Interaction between Corn Genotypes with Bt Protein and Management Strategies for Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Interaction between corn genotypes with Bt protein and management strategies for *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

Insect pests, including caterpillars, cause losses in maize (*Zea mays* L.; Poaceae) which is one of the most important agricultural crops in the world. The objective of this study was to evaluate the management of *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae) with transgenic and conventional maize genotypes. The experiments were conducted in the field in summer crops from the seasons 2014/2015 and 2015/2016 in a randomized complete block design with sub-subdivided plots represented by: control (no control), chemical control (methomyl + diflubenzurom), Integrated Pest Management–spinosade, and biological control (*Trichogramma pretiosum* Riley; Hymenoptera: Trichogrammatidae) with 3 maize genotypes (Impact VIP 3, P3862 HX, and BM 3061) and 4 replications. Control and reduction of *S. frugiperda* damage were higher in the Impact VIP 3 corn genotype. The crop yield was higher (11,838.59 kg per h^{-1}), and the damage to the ears was lower in the biological control with *T. pretiosum*.

Key Words: fall armyworm; egg parasitoids; host plant resistance to insects; *Zea mays*

Resumo

Pragas, incluindo lagartas, causam redução na produção em milho (*Zea mays* L.; Poaceae) uma das culturas mais importantes no mundo. O objetivo deste estudo foi avaliar o manejo de lepidopteras em genótipos de milho transgênico e convencional. Os experimentos foram conduzidos em campo, nas safras de verão nos períodos de 2014/2015 e 2015/2016 em delineamento de blocos casualizados em parcelas sub-subdividida representada por controle (sem controle), controle químico (methomyl + diflubenzurom), manejo integrado de pragas–spinosade), e controle biológico (*Trichogramma pretiosum* Riley; Hymenoptera: Trichogrammatidae) em três genótipos de milho (Impact VIP 3, P3862 HX, and BM 3061) em quatro repetições. Controle e redução de danos foi maior no genótipo Impact VIP 3. A produtividade foi alta (11,838.59 kg para h^{-1}) e os danos foram menores no controle biológico com *T. pretiosum*.

Palavras Chave: lagarta-do-cartucho; parasitoides; Resistência de plantas a insetos; *Zea mays*

Corn (*Zea mays* L.; Poaceae) is one of the most important agricultural crops in the world, but pests can compromise the yield and quality of this plant (Teixeira et al. 2014; Moraes et al. 2015). Lepidoptera larvae are among the most significant corn pests in Brazil, especially *Diatraea saccharalis* Fabricius sensu Guenée (Lepidoptera: Crambidae), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Mocis latipes* (Guenée) (Lepidoptera: Erebidae), and *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Pereira et al. 2000; Girón-Pérez et al. 2014; Paiva et al. 2016). *Spodoptera frugiperda*, the most important species of this group, is widely distributed, damaging the vegetative and reproductive stages of corn plants (Ramalho et al. 2011; Silva et al. 2013). Newly hatched larvae of this insect scrape the new and developed leaves, and also feed on the stem and corn ears (Farias et al. 2014; Paiva et al. 2016).

Chemical control is the main strategy used to manage *S. frugiperda*, which may cause environmental and human health problems, and to select for resistant pest populations (Jesus et al. 2014; Nascimento et al. 2016). Resistant plants represent an ideal method of integrated pest management, maintaining economic damage caused by the pest at lower levels (Seifi et al. 2013; Paiva et al. 2016). Additionally, the use of Bt hybrids is a major concern in the management of pests (Storer et al. 2012; Van den Berg et al. 2013; Farias et al. 2014; Omoto et al. 2016; Waquil et al. 2016).

The use of plant resistance and biological control with the lepidopteran egg parasitoid, *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae), can increase the control efficiency of *S. frugiperda* (Petacci et al. 2009) and other pests (Zhang et al. 2005; Soares et al. 2007). Damage to corn ears by *H. zea* was 26% lower with the release of *T. pretiosum*. The control of *S. frugiperda* was 19.4% higher

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with the release of *T. pretiosum* after the emergence of corn plants (Figueiredo et al. 2015). The parasitoids of *T. pretiosum* and *Telenonus podisi* (Ashmead) (Hymenoptera: Platygastridae) eggs complement pest control and reduce the use of insecticides in soybean crops (Bueno et al. 2011).

