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RESEARCH ARTICLE

Impact of Insecticide Seed Treatments on population dynamics of *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae)

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ABSTRACT

This study evaluated the effect of insecticides used in the treatment of soybean [(*Glycine max* (L.) Merrill)] seeds on the population dynamics of *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae), popularly known as the Neotropical brown stink bug. The experiment was conducted on 2015/2016 and 2016/2017 soybean crops in the experimental area of the Celeiro Seed Farm. Soybean seeds of the cultivar 'TMG 132RR®' were treated with the insecticides imidacloprid + thiodicarb, abamectin, fipronil and chlorantraniliprole. Two controls were used, the conventional cultivar 'TMG 132RR®' and the transgenic cultivar 'M 8644IPRO®', both without insecticide treatments. Crop I (2015/2016) had an IAD (insects accumulated daily) 2.9 times greater than Crop II (2016/2017); however, treatment with insecticides did not affect the IAD. For Crop I, there was a population peak of E. heros at R5.5 (from 75 to 100% of the full grains), since the soybean treatment with imidacloprid + thiodicarb had 2 and 2.5 times more insects than the conventional and transgenic controls, respectively. For Crop II, there was no population peak of E. heros. The IAD showed significant negative correlations, by F test, with mass of 1,000 seeds - MTS (-0.44112 **) and productivity (-0.45895 **). After analyzing the results, it was verified that there was no residual effect of the insecticides on the population dynamics of *E. heros* during the cycles and under the conditions of the soybean crops studied.

Keywords: Chemical control; *Glycine max;* Neotropical brown stink bug

INTRODUCTION

The yield of an agricultural crop is defined by the interaction between the plant and environment. This is the case for soybeans (*Glycine max* [L.] Merrill), where management is a key point in the production process. *Euschistus* heros (Fabricius) (Heteroptera: Pentatomidae) is the most abundant stink bug species in soybean crop (ABIOVE, 2018). It is an important polyphagous, multivoltine pest that exhibits diapause during unfavorable conditions (Smaniotto and Panizzi, 2015), and phytosanitary management of the species is complicated due to the "green bridge" that occurs during the offseason, since there are alternative hosts that promote the survival, longevity, fecundity and fertility of this insect (Tuelher et al., 2018).

Chemical products are the most common management method used to control *E. heros*. However, it is challenging to find products that selectively target pests and efficiently control *E. heros* (Pazini et al., 2019). Treating seeds with insecticides is a viable alternative, since it directly affects pests and may increase productivity by approximately 128.0 kg ha^{-1} (Hurley and Mitchell, 2017).

Many studies have discussed the impact of treating seeds on the physiological quality of seeds and the initial development of plants (BALDINI et al., 2018; Seraguzi et al., 2018; Bem Junior et al., 2019; Pereira et al., 2019), but rarely has research focused on grain yield and the association of micronutrients, herbivory, sucking insects, fungicides and insecticides (Burange et al., 2015; Calandra et al., 2016). Treating seeds may favor the initial development of plants and allow the plants to respond to sucking insects, decreasing the population density of these pests (Worrall et al., 2012). Exogenous application

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of such compounds, along with some synthetic chemicals, can also activate priming responses (Chiesa et al., 2016; Alford and Krupke, 2017), which may be the systemic mode of action in the plant (Kathiria et al., 2010; Boyko and Kovalchuk, 2011).

Studies that evaluated the effect of seed treatment with the insecticides Imidacloprid, Thiametoxam, Tiodicarb, Fipronil, Abamectin, Clothianidine and Acetamipride did not find a reduction in the population density of the insect *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) (Martins et al., 2009; Brustolin et al., 2012; Chiesa et al., 2016). However, the same cannot be assumed for *E. heros*. Therefore, the goal of this study was to evaluate the Impact of Insecticide Seed Treatments on population dynamics of *E. heros*

MATERIALS AND METHODS

Study site

The experiment was conducted in the experimental area of Celeiro Seed Farm, in the municipality of Monte Alegre do Piauí, Serra do Quilombo (09º21'12" S; 45º 07'42" W, 640 m), during the 2015/2016 and 2016/2017 growing seasons. The soil in the region is characterized as a yellow latosol (EMBRAPA, 1999) and the climate in the region is tropical Aw, with a dry season in the winter. Rainfall in the region was distributed mainly in the final and beginning months of each study year (Fig. 1).

