COMPARISON OF BT TRANSGENIC MAIZE IN CONTROL
OF *Elasmopalpus lignosellus* IN THE FIELD

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ABSTRACT - The Lepidoptera insects are responsible for large losses in maize production in Brazil, and stand out those that attack seedlings, such as lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). The objective of this work was to compare the performance of transgenic Bt maize in the control of the *E. lignosellus* caterpillar in the maize seedlings phase in two trials. In the first trial six treatments were tested: (1) Conventional Non-Bt maize; (2) Conventional Non-Bt maize with insecticide application; (3) transgenic maize expressing the Cry1Ab genes; (4) Cry1F; (5) Cry1A.105 + Cry2Ab2; (6) Cry1A.105 + Cry2Ab2 + Cry1F. The experimental design was randomized blocks, where each treatment was repeated 4 times in plots of 22.5 m2. Ten consecutive plants with third instar larvae of *E. lignosellus* in the seedling stage were artificially infested. Only the Non-Bt maize (Control) was affected by the *E. lignosellus* caterpillar, but all the treatments presented tillering, galleries and holes in the stem. In the second assay the genotypes used were seeded on 11/23/2012, and the damages of 3rd instar caterpillars of *E. ligosellus* (Zeller) were evaluated. The treatments were: (1) Conventional Non-Bt maize (Control); (2) transgenic maize expressing the Cry1F + Cry1A.105 + Cry2Ab2 genes; (3) Cry1A.105 + Cry2Ab2; (4) Vip3Aa20; (5) Vip3Aa20 + Cry1Ab; (6) Cry1F; (7) Cry1Ab + Cry1F. The plots were formed by a line spaced in 0.7 m of 2 m, with 10 plants, with barriers to prevent the exit of artificially infested insects. In the first and second assays, non-Bt maize with or without insecticide application were affected by *E. lignosellus* caterpillars. However, Bt transgenic maize was not harmed by *E. lignosellus* caterpillars, except the Vip3Aa20 treatment. Bt transgenic plants were poorly damaged by *E. lignosellus* in the seedling and leaf stage.
Keywords: crop pest, chemical control, genetically modified organism, lepidoptera.

COMPARAÇÃO ENTRE MILHOS TRANSGÊNICOS BT NO
CONTROLE DE *Elasmopalpus lignosellus* EM CAMPO

RESUMO - Os lepidópteros são responsáveis por grandes perdas na produção de milho no Brasil e destacam-se aqueles que atacam plântulas, como a lagarta-elasmo, *Elasmopalpus lignosellus* (Zeller). Este trabalho teve por objetivo comparar o desempenho de milhos transgênicos Bt no controle da lagarta *E. lignosellus* na fase de plântulas da cultura do milho em dois ensaios. No primeiro ensaio foram testados seis tratamentos: (1) milho convencional não transgênico; (2) milho convencional não transgênico com aplicação de inseticidas; (3) milho transgênico os genes Cry1Ab; (4) Cry1F; (5) Cry1A.105 + Cry2Ab2; (6) Cry1A.105 + Cry2Ab2 + Cry1F. O delineamento experimental foi realizado em blocos casualizados, onde cada tratamento foi repetido 4 vezes, em parcelas de 22,5 m^2. Foram infestadas artificialmente 10 plantas consecutivas com lagartas de 3º ínstar de *E. lignosellus* em fase de plântulas. Somente o milho controle não transgênico foi prejudicado pela lagarta *E. lignosellus*, mas todos os tratamentos apresentaram perfilhamento, galerias e furos no colmo. No segundo ensaio os genótipos utilizados foram semeados em 23/11/2012, sendo avaliados os danos de lagartas de 3º ínstar de *E. ligosellus*. Os tratamentos foram: (1) milho convencional não transgênico (Controle); (2) milho transgênico expressando os genes Cry1F + Cry1A.105 + Cry2Ab2; (3) Cry1A.105 + Cry2Ab2; (4) Vip3Aa20; (5) Vip3Aa20 + Cry1Ab; (6) Cry1F; (7) Cry1Ab + Cry1F. As parcelas foram formadas por uma linha espaçada em 0,7 m de 2 m, com 10 plantas, com barreiras para impedir a saída dos insetos infestados artificialmente. No primeiro e no segundo ensaio os milhos não transgênicos com ou sem aplicação de inseticidas, foram afetados pelas lagartas de *E. lignosellus*. Entretanto, os milhos transgênicos Bt não foram prejudicados pelas lagartas de *E. lignosellus,* exceto o tratamento Vip3Aa20. As plantas transgênicas Bt foram pouco danificadas pela *E. lignosellus* na fase de plântulas e nas folhas.
Palavras-chave: praga agrícola, controle químico, transgênico, lepidoptera.