The objective of this work was to evaluate the control of lepidopteran pests in transgenic and conventional maize genotypes using chemicals, biological insecticides, and the egg parasitoid *T. pretiosum*.

Materials and Methods

AREA STUDIED

The experiment was established at the Instituto Federal de Goiano, Campus Urutaí, Goiás, Brazil (17.4844444°S, 48.2127778°W) in the summer seasons of 2014/2015 and 2015/2016.

EXPERIMENTAL DESIGN AND TREATMENTS

The experimental design was a randomized block with sub-subdivided plots (3 × 4 × 4 m) with the corn genotypes BM 3061 (conventional), and P3862 HX and Impact VIP 3 (transgenic). The treatments were: control (no control), chemical control (methomyl + diflubenzurom), biological insecticide–spinosade, and biological control (*T. pretiosum*).

Each plot had eight 10-m rows with 0.50 m spacing totaling 40 m^2. The biological control subplot was installed approximately 500 m distant from the other treatments. The planting was fertilized according to the soil analysis and crop requirement.

Treatments were initiated when the plants had 10% of the leaves scraped by *S. frugiperda* (Cruz 1999). The chemical treatment subplot was sprayed at 13 and 27 d after emergence with 100 g per ha^{-1} of insecticide diflubenzurom (250 g kg^{-1}) and 0.6 L ha^{-1} of methomyl (215 g L^{-1}). The integrated pest management subplot received 0.08 L per ha^{-1} from the biological insecticide spinosade (480 g L^{-1}). The insecticides were applied with a CO_2 backpack sprayer and a spray volume of 200 L ha^{-1}.

PARASITOID REARING AND RELEASE

The parasitoid *T. pretiosum* was raised from adults obtained from the Rice and Beans National Research Center, Entomology Laboratory–EMBRAPA, Santo Antônio, Goiás, Brazil, and reared in the laboratory on eggs of the alternative host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Silva et al. 2013).

Anagasta kuehniella eggs, parasitized by *T. pretiosum*, were placed in the subplot of the biological control experiment, and distributed in the sheaths of corn plants every 7 d during the vegetative period at 13, 20, 27, and 34 d after emergence and 3 others in the reproductive period 66, 73, and 80 d after emergence. A total of approximately 4,800 *Trichogramma* was released per application, corresponding to 100,000 individuals per ha.

EVALUATION OF *SPODOPTERA FRUGIPERDA* DAMAGE AND GRAIN YIELD

In the vegetative stage, infestation and damage by *S. frugiperda* were evaluated at 14, 22, 29, and 36 d after emergence. Five corn plants were collected per plot, and the number of caterpillars in the leaves counted, assigning a damage score (0 to 9) based on a visual scale (Davis et al. 1992). The corn ears were collected 150 d after sowing at the central plants of 2 lines per plot, with 2.5 m at the ends considered to be a border, and with 10 linear m being evaluated. Thirty ears were evaluated randomly by quantifying the damage by caterpillars and the length, diam, and number of rows of grain in the ear, and the diam of the stem. The corn yield and the mass of 100 grains were calculated. The values were corrected for 13% moisture, and grain yield per plot extrapolated to kg ha^{-1}.

STATISTICAL ANALYSES

The data were submitted to analysis of variance with software R using the Shapiro-Wilk test to evaluate the normality of the residues. The averages were compared using the Tukey test at 5% probability. All analyses were performed using R vers. 3.2.2 software (R Core Team 2017).

Results

The number of *S. frugiperda* at 15 (F = 2.79; P = 0.0760), 22 (F = 3.28; P = 0.0502), and 29 d after emergence (F = 7.48; P = 0.0020) ranged between genotypes, and 22 d after emergence among control strategies (F = 5.79; P = 0.0027). The number of *S. frugiperda* increased with plant age and the evaluation period, being higher in treatment P 3862 HX and lower in Impact VIP 3 at 15 to 29 d after emergence. At 22 d after emergence, the infestation of *S. frugiperda* was lower in the chemical and integrated pest management treatments, and higher in the biological control (Table 1).

The damage by *S. frugiperda* varied between the genotypes at 15 (F = 23.49; P = < 0.0001), 22 (F = 28.76; P = < 0.0001), 29 (F = 81.87; P = 0.0001), and 36 d after emergence (F = 17.71; P = <0.0001), and between the control strategies at 29 d after emergence (F = 26.19; P = < 0.0001) (Table 2).

