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Effect of Fall Armywarm *Spodoptera Frugiperda* (J. E. Smith, 1797) (Lepidoptera: *Noctuidae*) on Transgenic Corn Hybrids and Their Conventional Isogenic Counterparts in Two Locations

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Abstract: Among the insects infecting the maize (*Zea mays* L.) crop, the fall armyworm (*Spodoptera frugiperda* Smith, 1797, *Lepdoptera: Noctuidae*) is considered one of the most important, because it causes the highest damage to yield. In this context, the use of Bt maize plants, resistents to insects atack, is a protection alternative to low down losses produced by pests. This study aimed to evaluate the effect of natural infestation and injuries of larvae of *S. frugiperda* under field conditions, on transgenic and conventional maize hybrids at different phonological stages, in two locations. The trials were established in the 2018 "off season" cultivation of the crop, in Natalio and General Artigas, Itapúa, Paraguay, in a randomized complete block design with eight treatments (hybrids) and four replications. Four transgenic hybrids were evaluated: DK910MG event MON 810 (Cry1Ab); 2B585HX event TC1507 (Cry1F); DK390VT3PRO event MON 89034 x MON 88017 (Cry1A105; Cry2Ab2; Cry3Bb1); event MIR162 (VIP3Aa19) and their respective non-*Bt isogenic*: DK910, 2B587, DK390, MIR162. Evaluated variables were: percentage of plants with damage in the whorl and intensity of the damage, weekly from the seventh days after emergence (DAE) to 49 DAE; number of alive larvae; and, grain yield. Significant differences were observed among the evaluation dates and the hybrids in both locations, in most of the variables. The transgenic hybrids with *Bt* Cry1A105 toxin; Cry2Ab2; Cry3Bb1 and *Bt* VIP3Aa19 toxin stood out for presenting lower percentage and intensity of damage, number of alive larvae and higher grain yield, in both locations. The Cry1Ab and Cry1F toxin hybrids showed similar behaviors to the conventional DK910, 2B587, DK390 and MIR162 hybrids, with the level of control of the larvae of *S. frugiperda* being low in the two tests.

Key words: *Spodoptera frugiperda*, Zea mays, Bacillus thuringiensis, Transgenic crops

1. Introduction

Maize is cultivated in the vast majority of agricultural properties in Paraguay, being exploited both in small properties, with low use of technology and as subsistence, and as much in large properties where high technology is used, with high productivity. The incidence of pests is one of the factors that affect crops, reducing the yield and quality of the product, resulting in a significant economic impact on productivity.

Among the most important pests in corn cultivation, the fall armywarm (*Spodoptera frugiperda*) stands out, which given the favorable climatic conditions can increase its population, destroying the leaves and the whorl, compromising the production of grains. *Spodoptera frugiperda* can cause losses in the production of corn crops between 17 and 38.7%, affecting the development stage of the attacked plants and the cultivar [1].

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To reduce the population of the pest below the economic damage level, the producer normally applies insecticides. The application of broad-spectrum chemicals affects biological agents, making it possible for pests to re-emerge. Therefore, the association of pest control techniques is recommended, mainly in the economic-environmental aspects [2].

With biotechnology events, a new form of pest control was developed consisting of genetically modified insect resistant plants. Through laboratory techniques, a gene from *Bacillus thuringiensis* Berliner was introduced into corn plants, giving rise to genetically modified corn, conferring high resistance patterns of the plant to some species of lepidopteran pests [3].

Genetically modified corn technology became available to US growers in 1996, with corn hybrids expressing the Cry1Ab protein (events MON 810, *Bt*-176, and *Bt*11) to control pests of global and economic importance, such as the European corn borer *Ostrinia nubilalis* (Hubner), the southwestern corn borer *Diatraea grandiosella* Dyar, *Heliothis virescens* (Fabr.) and the corn earworm *Helicoverpa zea* (Boddie) [4].

As reported by the Institute of Agricultural Biotechnology [5] Paraguay approved the first transgenic event in 2004, and by 2018 had 20 biotechnological events approved. Currently in Paraguay there are 21 released genetically modified materials that passed the regulatory process established in the country and are now available to producers. Among these technologies, 15 correspond to the corn species, 3 to soybean and 3 to cotton.

