academicJournals

Vol. 8(19), pp. 2232-2239, 23 May, 2013
DOI: 10.5897/AJAR12.2201
ISSN 1991-637X © 2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Agronomic efficiency of *Bacillus thuringiensis* (Bt) maize hybrids in pests control on Lucas do Rio Verde city, State of Mato Grosso, Brazil

Ricardo Shigueru Okumura[1]*, Daiane de Cinque Mariano[2], Rivanildo Dallacort[2], Thiago Ometto Zorzenoni[3], Paulo Vicente Contador Zaccheo[4], Cândido Ferreira de Oliveira Neto[1], Heráclito Eugênio Oliveira da Conceição[1] and Allan Klynger da Silva Lobato[1]

[1]Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia, Paragominas, Brazil.
[2]Departamento de Agronomia, Universidade do Estado de Mato Grosso, Tangará da Serra, Brazil.
[3]Engenheiro Agrônomo daDedini S/A Indústrias de Base, Piracicaba, Brazil.
[4]Pesquisadordo Instituto Agronômico do Paraná, Londrina, Brazil.

Accepted 10 May, 2013

The present study aimed to evaluate under field conditions, the effect of genetically modified maize hybridsin control of *Spodoptera frugiperda*, *Elasmopalpus lignosellus* and *Diatraea saccharalis*, to identify Trais which is more efficient in controlling this complex caterpillars. The experiment was conducted in city of Lucas doRio Verde, State of Mato Grosso, Brazil, the experimental design was randomized blocks with six treatments consist of transgenic materials: Cry1Ab, Cry1F, Cry1A.105+ Cry2Ab2 and Vip3Aa, and the conventional material with and without insecticide application. For the environmental conditions of this study, the genetic materials with biotechnology Cry1F, Cry1A.105 +Cry2Ab2 and Vip3Aa entered the hybrids were the highest yields of maize, as well as those with lower intensities damage from *S. frugiperda*. Regarding the control of *E. lignosellus*, only the conventional and hybrid technology Vip3Aa were susceptible to this insect pest incidence. All biotechnology inserted into maize hybrids were effective for control of *D. saccharalis*.

Key words: *Bacillus thuringiensis*, *Spodoptera frugiperda*, *Elasmopalpus lignosellus*, *Diatraea saccharalis*.

INTRODUCTION

Maize is the most cultivated cereal in Brazil, extends from North to South, one of the largest producers, according to information from the Food and Agriculture Organization of the United Nations (FAO, 2012), which according to the Instituto Brasileiro de Geografia e Estatística the State of Mato Grosso is among the States with higher production, 8.16 million megagrams in 2010 (Ibge, 2012). This high output is due to the importance of their grain for human consumption (Fufa et al., 2003) and animal (Callegaro et al., 2005).

One of the main factors that affect productivity and quality of output is the incidence of pests, which stresses the *Spodoptera frugiperda*, *Elasmopalpus lignosellus* and *Diatraea saccharalis* (Silva et al., 1968; Gallo et al., 2002). An important tool in the management of these insect pests is the use of genetically modified maize hybrids developed through biotechnology techniques (Waquil et al., 2002; Bobrowski et al., 2003). These

*Corresponding author. E-mail: ricardo.okumura@ufra.edu.br / Tel: +55 91 34682155.

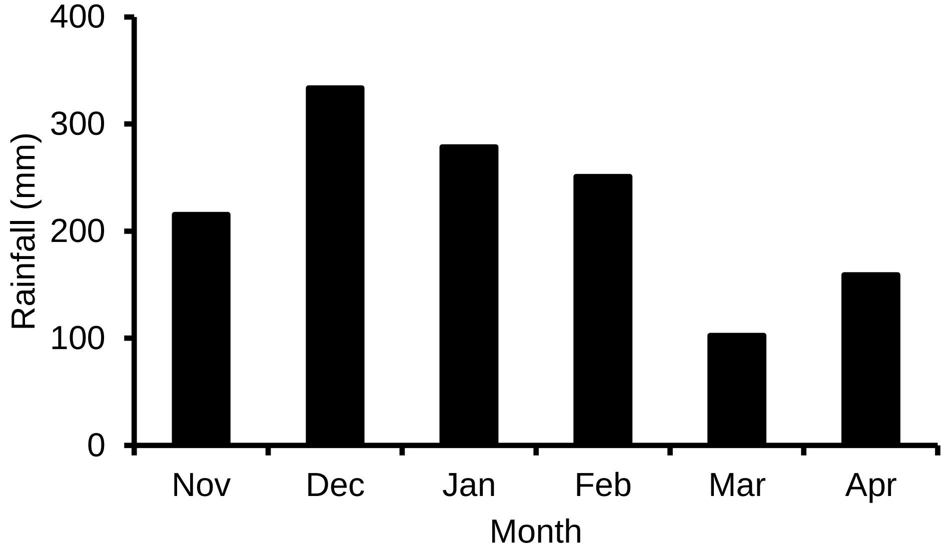

Figure 1. Rainfall (mm) in the period from November 2011 to April 2012 in city of Lucas doRio Verde, State of Mato Grosso, Brazil, in crop year 2011/2012.

plants expressing proteins of *Bacillus thuringiensis* (*Bt*) which has the characteristics of production of β-exotoxin and δ-endotoxin, both are highly specific toxic and therefore harmless to most other organisms, including beneficial insects (Ignoffo and Gregory, 1972; Herrero et al., 2001; Siegel, 2001; Ashfaq et al., 2010). The caterpillars, while feeding the leaf tissue of genetically modified maize, ingest this protein, which acts on epithelial cells of the gut of insects, thereby promotes osmotic disruption of these cells, which determines the death of the caterpillar, before the same occasion damage to the crop (Gill et al., 1992; Gill, 1995).

