

# Resistance of conventional and isogenic transgenic maize hybrids to *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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## Abstract

This study evaluated the effect of damage caused by *S. frugiperda* on yield of maize hybrids and their conventional and isogenic transgenic versions, with different *Bt* toxins, in field conditions. Experiments were conducted in the municipalities of Campinas and Mococa, São Paulo State, Brazil, in the growing seasons of 2010/2011 and 2011/2012, in a randomized complete block design, with 12 treatments and four replications. The variables evaluated were: grain productivity, one hundred grain weight and grain yield. For the assessment of damage caused by *S. frugiperda* was verified the intensity of leaf injuries through visual scale of notes, with variation of 0 and 9, from 15 to 60 days after sowing. Lower scores of damage caused by *S. frugiperda* were found in transgenic hybrids. Most conventional hybrids do not differ in grain productivity from at least one of isogenic transgenic versions. The same maize hybrid with different *Bt* toxins may have different productive behavior in field conditions. Different *Bt* toxins respond differently to damage caused by *S. frugiperda*.

**Key words:** *Zea mays* L., fall armyworm, genetically modified maize, yield.

## 1. INTRODUCTION

*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) stands out among the main pests of maize, whether by damage caused to crops, frequency of occurrence or difficult control with traditional methods (Mendes & Waquil, 2009). This pest feeds on the maize plant during all growth stages, but prefers young plants (Gallo et al., 2002), and may cause reductions in maize yield in the order of 34-40% (Fernandes et al., 2003).

The main strategy for the control of *S. frugiperda* has been the use of maize hybrids expressing *Bt* insecticidal protein (Céleres, 2013). In this context, *Bt* maize is characterized by the insertion of one or more genes of the bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) that induces the production of one or more insecticidal proteins toxic to certain species of lepidopteran pests. Thus, in the ear, *Bt* maize can reduce insect attack up to 90%, reducing

therefore the probability of fungal growth through the holes caused by insect pests (CIB, 2012).

According to the annual report of the International Service for the Acquisition of Agri-Biothec Applications (ISAAA), Brazil is the second largest producer of GM crops in the world, ahead of Argentina and behind the US (with 70.2 million). In Brazil, 90% of the planted maize area already uses some transgenic event (ISAAA, 2013).

In the 2013/2014 crop, it was found that the transgenic cultivars for the control of caterpillars, currently on the market, are the result of six transgenic events: TC 1507 event (*Bt* Cry1F toxin), Herculex I<sup>®</sup>; MON 810 event (*Bt* Cry1AB toxin), YieldGard<sup>®</sup>; Bt11 event (*Bt* Cry1AB toxin), Agrisure TL<sup>®</sup>; MIR162 event (*Bt* VIP3Aa20 toxin), TL VIP<sup>®</sup> and two transgenic events that confer resistance to glyphosate herbicide applied postemergence: NK603, Roundup Ready<sup>®</sup>, and the GA 21 -TG (EMBRAPA, 2014).

According to Mendes & Waquil (2009), the farmer can possibly find, under field conditions, different responses in the control of fall armyworm with the use of different *Bt* events. Within the same group of insects, the activity of each *Bt* toxin is different. Toxins Cry1A(b) and Cry1F have activity on lepidopteran maize pests and have high specificity for this group, although toxicological studies reveal significant differences in toxicity for each species.

Mendes et al. (2011) evaluated biological parameters of fall armyworm fed *Bt* maize hybrids expressing Cry1A(b) toxin, and their respective non-*Bt* isogenic, observed an interaction between the toxin *Bt* Cry1A(b) and the genetic basis of transgenic hybrids as to the survival and larval biomass of *S. frugiperda*. There are little information regarding the use of *Bt* technology and different events with other toxins in Brazilian's conditions; there is a need for further field studies to evaluate the efficacy of *Bt* technology compared to non-*Bt* commercial hybrids (Omoto et al., 2012).

Thus, considering the importance of *S. frugiperda* as maize pest and the lack of studies on this insect in maize containing different *Bt* toxins in Brazil, this study aimed to evaluate the effect of natural infestation of *S. frugiperda* on productivity of conventional maize hybrids and their isogenic transgenic versions with different *Bt* events in two environments under field conditions.

## 2. MATERIAL AND METHOD

Two experiments were conducted in the São Paulo State, Brazil: at the Agronomic Institute (IAC) in Campinas (22°53'20"S latitude 47°5'34"W longitude, 600 m altitude; and soil classified as moderate Oxisol, clayey, according to the Brazilian System of Soil Classification) (EMBRAPA, 2006), and at the Polo Regional Nordeste Paulista - APTA in Mococa (21° 28'S latitude 47° 01'W longitude, 665 m altitude; and soil classified as eutrophic Oxisol of medium texture) (EMBRAPA, 2006), in the 2010/2011 and 2011/2012 summer crops.

Soil was prepared with one plowing and two diskings. The experimental design was a randomized complete block design with four replications. Each plot was 63 m<sup>2</sup> area, consisting of eight rows of ten meters long, spaced 0.9 m apart. The useful area was only four central rows of hybrids, excluding 1.5 m from each end of the rows.

The treatments were twelve commercial maize hybrids, five in conventional version [P30F35, DKB390, DAS2B710, Maximus and Impacto] and seven in isogenic transgenic version [P30F35 YG (*Bt* Cry1AB toxin), P30F35 HX (*Bt* Cry1F toxin), DKB390 YG (*Bt* Cry1AB toxin), DKB390 PRO (*Bt* Cry1A105 (1AB, 1AC, 1F) + Cry2Ab2 toxin), DAS2B710 HX (*Bt* Cry1F toxin), Maximus Viptera (*Bt* VIP3Aa20 toxin) and Impacto Viptera (*Bt* VIP3Aa20 toxin)].