Studies carried out in countries of the region for these hybrids show the effective actions of *Bt* toxins in the control of lepidopteran larvae. Considering the importance of this pest in corn cultivation and the cost that the use of *Bt* technology implies for the producer, and the lack of national research results, it becomes necessary to carry out more detailed studies in the field, covering the behavior of these hybrids in the edaphoclimatic conditions of Paraguay.

2. Objective

The objective of this study was to evaluate the effect of natural infestation and injuries of larvae of *S. frugiperda* under field conditions, on transgenic and conventional maize hybrids at different phonological stages, in two locations.

3. Materials and Methods

3.1 Location and Research Period

Taking into account the maize growing areas and the behavior of the insect, two locations were selected to establish the trials: one in the district of Natalio (26º43'00"S and L 55º05'00"W) and the other in General Artigas (26º56'00"S and 56º13'00"W) during the 2018 "off season" planting of the crop.

The trial in General Artigas was established on a field with cassava cultivation as a predecessor. Soil analysis indicated a pH of 5.5; organic matter content, 1.5%; P, 2 ppm and K, 20 ppm. In Natalio, corn planting was carried out after harvesting of soybean crop planted at normal season. The soil analysis yielded a content of organic matter, 2.6%; P, 7 ppm and K, 40 ppm.

During the execution of the trial, a dry period of 15 days was recorded that coincided with the critical stage (flowering stage) of the crop in both locations. In sum, 1,160 mm were recorded in Natalio and 908 mm in General Artigas.

3.2 Corn Cultivars with Biotechnological Events and Their Respective Conventional Isogens

The genetic material consisted of eight simple corn hybrids, of which four were transgenic plants and four were conventional (treatments in Table 1). The hybrids and their *Bt* toxins were selected according to availability in the local market, and purchased directly from the supplying companies.

Table 1 *Transgenic Bt* maize cultivars and the non-*Bt isogenic* hybrids used in the experiment with *S. frugiperda* (J. E. Smith, 1797) under field conditions.

Hybrids	Type	Event	Brand (Acronym)	*Bt* Toxin
DK910MG	Simple	MON 810	YieldGard® (YG)	Cry 1Ab
MIR162	Simple	MIR 162	Viptera® (VIP)	VIP3Aa19
2B587HX	Simple	TC 1507	Herculex® (HX)	Cry 1F
DK390VT3P	Simple	MON 89034 x MON 88017 MON-89Ø34-3 x MON-88Ø17-3	GenuityVT Triple o VT3Pro®	Cry 1A105 (1Ab, 1Ac, 1F) + Cry2Ab2
DK910	Simple	-	-	-
MIR162	Simple	-	-	-

3.3 Methodological Design

Trials were conducted under a randomized complete block design with eight treatments and four replications. Each plot had six 5.0 m long rows of corn, with a separation of 0.9 m between rows and 0.2 m between plants, totaling 55,555 pl/ha. For the evaluation of whorl natural infestation, the four central rows (useful area of 18.72 m^2) with 104 plants were used; the grain yield evaluation was carried out in the two central rows (useful area 9.36 m^2). The total area of the trial corresponded to 1,305.2 m^2 (50.2 m × 26.0 m) per location. Separation between plots was 1.0 m and between blocks 2.0 m. Four rows of conventional corn were planted as a border on the sides.

3.4 Variables Evaluated

The evaluations were carried out in 20 plants per experimental unit, weekly from seven days after emergence (7 DAE) extending to the beginning of flowering, 49 DAE, commonly recorded in the "off season" development of corn.

Plants with damage to the whorl: all plants with damage in the whorl (with any type of injury and intensity) were counted from the total of 20 plants. It was expressed as percentage.

Damage intensity: it was determined in the same plant sample mentioned for plants with damage in the whorl. The visual damage scale of 0-9 (Table 2) was used, adapted from the methodology described by Davis et al. [6].

Table 2 Scale of notes (0 to 9) for evaluation of damage of *Spodoptera frugiperda* in the corn bud.

