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Comparative Evaluation of Biotechnologies for the Management of Lepidopteran Pests in Soybean Crop (*Glycine max* (L.) Merrill)

Adolar Freitag Junior
Doctor Scientiae

VIÇOSA - MINAS GERAIS
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ADOLAR FREITAG JUNIOR

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Thesis submitted to the Plant Production Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Aluizio Borem de Oliveira

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Assent:

Adolar Freitag Junior
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Adviser

I dedicate this thesis to my parents, because without their support and education, I would not be here today.

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To God, for all your love and guidance throughout my life.

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ABSTRACT

JUNIOR, Adolar Freitag, D.Sc., Universidade Federal de Viçosa, April, 2025. **Comparative Evaluation of Biotechnologies for the Management of Lepidopteran Pests in Soybean Crop (*Glycine max* (L.) Merrill)**. Adviser: Aluizio Borem de Oliveira.

Brazil is the largest soybean producer and exporter in the world. In harvesting season 2023/24, there were over than 46 million of hectares cultivated, with a final productivity average of 3,204 kilos per hectare (Conab, 2024). Nowadays, more than 90% of cultivated cultivars contain biotechnology, which represent an important role on the weeds control, protection against lepidopteran pests, and simplifying producers´ management. Nowadays in Brazil, besides cultivars non-Bt, there are 3 options of biotechnologies containing the Bt proteins (Bacillus thuringiensis) commercially approved, Cry1Ac, Cry1Ac+Cry1A.105+Cry2Ab2 and Cry1F+Cry1Ac. Thus, the objective of the current study was to evaluate the performance of available Bt biotechnologies, including non-Bt, and their protection against lepidopteran pests. The study was carried out in 15 locations during 2022/23 season and 10 locations during 2023/24 season, using repetitions randomized within treatments, collecting information of counting of different lepidopteran species per meter (through beating cloth), defoliation damage and productivity. For the statistical analysis, a Quasi-Poisson regression using a generalized linear model was performed through R (R Core Team, 2022). Results shown that the 3 most abundant species found were Spodoptera frugiperda (48,82%), followed by Rachiplusia nu (35,13%) and S. eridania (10,10%). Out of the total lepidopteran larvae collected, R. nu was the most predominant specie in the South (84,08%) and S. frugiperda in the North (75,80%). Relative insect abundance was statistically significant lower over Cry1Ac+Cry1A.105+Cry2Ab2 when compared to Cry1Ac+Cry1F and Cry1Ac, for both R. nu and S. frugiperda. As conclusion, Cry1Ac+Cry1A.105+Cry2Ab2 was superior in comparison with the other biotechnologies regarding protection against lepidopteran pests, and this protection is a very important aspect on the soybean production, which ultimately has a highly significant impact on farmers´ production.

Keywords: soybeans; biotechnology; protection; lepidopteran; caterpillars

RESUMO

JUNIOR, Adolar Freitag, D.Sc., Universidade Federal de Viçosa, abril de 2025. **Avaliação Comparativa de Biotecnologias para o Manejo de Lepidópteros na Cultura da Soja (*Glycine max* (L.) Merrill)**. Orientador: Aluizio Borem de Oliveira.

O Brasil é o maior produtor e exportador de soja no mundo. Na safra 2023/24, tivemos mais de 46 milhões de hectares cultivados, com uma média final de produtividade de 3,204 kg/ha (Conab, 2024). Atualmente, mais de 90% das cultivares plantadas contém biotecnologias, que apresentam um papel importante no controle de plantas daninhas, proteção contra lagartas, e na simplificação do manejo pelo produtor. Atualmente no Brasil, além das cultivares não-Bt, temos 3 opções de biotecnologia contendo as proteínas Bt (Bacillus thuringiensis) aprovadas comercialmente, Cry1Ac, Cry1Ac+Cry1A.105+Cry2Ab2 e Cry1F+Cry1Ac. Dessa forma, o objetivo do presente trabalho foi avaliar a performance dessas diferentes biotecnologias Bt disponíveis, incluindo não-Bt, na proteção contra lagartas. O experimento foi conduzido em 15 locais durante a safra 2022/23 e em 10 locais durante a safra 2023/24, utilizando repetições randomizadas dentro dos tratamentos, coletando informações de contagem de diferentes espécies de lagartas por metro (através dos panos de batida), danos de desfolha e produtividade. Para as análises estatísticas, foi realizada regressão de Quasi-Poisson usando um modelo linear generalizado, através do R (R Core Team, 2022). Resultados mostraram que as 3 espécies mais abundantes foram Spodoptera frugiperda (48,82%), seguida de Rachiplusia nu (35,13%) e S. eridania (10,10%). Do total das lagartas coletadas, R. nu foi a espécie mais predominante no Sul (84,08%) e S. frugiperda no Norte (75,80%). A abundância relativa das lagartas foi estatisticamente significativa inferior sobre Cry1Ac+Cry1A.105+Cry2Ab2 quando comparada com Cry1F+Cry1Ac e Cry1Ac, para ambas as espécies R. nu e S. frugiperda. Como conclusão, Cry1Ac+Cry1A.105+Cry2Ab2 se mostrou superior em comparação às outras biotecnologias na proteção contra lagartas, sendo essa proteção um aspecto muito importante na produção de soja, que ao final, tem um impacto altamente significante para o produtor.

Palavras-chave: soja; biotecnologia; proteção; lepidópteros; lagartas

SUMMARY

1 INTRODUCTION

Soybean, *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae) is one of the most important crop worldwide (MARQUES et al., 2017), and Brazil is currently its main producer and exporter. Soybeans is widely cultivated due to its importance as oil and protein source, for both human and animal consumption, and due to it increasingly demand globally, it is expected to grow its cultivated area in the next years. In this scenario, during 2023/24 season, an estimated area of 46 million hectares were cultivated with the crop in Brazil, with an average yield production around 3,204 kilograms per hectare (kg/ha) (CONAB, 2024), although, top producing farms can achieve average yield above 6,600 kg/ha under favorable conditions. Therefore, these high yields are not easily reached, and it depends on several factors, such as weather conditions, soil, agronomic practices, germplasm, biotechnology, among others that can influence final production.

Nonetheless, one of the central challenges in obtaining high yields in soybean crops is damage to plants by destructive entomological fauna (AGASYEVA et al., 2024). Hemiptera and Lepidoptera are the main orders of insect pests that attack the soybean crop and cause significant yield losses by reducing stand density (from plant death) and causing defoliation and feeding damage to pods (SOSA-GÓMEZ et al., 2014). Almost all major soybean-producing areas suffer significant crop losses from a complex of lepidopteran pests (MARQUES et al., 2016) and their populations often reach levels of economic damage to the crop (JUSTINIANO; FERNANDES; VIANA, 2014). Besides, crop rotation, sequential crops that can host the pests, irrigated farms, can have a green bridge effect, supporting the development, reproduction and increase of the pests, by providing access to viable host plants for longer periods during each year, thus resulting in higher rates of annual offspring and larger populations (SPECHT et al., 2019).

Moreover, climatic factors such as high temperature and high precipitation present positive correlation with specie abundance (SANTOS et al., 2021). Temperature, for instance, influences strongly the development rate and therefore, life history, distribution and abundance of an insect species (TOBIN; NAGARKATTI; SAUNDERS, 2003). Precipitations may produce a variable effect on pest populations, where species like Lepidoptera, might increase population density with higher rainfall or changes in precipitation patterns (SHRESTHA, 2019). Changes in solar radiation can alter the macro and microclimatic conditions of crops affecting the distribution, incidence, seasonality and circadian activity of insects pests (BARTON; TERBLANCHE, 2014). Therefore, climate change is expected to directly impact the distribution and relative intensity of pest pressure experienced in agronomic and specialty crops worldwide (HUSETH et al., 2021), so every efficient pest control strategy is extremely important to avoid additional losses on the production systems.

Controlling field pests can be done by insecticides (chemical or biological), enhancing the population of natural enemies, or adopting cultivars with biotechnology traits containing soil bacterium *Bacillus thuringiensis* (Berliner 1915) proteins (*Bt*), where these proteins are active against specific target species of insects. These Cry toxins from *Bacillus thuringiensis* have also been expressed in transgenic crops and substantially contributed to the efficient control of insect pests, dramatically reducing the use of chemical pesticides (BRAVO et al., 2011). Up to now, most of efforts developing new commercial biotechnologies are focused on protecting the crops against insect damage and conferring herbicide tolerance to the desirable crop, although, drought tolerance, nitrogen use efficiency, trans fat-free, and reduced saturated fat have also been developed, besides other potential future applications including additional nutritional enhancements, stress tolerance, disease resistance, among others (MORAIS; BORÉM, 2017).