After soil analysis, liming and fertilization for maize were performed according to van Raij et al. (1997). In Campinas, we used 450 kg ha<sup>-1</sup> of 4-14-8 (NPK) at planting, and 200 kg ha<sup>-1</sup> of ammonium sulfate as topdressing; in Mococa, 300 kg ha<sup>-1</sup> of 20-04-18 (NPK) at planting and 200 kg ha<sup>-1</sup> of ammonium sulfate as topdressing, 30 days after emergence. Weed control was performed when needed, using tembotrione (240 ml h<sup>-1</sup>) together with the adjuvant mixture of soybean oil methyl ester (1.0 l h<sup>-1</sup>) and atrazine (1,000 g i.a. ha<sup>-1</sup>).

Damage caused by *S. frugiperda* was evaluated every two weeks, from 15 days after sowing (DAS), until the beginning of the pre-flowering of maize plants (around 60 DAS). Visual estimates were made at random, ten plants per plot, through visual rating scale, ranging from 0 and 9, adapted from Wiseman et al. (1966), where: 0 = No visible damage; 1 = Small holes in a few leaves; 2 = Minor damage in the form of holes in a few leaves; 3 = Damage as holes on multiple leaves; 4 = Damage as holes on multiple leaves and injuries in a few leaves; 5 = Injuries on multiple leaves; 6 = Large injuries on multiple leaves; 7 = Large injuries on multiple leaves and portions eaten (torn) in a few leaves; 8 = Large injuries and large portions eaten (torn) on multiple leaves; 9 = Large injuries and large portions eaten (torn) in most of leaves. Three independent raters assigned scores of damage in each period to obtain final mean scores without any trend.

Around 150-160 DAS, we collected all the ears of the two central rows of each plot, when hybrids showed moisture around 18%. Subsequently, we evaluated grain productivity, one hundred grain weight and grain yield. For grain productivity, we obtained the weight in kg ha<sup>-1</sup> of grain from threshing all ears of the two central rows of each plot, with the values obtained later corrected to 13% on a wet basis. One hundred grain weight, in grams (g), was obtained by counting and weighing one hundred grains at random from each plot. Grain yield in percentage (%) was obtained by calculating the total grain weight after threshing each plot on the total mass of ears without the straw harvested in these plots.

Individual and joint analysis of variance was performed with the aid of the Statistical Software Genes (Cruz, 2001), in all locations measured, considering the fixed model and the means grouped by Tukey's test at 5% probability.

We also estimated the linear correlation coefficient (*r*) between grain productivity, one hundred grain weight and grain yield, and the scores of damage caused by *S. frugiperda* in four evaluation periods (15, 30, 45 and 60 days) in the twelve maize hybrids in the two growing seasons.

Orthogonal contrasts were performed between the means of the variables via the t-test values for grain productivity (GP), one hundred grain weight (100GW) and grain yield (GY), between conventional hybrids (P30F35, DKB390, DAS2B710, Maximus and Impacto) and their isogenic

transgenic (P30F35 YG, P30F35 HX, DKB390 YG, DKB390 PRO, DAS2B710 HX, Maximus Viptera and Impacto Viptera), of the 2010/2011 and 2011/2012 crops. For P30F35 and DKB390 hybrids, the first contrast tested refers to the comparison of the conventional hybrid with its two isogenic transgenic versions with different *Bt* events. Thus, the contrast of interest is  $Y_1 = 2m_1 - m_2 - m_3$ . The second contrast for these hybrids refers to the comparison between the two different *Bt* transgenic events, regardless of the conventional hybrid. Thus, the contrast of interest is  $Y_2 = m_2 - m_3$ . For DAS2B710, Maximus and Impacto, the only contrast tested refers to the comparison of the conventional hybrid with its isogenic transgenic. In this case, the contrast of interest is  $Y_1 = m_1 - m_2$ .

### 3. RESULTS AND DISCUSSION

The joint analysis showed interaction genotype  $\times$  location  $\times$  agricultural years, indicating that the results should be presented per crop and location.

### Crop 2010/2011

Damage caused by *S. frugiperda* was more severe in conventional maize hybrids than in their transgenic versions in both locations (Table 1). High scores of leaf damage were found for conventional hybrids P30F35, DKB390 and DAS2B710, both in Campinas as in Mococa at 60 DAS. These values were represented by plants (non-*Bt* hybrids) with large lesions and holes on multiple leaves, while the *Bt* hybrids showed few scraped leaves and small holes in a few leaves.

Mendes et al. (2008) analyzed the incidence and damage caused by fall armyworm in *Bt* and non-*Bt* maize in experimental plots under artificial infestation, and found significant differences between *Bt* and non-*Bt* versions of infested plants. The authors observed in hybrids with *Bt* versions, predominance of plants with scores of zero, while in non-*Bt* versions, prevailed scores above three and four in scales ranging from zero to five, in which zero indicates no damage and five indicates plants with many leaves and totally destroyed young leaves.