Despite the benefits of using *Bt* maize, potential risks should be considered (Capalbo and Fontes, 2004). The main concern is the placement of cultivars against the attack of insect control targets. For the control strategy works effectively, it is necessary to know which is the target of insect control for a given hybrid, since due to the specificity of these proteins and differences in susceptibility to pests, hybrids may be highly efficient for a given insect and almost no effect on other insects, though these are both from the same taxonomic class (Williams et al., 1997, 1998a, b; Vilella et al., 2002). However, there may be differential expression of the toxin in the different genotypes in which the gene is incorporated, this is, and the same crytoxin present in different hybrid variability may have opposite pest infestation (Waquil et al., 2002).

MacIntosh et al. (1990) showed a specificity of pure toxin for different species of insects. Thus, it has been recorded for the main species of lepidopteran pests of maize the following toxins with greater activity: Cry 1D and Cry 1F to control *S. frugiperda*, Cry 1A(c) for *Helicoverpazea*, Cry 1B to *D. saccharalis* and *D. grandiosella* (Bohorova et al., 1997) and Cry 1C for *Spodopteraexigua* (Visser et al., 1990). In study development by Williams et al. (1998a), which was added straw transgenic maize containing the toxin Cry 1A(b) showed 40% reduction in survival of 94% and biomass of larvae of *S. frugiperda*. As for the fall armyworm in maize plants expressing *Bt* toxins ranged from highly effective (Cry 1F), intermediate [Cry 1A(b) and Cry 1A(c)] and without any activity (Cry 9c) (Waquil et al., 2002). With respect to *E. lignosellus* all crytoxins [1F, 1A(b) 1A(c) and 9C] were effective in accordance with results reported by Vilella et al. (2002). This evaluate the field of transgenic plants with the *Bt* gene in Brazil is low (Waquil et al., 2004), which can cite the study of Fernandes et al. (2003) that studies conducted under field conditions for two years and in different places, and found that maize containing the protein Cry 1A(b) demonstrated an efficient control of armyworms. In this context, this study aims to evaluate the agronomic efficiency of different genetically modified maize hybrids in the complex control of caterpillars (*S. frugiperda*, *E. lignosellus* and *D. saccharalis*).

MATERIALS AND METHODS

Study site

The experiment was conducted in the season of 2011/2012, in city of Lucas doRio Verde, State of Mato Grosso, Brazil, located in the geographic coordinates of latitude 12°58'07"S and longitude 55°56'43"W with altitude mean of 390 m. According to Köppen classification, the climate is Aw, with the data of precipitation occurred during the experiment presented in Figure 1. The soil was classified as Typic Yellow eutroferric (Embrapa, 2006), with the

Table 1. Percentage of plants with leaf damage of any kind, regardless of the intensity of the lesion by *S. frugiperda* and percent control efficiency (%Ce) in conventional maize hybrids and genetically modified in the region of Lucas do Rio Verde, State of Mato Grosso, Brazil, crop year 2011 / 2012.

Hybrid	Growth stage							
	V_2	%Ce	V_4	%Ce	V_6	%Ce	V_8	%Ce
T_1[(1)]	59.2[b(2)]	40.8	87.7[b]	12.3	64.6[b]	35.4	62.5[b]	36.5
T_2	54.4[b]	45.6	64.3[c]	35.7	17.1[c]	82.9	17.9[c]	81.8
T_3	14.3[c]	85.7	49.4[d]	50.6	1.9[d]	98.1	1.1[d]	98.9
T_4	6.2[c]	93.8	47.1[d]	52.9	0.8[d]	99.2	0.6[d]	99.4
T_5	99.7[a]	0.3	99.1[a]	0.9	74.2[b]	25.8	89.8[a]	8.7
Control	100.0[a]		100.0[a]		100.0[a]		98.4[a]	
C.V. (%)	20.1		12.6		10.2		11.0	

[(1)]T_1: Cry1AB; T_2: Cry1F; T_3: Cry1A.105 + Cry2AB2; T_4: Vip3Aa; T_5: hybridwithconventionalinsecticide application; Control: conventional hybrid without insecticide application. [(2)]Average followed by the same lower case letter in the column do not differ at 5% probability by the test of Scott-Knott (1974).

following chemical and textural characteristics in 0 to 0.20 m: pH in water of 5.5, 14.2 mg dm^{-3} of $P_{Mehlich1}$, 34 mg dm^{-3} of K^+, 4.8 $cmol_c$ dm^{-3} of Ca^{2+}, 2.2 $cmol_c$ dm^{-3} of Mg^{2+}, 0.3 $cmol_c$ dm^{-3} of Al^{3+}; 35 g $kg^{-1}$of organic matter and 650 g kg^{-1} clay.

