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A 6-year field monitoring of fall armyworm, *Spodoptera frugiperda,* in transgenic Bt maize in Brazil

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ABSTRACT

A 6-year field monitoring study was designed to compare the presence of fall armyworm *Spodoptera frugiperda* (J. E. Smith), leaf injury, its parasitoids, 100 seed weight, and seed yield of Bt maize hybrids approved for commercialization in the Brazilian market. Field trials were planted in two municipalities, divided into two periods, from 2011 to 2014 (period I), and due to the approval of different Bt hybrids, from 2015 to 2016 (period II). Treatments were Bt, non-Bt maize and all non-Bt maize sprayed with insecticide methomyl. Six Bt-maize hybrids were planted, expressing the following proteins: Cry1Ab, Cry1F, Cry1A.105 (Cry1Ab +Cry1Ac +Cry1F), Cry2Ab2a and Vip3. In 2015 and 2016 Bt Powercore was planted due to its commercial approval. The number of S. frugiperda larvae for most Bt hybrids was lower than on non-Bt hybrids, except for Fórmula TL and Herculex in period II, in both municipalities. In period I, Bt hybrids VT, VT 2 and Viptera showed almost no injury on their leaves, despite the high number of fall armyworm larvae found in plants. Different parasitoids emerged from larvae from Bt and non Bt hybrids: *Archytas* sp., *Campoletis* sp., *Chelonus* sp., *Eiphosoma* sp. and *Ophion luteus*. The average 100 seed weight was higher in municipality from Sete Lagoas for all Bt hybrids, non-Bt hybrids and non-Bt hybrids sprayed with methomyl, when compared to Nova Porteirinha during period I. Fórmula TL, non-Bt Fórmula TL sprayed with methomyl, and non-Bt Fórmula TL showed the lowest seed yield in both municipalities and periods.

Introduction

Maize (*Zea mays* L.) is an important cereal crop cultivated in many parts of the world and may be used for human and animal diet. Brazil is the third largest maize producer in the world, with an area of 17.894.2 million hectares and a total production of 100,083 ton. (CONAB, 2020). Maize is attacked by many important insect pests and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is the most important pest of maize in Brazil (Cruz, 1995; Valicente, 2015). Transgenic maize was planted on 16.3 million hectares with an 89% adoption rate (https://www.sna.agr.br/eua-e-brasil-continuam-a-liderar-a-producao-de-transgenicos-no-mundo), however, if Bt maize is not used, fall armyworm control is achieved essentially with chemical pesticides.

Transgenic crops expressing *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) (Bt) proteins are planted worldwide, and most of them are resistant to herbicides and insects, or both (Parisi et al., 2016; Abbas, 2018). Transgenic Bt maize is an important tool in Integrated Pest Management (IPM) programs. Crop damage due to insect pests and diseases could account for up to 35% of total losses (Pardo-Lopéz et al., 2013), and *S. frugiperda* may account for 34 to 52% of yield loss in Brazil (Valicente, 2015). *Bacillus thuringiensis* maize was first approved for commercial use in Brazil in 2008/2009 and important insect pests have been controlled using this technology, decreasing the use of chemical insecticides (Storer et al., 2010, 2012). *Bacillus thuringiensis cry* and *vip* genes have been cloned and its proteins have been expressed in maize, promoting excellent field control of lepidopterans pests in the field. Brazil is the second largest grower of biotech crops in the world after the US, and these data are impressive because Brazil is essentially a tropical climate, with many new regions adapted for agriculture where the growing period is extended to include 3 different crops each year (Céleres, 2015; ISAAA, 2020).

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The agricultural landscape in Brazil is changing fast and insects have “green food” available all year round, due to the occurrence of a “green bridge” among the most important crops in this system: cotton (*Gossypium hirsutum*), maize (*Zea mays*) and soybeans (*Glycine max*). Some important concerns should be addressed because fall armyworm feeds on these crops which express some of the same *Bt* proteins (Cry1Ab, Cry1F, Cry1Ac, Cry2Ab2, and Vip3Aa20). The presence of this insect in these Bt crops all year round may influence this insect to develop resistance to Bt proteins in the field.

The presence of non-target organisms (NTO) are important tools in the ecosystem and in IPM. Most of these NTO are natural enemies and may feed on hosts that are directly or indirectly exposed to Bt proteins. The possible impact of these Bt crops with these NTO are extremely important (Ludy and Lang, 2006; Romeis et al., 2006, 2020; Arpaia et al., 2017), and may be monitored because some potential adverse effects of Bt maize could affect the presence of NTO in Bt crops.

Field monitoring of pests and natural enemies’ information is scarce after commercial approval for the market (Kleppin et al., 2011; Sanvido et al., 2011). Data about genetically modified (GM) crops are needed for consumers to better understand GM biotechnology. This 6-year field monitoring aimed to verify the presence of fall armyworm and its parasitoids, leaf injury, 100 seed weight and seed yield for all Bt maize hybrids approved for commercialization in the Brazilian market.

Materials and methods

Field trials were planted in two municipalities: Sete Lagoas at Embrapa Maize and Sorghum Research Center, Minas Gerais State (MG) (SL; 19°27’15.25” S. lat.; 44°10’23.75”O. long.) and in Nova Porteirinha (Embrapa research area), MG (NP; 15°45’28.34”S. lat.; 43°17’10.92”O. long.) Brazil for 6 years.