**Table 1.** Mean scores of damage caused by *Spodoptera frugiperda*, in four evaluation periods in conventional maize hybrids and their isogenic transgenic versions, in the 2010/2011 summer crop, in the municipalities of Campinas and Mococa

| Hybrid          | Campinas 2010/2011 <sup>(1)</sup>      |          |         |          |
|-----------------|--|----------|---------|----------|
|                 | Days after sowing (DAS) <sup>(1)</sup> |          |         |          |
|                 | 15                                     | 30       | 45      | 60       |
| P30F35          | 1.3 aC                                 | 3.9 bB   | 6.5 aA  | 6.6 aA   |
| P30F35 YG       | 0.5 bD                                 | 1.1 cC   | 2.0 cB  | 2.7 cdA  |
| P30F35 HX       | 0.4 bC                                 | 0.8 cC   | 1.3 dB  | 1.9 eA   |
| DKB390          | 1.4 aC                                 | 4.4 bB   | 6.5 aA  | 6.6 aA   |
| DKB390 YG       | 0.4 bD                                 | 1.2 cC   | 2.1 cB  | 3.1 cA   |
| DKB390 PRO      | 0.4 bC                                 | 0.6 cC   | 1.3 dB  | 2.8 cdA  |
| DAS2B710        | 1.7 aD                                 | 5.4 aC   | 6.2 aB  | 6.8 aA   |
| DAS2B710 HX     | 0.3 bB                                 | 0.7 cB   | 1.7 cdA | 2.1 deA  |
| Maximus         | 1.2 aC                                 | 4.5 bB   | 5.3 bA  | 5.7 bA   |
| Maximus Viptera | 0.3 bB                                 | 0.6 cB   | 1.3 dA  | 1.5 eA   |
| Mean            | 0.8 D                                  | 2.3 C    | 3.4 B   | 4.0 A    |
| Hybrid          | Mococa 2010/2011 <sup>(1)</sup>        |          |         |          |
| P30F35          | 2.4 aD                                 | 3.3 abC  | 4.3 aB  | 6.5 aA   |
| P30F35 YG       | 0.5 bC                                 | 0.8 dBC  | 1.4 cB  | 2.0 defA |
| P30F35 HX       | 0.6 bB                                 | 1.0 dB   | 1.0 cB  | 2.6 cdA  |
| DKB390          | 2.4 aD                                 | 3.9 aC   | 4.6 aB  | 6.6 aA   |
| DKB390 YG       | 0.9 bB                                 | 1.2 dB   | 1.2 cB  | 1.9 efA  |
| DKB390 PRO      | 0.4 bC                                 | 0.7 dC   | 1.4 cB  | 2.9 cA   |
| DAS2B710        | 2.3 aD                                 | 3.1 bC   | 4.2 abB | 6.8 aA   |
| DAS2B710 HX     | 0.8 bC                                 | 1.2 d BC | 1.5 cB  | 2.4 cdeA |
| Maximus         | 1.9 aC                                 | 2.4 cC   | 3.6 bB  | 5.2 bA   |
| Maximus Viptera | 0.4 bC                                 | 0.7 dBC  | 1.1 cB  | 1.7 fA   |
| Mean            | 1.3 D                                  | 1.8 C    | 2.4 B   | 3.9 A    |

<sup>(1)</sup> Different lowercase letters in the columns indicate statistical differences ( $p < 0.01$ ) by Tukey's test between hybrids and different capital letters in the rows indicate statistical differences ( $p < 0.01$ ) between the evaluation periods.

Among the transgenic hybrids, the lowest score of damage was found in the hybrids containing the Viptera<sup>®</sup> (*Bt* VIP3Aa20 toxin) and Herculex<sup>®</sup> (*Bt* Cry1F toxin) technology, in Campinas, and Viptera<sup>®</sup> (*Bt* VIP3Aa20 toxin) and YieldGard<sup>®</sup> (*Bt* Cry1ab toxin) technology in Mococa (Table 1).

Michelotto et al. (2011) evaluated the damage caused by the fall armyworm on commercial maize conventional and GM hybrids with different technologies to control lepidopteran pests, using visual scale of damage ranging from 0 and 9, observed that transgenic hybrids were less attacked by such worm and also that these hybrids were different with respect to the attack by this caterpillar. They also reported that the hybrids containing the Herculex<sup>®</sup> technology showed the lowest scores, therefore, lower attack compared to the scores reached by hybrids containing the Agrisure TL<sup>®</sup> and YieldGard<sup>®</sup> technologies.

In Campinas, in the 2010/2011 crop, conventional hybrids, with the exception of DAS2B710, showed no difference between 45 and 60 DAS in assessing damage caused by *S. frugiperda* (Table 1). As for GM, differences were detected between 15 and 30 DAS only in hybrids with YieldGard<sup>®</sup> technology. For Herculex<sup>®</sup> and Viptera<sup>®</sup> technologies, there were no differences between 45 and 60 DAS, demonstrating that the caterpillars feed on these hybrids during the crop cycle, but that this feeding does not increase, becoming stable since the beginning of cultivation of transgenic versions and not causing serious damage to plants.

According to Soberón et al. (2009), *Bt* toxin is continuously expressed in plant tissue, which explains the efficacy of this control technology throughout the cycle of the plant. The low scores obtained in this study with transgenic treatment agree with the literature (Fernandes et al., 2003; Waquil et al., 2002).

In general, both in Campinas and Mococa, we verified a significant increase in the scores of damage over time, but more pronounced in conventional hybrids than in their transgenic versions (Table 1).

Among conventional hybrids, Maximus stood out positively, with the lowest score of damage from the 45 DAS, differing from the other conventional hybrids in both locations (Table 1). This may be indicative of resistance, through preference of the caterpillar for feeding other type of resource, that is, this genotype shows signs of being less consumed by insects than other conventional maize genotypes. On the other hand, this hybrid could be carrying some factor that would confer antibiosis, killing the caterpillars earlier, thus preventing further damage. According to Panda & Khush (1995), in some cases it is very difficult to separate the non-preference for feeding from antibiosis by the fact that in case of pronounced effect of chemical compounds, both types of resistance will promote effects on the insect biology.

