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Toxicity of Bt Protein on *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) Fed With Corn Leaves in Paraguay

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Abstract

The fall armyworm *Spodoptera frugiperda* is the most destructive pest in corn cultivation in Paraguay with yield losses of 20 to 70%. The adoption of genetically modified crops (GMO) is a valid technique for managing this pest. The objective of this investigation was to evaluate the toxicity among Bt events used locally; determining mortality and important biological parameters. The *S. frugiperda* population previously collected from the field was raised under laboratory conditions fed with leaves from events MIR162™ (Vip3A19), VT3Pro™ (Cry1A105, Cry2Ab2, Cry3Bb), PowerCore™ (Cry1A105, Cry1F, Cry2Ab2) and a treatment with non-maize. Bt BR106™. The mortality of 120 larvae was evaluated for each treatment and the viability until the adult stage of those larvae that managed to survive the different treatments was observed. A high toxicity effect was verified with 100% mortality of neonatal larvae fed with the MIR162™ (Vip3A19) event; as for the other events, 86% and 61% VT3Pro™ (Cry1A105, Cry2Ab2, Cry3Bb), and PowerCore™ (Cry1A105, Cry1F, Cry2Ab2) respectively; with 13% mortality for the non-Bt control variety BR106™. The oviposition period, viability and number of eggs laid in adults surviving the PowerCore™ (Cry1A105, Cry1F, Cry2Ab2) and VT3Pro™ (Cry1A105, Cry2Ab2, Cry3Bb) events were reduced, affecting the fecundity of the surviving insect population.

Keywords: Bt corn, resistance management, *Spodoptera frugiperda*, corn pest

1. Introduction

Corn (*Zea mays*) is a traditional crop in Paraguay; currently it is an area of socio-economic relevance that involves small, medium, and large farmers; highly appreciated and used as a base in the preparation of traditional foods for human consumption; as well as a food base in animal production and industrial raw materials. In addition, it constitutes a factor of foreign exchange income from the export of grains that reached 750 million dollars to international markets, being the second with the largest planting area in Paraguay (CAPECO, 2023). Considering the importance and the production problems observed, especially the attack by *Spodoptera frugiperda*, new pest management technologies have been adopted; among them, resistance to pests through transgenic varieties, mainly reducing the use of insecticides, improving the yield and quality of production, as well as economic gains. The fall armyworm caterpillar *Spodoptera frugiperda* (Smith) is the key pest of corn in the region, a polyphagous species whose larvae are active at night and during the day; They attack corn plants by acting as cutters, defoliators and bud harvesters depending on the moment of their development. They cause direct damage when they feed on the grains of the ear and directly influence the yield and quality of the product, since there is a direct relationship between the leaf area at the time of stigma formation and grain filling (Sosa, 2003; Casmuz, 2010; Gomez et al., 2017). This pest has caused many losses to farmers from 20 to 70%; with insecticide management, which has generated massive applications of different active ingredients with diverse mechanisms of action; Even so, with difficult control due to the appearance of populations of resistant individuals (Gutierrez, 2019; CAPECO, 2023).

Decades of corn production have led science through biotechnology to the creation of genetically modified organisms (GMOs) called events with pest resistance characteristics; Important achievements have been obtained that result in increased production of the sector for the internal market, as well as the external market (Bortolotto et al., 2016). With the aim of improving corn production in Paraguay, the planting of transgenic Bt corn with expression of Cry1F proteins (Herculex™) has been released; Cry1A.105, Cry1F, Cry2Ab2