Experimental lay out was a randomized block design with four blocks and four replications. Plots were 10 m in length with 0.7-m row spacing, and 10m in width with a 50 seed planting density, with five seed per linear meter. Each treatment was planted in the same plot for 6 years. Treatments included Bt, non-Bt and non-Bt maize sprayed with methomyl. The treatment list, was as follows:

	Treatment specifications
T1	DKB 390 YieldGard (expressing Cry1Ab protein, abbreviated product ID YG)
T2	Bt11 (Fórmula TL expressing Cry1Ab)
T3	Herculex (Morgan 20A78Hx - expressing Cry1F protein - HX)
T4	DKB 390 Pro (Cry1A.105, Cry1Ab + cry1Ac and Cry1F and Cry2Ab2 - VT)
T5	DKB390 Pro2 (Cry1A.105, Cry2Ab2 and glyphosate tolerance - VT2)
T6	Impacto Viptera (expressing Vip protein - Viptera)
T7	Non-Bt DKB 390 + methomyl
T8	Non-Bt Fórmula TL + methomyl
T9	Non-Bt HX+ methomyl
T10	Non-Bt DKB 390 + methomyl
T11	Non-Bt DKB 390 + methomyl
T12	Non-Bt Viptera + methomyl
T13	Non-Bt DKB 390
T14	Non-Bt Fórmula TL
T15	Non-Bt HX
T16	Non-Bt DKB390
T17	Non-Bt DBK 390
T18	Non-Bt Viptera

DKB 390 was the non-Bt hybrid for YG, VT and VT 2 Bt hybrids, so it was included 3 times in the experiment. Insecticide used was methomyl (Lannate) at 215 g a.i. (active ingredient)/L and was applied with a sprayer equipped with a flat fan nozzle at spray angle of 65 degrees. The spray was applied at 120L/ha for all non-Bt treatments. The first methomyl application was done one week before the first fall armyworm evaluation and the second application was made one week before the second evaluation.

In 2015 and 2016, Bt Powercore was approved and planted (Cry1F, Cry1A.105, Cry2Ab2 – PC) during the period of this field experiment. The replacement in treatments is due to the commercial approval of Bt maize by CTNBio (National Technical Biosafety Committee). From 2016 to 2020 many Bt maize hybrids were not found in the market such as: YG, Bt11 and HX, and some new traits were approved for commercialization. Therefore, from 2011 to 2014 it was denominated period I, and from 2015 to 2016 period II.

Fall armyworm

The presence of armyworm larvae was evaluated by randomly sampling 30 maize plants per replicate, 120 plants per treatment twice during the growing season. This resulted in a total of 240 maize plants per season. The plants were harvested and put in plastic bags, transferred to the laboratory, and were evaluated for the presence of fall armyworm. The first sampling was 15-20 days (V1/V2) and the second 28 to 32 (V3/V4) days after plant emergence. All larvae found on the plants, regardless of their size, were counted and individually placed in 50 ml plastic cups with artificial diet (Valicente & Barreto 2003), sealed with an acrylic lid. Larvae were observed daily for detection of the emergence of parasitoids. The percentage of parasitism was calculated by dividing the number of parasitized larvae by the total number of larvae collected/treatment. Parasitoids were identified by species when possible.

The leaf injury rating developed by Davis et al. (1992) was adapted to a simpler scale rating, using a modified version where: 0 - no leaf injury, 1 - leaf injuries (small lesions) by first instar larvae that feed superficially on one side of the leaf, 2 - with elongated lesions (holes) on the leaves, 3 - when holes are observed on the leaves and in the whorls, 4 - when the caterpillars destroy the whorl of the maize plant and 5 - maize plants with many leaves and whorl destroyed (Mendes et al., 2008; Toepfer et al., 2021). Scores of 1 and 2 were considered low damage to the leaves, 3 was considered moderate, and 4 and 5 were considered high. Foliar damage ratings were summarized for all plants of the 2 central rows within each plot. The evaluations were performed from 2011 to 2014 (period I) and from 2015 to 2016 (period II) due to different Bt maize approved for commercial use in Brazil (e.g., Powercore - PC).

Plant parameters

The number of plants per treatment, number of ears, average ear size and weight, and average 100 seed weight (grams) were also recorded. These were considered as parameters that are characteristic of a specific genetic material, and an indicator of a general grain quality, seed yield (Kg) of all ears collected/ treatment.

Statistical analyses

Total number of larvae, percentage of larvae parasitism, average leaf injury rating, average 100 seed weight and seed yield were subjected to analysis of variance (ANOVA) and means were compared by the Scott-Knott test at 5% probability ($p \leq 0.05$), SISVAR program (Ferreira, 2011). Treatments, municipality, and year were modeled as fixed effects. The interactions between treatment x municipality, treatment x year and municipality x year were analyzed separately for each independent variable is shown as Supplementary Material. However, they are important for understanding the results.

Results

Fall armyworm

Overall, during period I, the number of fall armyworm larvae collected in Nova Porteirinha differed from Sete Lagoas ($F_{1,520} \geq 16.44$, $p < 0.0001$) (Fig. 1). During period I, the average number of larvae for most Bt hybrids (average of 48.75 larvae for YG; 39.19 larvae for Fórmula TL; 29.88 larvae for HX and 8.81 larvae for VT), and for all non-Bt hybrids tested in Nova Porteirinha (YG, $F_{1,520} = 3207.38$, $p < 0.0001$; Fórmula, $F_{1,520} = 1793.71$, $p < 0.0001$; HX, $F_{1,520} = 993.38$, $p < 0.0001$; VT, $F_{1,520} = 81.51$, $p < 0.0001$), was significantly higher than the average number of larvae in Sete Lagoas (Fig. 1). For period I in Nova Porteirinha, the average number of fall armyworm larvae collected in all non-Bt hybrids was higher than in all other treatments, with an average of 70.13 larvae for non-Bt VT ($F_{1,520} = 4787.48$, $p < 0.0001$). However, the average number of larvae found in Viptera (3 larvae) was lower ($F_{1,520} = 10.77$, $p = 0.0011$) when compared to all Bt hybrids and non-Bt hybrids and did not differ from the non-Bt hybrids sprayed with methomyl (Fig. 1).

For period II, the average number of fall armyworm collected in Bt hybrids was higher in Nova Porteirinha (HX, $F_{1,251} = 24.85$, $p < 0.0001$; Fórmula TL, $F_{1,251} = 18.78$, $p < 0.0001$) than in Sete Lagoas (Fig. 2). Larval numbers for HX (average of 104 larvae) and Fórmula TL (average of 92.5 larvae) were at least 3 times higher than all other Bt hybrids in Sete Lagoas (Fig. 2). All non-Bt hybrids in Nova Porteirinha showed a higher average number of larvae, at least 2.8 times, than the numbers of larvae collected in Sete Lagoas. Bt hybrids HX and Fórmula TL, and all other non-Bt hybrids were different from all other treatments in Nova Porteirinha ($F_{17,251} = 15.89$, $p < 0.0001$) and Sete Lagoas ($F_{17,251} = 2.00$, $p = 0.0118$) (Fig. 2).