Note	Description
0	Plant without damage
1	Plant with scores (more than one score per plant)
2	Plant with scores, 1 to 3 small circular lesions (up to 1.5 cm)
3	Plant with 1 to 5 small circular lesions (up to 1.5 cm); more than 1 to 3 elongated lesions (up to 1.5 cm)
4	Plant with 1 to 5 small circular lesions (up to 1.5 cm); more than 1 to 3 elongated lesions (greater than 1.5 cm and less than 3.0 cm)
5	Plant with 1 to 3 large elongated lesions (greater than 3.0 cm) in 2 or more leaves; more than 1 to 5 holes or lesions elongated up to 1.5 cm
6	Plant with 1 to 3 large elongated lesions (greater than 3.0 cm) on 2 or more leaves; more than 1 to 3 large holes (greater than 1.5 cm) on 2 or more leaves
7	Plant with 3 to 5 large elongated lesions (greater than 3.5 cm) on 2 or more leaves; 3-5 large holes (greater than 1.5 cm) on 2 or more leaves
8	Plant with many elongated lesions (more than 5) of all sizes on most leaves. Many holes of medium and large sizes (more than 5) greater than 3 cm in many leaves
9	Plant with many leaves almost completely destroyed

Number of alive larvae per plant: from five plants located on the edges of each plot, larvae were extracted with a tip forceps. The samples were placed in 70% alcohol; they were classified as small, those that were <10 mm long; in medians, those with a length > 10 < 20 mm; and in large ones those with length > 20 mm.

Yield (kg/ha): to determine the yield, having missing plants in the trials, the population correction was first made within each experimental unit using the adapted formula of LeClerg [7], that is presented below:

$P_{Corrected} = P_{Plot}$ [(52) - (0.3 x M) / (52 - M)], where M = number of missing plants

To calculate the yield per hectare, adjusted to a humidity of 13%, the following formula was used:

Yield in kg/ha = [(Plot weight in g / useful area) × 10 × (100 - Hum) / (87)]

3.5 Trial Management

Conventional soil preparation was carried out, firstly, with a harrow pass and a subsequent disk pass. The sowing was carried out manually, in humid soil, depositing 1 to 2 seeds per hole, to later thin out and leave one plant per hole. Before sowing, the seeds were treated with Imidacloprid insecticides (31%) + Thiodicarb (10%) at a rate of 2 L/100 kg of seeds.

In accordance with the recommendations of the soil analysis, corrections were made to the plots with dolomitic lime at 70% PRNT; 1,150 kg/ha in General Artigas and 900 kg/ha in Natalio. The sowing dates were January 16th and 17th, 2018 in General Artigas and Natalio, respectively.

Likewise, chemical fertilization was performed based on the results of the soil analysis and the nutritional requirement of corn for high yields [8]. So, 230 kg/ha of 04-30-10 as basic and 130 kg/ha of ammonium sulfate as cover, were applied in General Artigas; and, the same fertilizers were used in Natalio at a rate of 200 kg/ha and 130 kg/ha, respectively.

The trials were kept weed-free throughout the cultivation cycle by means of hoeing and without the use of insecticides for the control of the *S. frugiperda* or other pests present. At 150-160 days after planting, verifying grain humidity of 20%, the harvest was carried out.

3.6 Analysis of Data

The data obtained were subjected to analysis of variance tests at 5% probability of error with the statistical software ASSISTAT Version 7.7 beta-2016 [9]. The means of numbers of alive larvae and damages caused by *S. frugiperda* were transformed by the formula √(x+0.5) and subjected to comparison by the Tukey test at 5% probability of error.

4. Results and Discussion

4.1 Percentage of Plants with Damage to the Whorl

Fig. 1 shows the percentage of plants with damage caused by the fall armyworm larvae in the vegetative stage of the conventional and transgenic corn hybrids in the two locations.

