

# The potential of insecticides and genetically modified soybean in the control of *Spodoptera frugiperda*

## El potencial de los insecticidas y la soja genéticamente modificada en el control de *Spodoptera frugiperda*

## Potencial dos inseticidas e da soja geneticamente modificada no controle de *Spodoptera frugiperda*

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### Abstract

Brazil is the largest soybean producer in the world and soybean is the main crop in the country. The use of genetically modified soybean expressing Bt proteins represents a significant milestone in insect pest management in this crop. However, the isolated use of these biotechnologies is not sufficient to control the larvae of *Spodoptera frugiperda*, therefore, the use of insecticides in seed treatment becomes essential. Therefore, the objective of this study was to evaluate the efficiency of controlling *S. frugiperda* larvae through insecticides applied to seeds and different biotechnologies in soybean cultivation. The experiments were conducted in a laboratory, following a completely randomized block design in a factorial scheme with twelve treatments, consisting of combinations of soybean cultivars and insecticides in seed treatment. The mortality and leaf consumption of first and third instar *S. frugiperda* larvae were evaluated. Based on our studies, it was possible to verify that cyantraniliprole is an excellent molecule for controlling first and third instar *S. frugiperda* larvae. The biotechnology Intacta 2 Xtend, Cry1A.105/Cry2Ab2/Cry1Ac, and the insecticide cyantraniliprole protected soybean against damage caused by *S. frugiperda* larvae. As it is a pest present in almost the entire globe, the results obtained in this study can support studies in other locations of the globe, with different conditions and population of *S. frugiperda*.

### Resumen

Brasil es el mayor productor de soja del mundo y la soja es el principal cultivo del país. El uso de soja genéticamente modificada que expresa proteínas Bt representa un hito significativo en el manejo de plagas de insectos en este cultivo. Sin embargo, el uso aislado de estas biotecnologías no es suficiente para controlar las larvas de *Spodoptera frugiperda*, por lo tanto, el uso de insecticidas en el tratamiento de semillas se vuelve esencial. Por lo tanto, el objetivo de este estudio fue evaluar la eficiencia del control de las larvas de *S. frugiperda* a través de insecticidas aplicados a las semillas y diferentes biotecnologías en el cultivo de soja. Los experimentos se llevaron a cabo en un laboratorio, siguiendo un diseño de bloques completamente aleatorizado en un esquema factorial con doce tratamientos, que consisten en combinaciones de cultivares de soja e insecticidas en el tratamiento de semillas. Se evaluó la mortalidad y el consumo foliar de las larvas de *S. frugiperda* de primer y tercer instar. Basándonos en nuestros estudios, fue posible verificar que el ciantraniliprole es una excelente molécula para controlar las larvas de *S. frugiperda* de primer y tercer instar. La biotecnología Intacta 2 Xtend, Cry1A.105/Cry2Ab2/Cry1Ac, y el insecticida ciantraniliprole protegieron la soja contra los daños causados por las larvas de *S. frugiperda*. Dado que se trata de una plaga presente en casi todo el mundo, los resultados obtenidos en este estudio pueden fundamentar estudios en otros lugares del mundo, con diferentes condiciones y población de *S. frugiperda*.

### Resumo

O Brasil é o maior produtor de soja do mundo e a soja é a principal cultura do país. O uso de soja geneticamente modificada expressando proteínas Bt representa um marco significativo no manejo de pragas de insetos nesta cultura. No entanto, o uso isolado dessas biotecnologias não é suficiente para controlar as larvas de *Spodoptera frugiperda*, portanto, o uso de inseticidas no tratamento de sementes se torna essencial. Portanto, o objetivo deste estudo foi avaliar a eficiência do controle de larvas de *S. frugiperda* através de inseticidas aplicados às sementes e diferentes biotecnologias no cultivo de soja. Os experimentos foram conduzidos em laboratório, seguindo um delineamento de blocos completamente casualizado em um esquema fatorial com doze tratamentos, consistindo em combinações de cultivares de soja e inseticidas no tratamento de sementes. A mortalidade e o consumo foliar de larvas de *S. frugiperda* de primeiro e terceiro instares foram avaliados. Com base em nossos estudos, foi possível verificar que o ciantraniliprole é uma excelente molécula para controlar larvas de *S. frugiperda* de primeiro e terceiro instares. A biotecnologia Intacta 2 Xtend, Cry1A.105/Cry2Ab2/Cry1Ac, e o inseticida ciantraniliprole protegeram a soja contra danos causados por larvas de *S. frugiperda*. Por tratar-se de uma praga presente em quase todo o globo, os resultados obtidos neste estudo podem embasar estudos em demais locais do globo, com diferentes condições e populações de *S. frugiperda*.

