

Insecticides Management Used in Soybean for the Control of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae)

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Abstract

The soybean is the main agricultural product produced in Brazil. Among the States, Rio Grande do Sul was the third largest producer in the 2017/2018 harvest. However, insects interfere on the productivity and provide an increase in the cost of production. The velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae) is considered one of the main leaf stripper caterpillars. The occurrence can completely fade, reflected in the grain yield. The chemical method is the main control adopted, however, due to the inadequate management of the crop, failures are observed frequently on the field. In this context, the insecticide management is considered an alternative, still little adopted, due to a lack of knowledge between the movement action and the susceptibility of the insect. Therefore, the objective was to evaluate the performance of insecticides used in the soybean crop for the control of *A. gemmatalis* under laboratory conditions. In the residual and direct contact bioassays, eight treatments with five replicates were used in a completely randomized design, with 2nd and 5th instar caterpillars of *A. gemmatalis*. The results showed that by residual contact during 24 hours, all the treatments with exception of the control had effectiveness in both instar mortality. The treatments flubendiamide + zeta-cypermethrin and zeta-cypermethrin after four hours application reached 100% of effectiveness. Thus, aiming an integrated pest management program, where it advocated the association of different control tactics, the insecticide management with flubendiamide + zeta-cypermethrin were effective with 100% of control efficiency in both instars.

Keywords: chemical control, insecticide management and rationalization, insect resistance, soybean crop, application technology

1. Introduction

The soybean is a commodity of great economic value, and Brazil builds a large share in the world market. The state of Rio Grande do Sul is the third largest producer in the 2017/2018 harvest, with an average yield of 2,981 kg ha⁻¹, and its production is responsible for the generation of employment, supply of products and/or by-products for human and animal consumption, besides generating income and boosting the economy (CONAB, 2018).

The velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae), is considered one of the main defoliation caterpillars of the soybean crop, using for consumption, around 110 cm² of leaf. Due to the infestation level, it can reach losses of 3 to 75% productivity (Walker et al., 2000; Bortoli et al., 2005; Agostini et al., 2013; Franco et al., 2016). The chemical control is the main method of control adopted to the velvetbean caterpillar, but due to the inadequate crop management, faults are frequently observed in the field (Ramos & Pio, 2008; Brenha, 2015). The chemical insecticides used have caused financial losses, contamination for the man and non-target organisms, emergence of resistant populations, and elevation of secondary pests to the category of key pests (Agostini et al., 2013). The different chemical groups of insecticides with their specific modes of action provide distinct efficiencies. The main chemical groups of insecticides applied to the soybean crops are the pyrethroids, organophosphates and carbamates used for several decades (Tomquelski et al., 2015; Martins &

Tomquelski, 2015). The benzoylureas and diamide of phthalic acid have been researched in the last years, however, already with reports of resistant populations (Hannig et al., 2009; Guedes et al., 2012).

Thus, to mitigate the effects of the widespread use of insecticides on the soybean crop, Integrated Pest Management (IPM) contributes on the control of insects as *A. gemmatalis* by integrating various methods (Kogan, 1987). In this context, the management of insecticides is considered an alternative, that is still little adopted due to the lack of knowledge between the interaction of the mode of action and the susceptibility of the insect pest according to the development cycle.

Considering the importance of the velvetbean caterpillar, studies on the performance of the insecticides used in soybean crop are necessary, which will allow to define strategies of action, in order to delay and/or to reverse the evolution of resistance (Sosa-Gómez & Omoto, 2012). The objective of this study was to evaluate the performance of isolated and combined insecticides used in the soybean crop for the control of *A. gemmatalis* under laboratory conditions.

2. Materials and Methods

The experiments were conducted at Embrapa Temperate Climate Bioefficiency Nucleus in an air conditioned room at 25 ± 1 °C, RH of $70\pm 10\%$ and 12 hours of photophase.

2.1 Procurement of the Plants

The soybean seeds of the cultivar BMX Potência were sown in 20 liters plastic pots containing natural substrate based on humus, fiber and clay (West Garden®), and then kept in greenhouse. The plants by reaching the phenological stage V.4 (approximately 25 days) were used in laboratory experiments as food substrate to caterpillars.