Since its introduction in Brazil, soybean has undergone a process of technological modernization, receiving in the recent year´s new technologies that have provided a revolution in the production system, increasing mainly grain yield, as well as facilitated phytosanitary managements (insects, diseases, and weeds) and edaphoclimatic adaptation (NETO et al., 2020). Technological advances and commercial implementation of crop biotechnology for lepidopteran control offer a promising alternative and an additional tool to complement chemical insecticides in soybean (BERNARDI et al., 2014a). *Bt* crops have been adopted worldwide, representing one of the primary strategies to improve crop productivity (ABBATE et al., 2023), providing high-level protection from insect pests (HORIKOSHI et al., 2022). So, genetically modified (GM) crops expressing ‘plant-incorporated protectants’ have assumed a fundamental role in integrated pest management (STIRLE et al., 2024). Additionally, the use of *Bt* crops has reduced the need for insecticide applications in several cropping systems and survival of non-target arthropods has increased (NARANJO, 2009). Before adoption of *Bt* soybean, lepidopterans prevailed in the crop associated insect-pest community (PÁEZ JEREZ et al., 2023) and later on, with increased adoption, the *Bt* technology significantly reduced these target insect pests and favored populations of natural enemies (JUSTINIANO; FERNANDES; VIANA, 2014) and importantly, neither *Bt* soybean or insecticides sprays altered the structure of non-target arthropods communities in soybeans (MARQUES et al., 2017). Besides, GM crops that express insecticidal *Bt* proteins provided not only an effective alternative tool for pest management but also many social, environmental and economic advantages such as reducing the use of chemical insecticides, with benefits to the environment and biodiversity in particular and to human health, and increasing farm income (YU et al., 2013). Therefore, the use of *Bt* soybean as a tool for integrated pest management should be planned according to the major insect problems in each area (MURÚA et al., 2018).

Nevertheless, dealing with crop pests is though, and single *Bt* traits have suffered with the emergence of resistant populations, and so, multiple genes stacked in the same trait package have been seen as an effective solution. The effectiveness of pyramided traits against populations with potential *Bt* resistance is ostensibly conferred by the expression of multiple genes encoding *Bt* proteins in each plant (STIRLE et al., 2024), allowing the expression of different mechanisms of resistance, which can enhance the efficacy and durability of the trait, besides increasing the protection´s longevity.

The adoption of genetic modified crops started during 1990s and since then, the technology rapidly spread around the world including both developed and developing countries (GYANA RANJAN ROUT; RINNY SWAIN; DEBEE PRASAD SAHOO, 2023), and the main crops exploring biotechnology are soybeans, corn, cotton and canola. Genetic modified soybean, Roundup Ready, was first introduced in the USA in 1996 (MORAIS; BORÉM, 2017), and in the same year in South America, where its adoption started with the biotechnology being grown in Argentina. Two years later, in 1998, the Roundup Ready soybean was approved in Brazil, but only in 2005 the commercial approval was endorsed. The use of this technology impacted directly on weed control and non-tillage systems, providing alternative and efficient methods of weed control to the farmers. In 2013, the Intacta RR2 PRO technology was commercially approved, containing one *Bt* Cry1Ac insecticidal protein allied to the Roundup Ready (events MON87701 x MON89788), which besides glyphosate tolerance, added protection against 4 lepidopteran pests in soybeans (*Chrysodeixis includens* (Walker, 1858), *Anticarsia gemmatalis* (Hübner 1818), *Crocidosema aporema* (Walsingham, 1914) and *Chloridea virescens* (Fabricius, 1777)). In 2021, the commercial approval of Intacta 2 Xtend (herbicide events MON89788 x MON87708, in addition to insecticidal events MON87701 x MON87751), expressing three *Bt* insecticidal proteins Cry1Ac, Cry1A.105 and Cry2Ab2, which besides the benefits from Intacta RR2 PRO, added protection against 2 new pests (*Helicoverpa armigera* (Hübner, 1809) and *Spodoptera cosmioides* (Walker, 1858)), and herbicide tolerance to dicamba. Lastly, in 2022 the biotechnology Conkesta Enlist was launched (events DAS-81419-2 x DAS-44406-6), expressing two *Bt* proteins Cry1Ac and Cry1F, conferring protection against *Elasmopalpus lignosellus* (Zeller, 1848), *C. includens*, *A. gemmatalis*, *C. virescens* and *H. armigera*, and moderate protection against *S. cosmioides* and *S. eridania* (Stoll, 1782), and herbicide tolerant to glyphosate, glufosinate ammonium and 2,4-D.

Brazilian agricultural biotechnology has seen great advances in recent decades, especially in the development of GM crops, including soybean, cotton, and maize, which has placed Brazil in second place since 2013 in the ranking of countries with the greatest GM-cultivated area (FIGUEIREDO et al., 2019). Currently, in Brazil we have six soybean biotechnologies (three *Bt* traited plus herbicide tolerance, and three herbicide tolerant without *Bt* traits, besides conventional cultivars) commercially approved and available to the farmers. To provide some perspective, around 90% the total soybean planted in Brazil includes *Bt* cultivars. This highly adoption by the farmers demonstrate higher performance and cost-effective crop production, due to the improved weed and pest management systems. Besides, there are several other benefits proven by the adoption of GM crops. Carrying out specific analysis and studies to understand the impacts of GM vs non-GM crops including soybeans, corn and cotton, a meta-analysis showed that its adoption has increased crop yields by 22%, reduced chemical pesticide use by 37%, and increased farmer profits by 68% (KLÜMPER; QAIM, 2014). In another study with Cry1Ac soybeans biotechnology in South America considering its first five years of adoption, around 9.2% increase in yield and 15,1% reduction in pesticide spraying across Brazil, Paraguay, Argentina and Uruguay was observed (BROOKES, 2018).

Therefore, our intention was to evaluate the commercially available biotechnologies in soybeans (non-*Bt* and *Bt* traited), understanding their performance on the protection against lepidopteran species damaging the crop, and finally, their benefits to the soybean production.

2 OBJECTIVES

In this study carried out under field conditions during 2022/23 and 2023/24 seasons, four different biotechnologies were evaluated to assess their performance and efficacy against different species of lepidopteran soybean pests. The biotechnologies compared were the commercially approved non-*Bt*, Cry1Ac; Cry1Ac+Cry1A.105+Cry2Ab2; and Cry1Ac+Cry1F, including non-sprayed and sprayed treatments to control lepidopteran pests. The main objectives were to understand: 1) main lepidopteran species across Brazil and by region; 2) lepidopteran abundance by specie per season; 3) species distribution over individual biotechnologies; 4) differences on abundance of *R. nu* and *S. frugiperda* over biotechnologies; 5) differences on defoliation by biotechnology; 6) differences on yield by biotechnology.

3 MATERIAL AND METHODS

3.1 Locations

The field experiments were installed in different locations across different states, including Rio Grande do Sul (RS), Paraná (PR), Mato Grosso do Sul (MS), São Paulo (SP), Goiás (GO), Minas Gerais (MG), Mato Grosso (MT), Tocantins (TO) and Bahia (BA), and encompassing the 5 edaphoclimatic soybean´s macro regions, ensuring well coverage of trials across country. Soybean macro regions are based on agroecological zones, Köppen climate classification for Brazil and technical recommendations for soybean production, and they reflect environmental characteristics more precisely than do political (i.e., state) divisions (KASTER; FARIAS, 2012). The trials were carried out at research stations from universities, consulting companies and cooperatives, and the total number of locations were 15 in 2022/23 season and 10 locations in 2023/24 season (Table 1). More locations were initially planned, but due to environmental problems during the season, some of them were discarded from the analysis. The soybean varieties selected from the previously described different biotechnologies, were similar in maturity groups (not more nor less than 0.2 points in maturity), where each one was recommended, adapted and positioned for the planting region. Planting was carried out during ideal planting window for each region, varying from September through December, with harvesting varying between January through April.

Table 1. Locations across Brazil by macro and micro region, during 2022/23 and 2023/24 seasons.