**Palavras-chave:** manejo integrado de plagas, Glycine max, soja Bt, inseticidas químicos.

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## 1. Introduction

Soybean production is Brazil's main crop and ranks among the top crops worldwide. The country accounts for almost 35% of global output, surpassing the United States with 121.5 million tons (USDA, 2022). Intacta RR2 PRO®, a glyphosate-tolerant genetically modified soybean that expresses the insecticidal protein Cry1Ac, was first sown in Brazil in 2013 (Godoy et al., 2022; Barcellos et al., 2023). In the 2021/22 season, the new genetically modified soybean Intacta 2 Xtend began commercialization in Brazil, expressing insecticidal proteins Cry1A.105/Cry2Ab2/Cry1Ac and tolerance to glyphosate and dicamba (Barcellos et al., 2023).

The adoption of genetically modified soybean represents a historic milestone in integrated pest management in soybean cultivation, providing protection against major lepidopteran pests that attack the crop, such as *Anticarsia gemmatalis* (Hübner, 1818) (*Lepidoptera: Erebidae*), *Chrysodeixis includens* (Walker, [1858]), *Chloridea virescens* (Fabricius, 1781), and *Helicoverpa armigera* (Hübner, 1808) (*Lepidoptera: Noctuidae*) (Bacalhau et al., 2020). However, they are not efficient in controlling species of the *Spodoptera* genus, which are commonly found in soybean cultivation areas in Brazil. Moreover, Cry1Ac soybean is not effective in controlling *Spodoptera* species, including *Spodoptera frugiperda* (JE Smith, 1797), *Spodoptera eridania* (Stoll, 1782), *Spodoptera albula* (Walker, 1857), and *Spodoptera cosmioides* (Walker, 1858) (Godoy et al., 2022). On the other hand, Cry1A.105/Cry2Ab2/Cry1Ac soybean causes intermediate mortality in *S. eridania* but low lethality in *S. frugiperda*, for which resistance to Cry proteins is already widespread in Brazil (Bernardi et al., 2014; Machado et al., 2020a; Machado et al., 2020b; Horikoshi et al., 2021; Barcellos et al., 2023).

One of the main concerns in soybean production in Brazil and worldwide is *S. frugiperda*, which is found throughout the territory and considered a global problem. It is a polyphagous pest with a high number of host plants, affecting more than 350 plant species (Hoffmann-campo et al., 2000; Santos et al., 2005; Montezano et al., 2018; Czepak et al., 2019). Its importance increases every season, causing damage from the early stages of the crop, such as defoliation, to the reproductive period (Czepak et al., 2019), especially flowers and pods, leading to significant grain yield losses (Hoffmann-campo et al., 2000; Santos et al., 2005; Bueno et al., 2011; Farias et al., 2014; Horikoshi et al., 2021; Vivan & Ortega, 2023). The low susceptibility of these insects to Cry1Ac protein and the reduction in insecticide applications for caterpillar control in soybean may explain the higher occurrence of this pest (Horikoshi et al., 2021; Horikosh et al., 2022).

In this scenario, it is crucial to have information about the sensitivity of *S. frugiperda* to insecticides and biotechnologies for integrated pest management. This pest, which is an integral part of global agriculture, has a high population potential due to its polyphagy and large number of descendants, in addition to having various populations. The joint use of new biotechnologies and different types of insecticides can improve the control of this pest. In addition, this combined approach can prevent the pest's resistance to these strategies, ensuring the effectiveness of these tools for a longer period.

The novelty of this study lies in the comparison of different insecticides available on the market. Fipronil, an insecticide commonly used in seed treatment, and cyantraniliprole, a newer molecule widely used in aerial applications, are compared. Additionally, the study compares three different biotechnologies: RR, Intacta RR2 PRO (Cry1Ac), and Intacta 2 Xtend (Cry1A.105, Cry2Ab2, and Cry1Ac).

Therefore, the objective of this study is to evaluate the efficiency of larval control of *S. frugiperda* in response to the application of insecticides on seeds and different biotechnologies in soybean cultivation.