Cultivars and treatment of seeds

Seeds of the 'TMG132RR®' (determined habit) soybean cultivar, with a maturity group of 8.5, were treated with insecticides. Two controls, without the insecticide treatments, were also used: the 'TMG 132RR®' cultivar; and the 'M 8644IPRO®' cultivar (determined habit), with a maturity group of 8.6. The IPRO cultivar, popularly known as the Bt soybean, was used as an additional control because it was the cultivar most similar to the cultivar of the main treatment. There were six treatments, four with insecticides and two controls, for each crop. The insecticides used to treat the seeds were imidacloprid + thiodicarb, abamectin, fipronil and chlorantraniliprole (Table 1).

The seeds were treated in the Phytotechnology Laboratory at the Federal University of Piauí (UFPI/CPCE). The insecticides were diluted in distilled water to different doses (Table 1). The solution with the seeds was homogenized for 2 min in 2 kg plastic bags and the seeds were then dried in the shade. The seeds were sown soon after this procedure. In addition, the seeds were treated with a fungicide (30 g a.i. carbendazim + 60 g a.i. dimethyldithiocarbamate per 100 kg of seeds) and inoculated in a suspension of *Bradyrhizobium* (60 g/60 kg of seeds) to avoid problems with diseases and to guarantee biological fixation, respectively.

Implementing the experiment and phytosanitary management

The experiment was conducted in a randomized block, with four replicates, for two subsequent years. Seeds were

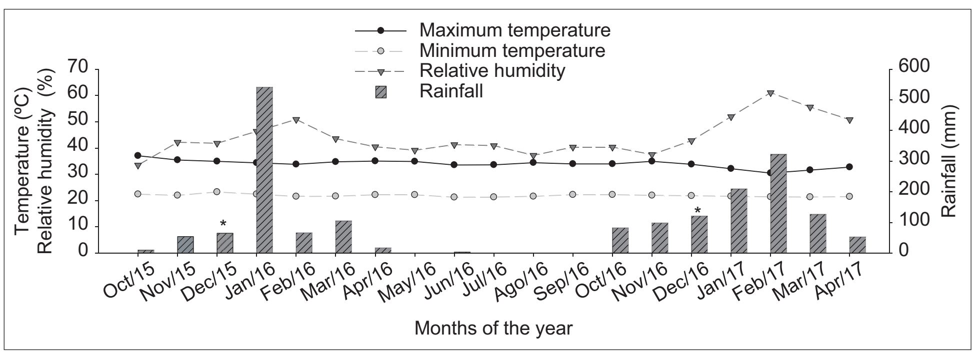

Fig 1. Rainfall, temperature (maximum, minimum and average) and relative humidity of the experimental area of Celeiro Seed Farm, Serra do Quilombo, Monte Alegre do Piauí, during the experiment for the 2015/2016 and 2016/2017 crops. *Start of experiment.

Table 1: Treatments used – Active ingredients applied via the soybean treatments, according to the recommendation for the crop by the Ministry of Agriculture, Livestock and Food Supply

Active ingredient	Chemical group	Mechanism of action	Conc. I. a. [1]	Dose of CP[2]
Imidacloprid +	Neonicotinoids	Nicotinic acetylcholine receptor agonists	150	700
Thiodicarb	Methylcarbamate	Acetylcholinesterase inhibitors	450	
Abamectin	Avermectin and milbemycins	Chloride channel activators	500	100
Fipronil	Phenylpyrazoles	Gaba-gated chloride channel antagonists	250	200
Chlorantraniliprole	Diamide	Ranodine receptor modules	625	100
Control	-	-	-	-
Bt soybean	-	-	-	-

[1]Concentration of active ingredient in liters of commercial product (CP). [2]Dose of CP in ml per 100 Kg^{-1} of seed.

sown with a no-till, four-row seeder on 23 December 2015 (Crop I) and 06 December 2016 (Crop II). Sowing density was 14 seeds per linear meter and 0.50 m between rows. Basic fertilization was carried out on the sowing line with 50 kg.ha^{-1} of P_2O_5 and 60 kg.ha^{-1} of K_2O based on a soil analysis. Phytosanitary management was conducted according the management of the farm (Table 2).

Monitoring *E. heros* and soybean harvesting

Starting at V_3 (vegetative stage), *E. heros* nymphs and adults were sampled. Sampling began during the third or fourth week after the plants emerged and included 13 sampling dates per crop based on the reproductive stage of the soybeans, which is the critical period when the crop is susceptible to attack by *E. heros* (Panizzi et al., 2012). Sampling was conducted using the beat cloth method, where a linear meter of soybeans (0.5 m²) was shaken over the cloth to collect the insects so they could be quantified. At the end of the crop cycle, two soybean lines per plot were manually harvested. The plants were collected and threshed at the site with a mechanical thresher (model LPR, ALMACO ©).