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INTRODUCTION

Brazil is the world's third largest producer of maize in the world, with a approximately 89.2 million ton in 2017/18 (BRASIL, 2017). High production area is given by the agricultural suitability and multiplicity of maize applications, whether in human or animal feeding, also assuming an important socioeconomic role (OLIVEIRA Jr. et al., 2006). Although maize occupies a large area of cultivated land in Brazil, its yield is one of the lowest in the world, but there are several factors that may contribute to low relative productivity (CONAB, 2018).

One of these factors may be related to the number of difficult-to-control pests that, lesser cornstalk borer, *Elasmopalpus lignosellus* (ZELLER, 1848) (Lepidoptera: Pyralidae) one of them, since it is sheltered near or inside the stem, or even in shelters of the web that they construct under the ground, and therefore it becomes a target of difficult reach (ZORZETTI et al., 2017).

The *E. lignosellus* is a polyphagous pest, and larvae attack crops of high economic value, in Brazil, maize, soybean and cotton crops are the main targets of the insect, but can also cause serious damage to crops of rice, sorghum, peanuts, sugar cane and common bean, as well as more than 60 species of cultivated plants (VIANA, 2004; GILL et al., 2010; SULEIMAN, 2010; SANDHU et al., 2011).

The occurrence of this insect is greater in sandy soils and in dry periods after the first rains. The larvae damage newly sprouted plants, initially causing damage by feeding on the leaves and then penetrating the bottom of the stem, close to the ground. Thereafter they cause damage due to the formation of galleries at the top of the corn plant, thus leading to the destruction of the apical bud, causing new leaves to dry and die, resulting in the so-called "dead heart" , which is used as pest monitoring (GALLO et al., 2002; MARTINS, 2009).

Data on losses caused by soil pests are few, but it is estimated that *E. lignosellus* in maize can cause losses ranging from 20% to total destruction of the crop, in high infestation condition. The most commonly control method used for *E. lignosellus* in Brazil is the preventive chemical control and seed treatment (VIANA, 2009). However, when chemical insecticides are applied indiscriminately, they can result in contamination of living organisms and environmental imbalance, leading to an increase in the pest population, including secondary insect pests (DEGUINE et al., 2009).

In the concept of integrated management, the goal is not simply to annihilate the pest, the most important is to reduce the population to a limit, compatible with the economic production of the crop and the consequent maintenance of the environmental quality (CRUZ, 1995). Therefore, the biological control of pests has increased its importance in Brazil for maize, and the use of bacteria, such as *Bacillus thuringiensis* (Berliner) (SILVA-WERNECK et al., 2000). The Entomopathogenic bacteria, such as *B. thuringiensis*, are among the alternatives to reduce the use of insecticides for pest control.

As a bioinsecticide, the bacterium *B. thuringiensis*, strain HD1, has been used for decades and is registered without limitation of use for the control of several species of Lepidoptera pests. One of the active fractions produced by *B. thuringiensis* Bt are proteins accumulated in the form of crystals inside the cells, called "cry", that can constitute more than 30% of the total proteins of the cell (FEITELSON et al., 1992, HERMSTADT et al., 1986, VIDAL-QUIST et al., 2009).