(PowerCore™); Cry1A.105, Cry2Ab2, Cry3Bb (VT3Pro™); Vip3Aa20 (MIR162™) (National Service for Plant and Seed Quality and Health SENAVE 2023). Despite the history of control of these transgenic events; The loss of effectiveness of these materials used is also mentioned due to inadequate handling, which generates a strong selection pressure for individuals with resistance characteristics in the insect population. In several regions of the world, initial effectiveness and failures after massive use are mentioned; with reports of good and low effectiveness (Williams et al., 1997; Waquil et al., 2002; Giaveno et al., 2010; Flores & Balbi 2014; Grandis de Lima & Assmann, 2015; Monnerat et al., 2015). In Puerto Rico, Brazil and the southern United States, resistance of *S. frugiperda* to Bt corn with the Cry1F or Cry1Ab proteins was detected (Storer et al., 2010; Farias et al., 2014; Huang et al., 2014; Omoto et al., 2016). At the level of Paraguay, Gómez et al. (2017) and Gamarra et al. (2022) report differences in susceptibility of *S. frugiperda* fed with bt corn released in Paraguay. More recent work in China at the laboratory level (Wang et al., 2022) mentions that there is variability in the susceptibility ranges of different populations of *S. frugiperda* to Bt corn events. These new technologies carry out pest control; However, it is necessary to implement studies related to their management, considering the relationship between pest and transgenic event in local conditions and in this way gain knowledge of the behavior in the agricultural ecosystem. Consequently, the main objective of this research is to evaluate the toxicity of the Bt protein expressed in the leaves of different corn events used in Paraguay on *S. frugiperda*. The specific objectives are: to calculate the percentage of mortality, and in the case of survival to detail the weight and length of pupae, the longevity of adults and the pest cycle. The study aims to determine the toxicity of Bt maize events proteins *S. frugiperda*.

2. Materials and Methods

2.1 Collection and Breeding of S. frugiperda

Manual collections of *S. frugiperda* larvae from *Triticum aestivum* wheat crops were carried out in the District of J. Eulogio Estigarribia, department of Caaguazú, Paraguay (latitude: 25°17′56″S, longitude: 57°31′47″W; altitude 272 meters above sea level. The larvae were placed in plastic containers with individual lids containing artificial diet (Parra, 2001; Da Silva & Parra, 2013), and transferred to the insect breeding laboratory within 48 hours. In the laboratory, the larvae were transferred to 100 plastic cups (50 ml) containing artificial diet (Parra, 1996) and placed in rearing rooms under controlled conditions of temperature (25±5 °C), relative humidity (60±10%), and photoperiod (2:10 p.m. L: W), until the pupal stage. The pupae were sexed (Butt & Cantu, 1962), removed and disinfested with 0.2% sodium hypochlorite in distilled water and then placed on filter paper in plastic plates covered with 250 cm^3 transparent glasses. The emerged adults (ten males and ten females) were transferred to mating cages that consisted of white PVC tubes, 24 cm high and 14.5 cm in diameter, lined internally with white paper as an oviposition substrate. The cages were placed on plastic plates, on filter paper, and covered on top with tulle-type fabrics secured with rubber bands to promote aeration. Adults were fed with a 10% solution of water and honey, presented on pieces of cotton, located at the top of the cage in containers 1.5 cm long and 2 cm in diameter. The eggs were removed every 48 h and placed in 50 ml plastic cups with a piece of moistened filter paper on top to prevent desiccation. The vessels were kept in BOD-type climate-controlled chambers (temperature 24±2 ºC), relative humidity 60±10%, and photoperiod 2:10 p.m. L:D. A part of the first generation of hatched larvae (F1) was used for experimental work (120 larvae per treatment, neonate larvae with a maximum of 24 h of age) and another part was separated for the maintenance of the population in the laboratory.

2.2 Vegetal Material

To obtain the leaves of the various treatments considered; In the greenhouse, sowing was carried out progressively in the month of November with the MIR162™ event (Vip3A19) and VT3Pro™ (Cry1A105, Cry2Ab2, Cry3Bb), in December with the PowerCore™ event (Cry1A105, Cry1F, Cry2Ab2) and the control variety BR106™ non-Bt. All corn events were planted with 15 cm between plants and 80 cm between rows. The leaves of the different corn events used in the experiments were obtained from plants 20 to 50 days old from emergence, which were cut from the upper part of the plant using a 3.0 cm diameter punch.