MACRO REGION	MICRO REGION	CITY	STATE	SEASON 2022/23	SEASON 2023/24
1	103 Baixa	Cruz Alta	RS	1	1
1	103 Alta	Passo Fundo	RS	1	
1	104	Guarapuava	PR	1	1
1	104	Itaberá	SP	1	
2	204	Taquarituba	SP	1	
2	201 Alta	Cafelândia	PR	1	1
2	202	Mandaguaçu	PR	1	
2	202	Maracaju	MS	1	1
3	401	Rio Verde	GO	1	
4	403	Primavera do Leste	MT	1	2
4	403	Rondonópolis	MT	1	
4	404	Canarana	MT	1	1
4	405	Sorriso	MT	1	1
4	405	Diamantino	MT	1	
4	405	Lucas do Rio Verde	MT	1	
5	408	Luis Eduardo Magalhães	BA		1
5	504	Lagoa da Confusão	TO		1
TOTAL				**15**	**10**

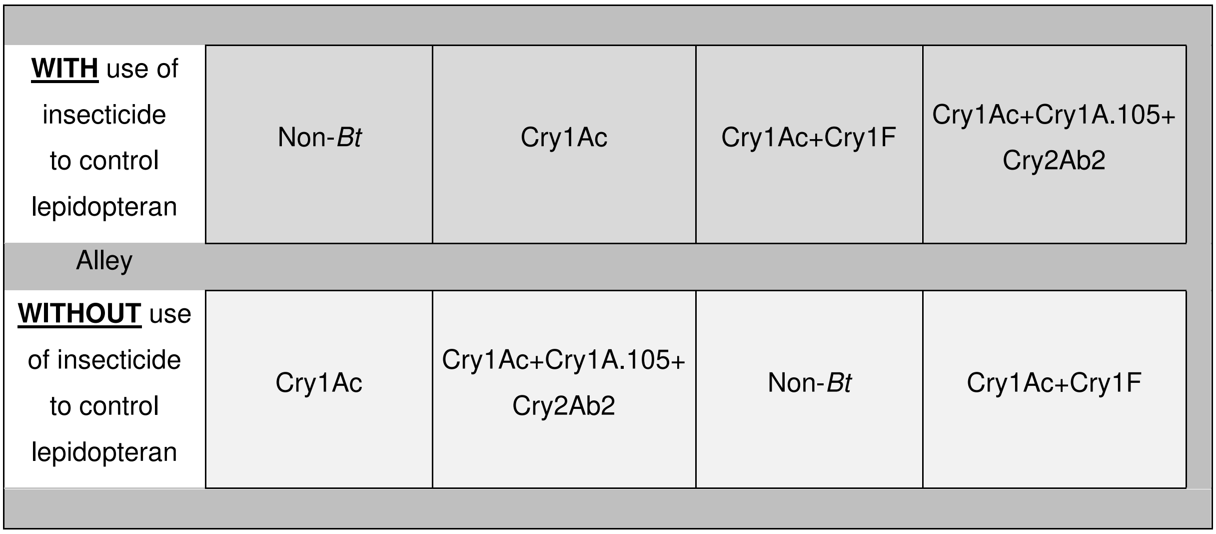
3.2 Treatments and field layout

Treatments included individual plots of each one of the four biotechnologies, with and without use of insecticides for lepidopteran control. So, in summary, a total of eight plots per location, being four plots (biotechnologies with control of lepidopteran pests) and four plots (same biotechnologies without control of lepidopteran pests).

On the plots with lepidopteran control with insecticides, the intention was to avoid damage and defoliation incidence by any kind of pests, for further comparison with the plots without control of lepidopteran, where no insecticides were used, allowing pests to develop, feed, and defoliate the biotechnologies under observation. Thus, the objective was to understand the individual effect of each biotechnology on the protection against different lepidopteran species.

However, for all other pests besides lepidopteran, such as aphids, stink bugs, mites, and whiteflies, chemical control was implemented uniformly across all treatments. Agronomic practices, including fertilization, weed management, fungal disease control, and non-lepidopteran pest management, adhered to the local recommendations provided by the research station.

Example of how field layout was planted is described in Figure 1, with the treatments allocated randomly within the blocks.



WITH use of insecticide to control lepidopteran	Non-*Bt*	Cry1Ac	Cry1Ac+Cry1F	Cry1Ac+Cry1A.105+ Cry2Ab2
Alley				
WITHOUT use of insecticide to control lepidopteran	Cry1Ac	Cry1Ac+Cry1A.105+ Cry2Ab2	Non-*Bt*	Cry1Ac+Cry1F

Figure 1. Field layout and treatments allocation. Source: prepared by the author.

3.3 Data collection

Each location had the plot size following a pattern of 625m^2 (25m length x 25m width), although, in some cases the plots were slightly reduced by length and width, according to the area availability. All data collection was targeted within the central 10m x 10m of each plot, avoiding border effects and potential migration of lepidopteran larvae on late development stages from one plot to the neighboring plot. Fields were sampled from early vegetative stages (V6) throughout the entire cycle until late reproductive stages (R6). Samplings were done using a beating sheet (1m length x 0,50m width), which was placed between two planted rows, and subsequently shaking the plants within this 1 meter against the surface of the cloth, dropping the insects initially on the plants, to fall onto the beating cloth. The beating sheet sampling activity included four repetitions randomly defined within each plot during each monitoring assessment. It was considered at least 8 different monitoring moments throughout the soybean cycle, at least 7 to 10 days apart from each other. Data collection consisted of identification of the larvae species (SOSA-GÓMEZ et al., 2014), counting the number of larvae per specie (average abundance of larvae per meter) and the defoliation level visually estimated and expressed in percentage (HAMMOND, RONALD B.; MICHEL; EISLEY, JAMES B., 2014). Yield data was collected by harvesting 4 random samples per treatment, consisting of 4 rows x 5 m each repetition. It was removed impurities and moisture was adjusted to 13%. The yield was then estimated, in kilograms per hectare (kg/ha).

4 STATISTICAL ANALYSIS

4.1 Lepidopteran abundance by location

A generalized linear model was performed on total (cumulative) insect counts separately for each location and species to estimate the insect abundance at each location, defined by insect counts on non-sprayed and non-traited control plots. Specifically, Quasi-Poisson regression was performed using the glm function in R (R Core Team, 2022), as follows:

```
glm(Count ~ Treatment/Rep + Insecticide -1, family=quasipoisson)
```

where Count is cumulative count over all collection times, Treatment is the *Bt* trait used (or control), Insecticide indicated whether insecticide was used, and Rep is the replicate nested within treatment, following experimental design.

Model output provides estimates for all combinations of *Bt* treatment and insecticide use; the estimate for non-traited, non-sprayed plot was used as estimate for insect abundance at the location, with approximate 95% confidence interval.

Poisson regression is commonly used to model count data, such as egg counts and insect counts; for these data, Gaussian-based linear models are often inappropriate because the variance increases with the mean, violating assumptions of linear models. For biological data, it is often the case that the variance is even greater than that predicted by Poisson models. This phenomenon is called overdispersion. Quasi-Poisson regression is a generalization of Poisson regression that accounts for overdispersion; specifically, when data are over dispersed, Quasi-Poisson regression will produce wider confidence intervals for parameter estimates than Poisson regression.

4.2 Impact of Bt traits on relative insect abundance

The impact of *Bt* traits on insect abundance was evaluated for the two most common species, *Rachiplusia nu* and *S. frugiperda*, using a generalized linear model applied to cumulative insect counts separately for each species across all locations. Quasi-Poisson regression was performed using the glm function in R (R Core Team, 2022), as follows:

glm(Count ~ Treatment/Rep + Insecticide + Treatment + Field, family=quasipoisson)

where Count is cumulative count over all collection times, Treatment is the *Bt* trait used (or control), Insecticide indicated whether insecticide was used, Field is the location, and Rep is the replicate nested within treatment, following experimental design. A separate model that included a term for the interaction of Insecticide and Treatment was also run to test for an interaction effect. [An interaction effect would mean that the *Bt* trait effect is different when an insecticide is used versus when it is not used]. For both species there is no interaction effect.

In this model our primary interest is the relative counts on traited plots compared to non-traited plots, so reported results show the relative comparison and approximate 95% confidence intervals.

For many locations the counts for *R. nu* and *S. frugiperda* are sometimes very low or even 0, so it might be tempting to exclude those locations from analysis, and if so, it can be challenging to decide which locations to exclude (i.e., how large do counts need to be to include location). Fortunately, when the appropriate generalized linear model is used, locations do not need to be excluded, even if there are no insects at that location: the model will weigh the locations appropriately. [This is not the case if count data were log-transformed then analyzed using a Gaussian-based linear model, which is why a generalized linear model is preferred to the alternative of first log-transforming count data then analyzing then using a Gaussian-based linear model].

4.3 Impact of Bt traits on defoliation

The impact of *Bt* traits on defoliation was evaluated using a generalized linear model applied to final defoliation observation across all species and locations. Quasi-Poisson regression was performed using the glm function in R (R Core Team, 2022), as follows:

glm(Defoliation ~ Treatment/Rep + Insecticide + Treatment + Field, family=quasipoisson)

In this model our primary interest is the relative defoliation on traited plots compared to non-traited plots, so reported results show the relative comparison and approximate 95% confidence intervals.

5 RESULTS

5.1 Lepidopteran abundance per location on non-Bt treatments

The total number of locations used in the analysis was 15 in 2022/23 and 10 in 2023/24 seasons. Not all locations had incidence of all species, and usually specific species were predominant over the others, depending on the location and region. Therefore, a larger number of locations had the incidence of *R. nu*, *S. frugiperda* and *S. eridania*. Other species were also detected, but in lower frequency and abundance. Figure 2 shows the average count of larvae found per location, considering only non-*Bt* plots without insecticide control, where each data point represents average larvae count for a single specie at a single location, which gives us an overview of the main species found during the study.