With the advent of biotechnology, a new pest control tactic was developed that consists of genetically modified (transgenic) insect resistant plants. By means of accurate laboratory techniques, a Bt gene was introduced into maize plants, giving rise to the genetically modified maize, conferring a high resistance standard of the plant to some species of lepidopteran pest (ARMSTRONG et al. 1995). The gene introduced encodes the expression of Bt proteins, with insecticidal action, effective in controlling lepidoptera such as *S. frugiperda*, as well as coleopterans and dipterans (HUANG et al., 2002; PARDO-LÓPEZ et al., 2013).

The caterpillars, feeding on the foliar tissue of genetically modified maize, ingest this protein, which acts on the epithelial cells of the digestive tract of insects. The protein promotes the osmotic breakdown of these cells, determining the death of the insects, before they can damage the culture (GILL, 1995; MEYERS et al., 1997).

Therefore, the aim of this work was comparing the performance of transgenic Bt maize in the control of the *E. lignosellus* caterpillar in the maize seedlings stage.

MATERIAL AND METHODS
Site locations and insect pest source

The two field assays were conducted at the Moura Lacerda University Center (Ribeirão Preto, São Paulo State, Brazil). The 3rd instar *E. lignosellus* caterpillars, used in the assays for artificial infestations were obtained from laboratory colonies maintained by Bug Agentes Biológicos (Charqueada, São Paulo State). The caterpillars were kept in artificial diet adapted to the species. All insect colonies were reared on artificial diet and maintained in a room with controlled conditions of temperature (25 ± 3°C), relative humidity (60 ± 5%) and photoperiod [14:10 (L:D) h].

First insect infestation

The first field assay was sown on January 31, 2012, with spacing of 75 cm between rows and maintaining five plants per meter, after thinning carried out on 02/13. The experimental design was with randomized block design, in which eight treatments were repeated four times in experimental plots of 3.75 (6 rows) x 6 m (22.5 m^2). A urea cover fertilization was performed at 80 kg ha^{-1} (03/05) and the weeds were controlled with manual weeding. The first assay was carried out with 6 treatments and the non-Bt isogenic maize hybrid (iso-hybrid) of the same genetic background was used as control (Table 1).

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The treatment where Non-Bt maize used insecticides to control caterpillars (2) was sprayed with spinosad (Tracer, 24 g i.a. ha^{-1}) on 02/20 and 03/01, and methomyl (Lannate BR, 129 g i.a. ha^{-1}) on 03/12. After germination, 10 consecutive seedlings of each plot were individually wrapped by a PVC tube 9 cm in diameter and 20 cm in height. The tube was pressed lightly so that it was buried 2 cm in the soil, thus forming a barrier around each seedling. The soil around the seedling was covered with a thin layer of vermiculite (less than 1 cm) and at 10 days after sowing an artificial infestation was performed with two caterpillars of 3rd instar per seedling, the dead caterpillars with no apparent reason up to the limit of two days after infestation were replaced when necessary.

TABLE 1 - Treatments (maize hybrids with the expressed Bt proteins if applicable), and corresponding Bt events.

Treatments	Event(s)
Non-Bt maize Iso-hybrid (Control)	None
Non-Bt maize with insecticides	None
Cry1Ab	MON810[a]
Cry1F	TC1507[b]
Cry1A.105 + Cry2Ab2	MON89034[c]
Cry1F+ Cry1A.105 + Cry2Ab2	TC1507 x MON89034 x NK603[d]

[a]Event MON810 expresses Cry1Ab + CP4EPSPS + GOXV247 proteins that confers glyphosate herbicide tolerance, Monsanto Company, St. Louis, MO. [b]Event TC1507 expresses Cry1F and PAT proteins. PAT protein confers glufosinate herbicide tolerance, Dow AgroSciences, Indianapolis, IN. [c]Event MON 89034 expresses Cry1A.105 + Cry2Ab2 proteins, Monsanto Company, St. Louis, MO. [d]Event NK603 expresses CP4EPSPS protein that confers glyphosate herbicide tolerance, Monsanto Company, St. Louis, MO.