Parameters evaluated

The population dynamics of *E. heros* along the phenological cycle of the crop were evaluated and, subsequently, the IAD (insects accumulated daily) was calculated and the yield components were quantified. The data for population density were converted to *E. heros* per square meter and an IAD index was calculated, which considered the accumulated daily population density of individuals using the equation IAD = [(0.5 x (Pn + Pn+1) x D], where: Pn refers to the number of individuals in sample n; Pn+1 refers to the number of individuals in the next sample; and D is the time in days between successive samples (Chiesa et al., 2016).

The yield components were evaluated after the harvest. Samples were weighed with an analytical precision balance (Shimadzu Auy 220) and the moisture content was then standardized to 13%, which was done using the drying method at 105°C ± 3°C for 24 hours (Brasil, 2009). The results were expressed in kg.ha^{-1}. To evaluate the mass of 1,000 seeds (MTS), eight subsamples of 100 seeds were separated based on the Rules for Analyzing Seeds (*Regras de Análise de Sementes* – RAS) (BRASIL, 2009). Seed mass was predetermined using a precision balance, seed moisture content was standardized to 13% and the results were expressed in grams.

Statistical analysis

An analysis of variance was conducted for each agricultural year for the yield variables, MTS and IAD. The IAD was transformed by the root of x + 0.5 to meet normal distribution. After the individual analyses, a combined analysis of the two agricultural years was conducted since they met the condition of uniformity of residual variances. When the interaction between the year and treatments was significant, the simple correlation between the variables measured (yield, MTS and IAD) was evaluated.

When significance was detected by F test for a variation source, the Tukey averages test was performed at 5% probability. Subsequently, a Pearson correlation analysis

Table 2: Phytosanitary management conducted on the 2015/2016 and 2016/2017 soybean crops

Phenological stage	Active ingredient	Class	Dose of CP applied[1]
Ve	Haloxyfop-P-methyl	Herbicide	1 L/ha
Ve	Mineral oil	Adjuvant	0.8L/ha
V2	Glyphosate	Herbicide	2.5 L/ha
V2	Mineral oil	Adjuvant	0.8 L/ha
V3	Glyphosate	Herbicide	4 L/ha
V3	Mineral oil	Adjuvant	0.4 L/ha
V5	Glyphosate	Herbicide	4 L/ha
V5	Mineral oil	Adjuvant	0.6 L/ha
V8	Pyraclostrobin+Epoxiconazole	Fungicide	0.5 L/ha
V8	Mineral oil	Adjuvant	0.3 L/ha
R1	Fluxapyroxad+Pyraclostrobin	Fungicide	0.25 L/ha
R1	Mineral oil	Adjuvant	0.3 L/ha
R4	Azoxystrobin+Benzovindiflupyr	Fungicide	0.2 kg/ha
R4	Mineral oil	Adjuvant	0.3 L/ha
R5.5	Pyraclostrobin+Epoxiconazole	Fungicide	0.5 L/ha
R5.5	Mineral oil	Adjuvant	0.3 L/ha
R6	Acetamiprid	Insecticide	0.3 kg/ha
R6	Imidacloprid	Insecticide	0.25 kg/ha
R6	Mineral oil	Adjuvant	0.3 L/ha
R6	Emamectin benzoate	Insecticide	0.035 kg/ha

[1]CP – Commercial products

was conducted between the characters studied and their significance was tested using a t-test.

The fluctuation of insects was analyzed in an exploratory way to see the significance of the effects of the interactions. All statistical analyses were made using SAS Institute software (2002).

RESULTS

There was significant interaction among the seed treatment (ST) products on the occurrence of *E. heros* in the 2015/2016 crop ($F = 1.53$, df = 60, 219, $P \leq 0.05$), which differed from the 2016/2017 crop ($F = 0.85$, df = 60, 231, $P > 0.05$ NS) where no significant effect of the products was observed in relation to phenological stage (Fig. 2A and B). Considering the crop in the evaluation according to the phenological stage, a significant interaction between the sources of variation ($F = 5.63$, df = 12, 75, $P \leq 0.05$ **) was observed (Fig. 2C).