Table 1. Treatment with corn events and the protein expressed by each event

Treatment	Protein	Event
1	Vip3A19	MIR162™
2	Cry1A105, Cry2Ab2, Cry3Bb	VT3Pro™
3	Cry1A105, Cry1F, Cry2Ab2	PowerCore™
4	Control	Variety BR106™ (non-Bt)

2.3 Bioassays

2.3.1 Percent Mortality of *Spodoptera frugiperda*

The experiment began once enough first instar larvae (L1) (120 larvae) had been obtained. They were fed with leaves from corn plants corresponding to each treatment. The leaves of the corn from 20 to 50 days of emergence were cut into a size of 3 cm with a punch, and then 2% agar-water (2 ml per container) was placed inside the disposable plastic containers (100 ml) previously self-nailed, a filter paper disc (4.5 cm in diameter) was placed on top of this medium, and the piece of corn leaf and one neonate larva (24 hours old) per container were placed on this paper, keeping them in a climate-controlled chamber at 27±1 °C, 60±10% RH and 14 hours of photophase. The leaves were replaced at first every two days, later with the increase in the size of the larva, its consumption increased, requiring a piece of leaf to be placed daily for those surviving larvae (the leaves of the bud were cut at the time of being used, from 20 days emergency reaching 50 days of emergency). 120 larvae were used per treatment. To determine the mortality percentage, the difference with the total number of individuals that did not complete their cycle was considered; corrected by the formula of Henderson and Tilton (1955), considering the non-Bt material BR106^{TM} as the control.

2.3.2 Duration of Larval and Pre-pupal Phase of Survivors

Through daily observations, the duration in days of the pre-pupal stages and then the pupal stages were determined. For the survival percentages, the total number of individuals that managed to complete each phase was used.

2.3.3 Duration, Weight, and Length of Pupae by Sex

The first day of pupa formation until the emergence of the adult was considered to determine the duration of the pupal phase, seeing individuals that managed to survive and complete this phase. After 24 hours of pupa formation, it was weighed with a precision balance (RADWAG WTB 200 Accuracy 0.001 g) and the length (mm) was determined with a millimeter ruler. The sex of everyone was also determined based on the morphological difference present in the last abdominal segment (Santos-Amaya et al., 2015).

Subsequently, the pupae were placed in a tray with moistened filter paper at the bottom and covered with plastic cups with respective numbers to identify them. They remained covered until the adult emerged.

2.3.4 Sex Ratio and Emergency Percentage

To evaluate these variables, the formulas according to García and Iannacone (2011) were used.

$$\%H = N°H \times 100/N°AE \quad (1)$$

$$\%M = N°M \times 100/N°AE \quad (2)$$

Where, %H = Percentage of females; %M = Percentage of males; N°H = Number of females; N°M = Number of males; N°AE = Number of adults emerged.

$$\%E = T - (PnE+AmE)/T \quad (3)$$

Where, %E = Emergency percentage; T = Total number of pupae; PnE = Pupae not emerged; AmE = Half-emerged adults.

2.3.5 Pre-oviposition Period, Post-oviposition Period (days), Female Fecundity and Egg Viability

Of the emerged adults; 10 pairs of insects were placed in a quantity of 1 pair per cage. From this the following variables have been measured:

Fertility, using the formula according to García and Iannacone (2011).

$$Fertility = N°HV/N°Hvs \quad (4)$$

Where, N°HV = Number of eggs laid; N°Hvs = Number of females.

Fertility or viability, according to the formula used by Hernández et al. (2010).

$$Fertility\ or\ Viability = N°Hvs/N°N \quad (5)$$

Where, N°Hvs = Number of eggs laid; N°N = Number of neonates.

2.3.6 Duration of the Total Cycle of *Spodoptera frugiperda*

Those individuals that managed to complete all the stages from larva to adult were considered.

2.3.7 Statistical Analysis

The data obtained were subjected to analysis of variance (ANAVA), a comparison of means was also carried out using the Tukey test (α = 5%), using the INFOSTAT-2014 Software (Di Rienzo et al., 2014).

3. Results

3.1 Percent Mortality of Spodoptera frugiperda

Neonate L1 larvae exposed to the different events show the average mortality percentages (Table 2). Seven days after the start of feeding L1 larvae with the leaves of the events considered; A total insect mortality was verified in the MIR162TM treatment of 100% (F = 89.3; p = 0.0001) with a significant statistical difference in relation to the other events. In the observations of the percentage of mortality of insects in development after 7 days; larva, prepupa, and pupa, the VT3Pro™ event presents higher mortality than PowerCore™ 83.9 percent with statistical differences (F = 353.2; p = 0.0001) and considering the mortality correction made by the Henderson and Tilton formula. The variety BR106™ (non-Bt) used as a control presented a lower percentage of mortality due to the lack of insecticidal proteins.