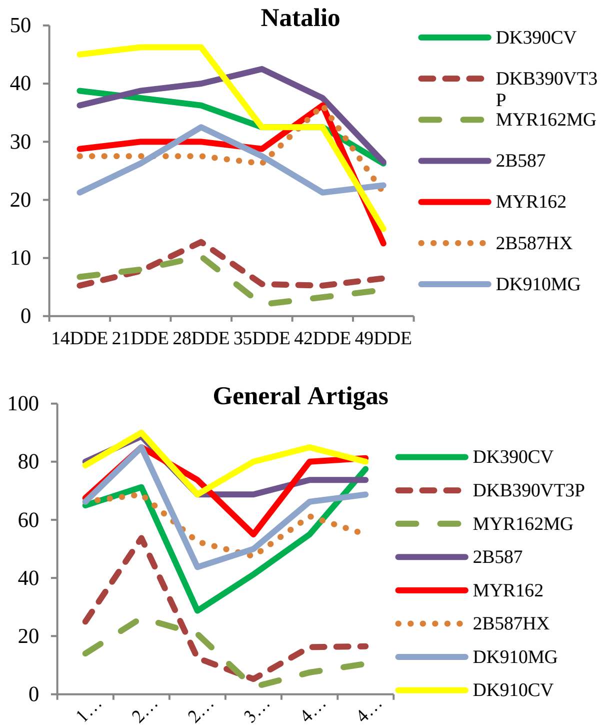

Fig. 1 Percentage of plants with whorl damaged by *S. frugiperda* in six evaluation moments in transgenic maize hybrids and their conventional isogens in the towns of Natalio and General Artigas, in 2018 "off season" planting.

The percentage of plants with damage in the Natalio locality was significant for the evaluation days and highly significant for the hybrids; however, the interaction did not reflect differences, as found in General Artigas, but with highly significant differences for the evaluation days and the hybrids.

In Natalio, a higher percentage of plants attacked at 28DAE was observed, with a subsequent decrease. Conventional materials DK910, 2B587, MYR162, DK390 and *Bt* maize DK910MG (Cry1Ab) and 2B587HX (Cry1F) presented the highest damage values in the whorl without differing between them. While the lowest percentages of damage and intensity of damage were observed in the Natalio locality with the *Bt* MYR162MG and DK390VT3PRO hybrids.

The highest percentage of damage in the whorl was observed at 21DAE in the General Artigas locality with the DK910 material. The *Bt* MYR162MG and DK390VT3PRO hybrids presented the lowest values. The results differ from that reported by Fernandes et al. [2] who found lower values of percentage of damage in the whorl in the transgenic corn *Bt* (Cry1Ab).

4.2 Damage Intensity

In the evaluations carried out at 7DAE, no damage or injury was observed in the plants, being affected from 14DAE to 49DAE in the two locations in 2018 "off season" planting. Table 3 shows the results of the intensity of damage caused by *S. frugiperda* with intensity scale 0 (no damage) to 9 (whorl totally destroyed).

Among the hybrids, the lowest values were found in the *Bt* MYR162MG and DK390VT3PRO maize, the highest intensities in the *Bt* and conventional DK390, 2B587, DK910, MYR162, DK910MG and 2B587HX hybrids, with no significant differences between these cultivars in the locality of Natalio.

Table 3 Intensity of damage caused by *S. frugiperda* in six evaluation moments in transgenic maize hybrids and their conventional isogens in Natalio in 2018 "off season" planting.

Hybrids	Natalio 2018						
	14DAE	21DAE	28DAE	35DAE	42DAE	49DAE	Average
DK390CV	1.3	1.6	2.2	2.1	1.7	1.4	1.7 a
DKB390VT3P	0.2	0.2	0.3	0.2	0.2	0.3	0.2 b
MYR162MG	0.1	0.1	0.2	0.0	0.1	0.1	0.1 b
2B587	1.5	2.0	2.6	2.7	1.9	1.5	2.0 a
MYR162	1.2	1.5	1.9	1.8	1.9	0.6	1.5 a
2B587HX	1.3	1.6	2.0	1.5	1.9	1.4	1.6 a
DK910MG	0.8	1.2	1.6	1.7	1.2	1.1	1.3 a
DK910CV	2.2	2.7	3.2	1.8	1.8	0.8	2.1 a
Hybrids	Natalio 2018						
	14DAE	21DAE	28DAE	35DAE	42DAE	49DAE	Average
Average	1.1 BC	1.4 ABC	1.8 ab	1.5 AB	1.3 ABC	0.9 C	
Days (A)							5.4 **
Hybrids (B)							16.6 **
Interaction A × B							0.6 ns
C.V.(%-a)							20.0
C.V.(%-b)							24.2

(1) Averages followed by lowercase letters in the column indicate statistical differences by Tukey's test between hybrids and different uppercase letters in the lines represent statistical differences between the times of damage assessment.
** Highly significant. * Significant. C.V. coefficient of variation.

However, in the General Artigas locality (Table 4) the intensity of damage was higher in the conventional hybrid DK910, and the lowest intensity of damage was observed in the conventional hybrids MYR162 and *Bt* 2B587HX, hybrids 2B587, DK390 and *Bt* DK910MG.