For grain productivity in the 2010/2011 crop, the conventional hybrid Maximus, its isogenic transgenic Maximus Viptera and the transgenic hybrid P30F35HX had the highest grain productivity, differing only from conventional hybrids P30F35, DKB390 and DAS2B710 (Table 2). These data reinforce the indicative of resistance of Maximus to fall armyworm, since even suffering damage from the pest, this hybrid showed a grain productivity similar to its isogenic transgenic but not different from the other transgenic hybrids, that is, even not having a *Bt* event, this hybrid did not lose productivity as the other conventional hybrids.

The conventional hybrids P30F35, DAS2B710 and Maximus did not differ from at least one of their isogenic transgenic versions in terms of grain productivity (Table 2). This demonstrates that not always a transgenic hybrid is advantageous over a conventional hybrid, in other words, under infestation of *S. frugiperda* some *Bt* hybrids could not cancel the pest effect so that the hybrid could express its full productive potential.

It is noteworthy that this *Bt* technology protects the productivity, that is, its use does not increase productivity, but is intended to protect the plant from pests so that they can express their maximum productive potential (Michelotto et al., 2011, 2013). Thus, the greater the attack of caterpillars, the greater their response compared to conventional hybrids and, in the absence of the pest, the transgenic hybrids will yield the same than their conventional hybrids.

The transgenic hybrid Maximus Viptera had the highest mean one hundred grain weight, only differing from the conventional hybrid P30F35 (Table 2). As to the grain yield, there were no significant differences ( $p < 0.05$ ) between the hybrids (Table 2), ranging from 80.5% for P30F35HX to 71.0% for DKB390 PRO.

There were significant linear correlations between grain productivity and one hundred grain weight, grain yield and scores of damage caused by *S. frugiperda* at 15, 30, 45 and 60 days (Table 3). From these correlations, it is observed that increased productivity is associated with the increase in relationships of one hundred grain weight and grain yield, and its reduction is related to the increase of the scores for damages caused by *S. frugiperda* in all periods (Table 3). All scores for damages caused by *S. frugiperda* showed highly significant correlation, showing consistency between assessments, i.e., the data show that the damage caused by *S. frugiperda* in one evaluation period influences the next period (Table 3).

The orthogonal contrast of the hybrid P30F35 shows that the conventional hybrid differs from its two isogenic transgenic versions for grain productivity and weight of one hundred grains, but not in grain yield (Table 4). When contrasted only the two hybrid transgenic events P30F35 (YG P30F35 P30F35 x HX), it was found that there were differences in grain productivity between the different

**Table 2.** Mean values of grain productivity, one hundred grain weight and grain yield in ten maize hybrids, in Campinas and Mococa, in the 2010/2011 summer crop

| Hybrid          | Grain productivity <sup>(1)</sup> | One hundred grain weight <sup>(1)</sup> | Grain yield <sup>(1)</sup> |
|-----------------|-----------------------------------|---|----------------------------|
|                 | kg ha <sup>-1</sup>               | g                                       | %                          |
| P30F35          | 7,105 cd                          | 34.4 b                                  | 76.6 a                     |
| P30F35 YG       | 7,931 abc                         | 38.1 ab                                 | 76.0 a                     |
| P30F35 HX       | 8,540 a                           | 38.1 ab                                 | 80.5 a                     |
| DKB390          | 6,810 d                           | 38.1 ab                                 | 71.6 a                     |
| DKB390 YG       | 8,001 abc                         | 38.8 ab                                 | 72.9 a                     |
| DKB390 PRO      | 8,217 ab                          | 37.5 ab                                 | 71.0 a                     |
| DAS2B710        | 7,336 bcd                         | 35.6 ab                                 | 72.9 a                     |
| DAS2B710 HX     | 7,895 abc                         | 38.1 ab                                 | 73.6 a                     |
| Maximus         | 8,716 a                           | 40.0 ab                                 | 79.6 a                     |
| Maximus Viptera | 8,888 a                           | 41.3 a                                  | 77.7 a                     |
| Mean            | 7,944                             | 38.0                                    | 75.3                       |
| C.V. (%)        | 8.0                               | 9.8                                     | 11.0                       |

<sup>(1)</sup> Different lowercase letters in the columns indicate statistical differences ( $p < 0.01$ ) by Tukey's test between hybrids.

**Table 3.** Linear correlation (r) between grain productivity (GP), one hundred grain weight (100GW) and grain yield (GY), and scores of damage caused by *Spodoptera frugiperda*, in four evaluation periods (days after sowing), in ten maize hybrids, in Campinas and Mococa in the 2010/2011 summer crop

|                    | GP      | 100GW  | GY    | Score of damage |        |        |    |
|--------------------|---------|--------|-------|-----------------|--------|--------|----|
|                    |         |        |       | 15              | 30     | 45     | 60 |
| GP                 | -       |        |       |                 |        |        |    |
| 100GW              | 0.68*   | -      |       |                 |        |        |    |
| GY                 | 0.73*   | 0.34   | -     |                 |        |        |    |
| Score of Damage 15 | -0.84** | -0.62  | -0.48 | -               |        |        |    |
| Score of Damage 30 | -0.91** | -0.65* | -0.58 | 0.96**          | -      |        |    |
| Score of Damage 45 | -0.85** | -0.60  | -0.55 | 0.99**          | 0.97** | -      |    |
| Score of Damage 60 | -0.83** | -0.64* | -0.53 | 0.98**          | 0.97** | 0.99** | -  |

\*, \*\* Significant by t-test at 5% and 1%, respectively.