The number of corn ears (F = 10.41; P = 0.0003), the percentage of damaged ears (F = 10.41; P = 0.0003), the weight of 100 grains (F = 128.27; P = < 0.0001), and yield (kg ha^{-1}) (F = 25.66; P = < 0.0001) differed between the genotypes. In the control strategies, the number (F = 5.77; P = 0.0027) and percentage of damaged ears (F = 5.77; P = 0.0027) and maize production (kg ha^{-1}) (F = 6.24; P = 0.0018) were similar (Table 3). The number of damaged ears was 13.44 and 16.18 in the genotypes P 3862 HX and BM 3061, which was 11.87% and 21.05% higher than in the Impact VIP 3 genotype, respectively. The weight of 100 grains was lower in the hybrid Impact VIP 3, and higher in the P 3862 HX and BM 3061 genotypes. Maize productivity was higher in hybrids P 3862 HX and BM 3061, and lower in Impact VIP 3. Control strategies showed the highest number and percentage of ears damaged in the control and lower in the biological control and integrated pest management, but the productivity was similar in integrated pest management, chemical, and biological controls.

The interaction of genotypes versus control strategies at 36 d after emergence showed that *S. frugiperda* did not infest the hybrid Impact VIP 3, and that the number of caterpillars was higher in the BM 3061 genotype in the chemical control. At 29 d after emergence, damage by *S. frugiperda* was higher in the BM 3061 and P 3862 HX genotypes in the control and the biological control. Regardless of the control strategy, less damage was found by *S. frugiperda* in the Impact VIP 3 genotype and the integrated pest management treatment. The damage in the BM 3061 genotype was higher in the control and Impact VIP 3, and lower in the biological control. The percentage of damaged ears was higher in the BM 3061 genotype in the control, and lower in Impact VIP 3 in the biological control. The grain yield was higher in the P 3862 HX genotype in the biological control, and lower in the Impact VIP 3 in the control (Table 4).

Table 1. Number of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), per 5 plants of 3 maize genotypes in different control strategies and different evaluation periods. Urutaí, Goiás, Brazil.

	Number of caterpillars			
Genotypes (G)	15 d after emergence	22 d after emergence	29 d after emergence	36 d after emergence
Impact VIP 3	0.02 b	0.22 b	0.37 b	0.18
P 3862 HX	0.07 a	0.73 a	1.05 a	0.15
BM 3061	0.31 ab	0.48 ab	1.04 a	0.26
F Test	2.79	3.28	7.48	1.57
P value	0.0760	0.0502	0.0020	0.2221
Control strategies (E)				
Control	0.06	0.67 ab	1.21	0.22
Integrated pest management – biological pesticide	0.01	0.13 b	0.50	0.10
Chemical	0.06	0. 18 b	0.91	0.25
Biological control	0.31	0.95 a	1.13	0.23
F Test	1.28	5.79	2.12	1.67
P value	0.2952	0.0027	0.1155	0.1931
Interaction (G × E)				
F Test	1.33	1.32	0.43	5.04
P value	0.2694	0.2758	0.8537	0.0009

[1]Means followed by the same letter within a column do not differ significantly by the Tukey test at 5% probability.

Discussion

Spodoptera frugiperda is the main caterpillar in conventional or transgenic corn crops in Brazil and integrated pest management-based strategies should be implemented for its control (Farias et al. 2014; Omoto et al. 2016; Bernardi et al. 2017).

Infestations by *S. frugiperda* were recorded in all corn genotypes, with the highest value in the conventional BM 3061 and in the transgenic P 3862 HX. The highest infestation in the hybrid P 3862 HX (Herculex®, Du Pont/Dow AgroScience, São Paulo, Brazil) by *S. frugiperda* confirms the resistance of this caterpillar to the Cry1F protein (Storer et al. 2012; Farias et al*.* 2014) and Cry1AB (Omoto et al. 2016). However, genotypes with the Vip3Aa20 insert (Impact VIP 3) efficiently controlled this pest, causing up to 100% mortality of *S. frugiperda* in Bt maize with this protein (Waquil et al. 2016). The lower level of infestation by *S. frugiperda* in the chemical control and integrated pest management, and the higher level in the biological control, in the first evaluations, was due to the first release of *T. pretiosum* after the leaves were scraped in the plant cartridge. This shows the importance of synchronizing the release of the *T. pretiosum* parasitoid with the first adults of *S. frugiperda* in the field (Petacci et al. 2009; Silva et al. 2013).