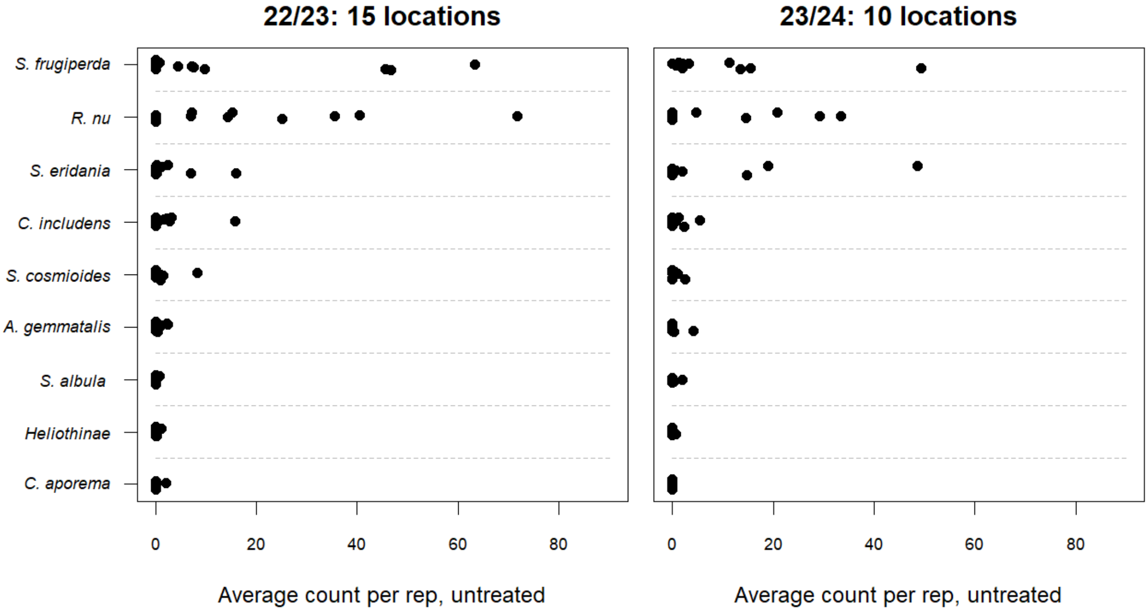


Figure 2. Average count per rep on non-treated plots (non-*Bt*) computed separately for each specie and each location. Each data point represents average count for a single specie at a single location. Main presence of *S. frugiperda*, *R. nu* and *S. eridania* across locations and seasons was observed.

5.2 Lepidopteran abundance across Bt treatments

Conversely, a total of 12541 lepidopteran pests were recorded across both insecticide and biotechnology treatments over the two cropping seasons. The beating sheet assessments identified 9 distinct species. *S. frugiperda* was the most abundant specie over both seasons combined, with 48,82% of the total individuals found, followed by *R. nu* and *S. eridania*, with 35,13% and 10,10%, respectively. The two most abundant species accounted for over 83% of the collected larvae. *C. includens*, *S. cosmioides* and *A. gemmatalis* appeared in less proportion, representing 2,34%, 1,81% and 0,60%, respectively (Figure 3). All other species including *S. albula*, *Heliothinae and C. aporema* represented less than 0,50% each.

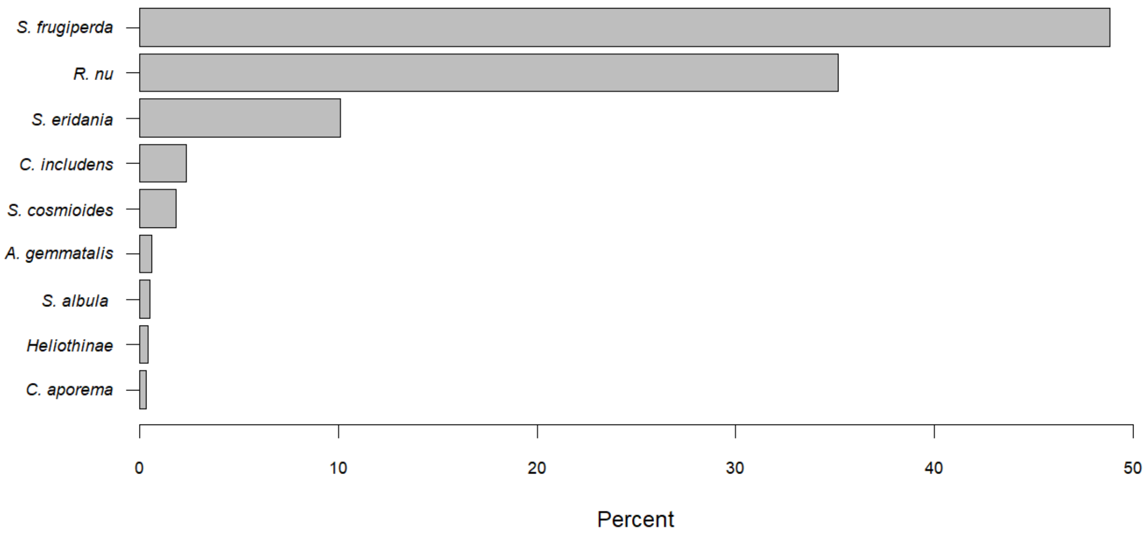


Figure 3. Percentage of total insect counts across all biotechnologies and seasons, attributed to species. *S. frugiperda* (48,82%), *R. nu* (35,13%), and *S. eridania*, (10,10%).

5.3 Proportion by specie per season

Observing seasons separately, the most abundant species did not change overall, but in 2022/23 season*, S. frugiperda* had 47,17% of the total individuals found, followed by *R. nu* with 40,26%, *S. eridania* with 5,96%, *C. includens* with 2,96%, *S. cosmioides* with 2,37%. On the other hand, in 2023/24 season, *S. frugiperda* was the most abundant with 51,38% of the total individuals found, *R. nu* with 27,15%, *S. eridania* with 16,56%, followed by *C. includens*, *S. albula, S. cosmioides, A. gemmatalis* and *Heliothinae*, with 1,37%, 1,10%, 0,94%, 0,82% and 0,60%, respectively (Table 2). Other species not mentioned represented less than 0,5% of the total abundance and were found in a reduced number of locations.

The three predominant species observed were consistent across seasons, with *S. frugiperda* and *R. nu*, and both accounted for over 78% of the total in both seasons, with similar prevalence. *S. eridania*, made up more than 5% in both seasons, showing a notable increase in abundance during 2023/24 season, reaching 16,56%.

Table 2. Number of individuals and abundance per specie (in %), accordingly to 2022/23, 2023/24 and with both seasons combined.

	2022/23		**2023/24**		**Both seasons**	
	Number	Proportion	Number	Proportion	Number	Proportion

A. gemmatalis	35	0,46	40	0,82	75	0,60
C. includens	226	2,96	67	1,37	293	2,34
C. aporema	30	0,39	7	0,14	37	0,30
Heliothinae	22	0,29	27	0,55	49	0,39
R. nu	3075	40,26	1331	27,15	4406	35,13
S. albula	11	0,14	54	1,10	65	0,52
S. cosmioides	181	2,37	46	0,94	227	1,81
S. eridania	455	5,96	812	16,56	1267	10,10
S. frugiperda	3603	47,17	2519	51,38	6122	48,82
TOTAL	7638	100,00	4903	100,00	12541	100,00

5.4 Proportion by specie per region

Therefore, different species prevalence can be observed when looking at different regions and considering North macro regions 3, 4, 5, which represents states of Goiás, Mato Grosso, Bahia and Tocantins, and South macro regions 1, 2, represents includes states of Rio Grande do Sul, Paraná, São Paulo and Mato Grosso do Sul. Out of the total larvae found across different species and seasons, 7596 individuals (60,57%) were found in the North and 4945 (39,43%) in the South region (Table 3). Therefore, the number differed drastically when looking at individual species abundance, where *S. frugiperda* was the predominant specie in the North, and *R. nu* was the predominant specie in the South (Figure 4), which was also noticed when listening to regional consultants and specialists working with soybean production systems in these regions. In the North, the main species found were *S. frugiperda* (75,80%), followed by *S. eridania* (16,02%), *R.nu* (3,26%) and *C. includens* (2,21%). On the other hand, in the South, the predominant specie was *R. nu* (84,08%) followed by *S. frugiperda* (7,36%), *S. cosmioides* (3,40%), *C. includens* (2,53%) and *S. eridania* (1,01%). All the other species not mentioned represented less than 1% of the total.

Table 3. Total insects count and proportion of the total across all treatments by species and regions.