Table 2. Average mortality percentage obtained in neonatal larvae of *S. frugiperda* fed with corn leaves with Bt events and non-Bt variety

Treatment	Mortality percentage and (SE)			
	Neonate larvae at 7 days	H&T Corrected Percentage	Larva, pre-pupa and pupa	H&T Corrected Percentage
MIR162™	100 (4.5)a*	100	-	
VT3Pro™	35 (4.5)b	34.3	86 (2.0)a	83.9
PowerCore™	21(4,5)b	20.2	61 (2.0)b	60.6
BR106™ (not Bt)	1 (4.5)c		13 (2.0)c	
Gl	3		3	
F	89.3		353.2	
P-value	0.0001		0.0001	
CV (%)	40.1		10.9	

Note. CV = Coefficient of Variation. EE = Standard Error.

Percentage corrected by the Henderson-Tilton formula.

* Means in the columns with the same letters are statistically similar at a 5% significance level ($p \leq 0.05$). Tukey test.

3.2 Duration and Percentage of the Larval and Prepupal Stages of Insects Surviving Bt and Conventional Corn

Larval mortality in the MIR162TM event was 100%, these variables are not considered; certainly, verified for the other events (Table 3), differentiated mean durations of the larval stage were recorded between the Bt events; VT3Pro™ (17.7 days) had a longer duration in the larva stage than PowerCore™ (14.9 days) and the variety BR106™ (13.3 days) with statistical differences (F = 105.5; p = 0.0001). In prepupa, a longer duration was detected in the insects fed with VT3Pro™ (2.0 days) and PowerCore™ (1.9 days) with a shorter duration in the non-Bt variety BR106™ (1.7 days) (F = 15.5; p = 0.0001).

Table 3. Average duration (days) of the larval stage and pre-pupae obtained in neonatal larvae fed with leaves from different events of Bt corn and a variety of non-Bt corn

Treatment	Larval stage (EE)	Pre-pupal stage (EE)
VT3Pro™	17.7 (0.2)a*	2.0 (0.1)a
PowerCore™	14.9 (0.2)b	1.9 (0.1)a
BR106™ (not Bt)	13.3 (0.2)c	1.7 (0.1)b
Gl	2	2
F	105.57	15.51
P-value	0.0001	0.0001
CV (%)	4.90	6.15

Note. CV = Coefficient of Variation. EE = Standard Error.

* In each column, different letters denote significant differences detected by the Tukey Test $P < 0.05$.

In the larval, prepupal and pupal stages (Table 4) despite not detecting significant differences ($F = 2.41$; $p = 0.1059$; $F = 5.45$; $p = 0.009$; $F = 0.13$; $p = 0.8759$) between the average percentages of survivors, the conventional variety BR106™ (non-Bt) presents a high percentage of survival (93% larva; 88% prepupa and pupa) in relation to the other events.

Table 4. Survival percentages in the larva, pre-pupa and pupa stages

Treatment	Larval stage	Pre-pupal stage	Pupal stage
VT3Pro™	31 a*	17b	14 a
PowerCore™	53 a	43b	39 a
BR106™ (not Bt)	93 a	88b	88 a
Gl	2	2	2
F	2.41	5.45	0.13
P-value	0.1059	0.009	0.8759
CV (%)	22.91	7.04	14.35

Note. * Means in columns with equal letters are statistically similar at a 5% significance level ($p \leq 0.05$). Tukey test.

3.3 Duration, Weight, and Length of Pupae by Sex

Values of the means of the variables separated by sex are observed in Table 5. For the length, weight and duration of female pupae, a difference was obtained ($F = 2.5$; $p = 0.0854$; $F = 14.6$; $p = 0.0001$; $F = 5.4$; $p = 0.0061$) between the VT3Pro™ event (16.3 mm) and the other PowerCore™ events (15.4 mm) and the BR106™ variety (non-Bt)(15.8 mm). In relation to male pupae, the VT3Pro™ event presented more length (17.3 mm), weight (0.3 g) and duration (9.6 days) in relation to PowerCore™ (15.8 mm; 0.2 g; 9.0 days) and BR106™ (15.8 mm; 0.2 g; 8.3 days) ($F = 14.8$; $p = 0.0001$; $F = 15.3$; $p = 0.0001$; $F = 24.6$; $p = 0.0061$).