**Table 4.** T-test values of orthogonal contrasts between the means of the variables: grain productivity (GP), one hundred grain weight (100GW) and grain yield (GY) of conventional maize hybrids and their isogenic transgenic versions in the 2010/2011 crop

| Hybrid   | Orthogonal contrasts     | Variables           |                     |                     |
|----------|--------------------------|---------------------|---------------------|---------------------|
|          |                          | GP                  | 100GW               | GY                  |
| P30F35   | $Y_1 = 2m_1 - m_2 - m_3$ | -7.53**             | -2.26*              | -0.56 <sup>ns</sup> |
|          | $Y_2 = m_2 - m_3$        | -4.76**             | -1.96 <sup>ns</sup> | 0.17 <sup>ns</sup>  |
| DKB390   | $Y_1 = 2m_1 - m_2 - m_3$ | -2.10*              | 0.18 <sup>ns</sup>  | -0.10 <sup>ns</sup> |
|          | $Y_2 = m_2 - m_3$        | -2.36*              | -0.32 <sup>ns</sup> | -0.33 <sup>ns</sup> |
| DAS2B710 | $Y_1 = m_1 - m_2$        | -2.05 <sup>ns</sup> | -1.31 <sup>ns</sup> | -1.89 <sup>ns</sup> |
| Maximus  | $Y_1 = m_1 - m_2$        | -0.40 <sup>ns</sup> | -0.60 <sup>ns</sup> | -0.40 <sup>ns</sup> |

\*\*, \* Significant at 1% and 5% probability; <sup>ns</sup>Non-significant; For the contrasts:  $m_1$  = mean of the conventional hybrid;  $m_2$  = mean of the transgenic hybrid;  $m_3$  = mean of the second transgenic hybrid (if any).

events of the same hybrid, but there were no differences in the weight of one hundred grains and the grain yield of these genotypes (Table 4).

For the hybrid DKB390, in the contrast between the conventional hybrid and its isogenic transgenic versions there were differences for grain productivity, with at least one transgenic version showing productivity greater than the conventional hybrid (Table 4). Regarding the weight of one hundred grains and grain yield, there is no difference by t-test between the conventional hybrid and its isogenic transgenic. When comparing only the two transgenic events of the hybrid DKB390 (DKB390 YG × DKB390 PRO), differences were registered between the different events for grain productivity, but not for one hundred grain weight and grain yield (Table 4).

The contrast between the conventional version and the transgenic version of the hybrid DAS2B710 and the hybrid Maximus showed no difference in grain productivity, the weight of 100 grains and grain yield of these genotypes, indicating that these hybrids really present an isogenic transgenic of its conventional hybrid (Table 4), since the characteristics of conventional and transgenic hybrids are the same, only added with the *Bt* event in the transgenic hybrid.

## Crop 2011/2012

In the 2011/2012 crop, as occurred in the 2010/2011 crop, the higher scores of leaf damage caused by *S. frugiperda* were observed in conventional hybrids DKB390 and DAS2B710, in Campinas, in the fourth evaluation (60 DAS) (Table 5). In Mococa, the highest scores were also assigned in the fourth evaluation in conventional hybrids P30F35, DKB390 and DAS2B710, and the lowest, in transgenic hybrids.

The conventional hybrid Impacto differed from the other conventional hybrids in the damage caused by the fall armyworm, in Campinas, from the second evaluation (45 DAS), and in Mococa 2011/2012, from the third evaluation (Table 5). There were infestations by *S. frugiperda* throughout the cycle, in all treatments (Tables 1, 5). Thus, it is expected some *scraping* damage in the leaves of transgenic maize, since, to be controlled, the insect has to ingest the Cry1Ab toxin, during the grazing (Waquil et al., 2002).

Mendes et al. (2008) reported that in *Bt* plants, even where there is initial survival of fall armyworm, the damage does not evolve to significant damage, when it exceeds three in the scale ranging from zero to five.

It is observed a difference between the damage caused by fall armyworm between different *Bt* events, at 60 DAS, in the two locations, in the 2011/2012 crop (Table 5). This data shows that the *Bt* events differently influence the damages caused by *S. frugiperda*. According to Mendes and Waquil (2009), the farmer can find, under field conditions, different responses in the control of fall armyworm with the

use of different events. In the same group of insects, the activity of each toxin is different. Cry 1A(b) and Cry 1F toxins have activity on lepidopteran maize pests and have high specificity for this group, although toxicological studies reveal significant differences in toxicity for each species.

Considering the two crops, the productivity of hybrids was higher in the 2011/2012 crop (9086 kg ha<sup>-1</sup>) than in 2010/2011 (7944 kg ha<sup>-1</sup>) (Tables 2, 6). The Viptera<sup>®</sup> technology (*Bt* VIP3Aa20 toxin) resulted in a high productivity also in the second crop; transgenic and conventional versions also presented similar grain productivities (Table 6). The hybrid Impacto Viptera showed higher grain productivity, differing from transgenic hybrids P30F35 YG, DKB390 YG and DAS2B710 HX, and from the conventional hybrids, P30F35, DKB390 and DAS2B710 (Table 6).

Moreover, studies in the US indicated that, when comparing the production of similar cultivars, transgenic and conventional, it is observed that when controlling for other factors, in conditions where there is no pressure of pests on crop development, the productivity of the conventional cultivation is equal to or slightly higher than in a transgenic

crop (Nill, 2003). This could be seen among the hybrids containing the Viptera<sup>®</sup> technology, in the two crops, where there was no difference in terms of productivity in these conditions of infestation by *S. frugiperda* (Tables 2, 6).