2.2 Collection and Breeding of Insects

The insects were collected at Terras Baixas Experimental Station in the municipality of Capão do Leão-RS, Brazil ($31^{\circ}49.268'$ S, $52^{\circ}27.472'$ W, altitude 7 m) in soybean crops in the 2015/2016 harvest, were kept on artificial diet of Greene et al. (1976), during 2 generations. From this, 200 eggs of *A. gemmatalis* were collected, packed in 9 cm Ø Petri dishes, with filter paper moistened with sterile distilled water at the bottom and at the top of the plastic film (PVC) pierced with a pin. The material was kept in an air conditioned room at 25 ± 1 °C, RH of $70\pm 10\%$ and photophase of 12 hours until the hatching of the caterpillars. For the residual contact and direct contact bioassays, we used 2nd and 5th instar caterpillars fed by natural diet.

2.3 Residual Contact Bioassay

The soybean leaves were cut into discs (4 cm Ø), immersed in the treatments for 20 seconds (Table 1). Treatments, which presented more than one product were placed on a bench, and evaporation of excess moisture was expected after being immersed in another insecticide. The leaves were conditioned in polystyrene containers with capacity of 100 ml and inoculated 1 caterpillar per container of 2nd or 5th instar, 5 replicates with 4 caterpillars, totaling 20 caterpillars per treatment. The treatments were kept in an air conditioned room at 25 ± 1 °C, RH of $70\pm 10\%$ and 12h of photophase.

2.4 Direct Contact Bioassay

The spraying was carried out in "Potter's Tower" (Burkard Scientific Uxbridge, UK), calibrated at a pressure of 10 lb pol^{-2} . In each treatment, a 1000 µL aliquot was used on the *A. gemmatalis* caterpillars of 2nd and 5th instar (Table 1). After spraying, 1 caterpillar was dispensed per 100 ml capacity polystyrene container containing discs (4 cm Ø) from the soybean leaf, being 5 replicates with 4 caterpillars, totaling 20 caterpillars per treatment. The treatments were kept in an air conditioned room at 25 ± 1 °C, RH of $70\pm 10\%$ and 12 h of photophase.

Table 1. Active ingredient, mode of action and dose of insecticides used in soybean crop on the control of *Anticarsia gemmatalis* caterpillars

Active ingredient	Mode of Action	Dose*
<i>B. thuringiensis</i>	Microbial disruptors of the mesentero membrane	250
<i>B. thuringiensis</i>	Microbial disruptors of the mesentero membrane	250
+	+	+
Chlorantraniliprole	Rannodyne receptor modulators	25
Chlorantraniliprole	Rannodyne receptor modulators	10
Chlorantraniliprole	Rannodyne receptor modulators	10
+	+	+
Flubendiamide	Rannodyne receptor modulators	25
Flubendiamide	Rannodyne receptor modulators	25
Flubendiamide	Rannodyne receptor modulators	25
+	+	+
Zeta-Cypermethrin	Sodium channel modulators	50
Zeta-Cypermethrin	Sodium channel modulators	50
Witness	Witness	-

Note. * mL ha⁻¹.

2.5 Experimental Design

The design was completely randomized (CRD) and the evaluations were performed 4, 24, 72, 120, 168 hours after the treatment (HAT). Data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% significance (SAS University, 2014). The control efficiency calculated through the corrected mortality determined by the formula according to Abbott (1925) described below:

$$CE (\%) = ([nT - nt]/nT) \times 100 \quad (1)$$

where, nT = living insects in the witness; nt = living insects in the treatment.

3. Results and Discussion

The second instar caterpillars of *Anticarsia gemmatalis* 4 hours after application (4HAT) showed significant mortality to the flubendiamide + zeta-cypermethrin and zeta-cypermethrin treatments, corresponding to a control efficiency of 95.0 and 85.0 (Table 2). It was observed that when combining insecticides with different modes of action, there was an increase in the efficiency of 10% compared to zeta-cypermethrin treatment. The insecticides, with a mode of action in the nervous system as zeta-cypermethrin (pyrethroids), present a marked shock action in the different orders of insects (Guedes et al., 2012). The flubendiamide regulates the release of intracellular Ca²⁺, the effect of which interferes on the muscle contraction, leading to cessation of feeding, lethargy, paralysis, and ultimately death (Boitano & Omoto, 1991).