Treatments and experimental design

The experimental plots consisted of six rows of plants, with 5.0 m length and spacing of 0.5 m. The experimental was conducted in randomized complete blocks designs, with six treatments and four replications, and the treatments by transgenic hybrids: Cry1Ab (T_1), Cry1F (T_2), Cry1A.105 + Cry2Ab2 (T_3), Vip3Aa (T_4); and hybrids with conventional insecticide application (T_5) and no application (Control). The installation of the experiment was conducted on the dates of November 5, 2011, with the aim of evaluating *E. lignosellus*, and November 25, 2011, for evaluation of *S. frugiperda* and *D. saccharalis*, both in no-tillage (Aguiar et al. 2008), with 0.50 m spacing between lines (Afférri et al., 2008), and population density of 70,000 plants ha^{-1} (Kappes et al., 2011).

Crop management

The maintenance of fertilization was performed with the application of 60 and 70 kg ha^{-1} P_2O_5 and K_2O in the furrow, and 90 kg ha^{-1} of nitrogen top dressing applications in the V_4 growth stage (Ritchie et al. 1993). Other cultural practices, fungicides and herbicides, were made in accordance with the technical recommendations for maize (FornasieriFilho, 2007).

Evaluated characteristics

Evaluations of *E. lignosellus* were performed every 2 days between the V_1 to V_5 growth stages (Ritchie et al., 1993). Plants with symptoms of caterpillar attack were removed from the plot, and in the end we calculated the percentage of loss (Silva et al., 1986). Evaluations of attack *S. frugiperda* and *D. saccharalis*, these growth stages were performed at V_2, V_4, V_6 and V_8 (Ritchie et al., 1993) recording the number of plants with either type of lesion intensity and damage (Fernandes et al., 2003), using the scale visual leaf damage by *S. frugiperda* (Davis et al., 1992). With this information, we calculated the index of damaged plants (Ceccon et al., 2004), in which the chemical control was performed as recommended by FornasieriFilho (2007), when leaf damage reached visual scale ≥ 4 (Gallo et al., 2002).

The damage caused by *D. saccharalis* were performed by opening the stem internodes for quantification of total and galleries, and its respective dimension (Araújo et al., 2011), then proceeded to the mean value, and this was converted to a percentage. Harvest was done manually on days 5 April 2012 (*E. lignosellus*) and on April 20, 2012 (*S. frugiperda* and *D. saccharalis*), with subsequent threshing, weighing of grain moisture content determination, and order conversion Mg ha^{-1}.

Data analysis

The experimental data, after being analyzed to verify normality and homoscedasticity waste by use of the Shapiro-Wilk test (Shapiro and Wilk, 1965) and Levene (Box, 1953) to 1% probability, by use of statistical software (Sas, 2008), were subjected to analysis of variance and treatment means compared by Scott and Knott (1974), with 5% significance, using the statistical software SISVAR (Ferreira, 2011), and the efficiency of these treatments was calculated by using the formula recommended by Abbott (1925).

RESULTS AND DISCUSSION

Effects of *S. frugiperda* in leaf damage in *Bt* hybrids

Assessments of leaf damage of any kind allowed to identify the intensity of damage to *S. frugiperda* treatments (Table 1). The conventional maize had higher intensity than genetically modified maize in all growth stages evaluated V_2, V_4, V_6 and V_8. According to the data in Table 1, it shows that there were some kind of damage "scraping" the leaves of *Bt* maize hybrids, since, to be controlled, the insect must ingest the toxin, at the herbivory (Buntin et al., 2001; Waquil et al., 2002). Thus, this caterpillar to feed maize containing *Bt* toxin, may have changed their biological cycle, with higher mortality of larvae, lower biomass and lower pupal mass (Fernandes et al., 2003). Buntin et al. (2001) studied the pattern of resistance to maize MON810 *S. frugiperda*,

Table 2. Percentage of damaged plants leaf equal or above 4 on a visual scale of Davis et al. (1992) for *S. frugiperda* and percent control efficiency (%Ce) in conventional maize hybrids and genetically modified in the region of Lucas do Rio Verde, State of Mato Grosso, Brazil, crop year 2011/2012.

Hybrid	Growth stage							
	V_2	%Ce	V_4	%Ce	V_6	%Ce	V_8	%Ce
T_1 (1)	11.6 b(2)	85.2	15.4 c	80.4	40.3 b	58.1	20.2 b	70.5
T_2	8.7 b	88.9	3.8 d	95.2	3.4 c	96.5	2.1 c	96.9
T_3	2.9 c	96.3	0.0 e	100.0	0.0 c	100.0	0.0 c	100.0
T_4	0.0 c	100.0	0.0 e	100.0	0.8 c	99.2	0.0 c	100.0
T_5	67.1 a	14.6	46.6 b	40.8	43.8 b	54.5	31.1 b	54.5
Control	78.6 a		78.7 a		96.2 a		68.4 a	
C.V. (%)	36.5		23.2		19.7		36.1	

(1) T_1: Cry1AB; T_2: Cry1F; T_3: Cry1A.105 + Cry2AB2; T_4: Vip3Aa; T_5: hybridwithconventionalinsecticide application; Control: conventional hybridwithoutinsecticide application. (2) Average followed by the same lower case letter in the column do not differ at 5% probability by the test of Scott-Knott (1974).

in different localities, observed a lower percentage of plants with damage to the cartridge of maize compared to conventional maize. The authors reported that in maize MON810, the percentage of plants with damage the cartridge reached a maximum of 35%, while inconventional maize were up 96.1% of damaged plants.