There was no significant difference between hybrids for weight of one hundred grains in this growing season. As to grain yield, the hybrids Impacto Viptera, Impacto, DKB390 PRO, DKB390, P30F35 HX and P30F35 were the genotypes that showed higher yield, differing from the hybrids P30F35 YG, DKB390 YG, DAS2B710 and DAS2B710 HX (Table 6).

Linear correlations were significant between productivity and other traits, except for weight of one hundred grains, in the 2011/2012 crop (Table 7). The increase in productivity is associated with the increase in the grain yield and its reduction is related to the increase in scores of damage caused by *S. frugiperda* in all periods of evaluation (Table 7). Scores for damage caused by *S. frugiperda* showed again high significant correlation, showing that the damage caused by the caterpillar during an evaluation period is reflected in the increased damage in the next period (Table 7).

In the orthogonal contrast of the hybrid P30F35, the conventional hybrid differed from its two isogenic transgenic versions (P30F35 YG and P30F35 HX) for grain productivity, but not for one hundred grain weight and grain yield (Table 8). When contrasted only the two transgenic events of the hybrid P30F35 (P30F35 YG × P30F35 HX), there were differences in grain productivity between the *Bt* events of the same hybrid, but there were no differences in the weight of one hundred grains and the yield of grains of these genotypes (Table 8).

**Table 5.** Mean scores of damage caused by *Spodoptera frugiperda*, in four evaluation periods in conventional maize hybrids and their isogenic transgenic versions, in the 2011/2012 summer crop, in the municipalities of Campinas and Mococa

| Hybrid          | Campinas 2011/2012 <sup>(1)</sup>      |         |          |         |
|-----------------|--|---------|----------|---------|
|                 | Days after sowing (DAS) <sup>(1)</sup> |         |          |         |
|                 | 15                                     | 30      | 45       | 60      |
| P30F35          | 2.2 aD                                 | 3.4 aC  | 4.1 aB   | 4.8 bcA |
| P30F35 YG       | 0.3 bC                                 | 1.4 cdB | 2.5 cdA  | 2.5 eA  |
| P30F35 HX       | 0.2 bC                                 | 1.3 dB  | 1.6 fB   | 2.6 eA  |
| DKB390          | 2.1 aD                                 | 3.5 aC  | 4.6 aB   | 5.4 abA |
| DKB390 YG       | 0.2 bC                                 | 1.6 cdB | 2.4 cdeA | 2.6 eA  |
| DKB390 PRO      | 0.1 bC                                 | 1.7 cdB | 2.2 deA  | 2.4 eA  |
| DAS2B710        | 2.0 aD                                 | 3.4 aC  | 4.3 aB   | 5.7 aA  |
| DAS2B710 HX     | 0.2 bC                                 | 2.0 bcB | 2.9 bcA  | 3.3 dA  |
| Impacto         | 1.8 aD                                 | 2.4 bC  | 3.4 bB   | 4.5 cA  |
| Impacto Viptera | 0.1 bC                                 | 1.5 cdB | 1.8 efB  | 2.3 eA  |
| Mean            | 0.9 D                                  | 2.2 C   | 3.0 B    | 3.6 A   |
| Hybrid          | Mococa 2011/2012 <sup>(1)</sup>        |         |          |         |
| P30F35          | 2.1 abcD                               | 3.3 abC | 4.6 aB   | 5.4 aA  |
| P30F35 YG       | 0.8 efC                                | 1.7 deB | 2.4 cdA  | 2.7 cdA |
| P30F35 HX       | 1.3 defB                               | 1.7 deB | 2.5 cdA  | 2.8 cdA |
| DKB390          | 2.4 abD                                | 3.6 aC  | 4.5 aB   | 5.3 aA  |
| DKB390 YG       | 1.4 cdeB                               | 2.2 cdA | 2.3 cdA  | 2.5 dA  |
| DKB390 PRO      | 0.6 fB                                 | 2.2 cdA | 2.5 cdA  | 2.7 cdA |
| DAS2B710        | 2.6 aC                                 | 3.3 abB | 4.8 aA   | 5.3 aA  |
| DAS2B710 HX     | 1.0 efC                                | 2.6 bcB | 3.0 cAB  | 3.3 cA  |
| Impacto         | 1.9 bcdC                               | 3.1 abB | 3.8 bA   | 4.3 bA  |
| Impacto Viptera | 1.0 efB                                | 1.3 eB  | 2.1 dA   | 2.2 dA  |
| Mean            | 1.5 D                                  | 2.5 C   | 3.2 B    | 3.6 A   |

<sup>(1)</sup> Different lowercase letters in the columns indicate statistical differences ( $p < 0.01$ ) by Tukey's test between hybrids and different capital letters in the rows indicate statistical differences ( $p < 0.01$ ) between the evaluation periods.