	North	**Proportion**	**South**	**Proportion**	**Both years**
A. gemmatalis	40	0,53	35	0,71	75

C. includens	168	2,21	125	2,53	293
C. aporema	7	0,09	30	0,61	37
Heliothinae	45	0,59	4	0,08	49
R. nu	248	3,26	4158	84,08	4406
S. albula	54	0,71	11	0,22	65
S. cosmioides	59	0,78	168	3,40	227
S. eridania	1217	16,02	50	1,01	1267
S. frugiperda	5758	75,80	364	7,36	6122
Total	7596	100,00	4945	100,00	12541

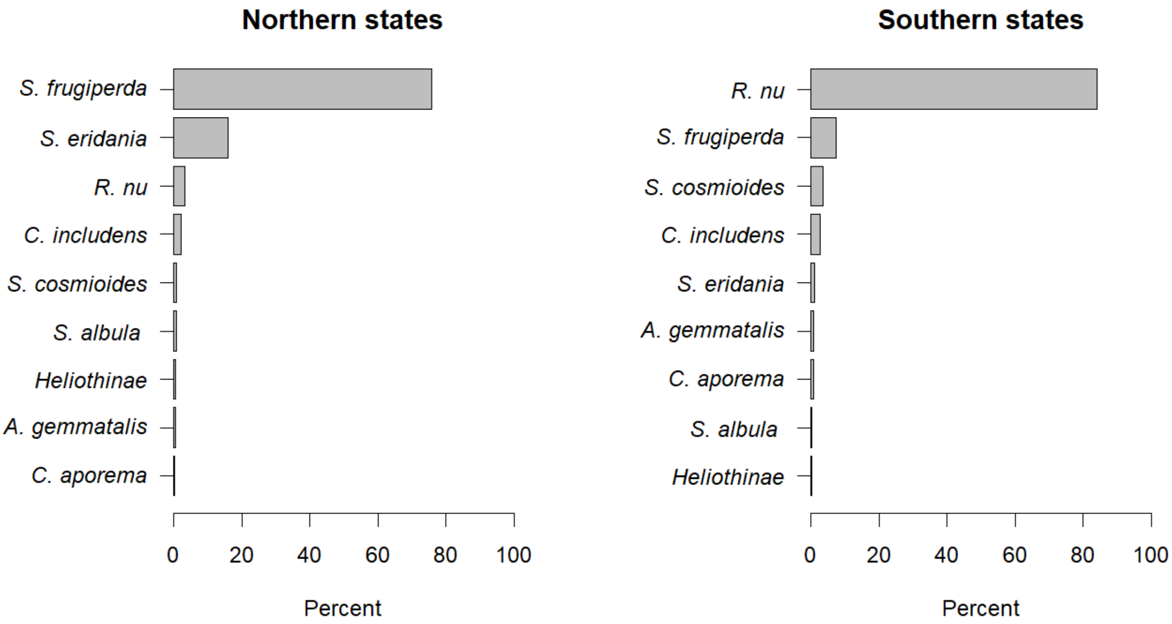


Figure 4. Percentage of total insect counts across all biotechnologies and seasons attributed to species for North (GO, MT, BA, TO) and South (RS, PR, SP, MS). Main abundance of *S. frugiperda* in the North (75,80%) and *R. nu* in the South (84,08%).

5.5 Species composition by biotechnology in non-treated plots

Considering all the lepidopteran larvae species count, collected over the non-sprayed plots (7889 individuals), 39,91% (or 3149 individuals) were over non-*Bt* treatment, 24,12% (or 1903 individuals) over Cry1Ac+Cry1F, 27,82% (or 2195 individuals) over Cry1Ac and 8,37% (or 642 individuals) over Cry1Ac+Cry1A.105+Cry2Ab2 (Table 4). Although, looking by individual biotechnologies, the three most abundant species across were always *S. frugiperda*, *R. nu*, and *S. eridania*, not necessarily in this order, with the first two varying as the most abundant within biotechnologies. For the non-*Bt* treatments, 40,58% of the total collected lepidopteran larvae were *R. nu*, followed by *S. frugiperda* and *S. eridania*, with 36,07% and 14,29%, respectively. Species such as *C. includens* was represented by 4,57% of the total individuals collected, followed by *S. cosmioides* (2,13%) and *A. gemmatalis* (1,40%). For the next treatment, Cry1Ac+Cry1F, the most abundant specie was *S. frugiperda* (56,81%), followed by *R. nu* (32,74%), *S. eridania* (6,78%), *S. cosmioides* (1,21%), *C. includens* (1,05%) and *S. albula* (1,00%). Cry1Ac was mostly represented by *S. frugiperda* (47,02%), *R. nu* (34,53%), followed by *S. eridania* (13,53%) and *S. cosmioides* (2,51%). For Cry1Ac+Cry1A.105+Cry2Ab2, the highest incidence was by *S. frugiperda* (84,42%), followed by *S. eridania* (9,97%) and *R. nu* (3,74%). All the other species not mentioned across different biotechnologies had less than 1% of representativity.

Table 4. Total insects count and proportion of the total across all seasons by species within biotechnologies, for treatments without insecticide control.

	Cry1Ac+ Cry1A.105+ Cry2Ab2	**%**	**Cry1Ac**	**%**	**Cry1Ac +Cry1F**	**%**	**non-*Bt***	**%**	**TOTAL**
A. gemmatalis	1	0,16	4	0,18	2	0,11	44	1,40	51
C. includens	1	0,16	13	0,59	20	1,05	144	4,57	178
C. aporema	0	0,00	7	0,32	2	0,11	8	0,25	17
Heliothinae	1	0,16	15	0,68	4	0,21	10	0,32	30
R. nu	24	3,74	758	34,53	623	32,74	1278	40,58	2683
S. abula	6	0,93	14	0,64	19	1,00	12	0,38	51
S. cosmioides	3	0,47	55	2,51	23	1,21	67	2,13	148
S. eridania	64	9,97	297	13,53	129	6,78	450	14,29	940
S. frugiperda	542	84,42	1032	47,02	1081	56,81	1136	36,07	3791
Total	642	-	2195	-	1903	-	3149	-	7889

5.6 Species composition across biotechnologies in non-treated plots

Nevertheless, biotechnologies treatments had different effect over the insects found. Regarding the treatments without insecticide control, and the most abundant specie *S. frugiperda*, with 3791 individuals found (or 48,05% of the total insects) across biotechnologies, where 29,97% of these individuals found were over non-*Bt* treatments, 27,22% over Cry1Ac+Cry1F, 28,51% over Cry1Ac, and 14,30% over Cry1Ac+Cry1A.105+Cry2Ab2. Therefore, *S. frugiperda* is not a target specie for any of the tested biotechnologies and has increased its incidence over soybean fields in the last years. For the second most abundant specie, *R. nu*, with 2683 individuals found (or 34,00% of the total insects) across biotechnologies, similar effects were found, but in different proportions, where non-*Bt* treatments had 47,63% of the insects found, Cry1Ac+Cry1F had 23,22%, Cry1Ac had 28,25%, and Cry1Ac+Cry1A.105+Cry2Ab2 had 0,89%. It showed what has been seen in the production fields, even not being target specie of any biotechnology tested, the last biotechnology showed positive benefits over this non-target specie. The third most abundant specie was *S. eridania*, with a total of 940 individuals found (equivalent to 11,91% of the total insects), where 47,87% were found over non-*Bt*, 13,72% over Cry1Ac+Cry1F, 31,60% over Cry1Ac and 6,81% over Cry1Ac+Cry1A.105+Cry2Ab2. This specie is only target for Cry1Ac+Cry1F, which is positioned against it with moderate protection. These 3 species summed over 93% of the total insects found across biotechnologies. The proportion of all the other insects are described in Table 5, and it is important to highlight that all biotechnologies continue effective regarding control of *A. gemmatalis* and *C. includens*, even after more than 10 years of launching the first *Bt* traited soybean (Cry1Ac). And the number of these two species found over the biotechnologies is so low across locations that does not require any special attention to resistance concerns up to now, and this low number is also explained due to the positive direct effect from to the high adoption of *Bt* traited soybeans, which has reduced the abundance of these species even on non-*Bt* soybean fields. Therefore, independently of the species, it is extremely important to manage pest control efficiently to avoid any crop losses due to insects´ herbivory and damages. In these comparisons, the intention was to understand the effect of each biotechnology over the different species found in this field study, and showing the effect of each individual biotechnology over non-*Bt* treatments, although, and as result, we saw high superiority of Cry1Ac+Cry1A.105+Cry2Ab2 when compared with the other biotechnologies tested.

Table 5. Total insects count and proportion of the total across all seasons by species and biotechnologies, for treatments without insecticide control.