With respect to genetically modified organisms, it was found that hybrids containing proteins Cry1A.105+Cry2Ab2 and Vip3Aa had the lowest incidence of *S. frugiperda*, which demonstrates the efficiency of these genes in these incorporated genetic material, with values near 100% efficiency control for these two hybrids (Table 1). Based on the data, it was found that genes Cry1F, Cry1A.105 + Cry2Ab2 and Vip3Aa showed resistance to *S. frugiperda* (Table 2), in which toxins were effective in protecting plants against infestation and damage promoted by this insect throughout the vegetative cycle. This fact demonstrates the occurrence of continuous expression of the toxin and its effectiveness on the pest, thereby adopting this technology ensured the best possible results and the reduction of insecticide application. The low intensity of leaf damage obtained in this study with treatment transgenic agree with the literature (Williams et al., 1998a, b; Buntin et al., 2001; Waquil et al., 2002; Michelotto et al., 2011).

Fernandes et al. (2003) studying the effect of genetically modified maize containing protein Cry 1A(b) observed that the percentage of plants with leaf damage caused by caterpillars throughout the growing cycle, was significantly higher in maize than in conventional transgenic maize in three experiments, with average values obtained for the conventional maize 72.7% of plants with damage, and the maize toxin Cry 1A(b) the average value was 33.8%. The insecticides were used to control the caterpillars when the percentage of leaf damage ≥4 scale Davis et al. (1992) were above 20% (Gallo et al., 2002). Thus, even the use of materials with *Bt* technology incorporated in their genetics, Cry1Ab, showed the need to use chemicals to aid in the control of caterpillars, which demonstrates the high population pressure *S. frugiperda* the experimental period (Table 2).

For conventional hybrids with insecticide application (T_5), these showed the need for control in all growth stages of evaluation, that is, until the emergence of the V_8 stage was performed applying chemicals for the control of caterpillars, increasing maize production cost. It is worth mentioning that it is between the V_8 to V_{10} growth stage that plants are more susceptible to attack the caterpillar, which can cause reduction of up to 18.7% of the grain yield of maize (Cross and Turpin, 1982). Despite the conventional hybrid seeds possess commercial value greater account than the *Bt* hybrids, these savings in price seed is relative, since in some regions is common to use more than five applications of pesticides during the season (Figueiredo et al., 2006). In the present study, it took four applications of insecticides for the control of *S. frugiperda*, and still control efficiency of this was still lower than that of materials containing *Bt* protein in their genes, as shown in Table 2. The lower control efficiency results in low yield due to pest damage by plant, which will impair its maximum yield potential.

Furthermore, the use of synthetic chemicals provides several problems as waste grains, destruction of natural enemies, poisoning of applicators and appearance of pest populations resistant to insecticides (Roel et al., 2000; Asogwa and Dongo, 2009). According to Table 2, it appears that conventional maize plants had leaves the cartridge with greater signs of damage from the start of ratings (growth stage V_2). In turn, the intensity of damage in the cartridge maize and conventional hybrids containing the gene Cry1Ab were significantly higher in the V_6 growth stage, denoting the initial feeding behavior *S. frugiperda*, with losses to plant development.

Zancanaro et al. (2012), studying different percentages of mixed seeds of transgenic plants with conventional, in

Table 3. Plant stand (PS), incidence of *D. saccharalis*(IDS), percent control efficiency (%Ce) and yield (YIELD) in conventional maize hybrids and genetically modified to *D. saccharalis* and *S. frugiperda*, in the region of Lucas do Rio Verde, State of Mato Grosso, Brazil, crop year 2011/2012.

Hybrid	PS (plants ha^{-1})	IDS (cm)	%Ce	Yield (Mg ha^{-1})
T_1[(1)]	59028[a(2)]	2.3[c]	94.9	6.7[b]
T_2	59722[a]	2.1[c]	95.3	7.6[a]
T_3	59167[a]	1.3[c]	97.1	7.8[a]
T_4	56944[a]	0.0[c]	100.0	7.7[a]
T_5	56805[a]	11.4[b]	74.6	5.8[b]
Control	51528[a]	44.8[a]		2.5[c]
C.V. (%)	3.1	22.9		2.8

[(1)]T_1: Cry1AB; T_2: Cry1F; T_3: Cry1A.105 + Cry2AB2; T_4: Vip3Aa; T_5: hybrid with conventional insecticide application; Control: conventional hybrid without insecticide application. [(2)]Average followed by the same lower case letter in the column do not differ at 5% probability by the test of Scott-Knott (1974).

which significant differences in the intensities of leaf damage caused by caterpillars, and the treatment of fully transgenic plants had the lowest intensity of damage, note the damage with 3.38, which agrees with the values of 2.10 and 2.64 reported by Fernandes et al. (2003) and Butin (2008), respectively. The low levels of damage notes obtained in this study agree with those reported by Williams et al. (1997), Buntin et al. (2001) and Waquil et al. (2002).