Evaluations were performed at 3 (02/13), 7, 14 and 28 days after artificial infestation. In the three initial evaluations, plants were noted for the presence of the "dead heart" symptom or if they were partially damaged. At the 28-day evaluation, all plants were minutely observed to be recorded if they were dead with a "dead heart" symptom or if tiller had been emitted, galleries were present in the stem and/or if holes were caused by caterpillars.

Second insect infestation

The second field assay was sown on November 23, 2012, spacing 0.7 m between rows and maintaining five plants per meter, after thinning performed on 12/02/2012. The experimental design was a randomized block design, in which seven treatments were repeated four times in experimental plots of 4.2 (6 rows) x 6.0 m (25.2 m2) being kept 0.70 m border cleaned. An ammonium sulphate cover fertilization of 500 Kg ha^{-1} equivalent (01/06/2013) was carried out and the weeds were controlled with the herbicide 2,4-dichlorophenoxyacetic (2,4-D). The second trial was carried out with 7 treatments and the non-Bt isogenic maize hybrid (iso-hybrid) of the same genetic background was used as control (Table 2).

TABLE 2 - Treatments (maize hybrids with the expressed Bt proteins if applicable), and corresponding Bt events

Treatments	Event(s)
Non-Bt maize Iso-hybrid	None
Cry1F	TC1507[a]
Cry1Ab + Cry1F	TC1507 x MON810[b] x NK603
Cry1F+ Cry1A.105 + Cry2Ab2	TC1507 x MON89034 x NK603[c]
Vip3Aa20	MIR162[d]
Vip3Aa20 + Cry1Ab	Bt11[e] x MIR162 x TC1507 x GA21[f]
Cry1A.105 + Cry2Ab2	MON89034[g]

[a]Event TC1507 expresses Cry1F and PAT proteins. PAT protein confers glufosinate herbicide tolerance, Dow AgroSciences, Indianapolis, IN. [b]Event MON810 expresses Cry1Ab + CP4EPSPS + GOXV247 proteins that confers glyphosate herbicide tolerance, Monsanto Company, St. Louis, MO. [c]Event NK603 expresses CP4EPSPS protein that confers glyphosate herbicide tolerance, Monsanto Company, St. Louis, MO. [d]Event MIR162 expresses Vip3Aa20 protein, Syngenta, Research Triangle Park, NC. [e]Event Bt11 expresses Cry1Ab and PAT proteins. Syngenta, Research Triangle Park, NC. [f]Event GA21 expresses MEPSPS protein that confers tolerance to glyphosate herbicides, Monsanto Company, St. Louis, MO. [g]Event MON 89034 expresses Cry1A.105 + Cry2Ab2 proteins, Monsanto Company, St. Louis, MO.

After germination, 10 consecutive seedlings of each plot were individually wrapped by a PVC tube 9 cm in diameter and 20 cm in height. The tube was pressed lightly so that it was buried 2 cm in the soil, thus forming a barrier around each seedling. The soil around the seedling was covered with a thin layer of sand (less than 1 cm) and at 10 days after sowing, an artificial infestation was performed with two *E. lignosellus* caterpillars of 3rd instar per seedling. The dead caterpillars with no apparent reason until two days after infestation were replaced when

necessary. The infestations were carried out in the morning, placing the caterpillars at the base of the seedlings.

Evaluations were performed at 7 (12/09), 10, 14, 21 and 28 days after artificial infestation. In the five evaluations, the plants were observed noting if they presented the symptoms of "dead heart", emission of tiller without affecting the development of the plant and emission of tiller affecting the growth of the plant.

