ab	2.59 a	
	T2	3.36 b	3.55 a	3.65 a	3.82 a	2.53 a	12.31
	Т3	4.25 a	3.82 a	3.52 a	2.93 b	2.67 a	
	Τ4	3.75 ab	3.58 a	4.02 a	2.90 b	2.80 a	

Table 1. Emergence and electrical conductivity of seeds and dry mass of the aerial part of seedlings from seeds treated with insecticides and stored in different periods.

Means followed by the same letter in the column do not differ statistically by Tukey's test (*P*<0,05). Control: no insecticide, T1: thiamethoxam, T2: imidacloprid, T3: fipronil, T4: fipronil + pyraclostrobin + methyl thiophanate.

Although bean seed treatment with thiamethoxam and imidacloprid insecticides has resulted in a significant reduction in the emergence of the seedlings, these insecticides have not compromised their emergence. According to current legislation, seed is characterized according to its category, being considered minimum germination of 70% for basic seed and 80% for certified (C1 and C2) or uncertified (S1 and S2) seed of first and second generations (BRASIL 2013). In this regard, in Figure 1 it is observed that the emergence rates remained above the ones established in the current legislation, even in the 60-day storage period, with an

average emergence above 90%. According to CASTELLANOS *et al.* (2017), the treatment of bean seeds cv. IAPAR Siriri with thiamethoxam, in doses between 200 and 300 mL 100 kg<sup>-1</sup> of seeds, enabled the expression of germination and vigor, as well as its potential for storage under controlled conditions, decreasing the rate of germination loss over time. It is also worth mentioning that synthetic products used in seed treatment may cause higher rates of seedling emergence, as reported by BARROS *et al.* (2005) for fipronil.

Regarding the electrical conductivity, there was a significant interaction between the insecticide factors and storage

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period (F<sub>16.75</sub>=3.38; P=0.0002). The regression models tested did not fit significantly (P>0.05) (Figure 2). However, in general, the conductivity of seeds treated with the insecticides T1 (thiamethoxam) and T3 (fipronil) was significantly higher than the other treatments in all storage periods (*P*<0.05, Table 1). These results indicate that thiamethoxam and fipronil-based products influenced the cellular degradation process of the seeds, once they caused the increase of leachates in the water-based solution used for electrical conductivity analysis (VAZQUEZ et al. 2014; HARTER et al. 2018). It is known that the "storage period factor" itself can cause an increase in electrical conductivity, indicating a reduction in physiological quality (FREITAS et al. 2016). However, in this research the electrical conductivity of treated seeds did not follow a statistical trend in relation to the storage period of treated seeds (P>0,05; Figure 2). On the other hand, VAZQUEZ et al. (2014) verified an increase in conductivity in insecticide-treated maize seeds stored for 35 days.

For the dry mass of the aerial part it was also found significant interaction between the insecticide and storage period ( $F_{16,75}$ =1.83; *P*=0.05). The quadratic polynomial model (y=a+bx+x<sup>2</sup>) was the one that best fitted the data for dry mass of the aerial in relation to the storage period, both for the control and for the insecticides T1 and T3 (*R*<sup>2</sup>≥0.96; *P*≥0.01) (Figure 3). None of the trend models fitted significantly for T2 and T4 treatments (*P*>0.05). In general, the dry mass of the aerial part tended to reduce with the increase in the storage period, generally presenting higher values of aerial dry mass in seedlings from seeds treated with systemic insecticides (Figure 3). Table 1 shows that T3 (fipronil) and T2 (imidacloprid) treatments caused significantly higher dry mass of the aerial part than the other treatments in storage periods of 0 and 45 days (*P*<0.05).