**Table 6.** Mean values of grain productivity, one hundred grain weight and grain yield in ten maize hybrids, in Campinas and Mococa, in the 2011/2012 summer crop

| Hybrid          | Grain productivity <sup>(1)</sup> | One hundred grain weight <sup>(1)</sup> | Grain yield <sup>(1)</sup> |
|-----------------|-----------------------------------|---|----------------------------|
|                 | kg ha <sup>-1</sup>               | g                                       | %                          |
| P30F35          | 8,587 cd                          | 37.5 a                                  | 82.3 a                     |
| P30F35 YG       | 8,939 bcd                         | 38.4 a                                  | 78.4 b                     |
| P30F35 HX       | 9,499 ab                          | 40.6 a                                  | 84.5 a                     |
| DKB390          | 8,336 d                           | 37.6 a                                  | 82.0 a                     |
| DKB390 YG       | 8,932 bcd                         | 37.1 a                                  | 78.9 b                     |
| DKB390 PRO      | 9,545 ab                          | 39.5 a                                  | 81.7 a                     |
| DAS2B710        | 8,556 cd                          | 38.9 a                                  | 78.2 b                     |
| DAS2B710 HX     | 9,015 bc                          | 35.6 a                                  | 78.9 b                     |
| Impacto         | 9,453 ab                          | 38.8 a                                  | 81.9 a                     |
| Impacto Viptera | 10,000 a                          | 41.3 a                                  | 87.5 a                     |
| Mean            | 9,086                             | 38.5                                    | 81.5                       |
| C.V. (%)        | 4.4                               | 8.2                                     | 6.1                        |

<sup>(1)</sup> Different lowercase letters in the columns indicate statistical differences ( $p < 0.01$ ) by Tukey's test between hybrids.

**Table 7.** Linear correlation (r) between grain productivity (GP), one hundred grain weight (100GW) and grain yield (GY), and scores of damage caused by *Spodoptera frugiperda*, in four evaluation periods (days after sowing), in ten maize hybrids, in Campinas and Mococa in the 2011/2012 summer crop

|                    | GP     | 100GW | GY    | Score of damage |        |        |    |
|--------------------|--------|-------|-------|-----------------|--------|--------|----|
|                    |        |       |       | 15              | 30     | 45     | 60 |
| GP                 | -      |       |       |                 |        |        |    |
| 100GW              | 0.59   | -     |       |                 |        |        |    |
| GY                 | 0.63*  | 0.55  | -     |                 |        |        |    |
| Score of Damage 15 | -0.73* | -0.25 | -0.28 | -               |        |        |    |
| Score of Damage 30 | -0.73* | -0.40 | -0.37 | 0.90**          | -      |        |    |
| Score of Damage 45 | -0.70* | -0.30 | -0.34 | 0.96**          | 0.97** | -      |    |
| Score of Damage 60 | -0.69* | -0.21 | -0.29 | 0.96**          | 0.95** | 0.97** | -  |

\*, \*\* Significant by t-test at 5% and 1%, respectively.

**Table 8.** T-test values of orthogonal contrasts between the means of the variables: grain productivity (GP), one hundred grain weight (100GW) and grain yield (GY) of conventional maize hybrids and their isogenic transgenic versions in the 2011/2012 crop

| Hybrid   | Orthogonal contrasts     | Variables |                     |                     |
|----------|--------------------------|-----------|---------------------|---------------------|
|          |                          | GP        | 100GW               | GY                  |
| P30F35   | $Y_1 = 2m_1 - m_2 - m_3$ | -5.11**   | -1.57 <sup>ns</sup> | 0.37 <sup>ns</sup>  |
|          | $Y_2 = m_2 - m_3$        | -2.47*    | -0.66 <sup>ns</sup> | 1.79 <sup>ns</sup>  |
| DKB390   | $Y_1 = 2m_1 - m_2 - m_3$ | -4.74**   | -0.51 <sup>ns</sup> | 0.65 <sup>ns</sup>  |
|          | $Y_2 = m_2 - m_3$        | -2.71*    | 0.32 <sup>ns</sup>  | 1.03 <sup>ns</sup>  |
| DAS2B710 | $Y_1 = m_1 - m_2$        | -2.47*    | 1.53 <sup>ns</sup>  | -0.31 <sup>ns</sup> |
| Impacto  | $Y_1 = m_1 - m_2$        | -3.71**   | -2.45*              | -3.20*              |

\*\*, \* Significant at 1% and 5% probability; <sup>ns</sup>Non-significant; For the contrasts:  $m_1$  = mean of the conventional hybrid;  $m_2$  = mean of the transgenic hybrid;  $m_3$  = mean of the second transgenic hybrid (if any).

In the hybrid DKB390, there were differences among the hybrids in grain productivity in the contrast between the conventional hybrid and its isogenic transgenic versions, with at least one of the transgenic events showing productivity higher than the conventional hybrid (Table 8). For the weight of one hundred grains and grain yield, there were no differences between the conventional hybrid and its isogenic transgenic versions. In the contrast between the two *Bt* transgenic events of the hybrid DKB390 (DKB390 YG × DKB390 PRO), there was a difference also between events for grain productivity, but not for weight of one hundred grains and grain yield (Table 8).

The contrast between the conventional hybrid and the transgenic hybrid DAS2B710 indicated differences between conventional and transgenic for grain productivity, but not for weight of one hundred grains and grain yield of these genotypes (Table 8). In the case of the hybrid Impacto, the conventional and transgenic version differed in grain yield, weight of one hundred grains and grain yield (Table 8).

## 4. CONCLUSION

There are different performances among hybrids in relation to the attack by *S. frugiperda*;

Minor scores of damage caused by *S. frugiperda* are found in transgenic hybrids;

Most conventional hybrids do not differ in grain productivity from at least one of its isogenic transgenic versions;

Higher productivity is reached by hybrids with the Viptera<sup>®</sup> technology, and lower ones with the conventional hybrid DKB390;

The conventional hybrids Maximus and Impacto, and the transgenic hybrids, hardly lose productivity when infested by *S. frugiperda*;

The same maize hybrid with different *Bt* toxins may have different productive behavior in field conditions;

Different *Bt* toxins respond differently to the damages caused by *S. frugiperda*.