	Cry1Ac+ Cry1A.105+ Cry2Ab2	**%**	**Cry1Ac**	**%**	**Cry1Ac +Cry1F**	**%**	**non-*Bt***	**%**	**TOTAL**
A. gemmatalis	1	1,96	4	7,84	2	3,92	44	86,27	51
C. includens	1	0,56	13	7,30	20	11,24	144	80,90	178
C. aporema	0	0,00	7	41,18	2	11,76	8	47,06	17
Heliothinae	1	3,33	15	50,00	4	13,33	10	33,33	30
R. nu	24	0,89	758	28,25	623	23,22	1278	47,63	2683

S. abula	6	11,76	14	27,45	19	37,25	12	23,53	51
S. cosmioides	3	2,03	55	37,16	23	15,54	67	45,27	148
S. eridania	64	6,81	297	31,60	129	13,72	450	47,87	940
S. frugiperda	542	14,30	1032	27,22	1081	28,51	1136	29,97	3791
Total	642	8,14	2195	27,82	1903	24,12	3149	39,92	7889

5.7 Species composition across biotechnologies in treated plots

A total of 7889 insects (62,90%) were collected over non-sprayed plots, and 4652 insects (37,10%) over sprayed plots. So, it showed the effect of the insecticides, and it reinforces the need of an efficient integrated pest management control. The three most abundant species described previously in Table 5, on the treatments without insecticide control, were also the three most abundant species found at the treatments with insecticide control, where they represented 94,04% of the total insects found, and followed similar proportion found over biotechnologies in both treatments, as described in Table 6.

Table 6. Total insects count and proportion of the total across all seasons by species and biotechnologies, for treatments with insecticide control.

	Cry1Ac+ Cry1A.105+ Cry2Ab2	**%**	**Cry1Ac**	**%**	**Cry1Ac +Cry1F**	**%**	**non-*Bt***	**%**	**TOTAL**
A. gemmatalis	1	0,26	0	0,00	3	0,28	20	1,06	24
C. includens	0	0,00	6	0,46	9	0,83	100	5,31	115
C. aporema	0	0,00	7	0,54	6	0,56	7	0,37	20
Heliothinae	0	0,00	1	0,08	1	0,09	17	0,90	19
R. nu	16	4,12	492	37,73	379	35,16	836	44,42	1723
S. abula	1	0,26	2	0,15	3	0,28	8	0,43	14
S. cosmioides	2	0,52	23	1,76	22	2,04	32	1,70	79
S. eridania	15	3,87	91	6,98	31	2,88	190	10,10	327
S. frugiperda	353	90,98	682	52,30	624	57,88	672	35,71	2331
Total	388	-	1304	-	1078	-	1882	-	4652

5.8 Distribution of species abundance by location and region

For the 6 most abundant species at individual locations, focusing exclusively on plots without insecticide spraying, an estimation was performed for each species using a generalized linear model with Quasi-Poisson error analysis. The results were presented with a 95% confidence interval (Figure 5). So, it was noticed that *A. gemmatalis* and *S. cosmioides* appeared only in a few locations across regions North and South. Therefore, even though with a relatively small number of individuals collected, most of *C. includens* were found in the North, with only one location in the South, and the same happened with *S. eridania*, where was mostly found in the North and only in 2 locations at the South. The presence of *S. frugiperda* was detected across various locations in the North and in the South, although, as seen in Table 3, *S. frugiperda* was predominant in the North, while *R. nu* was predominant in the South. The confidence interval by individual locations shows an estimate of the insects collected by location and in some cases, statistically differ among them.

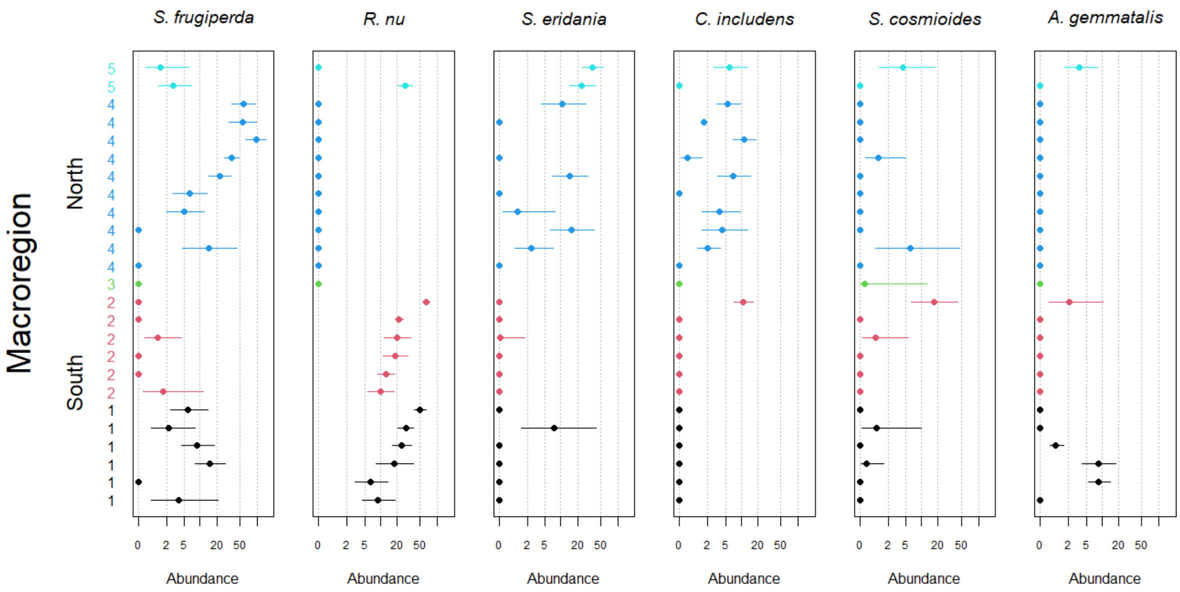


Figure 5. Estimated insect abundance over untreated plots by location for the six most prevalent species, color-coded by microregion, on log-scale. Estimation based on generalized linear model with Quasi-Poisson error term computed separately for each species (95% confidence interval). Each row represents one single location for each specie described, for example, high abundance of *R. nu* across locations in the South and just in one location in the North.

5.9 Differences on abundance of R. nu and S. frugiperda over biotechnologies

To compare the abundance of the two most prevalent species, *R. nu* and *S. frugiperda*, we used an estimation based on generalized linear model with Quasi-Poisson error term computed separately for each specie, comparing the abundance of insects found over each biotechnology in comparison with non-*Bt* treatment (control), including analysis of treatments with and without insecticide spraying. For both species, and both insecticide treatments, the biotechnology

Cry1Ac+Cry1A.105+Cry2Ab2 had lower insects´ abundance when compared with Cry1Ac and Cry1Ac+Cry1F, which had similar behavior among them and no statistical differences considering the 95% confidence interval (Figure 6). In addition, for both species the incidence of insects was lower for the treatments with insecticide spray in comparison with the treatments without insecticide spraying, as seen in Tables 5 and 6, respectively. So based on this analysis, Cry1Ac+Cry1A.105+Cry2Ab2 provided higher protection level for these two non-target species, when compared with Cry1Ac and Cry1Ac+Cry1F.

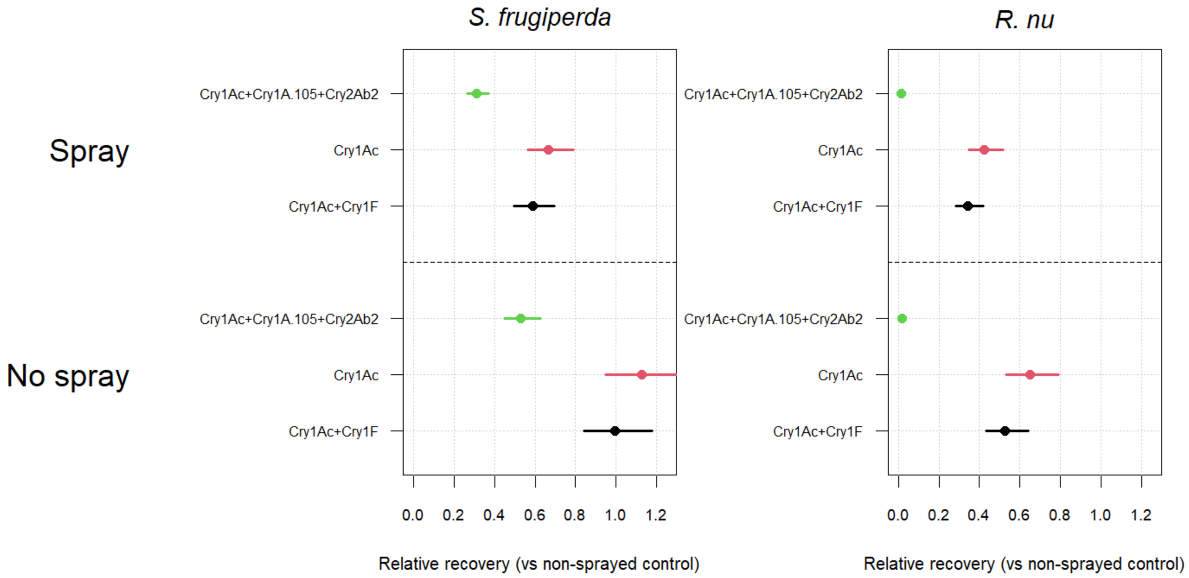


Figure 6. Impact of *Bt* traits on relative insect abundance, with and without insecticide spray. Estimation based on generalized linear model with Quasi-Poisson error term computed separately for each species (95% confidence interval). For example, on treatments without insecticide control, *S. frugiperda* showed 50% less incidence over Cry1Ac+Cry1A.105+Cry2Ab2 when compared to Cry1Ac and Cry1Ac+Cry1F, which were similar to non-*Bt* (Relative recovery=1). On the other hand, *R. nu* had close to null incidence over Cry1Ac+Cry1A.105+Cry2Ab2, and Cry1Ac and Cry1Ac+Cry1F had 60% incidence when compared to non-*Bt*.