The duration presents different values for the treatments; with lower value in the BR106™ (non-Bt) variety (8.3 days), and higher value in the VT3Pro™ event (9.6 days).

Table 5. Means of length, weight and duration of pupae by sex of *S. frugiperda*

Treatment	Females			Males		
	Length (mm) (EE)	Weight (g) (EE)	Duration (Days) (EE)	Length (mm) (EE)	Weight (g) (EE)	Duration (Days) (EE)
VT3Pro™	16.3 (0.3)a*	0.3 (0.1)a	8.7 (0.3)a	17.3 (0.3)a	0.3 (0.1)a	9.6 (0.2)a
PowerCore™	15.4 (0.2)b	0.2 (0.1) b	7.6 (0.2)b	15.8 (0.2)b	0.2 (0.1)b	9.0 (0.1)b
BR106™ (not Bt)	15.8 (0.1)b	0.2 (3.3)b	7.6 (0.1)b	15.8 (0.1)b	0.2 (3.8)b	8.3 (0.1)c
Gl	2	2	2	2	2	2
F	2.5	14.6	5.4	14.8	15.3	24.6
P-value	0.0854	0.0001	0.0061	0.0001	0.0001	0.0001
CV (%)	5.4	12.1	9.5	5.2	12.7	7.0

Note. CV = Coefficient of Variation. EE = Standard Error.

***** In each column, different letters denote significant differences detected by the Tukey Test $P < 0.05$.

3.4 Sex Ratio and Emergency Percentage

Among the means of the percentage of emerged females (Table 6), statistical differences are observed ($F = 14.2$; $p = 0.0001$) between the insects fed with the VT3Pro™ (12%), PowerCore™ (20%) and the BR106™ variety (non-Bt) (47.5%); In the means of the percentage of emergence of males, the results are similar to that of female insects ($F = 13.6$; $p = 0.0001$) (VT3Pro™ (12.2%), PowerCore™ (21.6%) and the variety BR106™ (non-Bt) (40.0 %). In adult emergence, all treatments differ from each other ($F = 222.3$; $p = 0.0001$) with VT3Pro™ (14.2%), PowerCore™ (38.3%) and the BR106™ variety (not Bt) (87.5%). %); in the proportion of female:male, the PowerCore™ event (0.5) and BR105™ (not Bt) (0.5) show statistical equality, differing from the VT3Pro™ event (0.8); ($F = 3.3$; $p = 0{,}0543$).

Table 6. Means of the percentages of females, males, adult emergence and female:male ratio of *S. frugiperda* in Bt and non-Bt corn under laboratory conditions

Treatment	% Females (EE)	% Males (EE)	% Emergency (EE)	Proportion ♀:♂ (EE)
VT3Pro™	12.0 (6.6)a*	12.2 (4.2)a	14.2 (2.5)a	0.8 (0.1) b
PowerCore™	20.0 (4.7)a	21.7 (3.6)a	38.3 (2.5)b	0.5 (0.1)a
BR106 ™ (not Bt)	47.5 (4.3)b	40.0 (3.6)b	87.5 (2.5)c	0.5 (0.1)a
Gl	2	2	2	2
F	14.2	13.6	222.3	3.3
P-value	0.0001	0.0001	0.0001	0.0543
CV (%)	48.4	48.7	18.5	43.0

Note. CV = Coefficient of Variation. EE = Standard Error.