Araújo et al. (2011) observed, regardless of the time of evaluation, a minor leaf damage in *Bt* hybrids compared to conventional. Regarding transgenic hybrids, these same authors found that the hybrid containing the toxin Cry 1F was more resistant to attack the caterpillar, in contrast, expressing Cry protein 1A(b) behaved as moderately resistant.

Effects of *S. frugiperda* and *D. saccharalis* in productivity of the hybrids

The incidence of *D. saccharalis* was higher in conventional hybrids, especially in control, no insecticide application, which demonstrates the need for pesticide use for the control of this insect pest, which can be seen from Table 3 that the use of pesticides obtained percentage of efficiency control over 70%. Regarding *Bt* hybrids, all showed resistance to this insect, independent of *Bt* protein embedded in their genes, this efficiency was above 94%, this is, the use of technology that effectively controls insects.

Table 3 shows the values of yields obtained in the present study, in which it appears that varied according to the hybrids tested, with the highest values were observed in genetic materials that express the *Bt* gene, whereas conventional hybrids without application chemicals for the control of caterpillars provided the lowest productivity. The minor leaf damage by *S. frugiperda*, and a lower incidence of *D. saccharalis* seen in hybrids containing the genes Cry1F, Cry1A.105 + Cry2Ab2 and Vip3Aa warrant the highest yield for these hybrids, since the attacks of these pests cause the death of the plants and reduction in the initial stand and/or leaf damage by feeding parenchyma of the leaves, the central bud of the plant and grain spike (Cruz and Turpin, 1982, 1983; Cruz et al., 1999; Sarmento et al., 2002), consequently, affects the number of production plants and the mass of spikes (Silveiraet al., 1998).

With respect to conventional hybrids with insecticide application, even the use of pesticides was not able to increase the productivity of maize grains to values statistically equal to *Bt* hybrids, which was to be expected, since the results presented in Tables 1 and 2 treatment with chemical control was lower in all parameters compared to treatment with genetic resistance (*Bt*). According to Koziel et al. (1993), Bobrowski et al. (2001, 2003), Pinto and Fiuza (2008) and Aziz et al. (2011), the use of insect-resistant plants is one of the ideal methods of control, due to maintenance of the pest population at levels below the economic injury. Moreover, this method does not cause harm to the environment and is compatible with other control methods (Gallo et al., 2002).

The hybrid transgenic Cry1F, Cry1A.105 + Cry2Ab2 and Vip3Aa showed a yield over other hybrids tested (Table 3). These results demonstrate that a significant increase in the production of maize can be achieved by incorporating the *Bt* gene expressing the toxin into genotypes with great potential for yielding. Thus, the most suitable for the producers are those who were less attacked by *S. frugiperda* and more productive.

In the study by Araújo et al. (2011), observed that the grain yield were higher with the use of *Bt* maize, with a superiority of 0.59 Mg ha^{-1} compared to conventional hybrid, and Bobrowski et al. (2003) report promotes the adoption of technology reduction of losses in yield of

Table 4. Mean percentage of dead plants (%DP) by the attack *E. slignosellus*, percent control efficiency (%Ce) and yield (YIELD) in conventional maize hybrids and genetically modified in the region of Lucas do Rio Verde, State of Mato Grosso, Brazil, year Farm 2011/2012.

Hybrid	%DP	%Ce	Yield (Mg ha^{-1})
T_1[(1)]	1.8[b(2)]	97.7	6.3[a]
T_2	1.2[b]	98.5	6.6[a]
T_3	0.3[b]	99.6	6.5[a]
T_4	68.4[a]	12.2	1.0[b]
Control	77.9[a]		1.1[b]
C.V. (%)	26.69		6.7

[(1)]T_1: Cry1AB; T_2: Cry1F; T_3: Cry1A.105 + Cry2AB2; T_4: Vip3Aa; Control: conventional hybrid without insecticide application. [(2)]Average followed by the same lowercase letter in the column do not differ at 5% probability by the test of Scott-Knott (1974).

about 20% due to better control of caterpillars that attack maize, besides providing reduction in the use of insecticides

According to James (2003a, b) in the field experiments conducted in Brazil, found an average gain in yield for *Bt* maize around 24% compared to conventional maize. While in Argentina, Trigo and Cap (2003) and James (2003a) founded that yield was on average 5 to 10% higher than conventional plants, respectively. In the United States the increase in yield of transgenic hybrids was approximately 0.33 to 0.94 Mg ha^{-1} (Marra et al., 2002). Gruère et al. (2007) observed that besides the increase in grain yield between 5 to 34% for transgenic maize, there is a reduction in the use of inputs that can reach 31.4%. Thus, the use of genetically modified crops provides increment in grain yield of maize and less use of chemical pesticides.