The major damage in the transgenic hybrid P 3862 HX and in the conventional BM 3061 confirms that plants without or with only 1 protein (Yieldgard®, Monsanto, São Paulo, Brazil; Total Liberty®, Bayer, São Paulo, Brazil; and Herculex®, Du Pont/Dow AgroScience, São Paulo, Brazil) are more vulnerable to *S. frugiperda*, whereas genotypes with more than 1 protein, such as Impact VIP 3, are less vulnerable (Michelotto et al. 2013; Moraes et al. 2015). Reduction of *S. frugiperda* damage with the interaction of integrated pest management and Impact VIP

Table 2. Damage level of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) by 5 plants of 3 corn genotypes in different control strategies and different evaluation periods. Urutaí, Goiás, Brazil.

	Caterpillar damage			
Genotypes (G)	15 d after emergence	22 d after emergence	29 d after emergence	36 d after emergence
Impact VIP 3	0.21 b	0.47 b	0.34 b	0.85 b
P 3862 HX	1.72 a	2.37 a	2.69 a	3.07 a
BM 3061	1.87 a	2.72 a	3.06 a	3.97 a
F Test	23.49	28.76	81.57	17.71
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Control strategies (E)				
Control	1.60	2.12	2.95 a	3.13
I ntegrated pest management – biological pesticide	1.00	2.00	1.07 b	2.03
Chemical	1.16	1.32	1.33 b	2.22
Biological control	1.31	2.00	2.77 a	3.15
F Test	1.35	1.96	26.19	1.79
P value	0.2744	0.1386	< 0.0001	0.1668
Interaction (G × E)				
F Test	1.98	0.57	4.28	2.06
P value	0.0973	0.7483	0.0027	0.0855

[1]Means followed by the same letter within a column do not differ significantly by the Tukey test at 5% probability.

Table 3. Number (NED) and percentage (PED) of damaged ears, weight of 100 grains (g), and grain yield (kg per ha^{-1}) of 3 corn genotypes in different control strategies. Urutaí, Goiás, Brazil.

Genotypes (G)	NED	PDE	Weight of 100 grains	Yield
Impact VIP 3	9.87 b	32.91 b	27.88 c	9,846.65 b
P 3862 HX	13.44 a	44.78 a	39.48 a	12,014.00 a
BM 3061	16.18 a	53.96 a	36.04 b	11,261.95 a
F Test	10.41	10.41	128.27	25.66
P value	0.0003	0.0003	< 0.0001	< 0.0001
Control strategies (E)				
Control	15.75 a	52.50 a	33.99	10,306.90 b
Integrated pest management – biological pesticide	13.58 ab	45.26 ab	33.54	10,980.17 ab
Chemical	14.00 a	46.67 a	34.62	11,037.81 ab
Biological control	9.33 b	31.11 b	35.71	11,838.59 a
F Test	5.77	5.77	2.41	6.24
P value	0.0027	0.0027	0.0843	0.0018
Interaction (G × E)				
F Test	2.89	2.89	0.72	3.32
P value	0.0223	0.0222	0.6327	0.0115

[1]Means followed by the same letter within a column do not differ significantly by the Tukey test at 5% probability.

Table 4. Deployment of corn genotype versus control strategies, referring to the number (36 d after emergence) and level of damage (29 d after emergence) of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and number and percentage of damaged ears and production of grains (kg per ha^{-1}). Urutaí, Goiás, Brazil.

	Control strategies / number of caterpillar				
Genotypes	Control	Integrated pest management	Chemical	Biological	*P* value
Impact VIP 3	0.45 Aa	0.20 aAB	0.10 bB	0.00 bB	0.0096
P 3862 HX	0.10 bA	0.05 aA	0.15 bA	0.30 abA	0.2602
BM 3061	0.10 bBC	0.05 aC	0.50 aA	0.40 aAB	0.0025
P value	0.0137	0.4163	0.0075	0.0109	—

	Control strategies / level of damage				
Genotypes	Control	Integrated pest management	Chemical	Biological	*P* value
Impact VIP 3	0.80 bA	0.15 bA	1.15 bA	0.25 bA	0.4470
P 3862 HX	3.65 aA	1.50 aB	1.75 aB	3.85 aA	< 0.0001
BM 3061	4.40 aA	1.55 aB	2.10 aB	4.20 aA	< 0.0001
P value	< 0.0001	0.0065	< 0.0001	< 0.0001	—