***** In each column, different letters denote significant differences detected by the Tukey Test $P = 0.05$.

3.5 Adults: Pre-oviposition, Oviposition, Post-oviposition Period (days), Fecundity and Egg Fertility

The pre-oviposition, oviposition, and post-oviposition periods are observed (Table 7). The duration of the pre-oviposition period in the PowerCore™ event (3.4 days) was significantly longer ($F = 3.3$; $p = 0.0543$) than in the VT3Pro™ event (2.5 days) and the treatment with conventional corn BR106™ (non-Bt) (2.2 days). The three treatments were statistically equal in the parameters studied: oviposition and post-oviposition periods; ($F = 1.6$; $p = 0.2189$) ($F = 0.4$; $p = 0.6735$).

Table 7. Average of the pre-oviposition, oviposition, and post-oviposition periods in days of *S. frugiperda* farms fed during the larval stage with Bt and non-Bt corn leaves under laboratory conditions

Treatment	Pre-oviposition (EE)	Oviposition (EE)	Post-Oviposition (EE)
VT3Pro™	2.5 (0.3)ab*	5.1 (0.6)a	1.0 (0.3)a
PowerCore™	3.4 (0.2)b	4.7 (0.5)a	1.1 (0.3)a
BR106™(not Bt)	2.2 (0.2)a	6.0 (0.5)a	1.3 (0.3)a
Gl	2	2	2
F	5.0	1.6	0.4
P-value	0.0157	0.2189	0.6735
CV (%)	31.9	30.6	59.9

Note. CV = Coefficient of Variation. EE = Standard Error.

* In each column, different letters denote significant differences detected by the Tukey Test P < 0.05.

3.6 Average Fertility: Number of Neonates per Birth and Percentage of Viability

n the three variables evaluated: fecundity, neonates/casual and viability (Table 8), there were significant differences between the VT3Pro™ and PowerCore™ events and non-BT corn (F = 8.6; p = 0.0016) (F = 24.5; p = 0. 0001) (F = 32; p = 0.0001), but without differentiating the events from each other. The variety BR106™ (non-Bt) presented a greater number of eggs (2180) per female per clutch and greater viability (93.2%) in relation to the events. These results indicate that leaf feeding from Bt events affected female fecundity and egg viability.

Table 8. Means of the number of eggs (fertility), numbers of hatchlings obtained and fertility or viability of eggs in the farms fed during the larval stage with Bt and non-Bt corn leaves under laboratory conditions

Treatment	Fertility1(EE)	Neonates/household (EE)	Feasibility %2 (EE)
VT3Pro™	1149.0 (215.1)a*	738 (18.1)a	64.2 (6.1)a
PowerCore™	1432.8 (166.5)a	816 (13.9)a	56.9 (4.7)a
BR106™(not Bt)	2180.2 (166.5)b	2033(13.9)b	93.2 (4.7)b
Gl	2	2	2
F	8.6	24.5	32.0
P-value	0.0016	0.0001	0.0001
CV (%)	36.4	34.9	23.2

Note. [1] Fertility calculated according to the formula of García and Iannacone (2011).

[2] Fertility or viability according to the formula of Hernández et al. (2010).

*Means in the columns with the same letters are statistically similar at a 5% significance level ($p \leq 0.05$). Tukey test.

3.7 Duration of the Total Cycle of Spodoptera frugiperda

The longevity of adult female insects can be seen in Table 9. The duration of the pre-pupal and adult stages was not statistically significant (F = 1.77; p = 0.1929); (F = 0.34; p = 0.7134). Regarding the duration of larva, pupa and total cycle, significant differences were observed between treatments (F = 242.3; p = 0.0001); (F = 11.27; p = 0.0004); (F = 36.41; p = 0.0001). The duration of the development of the stages of the individuals was significantly longer in those fed with VT3Pro™ (37.8 days) in relation to the PowerCore™ event (32.6 days) and the BR106™ variety (non-Bt) (30.4 days).

Table 9. Mean duration in days of instars in *S. frugiperda* females with Bt and conventional corn under laboratory conditions

Treatment	Females				
	Larva (EE)	**Pre-pupa (EE)**	**Pupa (EE)**	**Adult (EE)**	**Total (EE)**
VT3Pro™	18.5 (0.2)a*	2.0 (0.1)a	8.6 (0.2)a	8.6(0.8)a	37.8 (0.7)a
PowerCore™	14.1 (0.1)b	1.8 (0.1)a	7.3 (0.2)b	9.2 (0.6)a	32.60 (0.5)b
BR106™(not Bt)	11.8 (0.1)c	1.6 (0.1)a	7.1 (0.2)b	9.5 (0.6)a	30.40 (0.5)c
Gl	2	2	2	2	2
F	242.3	1.77	11.27	0.34	36.41
P-value	0.0001	0.1929	0.0004	0.7134	0.0001
CV (%)	4.1	23.5	8.8	21.2	5.4

Note. CV = Coefficient of Variation. EE = Standard Error.