During period I, the average number of larvae collected in all non-Bt hybrids and DKB 390 YG was higher in 2011 than what was collected in all other years, except for Herculex (2013 and 2014). For period II, the average number of larvae was higher in 2015 (Table S1, S2 and S3 showed in Supplementary Material).

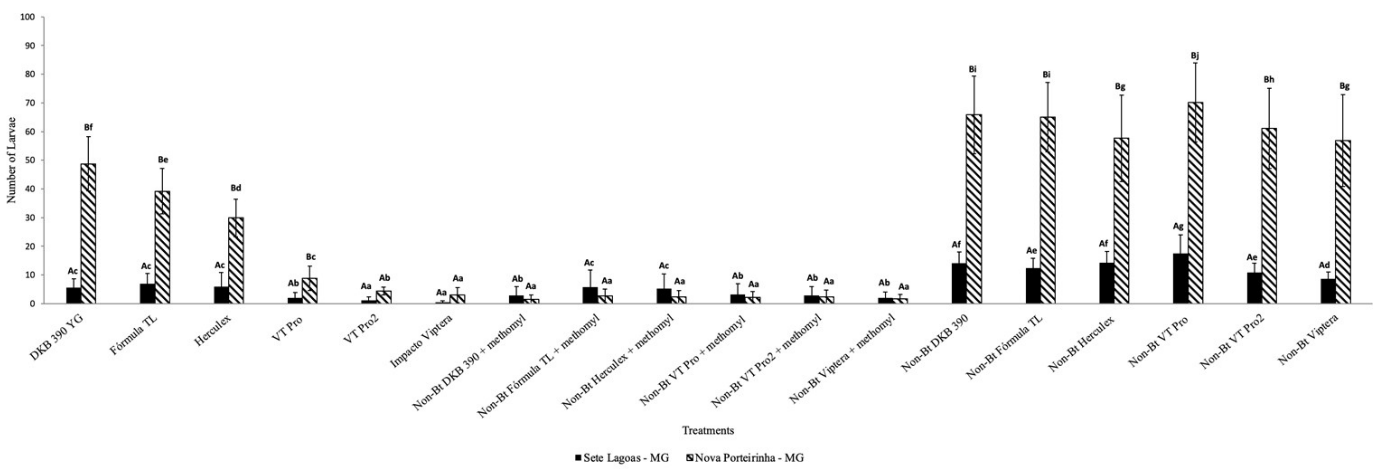

Figure 1 Average number of fall armyworm larvae, *Spodoptera frugiperda* collected in Bt hybrids, non-Bt hybrids and non-Bt hybrids sprayed with methomyl from 2011 to 2014 (period I), in Sete Lagoas and Nova Porteirinha. Average followed by the same upper-case letter between municipalities and same lower-case letter in each municipality do not differ statistically (Scott-Knott test at p ≤ 0.05).

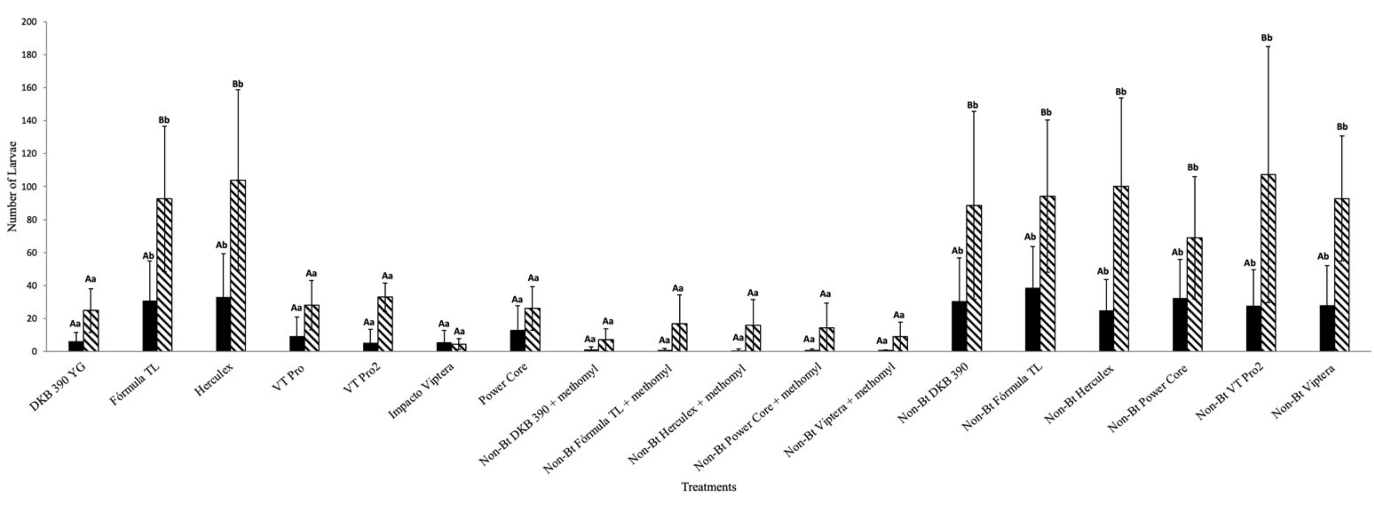

Figure 2 Average number of fall armyworm larvae, Spodoptera frugiperda, collected in Bt hybrids, non-Bt hybrids and non-Bt hybrids sprayed with methomyl in 2015 and 2016 (period II), in Sete Lagoas and Nova Porteirinha. Average followed by the same upper-case letter between municipalities and same lower-case letter in each municipality do not differ statistically (Scott-Knott test at p ≤ 0.05).