The results for aerial dry mass indicate that the systemic insecticides acted as bioactivators in the treated seeds, promoting greater accumulation of dry matter in relation to the



**Figure 2.** Electrical conductivity of seeds treated with systemic insecticides and stored for 60 days. The symbols represent the averages of the repetitions and the lines represent the tendency of the electrical conductivity in relation to the storage period. Control: no insecticide, T1: thiamethoxam, T2: imidacloprid, T3: fipronil, T4: fipronil + pyraclostrobin + methyl thiophanate.

control. Bioactivators are usually complex organic substances capable of acting on the transcription of plant DNA, gene expression, membrane proteins, metabolic enzymes and mineral nutrition (ALMEIDA *et al.* 2009). It is a known fact that germination bioactivators stimulate root growth in a faster way, favoring the increase of nutrient absorption by the plant (COSTA *et al.* 2017). The larger the leaf area, the greater the photosynthetic active surface, and consequently the greater the production of energy and leaf assimilates. Therefore, a plant that has a high capacity to accumulate foliar tissue can present a great competitive advantage, especially at the critical moment that represents its establishment in the field (TUNES *et al.* 2018).

Planting under field conditions revealed that the plant stand (plants ha<sup>-1</sup>) did not vary significantly between treatments (P>0.05) (Table 2). At 21 and 28 days after planting, the number of individuals of *C. arcuata* per plant was significantly higher in the control (P<0.05), compared to plants from seeds treated with insecticides T1 (thiamethoxam), T2 (imidacloprid), T3 (fipronil) and T4 (fipronil + pyraclostrobin + methyl thiophanate) (Table 2). In a general way, less leaf

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damage was found in plants from seeds treated with these products (*P*<0.05) (Table 2). The products based on systemic insecticides caused less leaf damage to plants and lower incidence of *C. arcuata*, corroborating with the results of KocH *et al.* (2005) for *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). According to BARBOSA *et al.* (2002), bean seed treatment with the insecticides imidacloprid and thiamethoxam also provided improvements in the agronomic characteristics of the crop, resulting in increased productivity.

Considering the importance of phytosanitary seed treatment, products based on thiamethoxam, imidacloprid and fipronil did not affect the germination potential of common bean seeds under laboratory conditions and were effective in controlling *C. arcuara* under field conditions. On the other hand, it is important to point out that recent studies indicate that the proportion of insecticides translocated in plants during the entire growth phase is low in relation to the amount applied to the seeds (GOULSON 2013). This observation may provide a basis to explain reports of neonicotinoid residues in the environment and justifies the use of seed treatment integrated with other control strategies during



**Figure 3.** Dry mass of the aerial part of seedlings sown with seeds treated with systemic insecticides and stored for 60 days. The symbols represent the averages of the repetitions and the lines represent the tendency of the dry mass of the aerial part in relation to the storage period. Control: no insecticide, T1: thiamethoxam, T2: imidacloprid, T3: fipronil, T4: fipronil + pyraclostrobin + methyl thiophanate.

	<b>Table 2.</b> Plant stand, incidence of <i>C. grcugtg</i> and leaf damage in bean plants sov	wn with seeds treated with systemic insecticides.
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Treatment —		21 days	28 days		
	Plants ha <sup>-1</sup>	Insects/plant	Foliar damage	Insects/plant	Foliar damage
Control	170,000 a	1.94 a	2.25 b	1.62 a	3.69 a
T1	185,000 a	1.00 c	2.31 b	0.56 c	3.37 b
T2	260,000 a	1.25 b	3.75 a	0.88 b	3.35 b
Т3	290,000 a	0.81 c	1.75 c	0.05 d	2.75 c
T4	297,500 a	0.94 c	1.44 d	0.19 d	2.69 c
CV (%)	26.26	33.18	16.26	53.93	12.32

Means followed by the same letter in the column do not differ statistically by Tukey's test (*P*<0,05). Control: no insecticide, T1: thiamethoxam, T2: imidacloprid, T3: fipronil, T4: fipronil + pyraclostrobin + methyl thiophanate.

plant establishment phase in the field, reducing risks of *C. arcuara* control failures, among other coexisting pests in the same environment (BONMATIN *et al.* 2015; ALFORD & KRUPKE 2017).

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