The trial result differed from the studies by Waquil et al. [1], where the hybrids expressing the Cry1F toxin were the most resistant (immune) and the hybrids expressing the Cry1Ab toxin were moderately resistant.

Nais et al. [4] concluded that the Cry1F toxin was the most effective in protecting the plant, in relation to the other toxic proteins expressed by the other *Bt* hybrids against infestation and the damage caused by this pest, regardless of the planting season.

4.3 Number of Larvae per Plant

Table 5 shows the ANAVA values of the average number of larvae of *S. frugiperda* in conventional and transgenic corn hybrids in the two locations, resulting in highly significant differences on the days of evaluation and hybrids, but without finding an effect of the interaction.

The lowest larvae averages were observed in the *Bt* MYR162MG and DK390VT3PRO hybrids; while the conventional hybrids DK390, DK910, 2B587, MYR162 and the *Bt* maize DK910MG and 2B587HX presented the highest values without differing between them. The highest average number of larvae was found in the Natalio locality at 14 and 28DAE.

In General Artigas at 14DAE the highest average number of larvae was presented in the conventional hybrids DK390, DK910, 2B587, MYR162 and the *Bt* DK910MG and 2B587HX maize, and without differing between them. While the lowest average number of larvae was observed in the *Bt* MYR162MG hybrid, differing from the *Bt* DK390VT3PRO.

4.4 Grain Yield

The yield of conventional transgenic and isogenic maize hybrid grains is shown in Table 6. Highly significant differences were found between the localities of Natalio and General Artigas as well as among the hybrids, and in the effect of the interaction of the two factors.

Higher yields were obtained for all hybrids in Natalio, compared to General Artigas. In Natalio *Bt* DKB390VT3P registered the highest yield, resulting statistically superior to almost all of the other hybrids. Although the hybrids showed low yield in General Artigas, the conventional hybrid MYR162 obtained a yield of 4,332 kg/ha, being similar to DKB390VT3P and MYR162MG.

Table 4 Intensity of damage caused by S. *frugiperda* in six evaluation moments in transgenic maize hybrids and their conventional isogens in General Artigas in 2018 "off season" planting.

Hybrids	General Artigas 2018						
	14DAE	21DAE	28DAE	35DAE	42DAE	49DAE	Average
DK390CV	2.6	2.9	2.1	1.6	2.4	3.9	2.6 bc
DKB390VT3P	0.3	1.2	0.2	0.0	0.2	0.3	0.4 d
MYR162MG	0.2	0.6	0.3	0.0	0.1	0.1	0.2 d
2B587	2.1	3.2	3.5	2.8	3.7	4.5	3.3 ab
MYR162	1.6	2.9	2.1	1.4	2.4	3.2	2.3 c
2B587HX	1.4	2.6	2.6	1.6	2.5	2.7	2.2 c
DK910MG	1.5	3.0	1.6	1.8	3.4	4.0	2.6 bc
DK910CV	2.3	2.6	4.0	3.4	4.8	5.4	2.1 a
Average	1.5 B	2.5 AB	2.0 AB	1.6 B	2.4 AB	3.0 A	
Days (A)							5.2 **
Hybrids (B)							55.1 **
Interaction A x B							1.5 ns
C.V.(%-a)							23.3
C.V.(%-b)							15.9

(1) Averages followed by lowercase letters in the column indicate statistical differences by Tukey's test between hybrids and different uppercase letters in the lines represent statistical differences between the times of damage assessment.
** Highly significant. * Significant. C.V. coefficient of variation.

Table 5 Average number of larvae of *S. frugiperda* in six evaluation moments in transgenic maize hybrids and their conventional isogenic in the localities of Natalio and General Artigas, in 2018 "off season" planting.