5.10 Differences on defoliation over biotechnologies

For defoliation across species, considering both treatments with and without insecticide spraying, there were no statistical differences comparing the biotechnologies, although, the damage was lower over Cry1Ac+Cry1A.105+Cry2Ab2 when compared with Cry1Ac and Cry1Ac+Cry1F (Figure 7). Lower defoliation incidence can be explained by the reduced incidence of insects during the two seasons, although during periods with higher incidence, the results should spread among biotechnologies following the same pattern seen here, but in higher proportions.

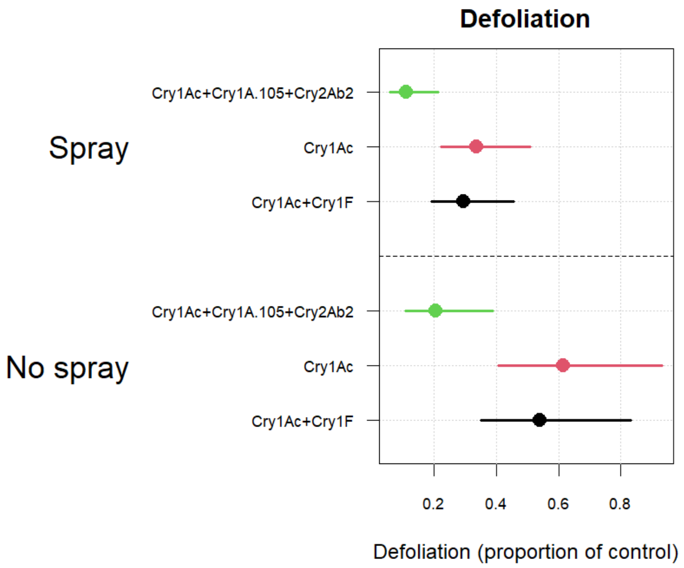


Figure 7. Impact of *Bt* traits on defoliation (95% confidence interval), with and without insecticide spray. Estimation based on generalized linear model with Quasi-Poisson error term computed separately for each species. Because impact of *Bt* traits will depend on insect abundance, 6 locations (out of 25) with low insect abundance were excluded from analysis. For example, on treatments without insecticide control, the average defoliation of Cry1Ac+Cry1A.105+Cry2Ab2 was 0.2, Cry1Ac 0.62 and Cry1Ac+Cry1F 0.55, when compared to the control non-*Bt*, which was 1.

5.11 Differences on yield over biotechnologies

To compare production per biotechnology, the yield increase over non-*Bt* treatments was observed. When the treatments were sprayed with insecticide, yield was higher for all technologies when compared with plots with no insecticide control, although, these differences were not statistically significant. On the other hand, comparing the biotechnologies, they had no differences as well in both treatments (with and without insecticides), but looking at the mean, it shows a difference from Cry1Ac+Cry1A.105+Cry2Ab2 to Cry1Ac over 80kg/ha and to Cry1Ac+Cry1F over 160kg/ha (Figure 8). Since the cultivars used across regions where different, due to their adaptability to regional conditions, these differences in yield could be related to the potential of them, although, it gives us an estimate production from each biotechnology over non-*Bt* treatments across Brazil.

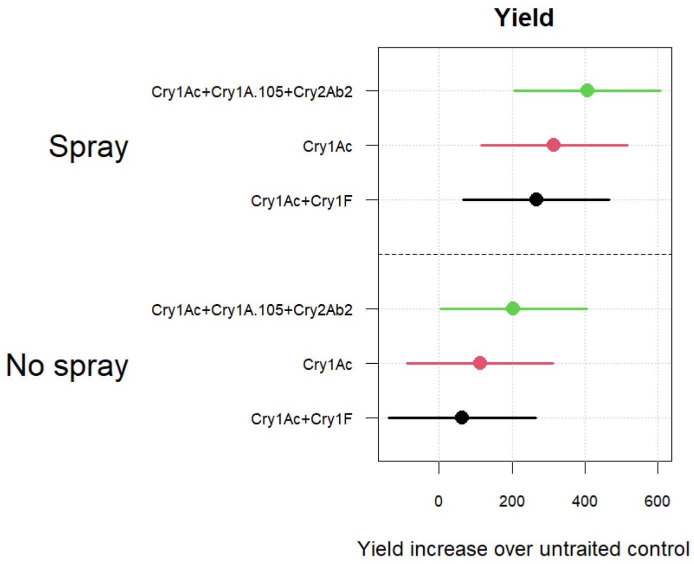


Figure 8. Impact of *Bt* traits on yield (95% confidence interval), with and without insecticide spray. Estimation based on generalized linear model with Quasi-Poisson error term computed separately for each species. Because impact of Bt traits will depend on insect abundance, 6 locations (of 25) with low insect abundance were excluded from analysis. For example, on treatments without insecticide control, yield increase of Cry1Ac+Cry1A.105+Cry2Ab2 was in average 200kg/ha more than non-*Bt* control.

6 DISCUSSION

Several studies have been carried out worldwide to understand the efficacy of *Bt* traits against specific species, using leaf disc bioassays, laboratory tests, trials in greenhouse and in the field. Therefore, it is noticed that in studies overtime, species that were susceptible feeding from specific *Bt* proteins, could become resistant and the protein might not have the same efficacy as initially seen, especially due to the evolution of resistance in these species.

In our study we focused on 9 lepidopteran species, mostly found in soybean production fields. Across all biotechnologies, the three most abundant species accounted for more than 94% of all individuals collected during samplings in the field, being represented by *R. nu*, *S. frugiperda* and *S. eridania*. Therefore, other studies to understand lepidopteran species composition in commercial fields in 2019/20 and

2020/21 cropping seasons, showed *C. includens*, *S. frugiperda* and *A. gemmatalis* as the three most abundant species over non-*Bt* soybeans and *S. frugiperda*, *S. eridania* and *S. cosmioides* over Cry1Ac soybeans (HORIKOSHI et al., 2021). This could be happening due to the methodology used, where Horikoshi (2021) sampled in large commercial fields and the current study was sampled in small plots, which are usually surrounded by commercial fields containing the different new biotechnologies available nowadays, and this could be causing a reduction on the incidence of such species. Still regarding the mentioned study from 3 years ago, important to reinforce that at that time *R. nu* was lightly detected on production fields, with less than 1% of incidence out of the total species found over non-*Bt* and zero incidence over Cry1Ac soybeans, and this is an important factor regarding the resistance breakage of *R. nu* over Cry1Ac, which increased the incidence of resistant populations of the specie across different regions in Brazil in the last 3 cropping seasons. Although these factors, climate and environmental conditions could be also affecting the species composition and their abundance across seasons and regions, and as example, *R. nu*, which was initially a pest with larger adaptation mainly in cold regions with lower temperatures, mostly subtropical and temperate regions, now is adapting over different regions up in the North, where it was not seen a couple years ago, and now it is a reality, even in small proportions up to now.

In Brazil, the species *C. includens* and *A. gemmatalis* continue to be the primary lepidopteran pests of soybean in Brazil and Cry1Ac continues to effectively manage the target lepidopteran pests, although an increase in the relative abundance of *Spodoptera* spp. was detected (HORIKOSHI et al., 2021). In our study, the low number of individuals from *C. includens* and *A. gemmatalis* collected over the biotechnologies does not represent any warning, and this could be related to the migration of adult larvae from non-*Bt* plots to the *Bt* traited plots. In addition, the low number from these two species showed that all *Bt* biotechnologies has been effective on their control, especially considering Cry1Ac, which was launched in 2013 and remains effective over these two species. Therefore, the increase of *Spodoptera* spp. was also detected in our study, mainly with *S. frugiperda* followed by *S. eridania* in the North region (Figure 4). On the other hand, *R. nu* was mostly detected on the South region, followed by *S. frugiperda*. Including different regions across Brazil was important to understand the main species present and causing damage to soybeans. New biotechnologies can also affect the dynamics of the insects, so including the four biotechnologies available commercially was fundamental to bring details on individual performance (and their comparisons) across different species.