* In each column, different letters denote significant differences detected by the Tukey Test $P < 0.05$.

The means of the stages in males are presented in Table 10, with similar results to females (Table 9). The duration of the pre-pupal and adult stages was verified without statistical differences ($F = 1.4$; $p = 0.2498$); ($F = 1.3$; $p = 0.2703$). Regarding the duration of larva, pupa, and total cycle, there are significant differences between the treatments ($F = 105.1$; $p = 0.0001$); ($F = 8.7$; $p = 0.0015$); ($F = 66.8$; $p = 0.0001$). The duration of the development of the stages of the individuals was significantly longer in those fed with VT3Pro™ (40.0 days) in relation to the PowerCore™ event (33.7 days) and the BR106™ variety (non-Bt) (31.7 days).

Table 10. Mean duration in days of instars in *S. frugiperda* males with Bt and conventional corn under laboratory conditions

Treatment	Males				
	Larva(EE)	**Pre-pupa(EE)**	**Pupa(EE)**	**Adult(EE)**	**Total(EE)**
VT3Pro™	17.5(0.3)a*	2.0(0.15)a	9.8(0.27)a	10.6(0.63)a	40.0(0.59)a
PowerCore™	13.4(0.2)b	1.9(0.11)a	8.9(0.21)b	9.4(0.49)a	33.7(0.46)b
BR106™(not Bt)	11.1(0.2)c	1.7(0.11)a	8.4(0.21)b	10.2(0.49)a	31.7(0.46)c
Gl	2	2	2	2	2
F	105.1	1.4	8.7	1.3	66.8
P-value	0.0001	0.2498	0.0015	0.2703	0.0001
CV (%)	6.3	19.5	7.4	15.5	4.2

Note. CV = Coefficient of Variation. EE = Standard Error.

* In each column, different letters denote significant differences detected by the Tukey Test $P < 0.05$.

The stages of *S. frugiperda* without discriminating sex presented the following results shown in Table 11. The duration of the larva stage shows the difference between all treatments ($F = 241.3$; $p = 0.0001$). In the pre-pupal stage, there was a difference ($F = 3.3$; $p = 0.0425$) between VT3Pro™ and the variety BR106™ (non-Bt). In the pupa; the VT3Pro™ event differs with the other treatments ($F = 9.3$; $p = 0.0004$).; and in adults there was no significant variation between treatments. In the comparison of Bt and non-Bt corn in the total insect cycle, a difference between the three treatments resulted ($F = 79.1$; $p = 0.0001$). The VT3Pro™ event had the longest duration (38.9 days) followed by PowerCore™ (33.1 days) and BR106™ (non-Bt) (31.0 days).

Table 11. Means of the duration in days of the stages of *Spodoptera frugiperda* with Bt and conventional corn under laboratory conditions

Treatment	Stages of *S. frugiperda*				
	Larva(EE)	**Pre-pupa(EE)**	**Pupa(EE)**	**Adult(EE)**	**Total(EE)**
VT3Pro™	18.0(0.2)a*	2.0(0.1)a	9.2(0.2)a	9.6(0.5)a	38.9(0.5)a
PowerCore™	13.7(0.1)b	1.8(0.1)ab	8.1(0.2)b	9.3(0.4)a	33.1(0.3)b
BR106™ (not Bt)	11.4(0.1)c	1.65 (0.1)b	7.7(0.2)b	9.8(0.4)a	31.0(0.3)c
Gl	2	2	2	2	2
F	241.3	3.3	9.3	0.4	79.1
P-value	0.0001	0.0425	0.0004	0.6199	0.0001
CV (%)	5.9	21.0	11.7	18.6	5.1

Note. CV = Coefficient of Variation. EE = Standard Error.