The population peaks of *E. heros* occurred during the grain filling period of the soybeans (R_5) **(**reproductive stage) (Fig. 2) and the density for the first crop was greater, with 1.5 insects.m^{-2}. For Crop I, the population peak of *E. heros* occurred at R5.5, when the soybeans treated with imidacloprid + thiodicarb had 2 and 2.5 times more insects than the control and Bt soybeans, respectively. Considering only the numbers, for Crop II the Bt soybean and conventional soybean treated with fipronil had a low incidence in the area, while the remaining treatments exhibited population outbreaks, except for the treatment with imidacloprid + thiodicarb that exhibited a constant population density **(**Fig. 2B). However, only the soybeans treated with abamectin and chlorantraniliprole had *E. heros* during the critical period of occurrence (Fig. 2A). In R6 **(**reproductive stage**)**, a decrease in the *E. heros* population dynamics was observed due to the application of insecticides (Acetamiprid, Imidacloprid and Emamectin benzoate**)**.

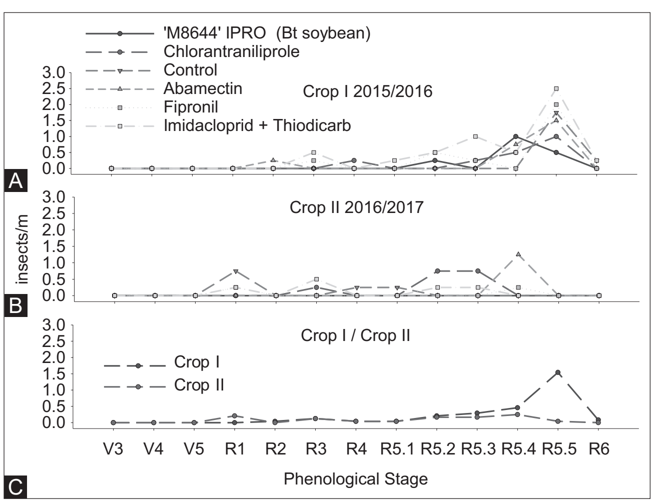

Fig 2. Population fluctuation of *Euschistus heros* for the different treatments. A. Crop I B. Crop II C. Both crops.

The daily-accumulated population density of *E. heros* (IAD) differed only between harvests (Table 2). It can be seen that Crop I had an IAD 2.9 times greater than Crop II; however, treatment with insecticides did not affect the IAD.

The crop factor was the only source of variation that affected the IAD ($F = 26.05$, df = 1.30, $P \leq 0.05$ **). In relation to the yield components, MTS was influenced in an isolated way by the harvest ($F = 46.67$, df = 1.30, $P \leq 0.05$ **) and the applied ST products ($F = 6.94$, df = 5, 30; $P \leq 0.05$ **.), and the Bt soybean of Crop II had the highest MTS value (188.13 g). Productivity was significantly affected by the interaction between product and crop ($F = 5.63$, df = 12, 75, $P \leq 0.05$ **). It is worth mentioning that productivity values were higher for all treatments of Crop II, especially the results obtained with abamectin that had a productivity of 2,954 kg.ha^{-1}.

According to the Pearson correlation coefficients, the IAD showed significant negative correlations, by F test, with MTS (-0.44112 **) and productivity (-0.45895 **).

DISCUSSION

Here, we show that treating the soybean seeds with insecticides did not have a sufficient residual effect on the control rates of *E. heros* on the soybean crops. Treating seeds with insecticides has been shown to help protect plants up to 47 days after planting. Applying insecticides can induce defense mechanisms in plants. In the study conducted by Worrall et al., (2012) it was observed that seed treatment reduced the performance of all three herbivores tested. Jasmonic acid JA is well-known for its role in defense against chewing insects (Zhang et al., 2017). Its role in defense against brown stink bug and other phloem feeding herbivores is less clear, since these insects tend to activate SA-dependent responses in the plant (Lin et al., 2019). Application of the insecticide Imidacloprid (Neonicotinoid) can induce defense mechanisms in plants, such as production of silicic acid (Ford et al., 2010), an important compound in the defense of plants against sucking insects (Pieterse et al., 2009; Alford and Krupke, 2017).

The persistence of insecticides in plant tissue, when applied to seeds, tends to decrease as the crop develops **(**Alford and Krupke, 2017). This helps explain the results of the present study, since the period of protection of the insecticides applied to the seeds did not coincide with the occurrence of *E. heros*. In a similar experiment with the stink bug *Dichelops melacanthus* (Dallas 1851) (Hemiptera: Pentatomidae) on soybeans, it was found that seeds treated with the insecticides Imidacloprid**,** Thiamethoxam**,** Thiodicarb**,** Fipronil and Abamectin did not affect the incidence of the insect (Chiesa et al., 2016).