Meanwhile, different studies either in laboratory or greenhouse, single *Bt* trait technology Cry1Ac have shown its efficacy against *A. gemmatalis*, *C. includens* (BERNARDI et al., 2012; CARPANE et al., 2022), *H. virescens* (BERNARDI et al., 2014a), and *H. armigera* (YU et al., 2013; AZAMBUJA et al., 2015; CARPANE et al., 2022). Therefore, studying different species, it was shown that Cry1Ac had low lethal effect over *S. cosmioides* and *S. eridania*, *S. frugiperda* (BERNARDI et al., 2014b; CARPANE et al., 2022; GODOY et al., 2022, 2023; BARCELLOS et al., 2023), *S. albula* (GODOY et al., 2022, 2023; BARCELLOS et al., 2023), *R. nu* (NARDON et al., 2021; RUTHES; ANJOS, 2021; BUENO; SOSA-GÓMEZ, 2022; LEITE; MORAES; CATHARIN, 2022; SZWARC et al., 2022; HILL et al., 2023; ÁVILA, 2023; ALVES et al., 2024; REIS et al., 2024), and *C. aporema* (RUTHES; ANJOS, 2021; BUENO; SOSA-GÓMEZ, 2022). On the other hand, earlier to these previous works, evaluating survivorship in greenhouse under artificial infestation, it was found that Cry1Ac had a suppressive effect on *S. frugiperda* and *S. albula*, however, *S. eridania* and *S. cosmioides* were not susceptible to the protein (MURÚA et al., 2018), and in this case, the suppression might be related to older studies, susceptible populations used, or development of resistant populations overtime, because nowadays most of the studies doesn´t show any suppression benefits of this single protein to the *Spodoptera* spp. The low susceptibility of *Spodoptera* species to Cry1Ac protein can be attributed to the inactivation of *Bt* proteins by proteases and low specific binding sites for Cry proteins in its midgut (HERRERO et al., 2016).

On the other hand, studying the dual *Bt* traits Cry1Ac+Cry1F, in field studies, with artificial infestation of different lepidopteran species, results demonstrated consistent efficacy for *A. gemmatalis, C. includens, H. virescens* e *S. cosmioides* (MARQUES et al., 2016) and also against secondary pests as *E. lignosellus* and *H. armigera*, and moderate level efficacy against *Agrotis ipsilon* (MARQUES et al., 2017). In different studies with leaf tissue diet, it was seen that Cry1Ac+Cry1F provided population suppression of *S. eridania* and *S. cosmioides*, however, had minimal effects on *S. albula*, reflecting their inherently low susceptibility to the Cry1Ac and Cry1F *Bt* proteins (MACHADO et al., 2020b). For the specie *S. frugiperda*, homozygous-resistant and heterozygous individuals were able to survive and emerge as fertile adults when fed by this dual *Bt* protein, suggesting cross crop resistance (MACHADO et al., 2020a).

Studying the three *Bt* protein trait Cry1Ac+Cry1A.105+Cry2Ab2 in different environments, including greenhouse and field trials, it was identified high efficacy against the species *C. includens*, *A. gemmatalis* and *H. armigera* (BACALHAU et al., 2020). In another study, larvae of *R. nu* did not survive on artificial diet using Cry1Ac+Cry1A.105+Cry2Ab2 leaves (ALVES et al., 2024), and demonstrated efficient control of this specie in soybean cultivars with the biotechnology (ÁVILA, 2023), with Cry2Ab2 likely ensuring the effectiveness and durability against this specie and being a valuable tool to manage Cry1Ac resistance (REIS et al., 2024). Studying other species, the same biotechnology caused high lethality in neonates of *S. cosmioides* and *S. albula*, intermediate mortality of *S. eridania*, however, it showed low lethality in resistant individuals of *S. frugiperda* (GODOY et al., 2022; BARCELLOS et al., 2023). In another study, on unsprayed Cry1A.105+Cry2Ab2+Cry1Ac soybean, only *S. frugiperda* showed around 60% mortality after 10 days feeding from leaves, whereas *S. cosmioides*, *S. eridania* and *S. albula* showed >81% mortality (GODOY et al., 2023).The reduced effectiveness controlling *S. frugiperda* can be explained by the widespread resistance of this insect to Cry proteins expressed in multiple *Bt* plant species and the large degree of cross-resistance between Cry proteins expressed in *Bt* maize, *Bt* cotton, and *Bt* soybean in Brazil (HORIKOSHI et al., 2016).

Since two of these biotechnologies were launched commercially in the last years, only a few studies were conducted comparing these four biotechnologies together. Therefore, in a laboratory study with plant tissue diets to understand the performance against *S. frugiperda*, *S. cosmioides*, and *S. eridania*, showed that Cry1Ac+Cry1A.105+Cry2Ab2 had improved performance considering larvae mortality level and leaves consumption when compared to Cry1Ac+Cry1F, Cry1Ac and non-*Bt* (SANTANA, 2023).

In our study, similar results from the previous work done with the biotechnologies were seen. The three *Bt* traited biotechnologies were effective against *C. includens* and *A. gemmatalis*, and Cry1Ac continue effective more than 10 years of its initial adoption. Very low incidence of *R. nu* was only observed on Cry1Ac+Cry1A.105+Cry2Ab2, demonstrating its benefits over this specie. For *S. frugiperda*, incidence was noticed on all biotechnologies. Therefore, for these two species, the incidence was around 50% lower over Cry1Ac+Cry1A.105+Cry2Ab2 when compared with Cry1Ac+Cry1F and Cry1Ac (Figure 6), showing its superiority against these species when compared with the other biotechnologies.

In addition, besides individual efficacy from each biotechnology according to the target and non-target species, additional benefits has been identified by the regional suppression of lepidopteran pests (specially *C. includens* and *H. armigera*) and reduced insecticide use (in *Bt* and non-*Bt* soybean cultivated area) with the wide-spread adoption of Cry1Ac soybean in Brazil, bringing economic, social and environmental benefits (HORIKOSHI et al., 2022). Furthermore, the three most abundant species observed during the study were *R. nu*, *S. frugiperda* and *S. eridania*, were previously considered secondary pests in soybeans just a few years ago. Studies in Argentina also detected similar trends, where the number of non-target lepidopterans (*Spodoptera* spp.) increased year by year, and they were the prevailing lepidopteran species on *Bt* soybean in 2020-21 (PÁEZ JEREZ et al., 2023).

It is important to mention all the biotechnologies consider protection against specific target species in their initial development, such as neonates. Therefore, advanced instars are presumably more tolerant to *Bt* proteins (BERNARDI et al., 2012), and in general, the more advanced the instar, the greater the difficulty of controlling it, for the larvae must ingest a larger amount of tissue to cause lethality, with consequent damage to the plant (AZAMBUJA et al., 2015).

Besides, constant studies regarding the main soybean species survival and resistance per *Bt* trait or biotechnology are needed, even done through laboratory diets, greenhouse trials or field trials, due to the appearance of resistant populations that can quickly expand and cause severe damage to the crop. The dynamics of the lepidopteran pests is fast and requires special attention on the production systems to ensure the durability and longevity of the biotechnologies.

7 CONCLUSION

The present study aimed to evaluate the efficacy of all commercially available *Bt* traits and their performance against different lepidopteran species during field evaluations carried out in the 2022/23 and 2023/24 seasons.

According to the results, the following conclusions were made: a) over the two seasons, the three most abundant species found were *S. frugiperda* (48,82%) followed by *R. nu* (35,13%), and *S. eridania* (10,10%); (b) out of the total lepidopteran larvae collected, *R. nu* was the most predominant specie in the South (84,08%) and *S. frugiperda* in the North (75,80%); c) relative insect abundance was statistically significant lower over Cry1Ac+Cry1A.105+Cry2Ab2 when compared to Cry1Ac+Cry1F and Cry1Ac, for both *R. nu* and *S. frugiperda*; d) even though not statistically significant, it was found lower defoliation on Cry1Ac+Cry1A.105+Cry2Ab2 when compared to Cry1Ac+Cry1F and Cry1Ac, and superior productivity of Cry1Ac+Cry1A.105+Cry2Ab2 over Cry1Ac (around 80kg/ha) and over Cry1Ac+Cry1F (around 160kg/ha).

Our findings suggest that even not considered target species for the biotechnologies studied, Cry1Ac+Cry1A.105+Cry2Ab2 shows additional benefits on protection specially against *R. nu*, but also against *S. frugiperda*, when compared with Cry1Ac+Cry1F and Cry1Ac. These benefits are close to full protection against *R. nu* and around 50% less incidence regarding *S. frugiperda*.

Therefore, the use of *Bt* soybean cultivars must be allied to the integrated pest management (IPM) control, and the deployment of refuge areas, using non-*Bt* soybean cultivars on at least 20% of the total cultivated area within 800 meters of *Bt* soybeans. It supports the longevity and durability of the traits, preventing the appearance of resistant populations and avoiding breakage of the *Bt* traits efficacy.

The comparisons made in this study provide reliable information regarding the commercially available biotechnologies and their performance, supporting important decisions for the farmers while deciding what are the cultivars they intend to plant in the upcoming seasons, seeking always for less issues in the season and finally, higher yields.

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