Hybrids	Natalio 2018						
	14DAE	21DAE	28DAE	35DAE	42DAE	49DAE	Average
DK390CV	0,75	0,75	0,80	0,65	0,40	0,50	0,64 a
DKB390VT3P	0,35	0,35	0,50	0,20	0,15	0,30	0,30 b
MYR162MG	0,15	0,20	0,35	0,05	0,00	0,00	0,12 b
2B587	0,90	0,75	0,85	0,80	0,60	0,35	0,70 a
MYR162	1,10	0,70	0,75	0,70	0,45	0,25	0,65 a
2B587HX	1,15	0,65	0,75	0,55	0,45	0,40	0,65 a
DK910MG	0,70	0,55	0,60	0,80	0,50	0,45	0,60 a
DK910CV	1,05	1,05	1,05	0,70	0,40	0,25	0,75 a
Average	0,76 A	0,62 AB	0,70 A	0,55 AB	0,36 BC	0,31 C	
Days (A)							8,0 **
Hybrids (B)							12,1 **
Interaction A x B							0,8 ns
C.V.(%-a)							17,0
C.V.(%-b)							14,3
Hybrids	General Artigas 2018						
	14DAE	21DAE	28DAE	35DAE	42DAE	49DAE	Average
DK390CV	1,15	0,75	1,00	0,70	0,65	0,70	0,82 a
DKB390VT3P	0,50	0,55	0,65	0,35	0,30	0,40	0,45 b
MYR162MG	0,40	0,20	0,40	0,15	0,05	0,10	0,21 c
2B587	0,90	1,05	0,80	0,90	0,70	0,65	0,83 a
MYR162	1,35	0,95	0,80	0,75	0,70	0,50	0,84 a
2B587HX	1,25	0,90	0,70	0,65	0,65	0,55	0,78 a
DK910MG	1,05	0,75	0,60	0,85	0,60	0,60	0,74 a
DK910CV	1,20	1,00	0,95	0,80	0,45	0,35	0,79 a
Average	0.97 A	0.76 AB	0,73 AB	0,64 AB	0,51 B	0,48 B	
Days (A)							5,61 **
Hybrids (B)							17,3 **
Interaction A x B							1,0 ns
C.V.(%-a)							18,0
C.V.(%-b)							10,9

(1) Averages followed by lowercase letters in the column indicate statistical differences by Tukey's test between hybrids and different uppercase letters in the lines represent statistical differences between the times of damage assessment.
** Highly significant. * Significant. C.V. coefficient of variation.

The corn hybrids that presented the best yields were *Bt* DKB390VT3P and MYR162MG and the conventional hybrid MYR162 without statistically differing among them.

The results of the trial agree with what was expressed by Moraes [10], who indicates that the majority of conventional hybrids do not differ in grain productivity, at least from one of their transgenic isogenic versions.

Table 6 Yield of grains in transgenic corn hybrids and their conventional isogens in the localities of Natalio and General Artigas, in the 2018 "off season" planting.

Hybrids	Natalio			General Artigas				
	kg/ha			kg/ha			Average	
DK390CV	5.429	abc	A	2.625	cde	B	4.027	b
DKB390VT3P	6.299	a	A	3.706	abc	B	5.002	a
MYR162MG	5.846	abc	A	4.332	ab	B	5.089	a
2B587	5.117	bcd	A	2.200	e	B	3.659	b
MYR162	5.991	ab	A	4.607	a	B	5.299	a
2B587HX	4.221	d	A	2.838	cde	B	3.530	b
DK910MG	4.859	cd	A	3.372	bcd	B	4.115	b
DK910CV	4.921	bcd	A	2.324	de	B	3.623	b
Average	5.335		A	3.250		B		
Days (A)							642.16	**
Hybrids (B)							16.72	**
Interaction A x B							3.85	**
C.V.(%-a)							7,67	
C.V.(%-b)							11.69	

(1) Averages followed by lower case letters in the column indicate statistical differences by Tukey's test among hybrids and different capital letters in the lines represent statistical differences between the localities.
** Highly significant. *Significant. C.V. coefficient of variation.

5. Conclusions

According to the results obtained, under the conditions in which the experiments were carried out, it is concluded that:

- The percentage of plants with damage to the whorl, the intensity of damage and the number of larvae vary depending on the different types of hybrids and phenological stage of the crops.
- The behavior of transgenic maize hybrids and conventional isogenic ones are very different in relation to the attack of the larvae of *S. frugiperda*.
- *Bt* hybrids (VIP3Aa19 toxin) and Cry1A.105 toxins; Cry2Ab; Cry 3Bb1 maintain low infestation levels and damage intensity in both locations, with almost no attack.
- In *Bt* maize hybrids (Cry1Ab and Cry1F) and conventional isogens, high infestation of larvae of *S. frugiperda* is observed in both locations.
- The highest grain productivity is obtained with the transgenic *Bt* hybrids (VIP3Aa19 toxin) and (Cry1A.105 toxin; Cry2Ab; Cry 3Bb1).

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