The lowest efficiency, in relation to the other treatments, did not differ significantly for *B. thuringiensis* (4HAT). Therefore, when combined with chlorantraniliprole the efficiency reached 50% in the control of 2nd instar caterpillars (Table 2). The increase in efficiency by 40% can be attributed to the difference between the mode of action of the insecticides. The bacterium *B. thuringiensis* acts in the middle intestine, from the ingestion of the protein crystals (δ -endotoxins), which are solubilized due to the alkaline pH of the digestive tract of the insect pest. The insecticidal proteins present the characteristics of being highly toxic and specific, however, they require more time to the effective control (Bobrowski et al., 2003; Bravo et al., 2017). Insecticides of the group of anthranilic diamidases, which the active ingredient chlorantraniliprole belongs, bind to the insect's ryanodine receptors in the muscle cells, promoting uncontrolled calcium release due to the canal opening, causing muscle paralysis and the insect death (Cordova et al., 2006; Lahm et al., 2007; Arrue et al., 2014).

The chlorantraniliprole + flubendiamide treatment when applied together had a 5% reduction in the control efficiency of the 2nd instar caterpillars when compared to the use of the isolated chlorantraniliprole, the same was not observed for the flubendiamide (Table 2). However, although diamens are tolerant, after several years of application in the agriculture and have the same site of action, it has been reported cases of resistance development for lepidopteran species, including cross-resistance between both (Thomas, 2013; Roditakis et al., 2015). The results indicate that due to the prolonged use in the field, insects have acquired resistance to flubendiamide and, when used in conjunction with chlorantraniliprole, there is competition to the same site of action, interfering on the efficiency of the pest insect control (Gonçalves et al., 2016).

From the 24 HAT evaluations, all the insecticides had 100.0% of effectiveness in the control of *A. gemmatilis* (Table 2). This effect was observed in the 2nd instar caterpillars of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), which received field leaves with 24 HAT. The treatments that responded significantly were chlorantraniliprole and flubendiamide with 100.0% of efficiency. In the treatment with *B. thuringiensis*, no effective control was observed because of the environmental conditions interfered on the pathogenicity. Therefore, the study demonstrated the importance of studying the sublethal effects, indicating considerable deaths of 60.0% in the pupae phase (Bortoli et al., 2012; Abbas et al., 2015; Kuss et al., 2016).

The control of 5th instar caterpillars of *A. gemmatilis* 4 HAT, achieved results similar to the previous one for the flubendiamide + zeta-cypermethrin, zeta-cypermethrin and *B. thuringiensis* + chlorantraniliprole treatments. However, when evaluating chlorantraniliprole, chlorantraniliprole + flubendiamide and flubendiamide the results had inversion, and the use of isolated flubendiamide was more advisable on the control of 5th instar caterpillars (Table 2). The insecticides beyond the action spectrum present a range to the control of the target insect, this is observed, due to the variation of the susceptibility of the species to the instar that is applied the insecticide (Storch et al., 2007; Guedes et al. 2012). Studies performed with insecticides to the control of *Grapholita molesta* (Busk, 1916) (Lepidoptera: Tortricidae) found that, according to the development of the caterpillars, there is reduction to the insecticide susceptibility (Arioli et al., 2004). In this way, it is evident that the control should be directed to the first instars caterpillars, not only because of the ease of the control, but because of the reduction of the damage on the crop (Silva et al., 2003).

In Rio Grande do Sul, reports of farmers regarding the control failures of *A. gemmatilis* and *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) with pyrethroids have been frequent. In addition, beyond *A. gemmatilis*, *C. includens* e *Rachiplusia nu* (Guenée, 1852) (Lepidoptera: Noctuidae) are species that occur in the Southern Region and for which there is no information on their response to insecticides under laboratory conditions in Brazil. The availability of information relative to the relative regional proportion of these species to rationalize their control is often unknown, because there are no systematic data collection, periodic surveys and during several crop cycles (Sosa-Gomes & Omoto, 2012).