Leaf injury ratings

The average leaf-feeding injury ratings caused by fall armyworm during period I to all Bt hybrids in Nova Porteirinha was higher (Fórmula TL 1.58) than in all Bt hybrids in Sete Lagoas (Fórmula TL 0.82) ($F_{1,520}$ = 205.08, p < 0.0001), except for VT, VT 2 and Viptera (VT, $F_{1,520}$ = 0.016, p = 0.9005; VT 2, $F_{1,520}$ = 0.045, p = 0.8312; Viptera, $F_{1,520}$ = 0.001, p = 0.9728) (Table 1). These Bt hybrids showed almost no injury on their leaves, with average rating of 0.16 for VT; 0.11 for VT 2 and 0.03 for Viptera, despite the high number of fall armyworm larvae present in the area. For the non-Bt hybrids in period I, the average leaf-feeding injury rating in Nova Porteirinha (average leaf injury rating 2.46 for non-Bt VT) was higher than the ratings of all non-Bt hybrids in Sete Lagoas (average leaf injury rating of 1.15 for for non-Bt Viptera) ($F_{1,520}$ = 205.08, p < 0.0001) (Table 1). When all treatments were considered, VT, VT 2 and Viptera showed lower injury levels than all other treatments in Nova Porteirinha ($F_{17,520}$ = 6.26, p < 0.0001) and Sete Lagoas ($F_{17,520}$ = 25.83, p < 0.0001) (Table 1).

For period II, the average leaf-feeding injury caused by fall armyworm to Bt hybrids was higher in Nova Porteirinha with rating of 1,10 for YG; 2,05 for Fórmula TL; 2.05 for HX and 0.97 for VT 2, than in Sete Lagoas with rating of 0.25 for YG; 0.68 for Fórmula TL; 0.71 for HX and 0.22 for VT 2 (YG, $F_{1,251}$ = 5.20, p = 0.0234; Fórmula, $F_{1,251}$ = 13.35, p = 0.0003; HX, $F_{1,251}$ = 12.87, p = 0.0004; VT 2, $F_{1,251}$ = 4.05, p = 0.0452), except for VT, Viptera and Power Core (Table 2). Viptera in Nova Porteirinha (average leaf injury rating 0,03 for Viptera), showed almost no injury on the leaves, and it was different from all other treatments ($F_{17,251}$ = 7.18, p < 0.0001). In Sete Lagoas the number of larvae present in this field experiment was incredibly low, and there was no difference among any treatments regarding the leaf injury level ($F_{17,251}$ = 0.69, p = 0.8110) (Table 2).

The years 2011, 2012 and 2014 (in period I) showed a lower injury level when compared to year 2013, except for Fórmula TL, Herculex and non-Bt Viptera. For period II the average leaf-feeding injury ratings caused by fall armyworm was higher in year 2015 (Table S4, S5 and S6 showed in Supplementary Material).

Parasitoids

The most frequent parasitoids that occurred in both municipalities were: *Archytas sp. (Diptera:Tachinidae), Campoletis sp. (Hymentoptera: Ichneumonidae), Chelonus sp. (Diptera: Braconidae), Eiphosoma sp. (Hymenoptera: Ichneumonidae), Ophion luteus (Ichneumonidae),* nematodes and some parasitoids that didn´t emerge as adults. For period I, the average percentage of parasitism of larvae in Nova Porteirinha, for most Bt hybrids tested, was higher than in Sete Lagoas ($F_{1,520}$ = 6.33, p = 0.0121), with an average high of 49.76% in larvae collected in HX, except for YG, VT 2 and Viptera with an average low of 6.25% (Table1). Average percentage of parasitism, among non-Bt hybrids, also was higher ($F_{1,520}$ = 6.33, p = 0.0121) in Nova Porteirinha with an average of 40.01% for HX, than the parasitism in *S. frugiperda* found in Sete Lagoas, except for non-Bt DKB390 and non-Bt VT (Table 1). In Sete Lagoas, for period I, the average parasitism of larvae found was lower ($F_{17,520}$ = 14.30, p < 0.0001) in Viptera and HX with an average of 14.06% and 15.58% of parasitism, respectively, when compared to parasitism of all other Bt hybrids. Larvae collected in Viptera showed a low average parasitism of 6.25% in Nova Porteirinha when compared to larvae collected in all other Bt and non-Bt hybrids, except for non-Bt hybrids sprayed with methomyl that showed an average percentage of parasitism of 0% (Table 1).

For period II in Nova Porteirinha, larvae collected in Bt hybrids showed a higher average of parasitism for VT 2 (16.73%), PC (19.28%) and VT (24.57%) when compared to Sete Lagoas (VT 2, $F_{1,251}$ = 6.74, p = 0.0099; PC, $F_{1,251}$ = 7.14, p = 0.0080; VT, $F1,251$ = 12.24, p = 0.0005) (Table 2). Average parasitism was low ($F_{17,251}$ = 2.64, p = 0.0006) for all Bt hybrids tested in Sete Lagoas during period II, for VT (2.61%), VT 2 (0.43%), Viptera

Table 1
Average leaf injury rating caused by *Spodoptera frugiperda*, parasitism of fall armyworm larvae, 100 weight seed and seed yield during period I (2011 to 2014), in Sete Lagoas (SL) and Nova Porteirinha (NP).