Statistical analysis

All data were submitted to analysis of variance (ANOVA). When the F-test of ANOVA indicated a significance of 5% of error probability, the complementary analyzes were carried out by means of the Tukey test, at 5% of probability, where the averages were compared. All statistical calculations were performed by Statistica for Windows (STATSOFT, 1996)

RESULTS AND DISCUSSION
First insect infestation result

The treatments with Bt transgenic maize presented different responses to the infestations of *E. lignosellus* caterpillars. At the 3rd day after the artificial infestation, only Non-Bt maize treatment (Control) presented damage caused by caterpillars, not significantly different from the other treatments, and there were no dead seedlings with a "dead heart" symptom.

However, at 7 days after infestation, more than 10% of the plants were dead in the Non-Bt maize treatments, and the one where the insecticides were still applied had the highest average percentage of dead plants, differing only from transgenic treatments (Figure 1).
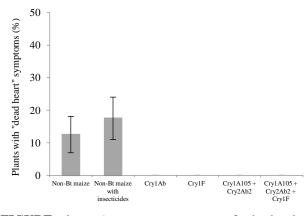

FIGURE 1 - Average percentage of dead plants manifesting the symptom "dead heart", in different transgenic Bt or conventional maize after 7 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *There were no significant differences between the Tukey test (p≤0.05).

On the same date, more than 20% of Non-Bt maize plants had partial damage caused by caterpillars, both differing from transgenic treatments (Figure 2). At 14

days after artificial infestation, the results for "dead heart" were still similar to those of the previous date, although numerically the average percentage of dead or partially damaged plants was higher in Non-Bt maize without application of insecticides (Figures 3 and 4). The Non-Bt maize treatment with chemical control had already undergone spraying with insecticide.
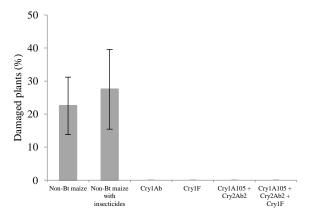

FIGURE 2 - Average percentage of partially damaged seedlings, in different transgenic Bt or conventional maize after 7 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).
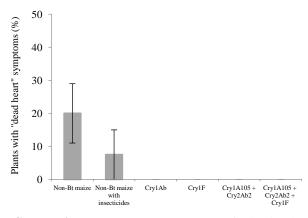

FIGURE 3 - Average percentage of dead plants manifesting the symptom "dead heart", in different transgenic Bt or conventional maize after 14 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).
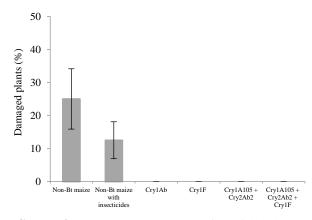
FIGURE 4 - Average percentage of partially damaged seedlings, in different transgenic Bt or conventional maize after 14 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).

FIGURE 6 - Average percentage of plants with emission of tiller, in different transgenic Bt or conventional maize after 28 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).

In the last evaluation, at 28 days after infestation, there were no significant differences between the treatments in the average percentage of dead plants (Figure 5), plants with tillering (Figure 6), with galleries in the stem (Figure 7) and the average number of holes in the stem (Figure 8), which did not exceed 0.4 holes per plant.
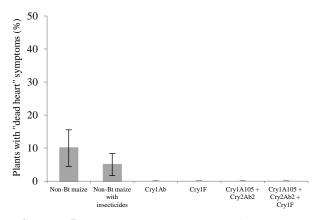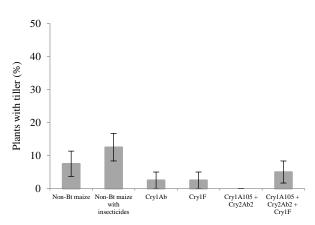

FIGURE 5 - Average percentage of dead plants manifesting the symptom "dead heart", in different transgenic Bt or conventional maize after 28 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).
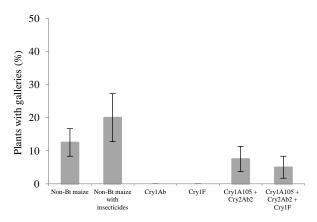

FIGURE 7 - Average percentage of plants with galleries, in different transgenic Bt or conventional maize after 28 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).

