Induction of resistance to fall armyworm (*Spodoptera frugiperda)* (Lepidoptera: Noctuidae) in transgenic and conventional corn plants

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Abstract

The fall armyworm (*Spodoptera frugiperda*) is an important pest of corn in Brazil. Insecticides are applied to reduce the losses caused by this pest. Integrated control methods, including resistance induction in plants using silicon and transgenic hybrids, must be used to reduce the impacts caused by insecticide application. The aim of this study was to assess the effects of calcium silicate application ($CaSi_4$) to the sowing furrow on *S. frugiperda* damage to and incidence on conventional and transgenic genotypes with different *Bt* technologies. The treatments were arranged in a randomized block design in a 3x4x4 factorial scheme with three corn hybrids, Morgan 30A91 PRO Power Core® (Cry1F, Cry1A105, Cry2Ab2 *Bt* toxin), Morgan 20A55 Herculex® (Cry1F *Bt* toxin) and AG 1051 (conventional); four silicon doses, 0, 300, 600 and 900 kg. ha^{-1} calcium and magnesium silicate (Agrosilicio Plus®, Ca = 25%, Mg = 6%, Si = 10.5%); and four assessment times, 15, 30, 45 and 60 days after emergence (DAE). Four replicates were performed. The mean number of caterpillars per plant, damage caused by *S. frugiperda*, and production components were assessed. Calcium and magnesium silicate application induced resistance in corn plants, thus reducing the damage caused by *S. frugiperda*. Corn responded to resistance induction with increasing doses of calcium and magnesium silicate. The transgenic hybrid with three proteins (Morgan 30A91 PRO) improved protection against *S. frugiperda*. The interaction between silicate fertilization and transgenics produced a synergistic effect, suggesting a viable alternative for *S. frugiperda* control.

Keywords: Silicate fertilization, integrated pest management, transgenic maize*, Zea mays.*

Introduction

Corn (*Zea mays* L.) is a tropical plant that requires heat and humidity for satisfactory production and rewarding yields as well as the interaction of a set of edaphoclimatic factors for proper growth. Despite its high production potential, corn is highly sensitive to biotic and abiotic stresses. Therefore, its cultivation requires planning and strict management (Andrade, 1995; Fancelli and Dourado Neto, 2004; Cruz et al., 2008). The fall armyworm (*Spodoptera frugiperda*) is a polyphagous species that attacks various economically important crops in several countries (Lopes et al., 2008; Aguirre et al., 2016). In Brazil, the fall armyworm is considered a key pest of corn because it causes enormous losses that can reduce production by more than 25%. The adults have a high dispersion capacity and can produce several generations per year (Waquil & Vilella, 2003).

Insecticides are applied to reduce the losses caused by this pest. The frequent use of this control method may impacts humans, the environment, the selection of resistant insects, and the resurgence of pests, in addition to leaving residues in food (Sosa-Gomez & Solva, 2010). The use of integrated control methods, including resistance induction in plants using silicon and transgenic hybrids, is necessary to reduce the impacts of insecticide application (Blanco et al., 2016; Reynolds et al., 2016). The use of transgenic corn plants expressing *Bacillus thuringiensis* (*Bt*) insecticidal proteins is one of the main alternatives for fall armyworm control in Brazil, reaching 80% of the planted area (Omoto et al., 2016). In the current corn production system in Brazil, multiple cropping with some *Bt* crops and the non-implementation of refuge areas have contributed to the evolution of resistance to *Bt* corn plants in the fall armyworm population (Farias et al., 2014; Horkoshi et al., 2016). Fall armyworm resistance to the Cry1F *Bt* protein has already been reported in Brazil (Farias et al., 2014).

The application of specific nutrients, including silicon, induces plant defenses against pests. Defense is induced in plants due to the formation of passive or active mechanical barriers by altering the plant biochemical responses to herbivore attack (Epstein, 1994; Marschner, 1995). Silicon application may reduce the population growth rate of the pest, increase caterpillar mortality, and stimulate growth and plant production through indirect effects possibly related to the presence of defense substances, which may have adverse effects on the insect's biology and behavior (Goussain et al., 2002; Gomes et al., 2005; Antunes, 2010).

Given the constant imbalances in *Bt*-corn fields and the benefits of applying nutrients such as silicon, plant resistance induction may be an alternative to delay the evolution of resistance to *Bt* corn in the fall armyworm. Accordingly, this study was conducted to assess the effects of calcium silicate

application to the sowing furrow on *S. frugiperda* damage to and incidence on conventional and transgenic corn genotypes with different *Bt* technologies.

Results and Discussion

The damage caused by *S. frugiperda* was affected by the time, hybrid and silicate dose. Significant interactions were observed between the factors of time and hybrid and between silicate dose and hybrid. The hybrids significantly differed from each other in the number of *S. frugiperda*, and a significant interaction was observed between silicate dose and hybrid (Table 1).

The damage caused by *S. frugiperda* caterpillars was highest at 30 DAE (3.84), and the conventional hybrid AG 1051 was the most damaged, followed by Morgan 20A55 (Herculex) and Morgan 30A91 PRO (Power Core). These results are attributable to the presence of genes of *B. thuringiensis* bacteria with insecticidal activity against *S. frugiperda* (Moraes et al., 2015).