Treatments	Leaf injury				Parasitism				Weight of 100 seeds (g)				Seed yield/Kg			
	SL		NP		SL		NP		SL		NP		SL		NP	
DKB 390 YG	0.90	Ab[1]	1.57	Bb[1]	27.67	Ab[2]	32.17	Ab[2]	32.50	Bc[3]	21.59	Ab[3]	11.39	Ac[4]	10.57	Ac[4]
Fórmula TL	0.82	Ab	1.58	Bb	22.39	Ab	39.12	Bb	23.63	Ba	17.59	Aa	6.43	Aa	7.64	Ab
Herculex	0.80	Ab	1.28	Bb	15.58	Aa	49.76	Bb	30.84	Bc	19.32	Aa	11.54	Ac	10.62	Ac
VT Pro	0.19	Aa	0.16	Aa	20.00	Ab	34.80	Bb	33.27	Bc	21.23	Ab	11.45	Ac	10.80	Ac
VT Pro2	0.16	Aa	0.11	Aa	26.52	Ab	28.45	Ab	31.10	Bc	19.89	Aa	12.55	Bc	10.17	Ac
Impacto Viptera	0.04	Aa	0.03	Aa	14.06	Ba	6.25	Aa	29.09	Bc	17.96	Aa	9.67	Bb	6.89	Aa
Non-Bt DKB 390 + methomyl	1.05	Ab	1.69	Bb	15.68	Ba	0.00	Aa	32.45	Bc	21.85	Ab	11.63	Ac	10.72	Ac
Non-Bt Fórmula TL + methomyl	1.06	Ab	2.06	Bc	7.79	Ba	0.00	Aa	24.02	Ba	19.03	Aa	6.35	Aa	6.74	Aa
Non-Bt Herculex + methomyl	1.03	Ab	1.89	Bb	14.80	Ba	0.00	Aa	31.28	Bc	19.78	Aa	12.29	Bc	10.72	Ac
Non-Bt VT Pro + methomyl	1.12	Ab	1.78	Bb	10.01	Ba	0.00	Aa	31.76	Bc	21.49	Ab	11.06	Ac	10.74	Ac
Non-Bt VT Pro2 + methomyl	1.02	Ab	1.84	Bb	10.97	Ba	0.00	Aa	31.57	Bc	20.95	Ab	12.39	Bc	10.01	Ac
Non-Bt Viptera + methomyl	0.78	Ab	1.52	Bb	7.34	Ba	0.00	Aa	30.94	Bc	18.85	Aa	11.57	Bc	8.86	Ac
Non-Bt DKB 390	1.20	Ab	2.42	Bc	32.97	Ab	38.44	Ab	31.75	Bc	20.73	Ab	11.37	Bc	9.91	Ac
Non-Bt Fórmula TL	1.16	Ab	2.42	Bc	30.34	Ab	36.76	Bb	25.13	Ba	18.89	Aa	6.34	Aa	6.08	Aa
Non-Bt Herculex	1.16	Ab	2.29	Bc	14.24	Aa	40.01	Bb	31.70	Bc	19.86	Aa	12.02	Bc	10.02	Ac
Non-Bt VT Pro	1.40	Ab	2.46	Bc	30.90	Ab	35.48	Ab	32.59	Bc	20.77	Ab	11.49	Ac	10.25	Ac
Non-Bt VT Pro2	1.18	Ab	2.35	Bc	23.07	Ab	38.09	Bb	30.69	Bc	19.77	Aa	12.51	Bc	9.86	Ac
Non-Bt Viptera	1.15	Ab	2.12	Bc	21.87	Ab	37.90	Bb	27.88	Bb	19.05	Aa	11.36	Bc	8.60	Ab

[1] Average score of leaf injury, [2] Parasitism, [3]100 seed weight and [4] Seed yield followed by the same upper-case letter in the row and same lower-case letter in the column do not differ statistically (Scott-Knott test at p ≤ 0.05).

Table 2
Average leaf injury rating caused by *Spodoptera frugiperda*, parasitism of fall armyworm larvae, 100 weight seed and seed yield during period II (2015 and 2016), in Sete Lagoas (SL) and Nova Porteirinha (NP).

Treatments	Leaf injury				Parasitism				Weight of 100 seeds (g)				Seed yield/Kg			
	SL		NP		SL		NP		SL		NP		SL		NP	
DKB 390 YG	0.25	Aa[1]	1.10	Bb[1]	12.50	Ab[2]	21.06	Ab[2]	32.47	Bc[3]	27.57	Ab[3]	7.13	Bc[4]	5.90	Ac[4]
Fórmula TL	0.68	Aa	2.05	Bc	22.25	Ab	19.97	Ab	22.87	Aa	21.31	Aa	4.86	Aa	4.31	Aa
Herculex	0.71	Aa	2.05	Bc	18.44	Ab	22.24	Ab	29.78	Ab	27.37	Ab	6.74	Bb	5.33	Ab
VT Pro	0.26	Aa	0.91	Ab	2.61	Aa	24.57	Bb	30.32	Bb	26.75	Ab	6.70	Ab	6.04	Ac
VT Pro2	0.22	Aa	0.97	Bb	0.43	Aa	16.73	Bb	31.34	Ab	28.83	Ac	7.13	Bc	6.26	Ac
Impacto Viptera	0.01	Aa	0.03	Aa	6.25	Aa	3.46	Aa	29.91	Bb	23.57	Aa	6.54	Bb	4.94	Ab
Power Core	0.36	Aa	0.98	Ab	2.51	Aa	19.28	Bb	32.90	Bc	29.44	Ac	8.00	Ac	7.40	Ad
Non-Bt DKB 390 + methomyl	0.51	Aa	1.49	Bb	0.00	Aa	0.96	Aa	33.85	Bc	29.93	Ac	7.19	Bc	5.92	Ac
Non-Bt Fórmula TL + methomyl	0.66	Aa	1.74	Bc	0.00	Aa	1.46	Aa	24.96	Aa	23.43	Aa	5.23	Ba	4.29	Aa
Non-Bt Herculex + methomyl	0.59	Aa	1.54	Bb	0.00	Aa	1.23	Aa	30.63	Bb	26.59	Ab	7.71	Bc	5.92	Ac
Non-Bt Power Core + methomyl	0.61	Aa	1.51	Bb	0.00	Aa	0.90	Aa	32.61	Ac	29.85	Ac	7.46	Bc	6.50	Ad
Non-Bt Viptera + methomyl	0.47	Aa	1.38	Bb	0.00	Aa	0.00	Aa	29.04	Ab	26.29	Ab	6.41	Bb	5.10	Ab
Non-Bt DKB 390	0.67	Aa	1.97	Bc	1428	Ab	21.17	Ab	34.06	Bc	27.82	Ab	6.87	Ab	6.02	Ac
Non-Bt Fórmula TL	0.81	Aa	2.07	Bc	10.95	Ab	22.88	Ab	24.12	Aa	21.82	Aa	5.33	Ba	3.62	Aa
Non-Bt Herculex	0.78	Aa	2.04	Bc	4.92	Aa	15.15	Ab	29.48	Ab	29.11	Ac	7.24	Bc	5.42	Ab
Non-Bt Power Core	0.60	Aa	1.86	Bc	4.29	Aa	19.18	Bb	31.65	Ac	30.33	Ac	7.27	Ac	6.75	Ad
Non-Bt VT Pro2	0.65	Aa	1.98	Bc	5.59	Aa	24.47	Bb	34.17	Bc	30.37	Ac	7.15	Bc	5.86	Ac
Non-Bt Viptera	0.64	Aa	3.41	Bd	15.98	Ab	17.88	Ab	29.08	Ab	2648	Ab	6.19	Bb	4.95	Ab

[1] Average score of leaf injury, [2] Parasitism, [3]100 seed weight and [4] Seed yield followed by the same upper-case letter in the row and same lower-case letter in the column do not differ statistically (Scott-Knott test at p ≤ 0.05).