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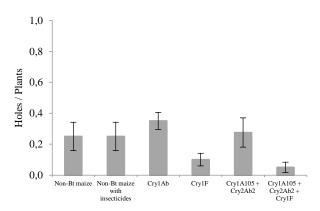

FIGURE 8 - Average number of holes in the plants caused by the caterpillar in different transgenic Bt or conventional maize after 28 days of the artificial infestation with 3rd instar caterpillars of *Elasmopalpus lignosellus* in the "safrinha" maize at Ribeirão Preto (São Paulo State, Brazil). *Mean values with followed by different letters were significantly different by Tukey's test (p≤0.05).

The tillering occurred in almost all treatments, except for Cry1.A105 + Cry2Ab2 (Figure 6). The treatments Cry1Ab and Cry1F did not show galleries in the stalks and the others did not reach 15% of the plants with galleries (Figure 7).

Second insect infestation result

After 7 days of infestation only Non-Bt maize (Control) and the transgenic Vip3Aa20 treatments showed "dead heart" symptoms, differing significantly from the other treatments (Table 3). On the other evaluation dates, the results were repeated, with Non-Bt maize and Vip3Aa20 treatments presenting, respectively, 40.0 ± 17.3 and $32.5 \pm 12.5\%$ of plants with a "dead heart" symptom at 28 days after infestation (Table 3). There was a harmful tillering to the plant only at 15 days after infestation in Vip3Aa20 + Cry1Ab treatment, $2.5 \pm 2.5\%$, without significant difference between treatments.

TABLE 3 - Average percentage of plants with "dead heart" symptoms due to feeding of *Elasmopalpus lignosellus* in Bt or Non-Bt maize plants.

Treatments	Days after infestation				
	7	10	14	21	28
Non-Bt maize (control)	32,5±10,3 b [*]	37,5±14,9 b	40,0±17,3 b	40,0±17,3 b	40,0±17,3 b
Cry1F	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a
Cry1Ab + Cry1F	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a
Cry1F+ Cry1A.105 + Cry2Ab2	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a
Vip3Aa20	30,0±12,9 b	32,5±12,5 b	32,5±12,5 b	32,5±12,5 b	32,5±12,5 b
Vip3Aa20 + Cry1Ab	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a
Cry1A.105 + Cry2Ab2	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a	0,0± 0,0 a

*Means followed by the same letter in the column do not differ by Tukey test (p≤0.05).

Ivan et al. (2012) also compared transgenic maize which expressed the Cry1F + pat + Cry1A.105 + Cry2Ab2 + cp4 + EPSPS, Cry1Ab, Cry1F + pat, Cry1A.105 + Cry2Ab2 genes and Non-Bt maize for the attack of *E. lignosellus* and verified that only the Non-Bt maize, with or without applications of insecticides, were damaged by the pest.

There are few studies that tested transgenic Bt maize on the control of *E. lignosellus*. However, the results obtained agree with Vilella et al. (2002), who verified resistance of Bt transgenic maize expressing the toxins Cry1Ab, Cry1Ac, Cry1F and Cry9C to the *E. lignosellus* caterpillar, in the laboratory.

The damage caused by *E. lignosellus* on seedlings could be tested by varying the number of caterpillars per plant, in order to evaluate the pressure that this lepidopterus exerts on these technologies. The impact of different transgenics on non-target organisms, such as bees and soil surface organisms, could be evaluated in future assays in larger plots. Therefore, almost all transgenic maize tested were not damaged by *E. lignosellus* caterpillar, except Vip3Aa20.

CONCLUSIONS

The Non-Bt maize treatment with or without insecticide application were affected by the *E. lignosellus*

caterpillar in the two trials. However, Bt transgenic maize was not harmed by *E. lignosellus* caterpillars, except for the Vip3Aa20 treatment. Bt transgenic maize plants were little damaged by *E. lignosellus* in the seedling and leaf phase.

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