* In each column, different letters denote significant differences detected by the Tukey Test $P < 0.05$.

4. Discussion

In various regions, studies carried out by Mendes and Waquil (2009), Bernardi et al. (2016), Gamarra et al. (2022), and Wang et al. (2022) mention differences in susceptibility in the control of *S. frugiperda* due to the differentiated expression of proteins present in the events. In this research, the difference in mortality can also be explained by the type of protein that the hybrids express; Bt corn, event MIR162™, containing the most recently released and planted Vip3A19 protein, was the one that presented total larval mortality; This is also verified in studies conducted by Adamczyk and Mahaffey (2008), Sena et al. (2009), Figueiredo et al. (2013), and Santos-Amaya et al. (2015) who observed similar results in sensitivity studies of *S. frugiperda* to the Vip3A19 protein (MIR162™) versus the Cry1A and Cry1F proteins. In studies carried out by Bernardi et al. (2016) in Brazil, there was complete mortality of *S. frugiperda* neonates in YieldGard VT PRO (Cry1A.105/Cry2Ab2) and PowerCore (Cry1A.105/Cry2Ab2/Cry1F) maize leaf discs; On the contrary, in corn without Bt the mortality was less than 15%. In related of work carried out in Paraguay, Gomez et al. (2017) show that neonate larvae of *S. frugiperda* fed with leaves from different events; VT3PRO™ produced 100% mortality; however, in larvae fed with leaves of 2B587HX™ or Formula TL™, mortality was 58 and 56%, respectively2B587HX™; Considering these results at the local level and those obtained in this research, a loss of sensitivity of the VT3PRO™ event to *S. frugiperda* is verified, the main cause being the inadequate management given to the transgenic events released in Paraguay, and already mentioned by Fatoretto et al. (2017) that at the level of Brazil and the southern cone of America the lack of implementation of shelters and other recommended tactics; as well as the ecological and evolutionary characteristics of *S. frugiperda* are driving the rapid evolution of resistance to Bt corn in Brazil and possibly in the region with similar ecological conditions.

The longest duration in days of the larva and prepupa phase observed in the insects fed with the bt corn events; the lowest survival percentage; the longest duration in days of pupae in males; the lower percentage of emergence in insects fed with the transgenic events, the difference in oviposition and viability verified with lower posture in the females fed with the events in relation to the control variety BR106™ (non-Bt) with a significant reduction in fecundity; verified in this study; as well as the longer duration of the cycle in females, males and the averages of both with statistical differences show an antibiosis effect of the materials with events studied on this pest. The transgenic bt corn events have effects on the biology and development of the studied pest; However, it denotes a dilution of control efficacy compared to the more recently planted events such as MIR162™ that contains the Vip3A19 protein, and a selection pressure for resistant individuals cannot be ruled out. Researchers (Cassio, 2002; Bernardi et al., 2016; Gamarra et al., 2022) mention similar effects in studies carried out. The effect of antibiosis will undoubtedly affect the population dynamics of *S. frugiperda* under field conditions, according to the material or event used in sowing; However, it is not the objective of transgenic materials, which rather seek varietal resistance to this pest.

This work, as well as the data obtained, serves to continue an important line of research with the aim of providing knowledge that contributes to the management of bt crops at a local and regional level; especially in corn cultivation.

5. Conclusion

The MIR162™ event that contains the Vip3A19 protein presents high expression in the leaves and high mortality in *S. frugiperda* larvae fed with leaves from corn material that expresses the protein. The VT3Pro™ (Cry1A.105,

Cry2Ab2, Cry3Bb) and PowerCore™ (Cry1A.105, Cry1F, Cry2Ab2) events present lower toxicity in the expression of the plant leaves. The VT3Pro™ (Cry1A.105, Cry2Ab2, Cry3Bb) and PowerCore™ (Cry1A105, Cry1F, Cry2Ab2) event may affect the fecundity of the surviving insect population.

It is recommended to continue research in order to identify the degree of toxicity of the proteins used and released locally, since there is a large production area with bordering and productive areas such as Argentina and Brazil, taking into account a possible migration of *Spodoptera frugiperda*.

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