(6.25%) and PC (2.51%), except for YG (12.50%), Fórmula TL (22.25%) and HX (18.44%). In Nova Porteirinha, only Bt hybrid Viptera showed a low average parasitism of 3.46% when compared to all Bt hybrids and non-Bt hybrids tested, except for non-Bt hybrids sprayed with methomyl that showed an average percentage of parasitism also low (Table 2).

In general, 2011 showed low average parasitism for all treatments in period I. The average percentage of parasitism of fall armyworm larvae was higher in year 2016 (period II), except for Viptera and non-Bt hybrids sprayed with methomyl (Table S7, S8 and S9 showed in Supplementary Material).

100 seed weight

A 100 seed weight is a characteristic for a specific genetic material, and it was measured for all traits. During Period I, the average 100 seed weight for all Bt hybrids, non-Bt hybrids and non-Bt hybrids sprayed with methomyl was lower in Nova Porteirinha than in Sete Lagoas ($F_{1,520} \geq 16.44$, $p < 0.0001$) (Table 1). Bt hybrid Fórmula TL, non-Bt Fórmula TL and non-Bt Fórmula TL sprayed with methomyl showed the lowest weight of seeds ($F_{17,520} = 15.45$, $p < 0.0001$) in Sete Lagoas, when compared to all other hybrids tested (Table 1). In Nova Porteirinha, Bt hybrids (YG and VT), non-Bt hybrids (DKB390 and VT) and non-Bt hybrids (DKB390 and VT) sprayed with methomyl showed a high average weight of seeds ($F_{17,520} = 2.72$, $p = 0.0002$) when compared to all other hybrids tested, except for the non-Bt hybrid VT 2 sprayed with methomyl (Table 1).

For period II, maize in Sete Lagoas showed a higher average weight of seeds than in Nova Porteirinha for non-Bt hybrids (DKB390 and VT 2), Bt hybrids (YG, VT, Viptera and PC) and non-Bt hybrids (DKB 390 and HX) sprayed with methomyl ($F_{1,251} = 81.30$, $p < 0.0001$) (Table 2). When all treatments were compared for Sete Lagoas, Bt hybrids (YG and PC), non-Bt hybrids (DKB390, PC and VT 2) and non-Bt hybrids (DKB390 and PC) sprayed with methomyl showed a higher average weight of seeds ($F_{17,251} = 10.14$, $p < 0.0001$) than found in all other treatments tested (Table 2). In Nova Porteirinha during period II, Bt hybrid Fórmula TL, non-Bt hybrid Fórmula TL and the non-Bt hybrid Fórmula sprayed with methomyl showed the lowest weight of seeds ($F_{17,251} = 7.50$, $p < 0.0001$) when compared to all other treatments, except for Bt hybrid Viptera (Table 2).

During Period I, the average 100 seed weight was higher in 2014, when compared to all other years. The average 100 seed weight for all treatments in 2015 and 2016 (period II) were not different, except for VT 2, Viptera, Power Core and Non-Bt DKB 390 (Table S10, S11 and S12 showed in Supplementary Material).

Seed yield

On average, seed weight obtained in Sete Lagoas was higher when compared to Nova Porteirinha in period I ($F_{1,520} = 78.63$, $p < 0.0001$) and II ($F_{1,251} = 125.36$, $p < 0.0001$) (Table 1 and 2). Fórmula TL, non-Bt Fórmula TL sprayed with methomyl and non-Bt Fórmula TL showed the lowest seed yield in both municipalities and periods, when compared to all other treatments (Table 1 and 2). Viptera, non Bt-Fórmula TL and non-Bt Fórmula TL sprayed with methomyl showed the lowest seed yield in Nova Porteirinha in period I with 6.89, 6.08 and 6.74 kg, respectively ($F_{17,520} = 11.65$, $p < 0.0001$) (Table1). VT 2 showed the highest seed yield of 12.55kg in Sete Lagoas in period I. During Period II, Fórmula TL, non-Bt Fórmula TL sprayed with methomyl and non-Bt Fórmula TL in Nova Porteirinha showed the lowest seed yield of 4.31, 4.29 and 3.62kg, respectively (Table 2).

During period I, the years 2013 and 2014 showed the highest and lowest seed yield, respectively, for all treatments tested. In period II, seed yield of all treatments in years 2015 and 2016 were not significantly different, except for Herculex, Viptera and Power Core (Table S13, S14 and S15 showed in Supplementary Material).

Discussion

Our data showed a more intensive attack of fall armyworm in Bt hybrids, during periods I and II in these field experiments in Nova Porteirinha and Sete Lagoas. It was observed that on average the number of larvae collected in Nova Porteirinha was higher than in Sete Lagoas in all treatments (Fig. 1 and 2), and the average number of larvae present in the field increased year after year, in all treatments, during the 6 years of this research (Table S1, S2 and S3 of the Supplementary Material). On average, the number of larvae in Bt hybrids and non-Bt hybrids in Nova Porteirinha was 7.0 and 5.5 times higher than the number of larvae in Sete Lagoas, respectively. This consistent difference in the number of larvae may be due to some important factors, and it is worth mentioning the toxic effect of the Bt protein used in each trait, the expression of the protein in the plant tissue and cross resistance among Bt proteins. It is also worth mentioning the extreme weather of these two locations, where in Nova Porteirinha the temperature is between 16 and 33ºC, with high of 37ºC, high humidity and precipitation of 830mm/year, while in Sete Lagoas the temperature is between 13 to 29ºC with a high of at most 33ºC, with precipitation of 1,335 mm/year (Weather spark, 2020).