Effect of *E. lignosellus* in productivity of the hybrids

The damage caused by *E. lignosellus* in maize ranged between hybrid and conventional transgenics. For all hybrids containing the *Bt* gene the percentage of dead plants was less than 2% (Table 4), with the exception of the hybrid gene containing the Vip3Aa. The material showed a higher incidence of susceptibility to *E. lignosellus* was the hybrid gene containing the conventional Vip3Aa and without addition of *Bt* protein, the values of dead seedlings were raised with the percentage of damage of 68.4 and 77.9%, respectively. Thus, it is observed that only 31.6 and 22.1% of desired plants can, if others do not suffer any direct and/or indirect, produce ears of maize.

In general, the major damage caused by *E. lignosellus* occur during drought periods (All et al., 1979; Viana and Costa, 1995), in turn, despite a cumulative rainfall of 216 mm in the month of November, there was a high population pressure in this insect pest in this study.

Conclusions

Biotechnology, Cry1F, Cry1A.105+ Cry2Ab2 and Vip3Aa, inserted in hybrids are the ones with better control to *S. frugiperda* and higher yields of maize compared technologies treated with insecticides and, with respect to the control of *D. Saccharalis* all hybrids containing *Bt* genes are efficient. The hybrid technologies Cry1Ab, Cry1F and Cry1A.105 +Cry2Ab2 are highly effective for controlling *E. lignosellus*, while the conventional hybrid, as the technology Vip3Aa are susceptible to incidence of *E. lignosellus*.

REFERENCES

Abbott WSA (1925). Method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18(1):265-267.

Afférri FS, Martins EP,Peluzio JM, Fidelis RR, Rodrigues VM (2008). Row spacing and sowing densities for late cropping maize in the Tocantins State, Brazil. Pesquisa Agropecuária Trop. 38(2):128-133.

Aguiar RA, Silveira PM, Moreira JAA, Wander AE (2008). Economic analysis of different cultural practices for corn (*Zea mays* L.) cultivation.Pesquisa Agropecuária Trop. 38(4):241-248.

All JN, Gallaher RN, Jellum MD (1979). Influence of planting date, preplanting weed control, irrigation, and conservation tillage practices on efficacy of planting time insecticide applications for control of lesser cornstalk borer in field corn. J. Econ. Entomol. 72(2):265-268.

Araújo LF, Silva AG, Cruz I, Carmo EL, Horvath Neto A, Goulart MMP, Rattes JF (2011). Population dynamics of *Spodoptera frugiperda* (J. E. Smith), *Diatraea saccharalis* (Fabricius) and *Doru luteipes* (Scudder) in conventional and Bt transgenic maize. Revista Brasileira de Milho e Sorgo 10(3):205-214.

Ashfaq M, Ane MN, Zia K, Nasreen A, Hasan M (2010). The correlation of abiotic factors and physic-morphiccharateristics of (*Bacillus thuringiensis*) Bt transgenic cotton with whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) and jassid, *Amrasca devastans* (Homoptera: Jassidae) populations. Afr. J. Agric. Res. 5(22):3102-3107.

Asogwa EU, Dongo LN (2009). Problems associated with pesticide usage and application in Nigerian cocoa production: A review. Afr. J. Agric. Res. 4(8):675-683.

Aziz A, Akhtar N, Afzal M, Ashraf M, Tanveer A, Ahmad R, Safdar ME, Ahmad S (2011). Comparative performance of Bt cotton with some elite conventional cotton cultivars under arid to semi-arid conditions. Afr. J. Agric. Res. 5(6):1600-1606.