## 2. Materials and methods

### 2.1. Insects

The *S. frugiperda* caterpillars used in this study were obtained from a laboratory colony maintained at the Entomology Laboratory of the School of Agricultural Sciences, Innovation, and Business (ESAN) at the University of Passo Fundo (UPF). The caterpillars used were from the fifth generation and reared on an artificial diet (Greene et al., 1976) under controlled conditions ( $25 \pm 2$  °C, relative humidity of  $60 \pm 10\%$ , and a 12-hour photoperiod). The experiments were conducted using first and third instar larvae, which were collected from a local corn field.

### 2.2 Soybean plants and insecticides

Soybean plants were grown in a greenhouse, in pots with a soil capacity of 8 liters, maintained in trays where water was provided to the plants through flooding. All seeds were treated with fungicide. Four soybean cultivars were used: Conventional, BMX Ativa (RR), BMX Zeus (Intacta RR2 PRO, Cry1Ac), BMX Trovão (Intacta 2 Xtend, Cry1A.105, Cry2Ab2, and Cry1Ac).

The evaluated insecticides were fipronil (Standak Top 100 ml/100kg) - GABA antagonists (IRAC MoA group 28); and ciantraniliprole (Fortenza 160 ml/100kg) - ryanodine receptor modulator (IRAC MoA group 2B).

### 2.3 Evaluation of mortality and consumption

In a completely randomized block design with a factorial arrangement, twelve treatments were evaluated, consisting of combinations between soybean cultivars (BMX Trovão, BMX Zeus, BMX Ativa, and Conventional) and seed treatment insecticides (control; fipronil; ciantraniliprole).

To obtain a uniform distribution, each insecticide was applied to 1.0 kg of seeds in a polyethylene bag with a small amount of water (0.3 L/100 kg of seeds). All seeds were also treated with the fungicides carbendazim + thiram. Leaf discs (1 or 4 cm<sup>2</sup>, as needed) were collected from the plants at the V1 stage (completely developed unifoliate leaves). The treatments were evaluated using infestations in 9 cm<sup>2</sup> Petri dishes, with 30 individualized larvae per treatment. Leaf sections were provided based on consumption and/or every 48 hours.

An infestation was conducted for each larval instar at 14 days after plant emergence (DAE) for both experiments. The number of dead larvae was evaluated daily, and the eaten leaf area was measured seven days after each infestation. Three replicates of ten larvae were used for mortality assessment, and 30 replicates for consumption evaluation.

All generated data were subjected to analysis of variance, and means were compared using the Tukey test at a 5% probability of error.

## 3. Results and discussions

Regarding the mortality of first instar *S. frugiperda* larvae, we can observe that there was no significant difference among the BMX Trovão, BMX Zeus, BMX Ativa, and Conventional cultivars in any of the evaluations on the 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> DAI (Table 1). As for the evaluated seed treatments, ciantraniliprole resulted in a mortality rate of 96.6% on the 3<sup>rd</sup> DAI, reaching 100% on the 5<sup>th</sup> DAI, significantly differing from the active ingredient fipronil and the control (without insecticide).

Concerning leaf consumption by the larvae, there was a significant interaction between cultivars and seed treatments (Table 1 and 2). We can observe that the BMX Trovão cultivar had the lowest leaf consumption, even without the use of seed treatments, demonstrating its ability to mitigate the damage caused by the larvae and showing a significant difference

compared to the other cultivars. As for the seed treatments, ciantraniliprole once again stands out, demonstrating efficiency in pest control by reducing leaf consumption by 100% compared to the use of fipronil and the absence of insecticide.

**TABLE 1** – MORTALITY (%) AND CONSUMPTION (CM<sup>2</sup>) OF FIRST INSTAR LARVAE OF *SPODOPTERA FRUGIPERDA* ON SOYBEAN CULTIVARS WHOSE SEEDS WERE TREATED WITH INSECTICIDES. PASSO FUNDO, RS, 2022.

Cultivar	1 <sup>st</sup> DAI	3 <sup>rd</sup> DAI	5 <sup>th</sup> DAI	Consumption (cm <sup>2</sup> )	
BMX Trovão	0 <sup>ns</sup>	33.33 <sup>ns</sup>	33.33 <sup>ns</sup>	1.21	a
BMX Zeus	0	33.33	33.33	3.22	bc
BMX Ativa	0	31.11	33.33	2.78	b
Conventional	0	31.11	33.33	3.42	c
Seed Treatment					
Water	0 <sup>ns</sup>	0	b	0	b
Fipronil	0	0	b	0	b
Ciantraniliprole	0	96.66	a	100	a
Interaction	ns	ns	ns	***	
C.V(%)	0	11.97	0	46.07	

Means followed by the same letter indicate statistical equality according to Tukey's test (p<0.05). ns: not significant; CV: coefficient of variation.