Another important factor is that field resistance of *S. frugiperda* to different Bt maize hybrids that has been reported during the period of this field experiment (Storer et al., 2010, 2012; Farias et al., 2014; Bernardi et al., 2014, 2015; Yang et al., 2017), and this factor may have influenced the increasing number of fall armyworm collected every year. Non-Bt hybrids in Nova Porteirinha showed the highest average of larval number of 70.13 larvae in period I and the highest average of 107.25 larvae in period II, when compared to 17.44 and 38.63 larvae in Sete Lagoas, with a difference of 302.12% and 177.63% in period I and II, respectively (Fig. 1 and 2). In both municipalities, Bt hybrids YG, Viptera, VT, VT 2 and PC (period II only) showed the lowest average number of larvae even when compared to the methomyl treatments (Fig. 1 and 2). In both municipalities and both periods, Fórmula TL and HX always showed a strikingly high number of fall armyworm larvae, being similar to non-Bt maize.

One important factor to be considered is the overlaid generations throughout the year, favoring the occurrence of severe infestations in each season and phenological stage of maize (Bernardi et al., 2015). When transgenic hybrids were analyzed separately, the difference of the highest average number of larvae in Nova Porteirinha of 48.75 compared to the average lowest number of larvae in Sete Lagoas of 0.5 was 9650% during period I, and 1881% during period II. However, when the average of the highest number of larvae in both locations is considered, this difference is 602.45% and 212.78% in period I and II respectively. This reduced effectiveness for all Bt maize during 6 years of field experiments was coincident with reports of field-evolved resistance to Bt maize in Brazil (Farias et al., 2014; Bernardi et al., 2015). These striking differences can be viewed in Figures 1 and 2. During period I and II the average number of fall armyworm larvae found in all non-Bt hybrids was higher than in all Bt hybrids, except for Fórmula TL and Herculex. This striking difference between non-Bt hybrids (17.44 larvae) and Bt hybrids (0.5 larvae) ranged from 3388% in Sete Lagoas to 1480% (70.13 larvae) and Bt hybrids (4.44 larvae) respectively, in Nova Porteirinha during period I. During period II, Sete Lagoas had a difference of 635.8% between non -Bt hybrids (38.63 larvae) and Bt hybrids (5.25 larvae). Nova Porteirinha had a difference of 2283% with an average of 107.25 (non Bt-hybrids) to 4.5 larvae (Bt hybrids) (Fig. 1 and 2). The genetic variability of fall armyworm across different crops and regions is an important factor in the differences of larval numbers among different commercial maize hybrids. Busato et al. (2004) found that the genetic variation of *S. frugiperda* populations are associated to host plants, confirming the presence of the "corn" and "rice" biotypes of *S. frugiperda* in Rio Grande do Sul State, in Brazil. Martinelli et al. (2006) found considerable gene flow between *S. frugiperda* populations from maize and cotton fields located in the same region in Brazil. This factor may occur with fall armyworm in Nova Porteirinha because of specific weather conditions.

The average number of fall armyworm larvae found among the Bt hybrids, DKB390 YG, Fórmula TL, HX and VT during period I, in Sete Lagoas, was not significantly different, however VT 2 and Viptera were different from all other Bt hybrids and showed the lowest number of larvae (Fig. 1). Bernardi et al. (2014) found that the frequency of resistance to Vip3Aa20 toxin in Brazilian populations of *S. frugiperda* was low. The average number of *S. frugiperda* larvae found in VT, VT 2 and Viptera was lower than the number of larvae found in all other Bt hybrids and non-Bt hybrids (Fig. 1). Bt hybrids Fórmula TL and YG expressing Cry1Ab and HX expressing Cry1F protein, were the most attacked.

Figure 1 shows the average number of *S. frugiperda* larvae collected in Sete Lagoas and Nova Porteirinha for 4 years (period I). It may also be observed that the number of larvae found in VT 2 and Viptera was low and very similar to larvae found in non-Bt hybrids sprayed with methomyl, providing the same level of control. Blanco et al. (2010) found that the susceptibility to Cry1Ac and Cry1Fa proteins of Bt in 133 isofamilies from five regions of three contries was similar to the susceptibiliy of two Bt-susceptible laboratory colonies to these proteins.

Overall, single-Bt gene maize hybrids showed consistently a higher leaf injury than with the pyramided Bt maize hybrids. The average leaf injury ratings caused by *S. frugiperda* was consistently greater in Nova Porteirinha than in Sete Lagoas in both periods. In period I, Bt maize VT, VT 2 and Viptera showed a difference of 3.85% in injury ratings, and in period II, VT, Viptera and PC showed a difference of 20.40% in injury ratings, when compared single and pyramided events (Table 1 and 2). Niu et al. (2014) found the same results with non-Bt and Bt maize plants containing single or multiple Bt genes, although they used susceptible and resistant fall armyworm populations. It may be observed that the leaf injury on Table 1 shows the injury ratings during period I, and these ratings of the injured leaves may be due to the high pressure of fall armyworm in Nova Porteirinha, that can be observed in Figures 1 and 2.

During period I, the pyramided Bt hybrids VT, VT 2 and Viptera (non-pyramided) showed the lowest injury ratings when compared to the non-Bt hybrids in both municipalities (Table 1). Single Bt hybrids YG, Bt11 and HX showed the same level of leaf injury than all the non-Bt hybrids during period I (Table 1). During period II, Viptera showed little or no leaf injury and it was different from all other treatments in Nova Porteirinha (Table 2). Zhu et al. (2019) also found that Bt maize expressing Vip proteins were resistant to fall armyworm. The ratings of the leaf injury for Bt11 and HX in Nova Porteirinha was higher than all other Bt hybrids with pyramided genes and YG in both municipalities, and the injury ratings were similar to all non-Bt hybrids in Nova Porteirinha, during period II (Table 2). The high number of larvae and high leaf injury in Nova Porteirinha may have affected 100 seed weight. In period I there was a difference between the two municipalities for all treatments, and on average, in Sete Lagoas produced a higher 100 seed weight than in Nova Porteirinha, with the highest weight of 33.27g of VT in Sete Lagoas (Table 1). The lowest average 100 seed weight in Sete Lagoas was Fórmula TL, non-Bt Fórmula TL sprayed with methomyl and Non-Bt Fórmula TL, with 23.63, 24.02 and 25.13g/100 seeds, respectively (Table 1).