Bobrowski VL, Fiuza LM, Pasquali G, Bodanese-Zanettini MH (2003). Bacillus thuringiensis genes: an approach to confer insect resistance to plants. Ciência Rural 34(1):843-850.
Bobrowski VL, Pasquali G, Bodanese-Zanettini MH, Fiuza LM (2001). Detection of cry1 genes in *Bacillus thuringiensis* isolates from south of Brazil and activity against *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Braz. J. Microbiol. 32(2):105-109.
Bohorova N, Cabrera M, Abarca C, Quintero R, Maciel AM, Brito RM, Hoisington D, Bravo A (1997). Susceptibility of four tropical Lepidopteran maize pests to *Bacillus thuringiensis* Cry I-type insecticidal toxins. J. Econ. Entomol. 90(2):412-415.
Box GEP (1953). Non-normality and tests on variances. Biometrika 40(3-4):318-335.
Buntin GD, Lee D, Wilson DM, Mcpherson RM (2001). Evaluation of YieldGard transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidade) on corn. Florida Entomol. 84(1):37-42.
Butin GD (2008). Corn expressing Cry 1Ab or Cry 1F endotoxin for fall armyworm and corn earworm (Lepidoptera: Noctuidae) management in field corn for grain production. Fla. Entomol. 91(4):523-530.
Callegaro MGK, Dutra CB, Huber LS, Becker LV, Rosa CS, Kubota EH,Hecktheur LH (2005). Determination of insoluble, soluble, and total dietary fiber of corn products. Ciência e Tecnologia de Alimentos 25(2):271-274.
Capalbo DMF, Fontes EMG (2004). GMO guidelines Project. Jaguariúna: Embrapa Meio Ambiente. P. 56. (Embrapa Meio Ambiente. Documents, 38).
Ceccon G,Raga A, Duarte AP,Siloto RC (2004). Effect of inseticides at sowing on seedling pests and yield off-season maize crop under no-tillage system. Bragantia 63(2):227-237.
Cruz I, Figueiredo MLC, Oliveira AC, Vasconcelos CA (1999). Damage of Spodopterafrugiperda (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. Int. J. Pest Manag. 45(4):293-296.
Cruz I, Turpin FT (1982). Effect of Spodopterafrugiperda on different growth stages of corn.Pesquisa Agropecuária Brasileira 17(3):355-359.
Cruz I, Turpin FT (1983). Yield impacto f larval infestation of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) to mid-whorl growth stage of corn. J. Econ. Entomol. 76(5):1052-1054.
Davis FM, Ng SS, Williams WP (1992).Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. Mississippi Agricultural and Forestry Experiment Station. P. 9. (Tech. Bull. P. 186).
Embrapa-Empresa Brasileira de Pesquisa Agropecuária (2006). Brazilian system of soil classification.2th ed. Rio de Janeiro, Embrapa Solos. P. 306.
FAO-Food and Agriculture Organization of the United Nations (2012). FAOSTAT. Available in: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor >.
Fernandes OD, Parra JRP, Ferreira Neto A,Pícoli R,Borgatto AF, Demétrio CGB (2003). Effect of the genetically modified corn MON810 on fall armyworm Spodopterafrugiperda (J.E. Smith, 1979) (Lepidoptera: Noctuidae). Revista Brasileira de Milho e Sorgo 2(2):25-35.
Ferreira DF (2011). Sisvar: a computerstatisticalanalysis system. Ciência e Agrotecnologia 35(6):1039-1042.
Figueiredo MLC, Martins-Dias AMP, Cruz I (2006). Relationship between fall armyworm and their natural biological control agents in the maize crop. Pesquisa Agropecuária Brasileira 41(12):1693-1698.
FornasieriFilho D (2007). Handbook of maize. Jaboticabal: Funep. P. 576.
Fufa H, Akalu G, Wondimu A, Taffesse S, Gebre T, Schlosser K, Noetzold H, Henle T (2003). Assessment of protein nutritional quality and effects of traditional processes: a comparison between Ethiopian quality protein maize and five Ethiopian adapted normal maize cultivars. Nahrung 47(4):269-273.
Gallo D, Nakano O, Silveira Neto S, Carvalho RPL, Baptista GC, Berti Filho E, Parra JRP, Zucchi RA, Alves SB, Vendramin JD, Marchini LC, Lopes JRS, Omoto C (2002). Agric. Entomol. Piracicaba: FEALQ. P. 920.
Gill SS (1995). Mechanism of action of Bacillus thuringiensis toxins.Memórias do Instituto Oswaldo Cruz. 90(1):69-74.
Gill SS, Cowles EA, Pietrantonio PV (1992). The mode of action of Bacillusthuringiensis endotoxins. Annu. Rev. Entomol. 37:615-636.
Gruère G, Bouet A, Mevel S (2007). Genetically modified food and international trade.Washington: International Food Policy Research Institute. (IFPRI Discussion Paper, 00740) P. 60.
Herrero S, Oppert B, Ferré J (2001). Different mechanisms of resistance to *Bacillus thuringiensis* toxins in the indianmeal moth. Appl. Environ. Microbiol. 67(3):1085-1089.
Ibge-Instituto Brasileiro de Geografia e Estatística (2012). Agricultural production. Available in: <http://www.ibge.gov.br/home/download/estatistica.shtm>.
Ignoffo CM, Gregory B (1972). Effects of Bacillus thuringiensisβ-exotoxina on larval maturation, adult longevity, fecundity, and egg viability in several species of Lepidoptera. Environ. Entomol. 1(1):269-271.
James C (2003a). Biotech corn can boost yields to help growing world food demands. Ithaca: International Service for the Acquisition of Agri-biotech Applications.
James C (2003b). Global status of commercialized transgenic crops: 2003. Ithaca: International Service for the Acquisition of Agri-biotech Applications.
Kappes C, Andrade JAC, Arf O, Oliveira AC, Arf MV, Ferreira JP (2011). Plants spatial arrangement for different maize hybrids. Pesquisa Agropecuária Trop. 41(3):348-359.
Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwll S, Launis K, Lewis K, Maddox D, McPherson K, Meghji MR, Merlin E, Rhodes R, Warren GW, Wright M, Evola SV (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Nat. Biotechnol. 11(2):194-200.
MacIntosh TB, Stone SR, Hunst PL, Greenplate JT, Marrone PG, Perlak FJ, Fischhoff DA, Fuchs RL (1990). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56(2):258-266.
Marra MC, Pardey P, Alston J (2002). The pay-offs of agricultural biotechnology: an assessment of the evidence. International Food Policy Research Institute, Washington.
Michelotto MD, Finotto EL, Martins ALM, Duarte AP (2011).Interaction between transgenic (Bt) insecticides and pest control in maize hybrids. Arquivos do Instituto Biológico 78:71-79.
Pinto LMN, Fiuza LM (2008).Cry genes from Bacillus thuringiensis applied to crop genetic engineer, for insect resistance improvement. Neotrop. Biol. Conserv. 3(3):159-168.
Ritchie SW, Hanway JJ, Benson GO (1993). How a corn plant develops. Ames: Iowa State University of Science and Technology, Cooperative Extension Service. (Special Report, 48) P. 26.
Roel AR, Vendramim JD, Frighetto RTS, Frighetto N (2000). Effect of ethyl acetate extract of *Trichilia pallida* Swartz (Meliaceae) on development and survival of fall armyworm. Bragantia 59(1):53-58.
Sarmento RA, Aguiar RWS, Aguiar RASS, Vieira SMJ, Oliveira HG, Holtz AM (2002). Biology review, occurrence and control of *Spodoptera frugiperda* (Lepidoptera, Noctuidae) in corn in Brazil. Biosci. J. 18(2):41-48.
Sas (2008). SAS/STAT® 9.2 User´s Guide. Version 9.2, Cary, NC: SAS Institute Inc. P. 584.
Scott A, Knott M (1974). Cluster-analysis method for grouping means in analysis of variance. Biometrics 30(3):507-512.
Shapiro SS, Wilk MB (1965). An analysis of variance test for normality (complete samples). Biometrika 52(3-4):591-611.
Siegel JP (2001). The mammalian safety of Bacillus thuringiensis-based insecticides. J. Invertebr. Pathol. 77(1):13-21.
Silva AGA, Gonçalves CR, Galvão DM, Gonçalves AJL, Gomes J, Silva MN, Simoni L (1968). Fourth catalog of insects that live on plants of Brazil: Their parasites and predators. 1° Tomo, 2th Parte. Rio de Janeiro, Ministério da Agricultura. P. 622.
Silva AL, Gonçalves CS, Oliveira Júnior LCC (1986). Assay of the armyworm control of *Elasmopalpus lignosellus* (Zeller, 1984) and termites Syntermes sp., *Procornitermes* spp. and *Cornitermes* spp.,