**TABLE 2** – BREAKDOWN OF THE INTERACTION OF CONSUMPTION (CM<sup>2</sup>) OF FIRST INSTAR *SPODOPTERA FRUGIPERDA* LARVAE UNDER SOYBEAN CULTIVARS WHOSE SEEDS WERE TREATED WITH INSECTICIDES. PASSO FUNDO, RS, 2022.

Cultivar	Seed treatment								
	Water			Fipronil			Ciantraniliprole		
BMX Trovão	A	1.89	c	A	2.03	b	B	0	a
BMX Zeus	A	4.54	a	A	5.12	a	B	0	a
BMX Ativa	A	4.12	b	A	4.6	a	B	0	a
Conventional	A	5.46	a	A	5.05	a	B	0	a

Means followed by the same uppercase letter in the column and lowercase letters in the row do not differ from each other, according to the Tukey test (p>0.05).

For the mortality of third instar *S. frugiperda* larvae, we observed no significant difference among cultivars on any of the evaluated days. However, in seed treatments, the active ingredient ciantraniliprole exhibited high mortality at the 3<sup>rd</sup> DAI, distinguishing it from the others, and reached 100% mortality at the 5<sup>th</sup> DAI (Table 3).

**TABLE 3** – MORTALITY (%) AND CONSUMPTION (CM<sup>2</sup>) OF THIRD INSTAR LARVAE OF *SPODOPTERA FRUGIPERDA* ON SOYBEAN CULTIVARS WHOSE SEEDS WERE TREATED WITH INSECTICIDES. PASSO FUNDO, RS, 2022.

Cultivar	1 <sup>st</sup> DAI	3 <sup>rd</sup> DAI	5 <sup>th</sup> DAI	Consumption (cm <sup>2</sup> )	
BMX Trovão	0.0 <sup>ns</sup>	23.33 <sup>ns</sup>	33.3 <sup>ns</sup>	2.85	b
BMX Zeus	0.0	20.00	33.3	4.40	a
BMX Ativa	0.0	20.00	33.3	4.25	a
Convencional	1.11	18.88	33.3	3.84	a
Seed Treatment					
Water	0	0	b	0	b
Fipronil	0	0	b	0	b
Ciantraniliprole	0.83	61.66	a	100	a
Interaction	ns	ns	ns	**	
C.V. (%)	600	39.76	2.98	24.70	

Means followed by the same letter indicate statistical equality according to Tukey's test ( $p < 0.05$ ). ns: not significant; CV: coefficient of variation.

Regarding the leaf consumption of third instar larvae, there was a significant interaction between cultivars and insecticides applied as seed treatments (Table 3). By observing the interaction breakdown, it is possible to verify that among the cultivars, even without seed treatment, the leaf consumption was lower in the BMX Trovão cultivar, differing only from BMX Zeus. When the seeds were treated with fipronil, the leaf consumption was lower in the BMX Trovão cultivar, differing from the others. Furthermore, when treated with ciantraniliprole, a 100% reduction in leaf consumption was observed for all cultivars (Table 3).

#### 4. Discussion

Bt plants and diamide-based insecticides are important tools for managing pest insects in the agricultural system. Based on our studies on mortality and foliar consumption, it was possible to observe that the diamide ciantraniliprole is an excellent molecule for controlling *S. frugiperda*. Moreover, the new transgenic soybean, Intacta 2 Xtend, despite not having a recommendation for the control of *S. frugiperda*, leads to a reduction in foliar consumption by first instar larvae.

It can be observed that the Cry1A.105/Cry2Ab2/Cry1Ac proteins, isolated in the Intacta 2 Xtend, were not sufficient for controlling first and third instar *S. frugiperda* larvae, whereas ciantraniliprole showed a high level of mortality in all four evaluated cultivars. These results are consistent with other studies that have demonstrated the efficacy of ciantraniliprole in seed treatment for *S. frugiperda* control (Pes et al., 2020). It is worth mentioning that in the present study, by the second day after infestation (DAI), the larvae that consumed soybean leaves treated with ciantraniliprole already began to show symptoms of intoxication, as this compound acts on ryanodine receptors in the insect's muscle cells, causing an uncontrolled release of calcium ions from internal stores, leading to muscular paralysis and death (Selby et al. 2013).