Regardless of the fall armyworm injury to the leaves, there was no difference in 100 seed weight for all Bt and non-Bt hybrids and those sprayed with methomyl in Sete Lagoas, except for Fórmula TL that had the lowest weight. However, in Nova Porteirinha the intense presence of fall armyworm during period I decreased 100 seed weight and most of the seed yield when compared to Sete Lagoas. In Nova Porteirinha the highest 100 seed wight was 21.85g/100 seeds of DKB390 sprayed methomyl, and when compared to 33.27g/100 seed of VT in Sete Lagoas, with a difference of 65.67% (Table 1). The seed yield didn´t follow the same patterns of the 100 seed weight in period I.

In period II, for the Bt hybrids PC, DKB390, VT and Viptera showed that 100 seed weight in Sete Lagoas, on average was higher when compared to the weight in Nova Porteirinha, 32.90, 32.47, 30.32 and 29.91g/100 seeds, respectively (Table 2).

The striking presence and differences in number of fall armyworm larvae were reflected in the leaf injury to maize plants in both municipalities. These factors are important in the decision making when considering IPM actions and the use of a chemical pesticide or not. In most cases, not considering the non-Bt hybrids sprayed with methomyl, the presence of parasitism was above 20% reaching up to 49.76% of natural control (Table 1 and 2). If a chemical pesticide is sprayed, parasitized larvae will be killed disrupting the environment, killing parasitoids that would be useful in the field, and it should be remembered that in fact 50% of larvae control was favored by nature at no cost.

Overall, the percentage of parasitism was lower in all non-Bt hybrids sprayed with methomyl because most larvae died before being harvested, and parasitism was 0 (zero) (Table 1 and 2). When non-Bt hybrids sprayed with methomyl are not considered, during period I in Sete Lagoas, all larvae collected in Bt hybrids and non-Bt hybrids showed no difference in the presence of parasitoids, except Viptera, HX and Non-Bt HX, with an average low between14% and 15% parasitism, and the highest was 32.97% with larvae collected on DKB390 (Table 1). During period I in Nova Porteirinha, all treatments showed high percentage of parasitism (from 28.45% to 49.76%), and only Viptera showed a significantly low percentage of parasitism (6.25%) when compared to all other treatments (Table 1). This may be due to the fact the on average only 3.0 larvae were found during this period. The highest average percentage of parasitism occurred in larvae collected in HX (49.76%) in Nova Porteirinha, and no significant difference was found in the percent of parasitism among all Bt and non-Bt hybrids (Table 1).

Period II showed on average a lower percent of parasitism in both locations, when compared to period I. The Bt hybrid VT showed the highest percent of parasitism and the Bt hybrid VT 2 showed the lowest percent, 24.57% (Nova Porteirinha) and 0.43% (Sete Lagoas), respectively. The incidence of parasitoids in all treatments, especially in most Bt hybrids indicates that these parasitoids did not discriminate or did not show any preference between larvae fed on Bt and non-Bt maize (Table 2). Host-parasitoid relationships are usually tight, and parasitoids are sensitive to changes in host quality (Godfray, 1994). Romeis et al. (2006), states that field studies have confirmed that the abundance and activity of parasitoids and predators are similar in Bt and non-Bt crops. All parasitoids were dipterans and hymenopterans, and some of them didn´t reach the adult phase.

Conclusion

Brazilian agricultural landscape has changed fast with three crops a year, and insects have "green food" available all year round, creating a "green bridge" among the most important crops in this system: cotton, maize, soybeans. Fall armyworm is one of the most important pests in this system. Our data showed a more intense and increasingly higher attack of fall armyworm in Bt hybrids during the field trials year after year. This striking presence and differences in the number of fall armyworm larvae were reflected in the level of leaf injury in both locations. We can see that single-Bt gene maize hybrids showed consistently greater leaf injury than the pyramided Bt maize. In Brazil structured refuge areas are not mandatory, and it is estimated that fewer than 20% of the Brazilian farmers adopt refuge areas (Bernardi et al., 2017). This is an important factor that, if adopted, could delay resistance and fall armyworm population explosion in many areas where transgenics are cultivated could be suppressed.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

FHV contributed to the concept, design of the field trials, interpretation, and manuscript preparation. CLD, JPVR, PNP, CFS, PTN, CRO, KGBB, FARD contributed to data field collection and laboratory evaluations, FMA contributed to all data analysis and the supplemented material.

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Supplementary material

The following online material is available for this article:

Table S1 - Interaction between treatment and year on mean number of fall armyworm larvae (Spodoptera frugiperda) during period I (2011 to 2014).

Table S2 - Interaction between treatment and year on mean number of fall armyworm larvae (Spodoptera frugiperda) during period II (2015 and 2016).

Table S3 - Interaction between municipalities and year on mean number of fall armyworm larvae (Spodoptera frugiperda) during period I (2011 to 2014) and period II (2015 and 2016).

Table S4 - Interaction between treatment and year on average leaf injury rating during period I from 2011 to 2014.

Table S5 - Interaction between treatment and year on average leaf injury rating during period II (2015 and 2016).

Table S6 - Interaction between municipalities and year on average leaf injury rating during period I (2011 to 2014) and period II (2015 and 2016).

Table S7 - Interaction between treatment and year on parasitism of fall armyworm larvae (Spodoptera frugiperda) during period I from 2011 to 2014.

Table S8 - Interaction between treatment and year on parasitism of fall armyworm larvae (Spodoptera frugiperda) during period II (2015 and 2016).

Table S9 - Interaction between municipalities and year on parasitism of fall armyworm larvae (Spodoptera frugiperda) during period I (2011 to 2014) and period II (2015 and 2016).

Table S10 - Interaction between treatment and year on mean 100 seed weight during period I from 2011 to 2014.

Table S11 - Interaction between treatment and year on mean 100 seed weight during period II (2015 and 2016).

Table S12 - Interaction between municipalities and year on mean 100 seed weight during period I (2011 to 2014) and period II (2015 and 2016).

Table S13 - Interaction between treatment and year on seed yield during period I from 2011 to 2014.

Table S14 - Interaction between treatment and year on seed yield during period II (2015 and 2016).

Table S15 - Interaction between municipalities and year on seed yield during period I (2011 to 2014) and period II (2015 and 2016).