in maize, through seed treatment. Pesquisa Agropecuária Trop. 16(1):19-26.
Silveira LCP, Vendramim JD, Rossetto CJ (1998). No feeding preference of fall armyworm larvae for corn genotypes. Bragantia 57(1):105-111.
Trigo EJ, Cap EJ (2003). The impact of the introduction of transgenic crops in Argentinean agriculture. AgBio Forum 6(3):87-94.
Viana PA, Costa EF (1995). Effect of soil moisture on lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) damage to corn.Anais da Sociedade Entomológica do Brasil 24(2):209-214.
Vilella FMF, Waquil JM, Vilela EF, Siegfried BD, Foster JE (2002). Selection of the fall armyworm, Spodopterafrugiperda (Smith) (Lepidoptera: Noctuidae) for survival on Cry 1A(b) Bttoxin. Revista Brasileira de Milho e Sorgo 1(3):12-17.
Visser B, Munsterman E, Strokes A, Dirlese WG (1990). A novel Bacillus thuringiensis gene encoding a Spodopteraexigua specific crystal protein. J. Bacteriol. 172(12):6783-6788.
Waquil JM, Vilella FMF, Siegfried BD, Foster JE (2004). Biological activity of Bt toxins, Cry 1A(b) and Cry 1F on Spodopterafrugiperda (Smith) (Lepidoptera: Noctuidae). Revista Brasileira de Milho e Sorgo 3(2):161-171.
Waquil JM, Villela FMF, Foster JE (2002). Resistance of Bt transgenic maize (Zea mays L.) to fall armyworm, Spodopterafrugiperda (Smith) (Lepidoptera: Noctuidae). Revista Brasileira de Milho e Sorgo 1(3):1-11.
Williams WP, Buckley PM,Sagers JB,Hanten JA (1998b). Evaluation of transgenic corn for resistance to corn earworm (Lepidoptera: Noctuidae), fall armyworm (Lepidoptera: Noctuidae), and southwestern corn borer (Lepidoptera: Crambidae) in a laboratory bioassay. J. Agric. Entomol. 15(1):105-112.
Williams WP, Davis FM, Buckley PM, Hedin PA, Baker GT, Luthe DS (1998a). Factors associated with resistance to fall armyworm (Lepidoptera: Noctuidae), and southwestern corn borer (Lepidoptera: Crambidae) in corn at different vegetative stage. J. Econ. Entomol. 91(6):1471-1480.
Williams WP, Sagers JB, Hanten JA, Davis FM, Buckley PM (1997). Transgenic corn evaluated for resistance to fall armyworm and southwestern corn borer. Crop Sci. 37(3):957-962.
Zancanaro PO, Buchweitz ED, Boiça Junior AL, Moro JR (2012). Evaluation of refuge technologies in transgenic maize crop. Pesquisa Agropecuária Brasileira 47(7):886-891.