For the first crop (2015/2016), the population density of *E. heros* increased along the phenological cycle of the plant and peaked at R5.5. However, for the second crop (2016/2017), a significant population peak was not verified for the grain filling period of the soybeans **(**R5) because, in addition to the effect of the treatments, climatic elements affected the occurrence of *E. heros*. While cultivating the first crop, rainfall was mostly in January (541 mm); however, for the second crop it was distributed throughout the cycle (Fig. 1). It can be affirmed that there is an inversely proportional contrast between rainfall and the occurrence of *E. heros*. This finding suggests that in years with well-distributed rainfall the occurrence of the brown stink bug would stay below the control level. This is supported by Ramos et al., (2017)**,** who found a negative correlation between relative humidity and population density of *E. heros* on the common bean. The fruits start to form at R3, which is when *E. heros* is expected to migrate from alternative hosts to the soybeans. The insects can also come out of diapause and migrate to the main crop; consequently, there is a progressive increase in the population during the reproductive phase. As discussed, this only occurred for first crop in the present study.

For the analysis of the treated seeds on the number of insects accumulated daily (IAD), it was found that the insecticides did not control *E. heros* via the seed treatments. The lower numerical IAD value for the Bt soybean contradicts the premise that *E. heros* lacks a preference for other soybean cultivars, which was observed in studies of the spatial distribution of *E. heros* adults and nymphs on Bt and non-Bt soybeans (Fonseca et al., 2014). However, in relation to the resistance mechanisms of plants (e.g. antibiosis and antixenosis), it is necessary to conduct additional studies to characterize and delimit the interference of transgenic plants on the population dynamics of pests.

For the first crop, the components of MTS yield and yield were not altered by the treatments. The productivity found in the present study is similar to the average estimated for the region (CONAB, 2020). The low MTS and productivity values for the first crop are related to the concentrated rainfall during the growing period. The population peak of *E. heros* during the grain filling period was below the level that causes economic damage, since soybeans tolerate up to 4 stink bugs/linear meter without suffering from a reduction in MTS and productivity. In the present study the maximum density of insects was 2.5 insects.m^{-1}, less than the level of economic damage, and even then, a negative correlation was found between the IAD and yield components (MTS and Productivity). However, the Crop I treatment with Imidacloprid + Thiodicarb, which had 2.5 insects.m^{-1}, did not exhibit a significant reduction in MTS and Productivity values.

Crop II, compared to the first crop, had rainfall distributed throughout the phenological cycle with low incidence of stink bugs, and high MTS and Productivity values were found when using the active ingredient Abamectin**.** It is important to carry out studies about the persistence of pesticides applied to soybean seeds to identify possible biochemical and physiological changes in the plant.

Our experiments do not distinguish between a requirement for residual effects in perception of the seed treatment and subsequent expression of defense in infected leaves. Constitutive activation of plant defense is commonly associated with reductions in growth and reproductive fitness. Priming of defenses on the other hand minimizes these costs whilst improving future resistance to attack. However, it was not possible to associatesuch effects with the seed treatments employed here.

Further work is required to establish a causal effect for the residual effect of treating seeds with insecticides to combat pests. This can be done by testing plants with the metabolism modified in response to the molecules of insecticides (i.e. in some cases, insecticides have been reported to enhance plant vigor and abiotic stress tolerance, independent of their insecticidal function).

CONCLUSIONS

In this work the treating the seeds with insecticides did not change the population dynamics of *E. heros* for the soybean crops under the conditions evaluated. Since seed treatments are economically more attractive than chemical application to plants in the field and require no action by growers, the approaches we have described here may have useful applications in agriculture in future research.

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AUTHORS' CONTRIBUTIONS

Thiago Ferreira Rodrigues and Luciana Barboza Silva conceived research. Thiago Ferreira Rodrigues, Raimundo Henrique Ferreira Rodrigues, Lorrana Francisca Oliveira Almeida and Kaleb Sousa conducted experiments. Ciro Humberto Almeida Alvares contributed material. Bruno Ettore Pavan analysed data and conducted statistical analyses. Thiago Ferreira Rodrigues, Luciana Barboza Silva and Raimundo Henrique Ferreira wrote the manuscript. Ciro Humberto Almeida Alvares secured funding. All authors read and approved the manuscript.

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