	Control strategies / number of damaged ears				
Genotypes	Control	Integrated pest management	Chemical	Biological	*P* value
Impact VIP 3	15.25 abA	8.00 bAB	10.25 aAB	6.00 bB	0.0138
P 3862 HX	12.00 bAB	18.00 aA	16.25 aA	7.50 bB	0.0029
BM 3061	20.00 aA	14.75 abA	15.50 aA	14.50 aA	0.1809
P value	0.0236	0.0035	0.0769	0.0097	—

	Control strategies / percentage of damaged ears				
Genotypes	Control	Integrated pest management	Chemical	Biological	*P* value
Impact VIP 3	50.82 abA	26.65 bAB	34.20 aAB	20.00 bB	0.0138
P 3862 HX	40.00 bAB	60.00 aA	54.15 aA	24.97 bB	0.0029
BM 3061	66.67 aA	49.15 abA	51.67 aA	48.35 aA	0.1806
P value	0.0235	0.0034	0.0776	0.0097	—

	Control strategies / yield (kg per h^{-1})				
Genotypes	Control	Integrated pest management	Chemical	Biological	*P* value
Impact VIP 3	8,744.29 bB	10,587.71 aA	9,707.36 bAB	10,347.24 bAB	0.0237
P 3862 HX	10,915.38 aB	11,469.09 aB	11,800.98 aB	13,870.56 aA	< 0.0001
BM 3061	11,261.03 aA	10,883.71 aA	11,605.08 aA	11,297.98 bA	0.7102
P value	< 0.0001	0.3560	0.0028	< 0.0001	—

[1]Means followed by the same letter, lowercase by column or uppercase by line, do not differ significantly by the Tukey test at 5% probability.

3 demonstrates that genotypes with a single protein, such as P 3862 HX (Cry1F), should not be used as an isolated strategy in *S. frugiperda* integrated pest management, and that combinations of these are the best alternative for pest management in agricultural crops (Bueno et al. 2011; Ramalho et al. 2011).

The highest number and percentage of damaged ears in the transgenic genotype P 3862 HX and in the conventional BM 3061, and the lower values in the Impact VIP 3 show the low effectiveness of the Cry1F protein in the management of this pest. The higher efficiency of biological control with *T. pretiosum*, and the reduction of *S. frugiperda* damage in corn ears corroborates the high percentages of those genotypes with Herculex® and Yieldgard® technologies (Michelotto et al. 2013), and damage reduction by *H. zea* Boddie in areas with 100,000 parasitoids per ha (Foresti et al. 2012). Maize productivity was higher with the interaction of the P 3862 HX genotype and the biological control. The integrated pest management in agricultural crops should be implemented with practices compatible with biological control, because isolated use of insecticides may not be sufficient to increase crop productivity.

The efficiency of the interaction of the transgenic genotype Impact VIP 3 and the biological control at 36 d after emergence shows the importance of these combined control methods in the management of *S. frugiperda* in Bt plants. This agrees with the fact that the egg parasitoids *T. pretiosum* and *T. podisi* have complemented the pest control for soybean (Bueno et al. 2011). In addition, the release of *T. pretiosum* maintained the population and reduced the damage of *S. frugiperda* below the level of economic damage in corn (Martinazzo et al. 2007). This demonstrates the importance of using integrated pest management compatible practices to minimize the risk of selecting Bt protein-resistant individuals, and to increase efficiency for the control of *S. frugiperda* (Matos-Neto et al. 2004; Zanuncio et al. 2008; Jesus et al. 2014). This is consistent with the results from the literature on the use of Bt proteins for the control of *S. frugiperda* (Zanuncio et al. 2008).

Biological control or interaction of control strategies in integrated pest management are the best alternatives for pest management in agricultural crops. The interaction of biological control with Bt plants increases pest control efficiency.

Control efficiency and reduction of *S. frugiperda* damage were higher with Viptera 3 technology (Cry1Ab and Vip3Aa20). The caterpillar infestation and pest damage were similar in corn P 3862 with Herculex technology (Cry1F) and in the conventional hybrid BM 3061, indicating the limited efficiency of this protein for the management of *S. frugiperda*. The egg parasitoid *T. pretiosum* diminished the damage to the ears and increased the productivity of the corn crop.

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