Table 2. Mean (X±SE) of living caterpillars and control efficiency (CE%) of *Anticarsia gemmatilis* caterpillars at 4, 24, 72, 120, 168 hours after the treatment (HAT) via residual contact

Treatments	4 HAT	CE (%)	24 HAT	CE (%)	72 HAT	CE (%)	120 HAT	CE (%)	168 HAT	CE (%)
<i>2nd instar</i>										
<i>B. thuringiensis</i>	3.6±0.40 ¹ ab ²	10	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
<i>B. thuringiensis</i> +Chlorantraniliprole	2.0±0.55 bcd	50	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Chlorantraniliprole	2.2±0.49 abc	45	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Chlorantraniliprole+Flubendiamide	2.4±0.68 abc	40	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Flubendiamide	3.0±0.32 ab	25	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Flubendiamide+Zeta-Cypermethrin	0.2±0.20 d	95	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Zeta-Cypermethrin	0.6±0.40 cd	85	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Control	4.0±0.00 a	0	4.0±0.00 a	0	4.0±0.00 a	0	4.0±0.00 a	-	4.0±0.00 a	0
CV (%)	6.43		3.16		3.16		3.16		3.16	
<i>5th instar</i>										
<i>B. thuringiensis</i>	3.2±0.37 ¹ ab	20	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
<i>B. thuringiensis</i> +Chlorantraniliprole	2.0±0.32 c	50	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Chlorantraniliprole	3.8±0.20 ab	5	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Chlorantraniliprole+Flubendiamide	3.4±0.24 ab	15	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Flubendiamide	3.0±0.37 bc	25	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Flubendiamide+Zeta-Cypermethrin	0.0±0.00 d	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Zeta-Cypermethrin	0.0±0.00 d	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100	0.0±0.00 b	100
Control	4.0±0.00 a	0	4.0±0.00 a	0	4.0±0.00 a	0	4.0±0.00 a	0	4.0±0.00 b	0
CV (%)	5.82		3.18		3.18		3.18		3.18	

Note. ¹(X±SE) = Average number of caterpillars±Standard error. ²Means followed by the same letter in column do not differ by Tukey test (P < 0.05). CV (%) = Coefficient of variation.

With reference to the insecticides applied by direct contact, the results obtained were very close to the previous experiment (residual contact). The treatments flubendiamide + zeta-cypermethrin and zeta-cypermethrin reached

100.0% of efficiency in the control of 2nd instar caterpillars of *A. gemmatalis* 4 HAT. However, the other treatments obtained a gradual evolution, not exceeding a 25% increase on the insect control over 168 HAT (Table 3).

Bacillus thuringiensis was the treatment that obtained the highest average of surviving insects and lower control efficiency, due to the insecticide having the mode of action by ingestion (Bobrowski et al., 2003). When the treatment between *B. thuringiensis* and chlorantraniliprole was combined, the results indicated an increase in the control of *A. gemmatalis* caterpillars. Thus, even with the insecticide combination, the control efficiency was lower due to the application method (Table 3).

In order to achieve effectiveness in the control, in addition to the choice of product, it is necessary to determine the application technology, with the proper placement and distribution of the product in the target, in a necessary quantity, in an economical way and minimum environmental contamination (Matuo, 1998; Bonadiman, 2008; Costa et al., 2017).

The flubendiamide treatment isolated throughout the experiment was highlighted in the control of *A. gemmatalis* in comparison to the chlorantraniliprole and the combination between both (Table 3). The chemical management for the adequate control of insects should include the technique of rotation of insecticides with different modes of action to avoid the emergence of resistant populations and to increase the useful life of the product (Castelo-Branco & Amaral, 2002; Roditakis et al., 2015; Gonçalves et al., 2016). It should be noted that in the southern region of Rio Grande do Sul, the active chlorantraniliprole is used in the crops such as rice, soybean and corn to the control of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), *A. gemmatalis*, *C. includens*, *H. armigera* and *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) (Agrofit, 2018).

The observed efficiency for *A. gemmatalis* 5th instar caterpillars to flubendiamide + zeta-cypermethrin and zeta-cypermethrin treatments reached 100.0% 4 HAT, differing significantly. The other insecticides had a control efficiency of less than 80%, however, they were more effective to the 5th instar caterpillars than the 2nd instar caterpillars (4 HAT-168 HAT) (Table 3) (MAPA, 2018; Embrapa, 2018). The results evidenced a pattern in mortality, with emphasis on flubendiamide + zeta-cypermethrin and zeta-cypermethrin treatments, independently of the mode of the application and insect instar. However, the study emphasizes the producer's concern with the mode of action of each product, besides equipment calibration, droplet diameter that contribute to the uniform distribution and effective control of the target (Gonçalves et al., 2016; Souza et al., 2016).