## REFERENCES

- Céleres (2013). Os benefícios econômicos da biotecnologia agrícola no Brasil: 1996/97 a 2012/13. Recuperado em 10 de dezembro de 2013, de [http://celeres.com.br/wordpress/wp-content/uploads/2014/01/PressRelease2013\\_Economico.pdf](http://celeres.com.br/wordpress/wp-content/uploads/2014/01/PressRelease2013_Economico.pdf).
- Conselho de Informações sobre Biotecnologia – CIB. (2012). O que você precisa saber sobre transgênicos. Recuperado em 17 de janeiro de 2014, de [http://cib.org.br/wp-content/uploads/2012/08/Guia\\_Transgenicos\\_2012.pdf](http://cib.org.br/wp-content/uploads/2012/08/Guia_Transgenicos_2012.pdf).
- Cruz, C. D. (2001). Aplicativo computacional em genética e estatística – Programa Genes. Viçosa: UFV.
- Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA. (2006). Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos (2nd ed.). Rio de Janeiro: EMBRAPA.
- Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA. (2014). Milho - cultivares para 2013/2014. Recuperado em 8 de maio de 2014, de <http://www.cnpms.embrapa.br/milho/cultivares/>.
- Fernandes, O. D., Parra, J. R. P., No., A. F., Pícoli, R., Borgatto, A. F., & Demétrio, C. G. B. (2003). Efeito do milho geneticamente modificado MON810 sobre a lagarta-do-cartucho *Spodoptera frugiperda* (J. E. SMITH, 1797) (Lepidoptera: Noctuidae). Revista Brasileira de Milho e Sorgo, 2, 25-35.
- Gallo, D., Nakano, O., Silveira, S., No., Carvalho, R.P.L., Batista, G.C., Berti, E., Fo., Parra, J.R.P., Zucchi, R.A., Alves, S.B., Vendramin, J.D., Marchini, L.C., Lopes, J.R.S., & Omoto, C. (2002). Entomologia Agrícola. Piracicaba: FEALQ.
- International Service for the Acquisition of Agri-Biothec Applications – ISAAA. (2013). Brief 46: Global Status of Commercialized Biotech/ GM Crops: 2013. Recuperado em 13 de abril de 2014, de <http://www.isaaa.org/default.asp>.

- Mendes, S. M., & Waquil, J. M. (2009). Uso do milho Bt no manejo integrado de lepidópteros-praga: recomendações de uso (Comunicado técnico, 170). Sete Lagoas: Embrapa Milho e Sorgo.
- Mendes, S. M., Boregas, K. G. B., Lopes, M. E., Waquil, M. S., & Waquil, J. M. (2011). Respostas da lagarta-do-cartucho a milho geneticamente modificado expressando a toxina Cry 1A(b). *Pesquisa Agropecuária Brasileira*, 46, 239-244.
- Mendes, S. M., Marucci, R. C., Moreira, S. G., & Waquil, J. M. (2008). Milho *Bt*: avaliação preliminar da resistência de híbridos comerciais à lagarta do cartucho, *Spodoptera frugiperda* (J. E. Smith, 1797) (Comunicado técnico, 157). Sete Lagoas: Embrapa Milho e Sorgo.
- Michelotto, M. D., Crosariol, J. No., Freitas, R. S., Duarte, A. P., & Busoli, A. C. (2013). Milho transgênico (*Bt*): efeito sobre pragas-alvo e não-alvo. *Nucleus*, 3, 67-82. <http://dx.doi.org/10.3738/nucleus.v0i0.903>.
- Michelotto, M. D., Pereira, A. D., Finoto, E. L., & Freitas, R. S. (2011). Controle de pragas em híbridos de milho geneticamente modificados. *Pesquisa & Tecnologia*, 8, 36-38.
- Nill, K. (2003). *Correcting the myths: presenting the truth about why U.S. farmers have adopted biotechnology*. St. Louis: American Soybean Association.
- Omoto, C., Bernardi, O., Salmeron, E., Farias, J. R., & Bernardi, D. (2012). Estratégias de manejo da resistência e importância das áreas de refúgio para tecnologia Bt. In M. E. A. G. Z. Paterniani, A. P. Duarte, & A. Tsunechiro. *Diversidade e inovações na cadeia produtiva de milho e sorgo na era dos transgênicos* (p. 303-314). Campinas: Instituto Agrônomo. Associação Brasileira de Milho e Sorgo.
- Panda, N., & Khush, G. S. (1995). Host plant resistance to insects (*Bulletin of Entomological Research*, 86). Wallingford: CAB International.
- Soberón, M., Gill, S. S., & Bravo, A. (2009). Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells? *Cellular and molecular life sciences: CMLS*, 66, 1337-1349. <http://dx.doi.org/10.1007/s00018-008-8330-9>. PMID:19132293
- van Raij, B., Cantarella, H., Quaggio, J. A., & Furlani, A. M. C. (1997). *Recomendações de adubação e calagem para o Estado de São Paulo* (Boletim técnico, 100). Campinas: Instituto Agrônomo.
- Waquil, J. M. L., Villela, F. M. F., & Foster, J. E. (2002). Resistência do milho (*Zea mays* L.) transgênico (Bt) à lagarta-do-cartucho, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Revista Brasileira de Milho e Sorgo*, 1, 1-11.
- Wiseman, B. R., Painter, R. H., & Wasson, C. E. (1966). Detecting corn seedling differences in the greenhouse by visual classification of damage by the fall armyworm. *Journal of Economic Entomology*, 59, 1211-1214.