Based on the assays conducted with first and third instar larvae, the high efficiency of the ciantraniliprole-based insecticide used in seed treatment for *S. frugiperda* control becomes evident. The results are consistent with those found in previous studies, which demonstrated the efficacy of ciantraniliprole seed treatment in soybean for controlling first and third instar

larvae of *H. armigera* (Suzana et al., 2017). Anthranilic diamides are important insecticide molecules in managing armyworm in corn and have been gaining prominence in other crops such as soybean, owing to their excellent translocation performance when used in seed treatment (Pes et al., 2020).

Research conducted with Chlorantraniliprole and Cyantraniliprole in the treatment of corn seeds for the control of *Spodoptera frugiperda* revealed interesting results. First instar larvae showed a mortality rate that varied from 100% at 7 days after hatching (DAH) to 6% at 42 DAH when treated with cyantraniliprole 19.8% + thiamethoxam 19.8% FS. In the case of chlorantraniliprole 62.5% FS, larval mortality was 100% at 7 DAH and decreased to 10% at 42 DAH. When it comes to third instar larvae of *S. frugiperda*, larval mortality varied from 100% at 7 DAH to 2% at 42 DAH in the case of cyantraniliprole 19.8% + thiamethoxam 19.8% FS. Seeds treated with chlorantraniliprole 62.5% FS exhibited 100% larval mortality at 7 DAH, which decreased to 6% at 42 DAH. These results, documented by RK (Behera; Muralimohan, 2024), are in line with the results obtained in this study (Tables 1 and 3).

In similar studies with the species *Spodoptera eridania*, the use of soy Cry1A.105/Cry2Ab2/Cry1Ac resulted in a significantly higher mortality rate (60%-83%) in neonates of *S. eridania* compared to soy Cry1Ac and non-Bt soy (<11%). However, this difference was not observed at all growth stages. The surviving larvae of *S. eridania* on soy Cry1A.105/Cry2Ab2/Cry1Ac showed a notable delay in growth, with more than 94% of the larvae not progressing beyond the second instar, and a reduced larval weight ( $\leq 1.4$  mg/larva), representing a growth inhibition of over 60% compared to non-Bt. In contrast, *S. eridania* on soy Cry1Ac and non-Bt soy showed similar larval weights, despite the observed dwarfism (Barcellos et al., 2023) which differed from the results found in this study (Table 1). Nevertheless, this biotechnology was effective in controlling *Spodoptera cosmioides* and *Spodoptera albula* and showed intermediate control for *Spodoptera eridanea* (Godoy et al., 2022). The low control of *S. frugiperda* by Cry1A.105/Cry2Ab2/Cry1Ac soybean can be explained by the widespread resistance of this insect to Cry proteins expressed in multiple Bt plant species and the high degree of cross-resistance among Cry proteins expressed in Bt corn, cotton, and soybean in Brazil (Hernández-rodríguez et al., 2013; Carrière et al., 2015; Horikoshi et al., 2016; Godoy et al., 2022).

Hence, integrated pest management tactics, including the use of Bt plants and insecticides in seed treatment, are important strategies for *S. frugiperda* management.

#### 4. Conclusion

Some of the challenges encountered in this study were the variety of populations present in Brazil and around the world, as the population found in the south of Brazil may present different results when compared with larvae from other states and countries, showing a greater or lesser sensitivity to the treatments used in this study. In addition to reducing the damage caused by the larvae, the insecticide cyantraniliprole (160 g a.i./100kg of seeds) controls the larvae of *S. frugiperda* in the first and third instar. The biotechnology Intacta 2 Xtend, Cry1A.105/Cry2Ab2/Cry1Ac, and the insecticide cyantraniliprole (160 g a.i./100kg of seeds) protect soybeans against damage caused by the larvae of *S. frugiperda*. Future research in this field can explore various populations, and new molecules and emerging biotechnologies.

#### Credit author statement

Vítor Schmaedecke: Conceptualization, Original draft preparation, Methodology, Data curation, Writing. Cristiano Barreto Da Costa Hoffmann: Conceptualization, Original draft preparation, Methodology, Data curation, Writing. Talison Roberto Maurer: Visualization, Investigation, Original draft preparation, Writing. Crislaine Sartori Suzana-Milan: Conceptualization, Original draft preparation, Methodology, Data curation, Writing, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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