Thus, the management of insecticides should also be considered, if the producer does not adopt, there will be selection of the resistant individuals. As they multiply, the genes responsible for the resistance are transferred to the offspring, and gradually the population becomes less sensitive, as observed to the insecticide chlorantraniliprole. The result is the early withdrawal of the technology from the market, with losses for both industry and producers.

Table 3. Mean (X±SE) of living caterpillars and control efficiency (CE%) of *Anticarsia gemmatalis* caterpillars at 4, 24, 72, 120, 168 hours after the treatment (HAT) via direct contact

Treatments	4 HAT	CE (%) 24 HAT	CE (%) 72 HAT	CE (%) 120 HAT	CE (%) 168 HAT	CE (%)
<i>2nd instar</i>						
<i>B. thuringiensis</i>	3.8±0.20 ¹ ab ² 5	3.0±0.55 ab 25	3.0±0.55 ab 25	2.8±0.73 ab 30	2.8±0.73 ab 30	
<i>B. thuringiensis</i> +Chlorantraniliprole	3.4±0.24 abc 15	2.6±0.24 ab 35	2.6±0.24 ab 35	2.6±0.24 ab 35	2.6±0.24 ab 35	
Chlorantraniliprole	2.8±0.37 bc 30	2.8±0.37 ab 30	2.6±0.51 ab 35	2.6±0.51 ab 35	2.6±0.51 ab 35	
Chlorantraniliprole+Flubendiamide	3.4±0.24 abc 15	2.8±0.49 ab 30	2.4±0.68 ab 40	2.4±0.68 ab 40	2.4±0.68 ab 40	
Flubendiamide	2.6±0.40 c 35	1.8±0.37 b 55	1.6±0.51 bc 60	1.6±0.51 bc 60	1.6±0.51 bc 60	
Flubendiamide+Zeta-Cypermethrin	0.0±0.00 d 100	0.0±0.00 c 100	0.0±0.00 c 100	0.0±0.00 c 100	0.0±0.00 c 100	
Zeta-Cypermethrin	0.0±0.00 d 100	0.0±0.00 c 100	0.0±0.00 c 100	0.0±0.00 c 100	0.0±0.00 c 100	
Control	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	
CV (%)	9.01	7.90	8.19	6.63	6.63	
<i>5th instar</i>						
<i>B. thuringiensis</i>	3.2±0.37 ¹ ab ² 20	2.8±0.37 ab 30	2.8±0.37 abc 30	2.8±0.37 ab 30	2.6±0.37 ab 35	
<i>B. thuringiensis</i> +Chlorantraniliprole	2.0±0.32 c 50	1.8±0.49 b 55	1.8±0.49 bcd 55	1.4±0.40 bc 65	1.4±0.40 bc 65	
Chlorantraniliprole	3.8±0.20 ab 5	3.0±0.32 ab 25	3.0±0.32 ab 25	2.8±0.84 ab 30	2.6±0.24 ab 35	
Chlorantraniliprole+Flubendiamide	3.4±0.24 ab 15	1.6±0.51 bc 60	1.0±0.55 de 75	1.0±0.55 c 75	1.0±0.55 c 75	
Flubendiamide	2.8±0.37 bc 30	1.8±0.20 b 55	1.4±0.24 cde 65	1.0±0.32 c 75	1.0±0.32 c 75	
Flubendiamide+Zeta-Cypermethrin	0.0±0.00 d 100	0.0±0.00 c 100	0.0±0.00 e 100	0.0±0.00 c 100	0.0±0.00 c 100	
Zeta-Cypermethrin	0.0±0.00 d 100	0.0±0.00 c 100	0.0±0.00 e 100	0.0±0.00 c 100	0.0±0.00 c 100	
Control	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	4.0±0.00 a 0	
CV (%)	5.86	7.43	9.01	6.54	4.31	

Note. ¹(X±SE) = Average number of caterpillars±Standard error. ²Means followed by the same letter in column do not differ by Tukey test (P < 0.05). CV (%) = Coefficient of variation.

4. Conclusion

Aiming an integrated pest management program (IPM), where the use of different control tactics is recommended, insecticide management with flubendiamide + zeta-cypermethrin are effective on the control of *A. gemmatalis* caterpillars, for both 2nd and 5th instar.

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