A significant response to the silicon doses was observed, and the damage caused by *S. frugiperda* decreased with increasing silicate dose. This decrease in damage caused by *S. frugiperda* resulted from the formation of a protective silicon layer in leaves, which acted as a mechanical barrier impairing caterpillar feeding (Goussain et al., 2002; Keeping & Kevedaras 2008). The number of *S. frugiperda* caterpillars was higher in the conventional hybrids AG 1051 and Morgan 20A55 (Herculex) than in the Morgan 30A91 PRO (Power Core) hybrid. The Morgan 30A91 PRO hybrid has three *Bt* proteins (Cry1F, Cry1A105, Cry2Ab2), whereas the Morgan 20A55 hybrid has only the Cry1F *Bt* protein. *Bt protein* pyramiding achieves better results in *S. frugiperda* control compared with a hybrid expressing the Cry1F protein as the only *Bt* protein (Farias et al., 2016); susceptibility of the latter type of hybrids to *S. frugiperda* has been reported (Farias et al., 2014; Bernardi et al., 2015). This explains why the performance of this hybrid is similar to that of a conventional hybrid.

A significant interaction between time and corn hybrid was observed for damage caused by *S. frugiperda* (Figure 1). The conventional hybrid AG 1051 exhibited more damage than the transgenic hybrids, with the highest values from 30 to 45 DAE. The corn hybrids exhibited a decreasing linear response to the doses of calcium and magnesium silicate (Figure 2). *S. frugiperda* damage decreased with increasing dose due to silicon accumulation in the plants, which caused the caterpillars to feed less on the plants that received the highest doses. Corn is a silicon-accumulating plant (Andrade et al., 2014), and this chemical element is associated with induction of plant resistance to pest attacks (Souza et al., 2014; Almeida et al., 2015, Nikpay et al., 2015). Silicon application in corn plants increases *S. frugiperda* larval mortality, caterpillar cannibalism and mandible wear from plant feeding (Goussain et al., 2002).

The number of caterpillars remained constant in the Morgan 20A55 (Herculex) hybrid with increasing silicate dose, whereas the increasing dose reduced the number of *S. frugiperda* caterpillars in the conventional hybrid AG 1051 (Figure 3). This decrease in the number of caterpillars in the conventional hybrid indicates plant resistance induction due to the application of a silicon source and suggests an alternative for *S. frugiperda* control and/or a control alternative in the refuge area in corn to disfavor the selection of pest resistance to *Bt* organisms (Zancanaro et al., 2012; Horkoshi et al., 2016).

The hybrids exhibited significant differences in ED, NRPE, NGPR, and 100W. The calcium and magnesium silicate doses affected EL and NGPR (Table 2).

The corn hybrids exhibited significant differences in dry phytomass, yield and stand (Table 3). Only yield differed significantly with increasing silicon dose, with no interaction between the factors of hybrid and dose. Yield was higher in the transgenic hybrids than in the conventional hybrids, and the calcium and magnesium silicate dose of 600 kg.ha^{-1} increased the yield. These data further support resistance induction and the use of transgenic hybrids for *S. frugiperda* control, as the grain yield increased with increasing dose. Corn plant yield also increased with increasing silicon dose. This increase in yield was related to the increase in plant chlorophyll content and number of chloroplasts upon silicate fertilization, which improved the photosynthetic efficiency and reduced the transpiration rate, thus avoiding water loss through the stomata. These changes may also alter the architecture of the plants, rendering them more upright and thus reducing self-shading (Ma & Takahashi, 2002; Xie et al., 2014)

Materials and Methods

Experimental conditions

The experiment was conducted in the experimental area of the Federal Institute of Goiás (Instituto Federal Goiano) - Urutaí Campus in the municipality of Urutaí, Goiás (17°48'15.9''S – 50°54'19.5''W), from November 2014 to May 2015. The experimental area was managed for two years by rotation of corn in the spring/summer with millet in the autumn/winter. Before starting the experiment, soil samples were collected at a depth of 0 to 0.20 m for chemical and physical analysis. The soil (Red-Yellow Latosol) had the following physicochemical characteristics: pH in water: 5.5; Ca, Mg, Al, H+Al: 2.3, 0.8, 0.0 and 1.4 cmol/dm^3, respectively; K and P: 141.0 and 4.7 mg/dm^3, according to the anion exchange resin method; organic matter: 18.0 g/dm^3; base saturation: 71.19%; and texture: 35, 12 and 53 g/kg clay, silt and sand, respectively.

Pre-sowing management was conducted using a conventional system with one plowing and one harrowing. A total of 3.5 seeds were sown per linear meter, and 420 kg ha^{-1} of fertilizer (10-29-20, NPK) was used in fertilization at planting. Topdressing with 120 kg ha^{-1} nitrogen was applied at the V4-V5 stage.

Treatments and assay

The treatments were arranged in a randomized block design in a 3x4x4 factorial scheme with three corn hybrids, Morgan 30A91 PRO Power Core® (Cry1F, Cry1A105, Cry2Ab2 *Bt* toxin), Morgan 20A55 Herculex® (Cry1F *Bt* toxin) and AG 1051 (conventional); four silicon doses, 0, 300, 600 and 900 kg. ha^{-1} calcium and magnesium silicate (Agrosilicio Plus®, Ca = 25%, Mg = 6%, Si = 10.5%); and four assessment times, 15, 30, 45 and 60 days after emergence (DAE). Four replicates were performed. The plots consisted of six rows (0.5 m) 6 m in length with a total area of 18 m^2. The four central rows of each plot were assessed, excluding 0.5 m from each end, resulting in a total useful area of 10 m^2 per plot. The other cultivation practices were performed according to Francelli & Dourado Neto (2004).

Table 1. Effect of hybrids, time, and calcium and magnesium silicate dose on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) damage to and mean number on corn.

Factors[1]	Evaluation	
	Damage to *S.frugiperda*	Number of *S.frugiperda*
Time (T)		
15 DAE	2.96	1.00
30 DAE	3.84	1.03
45 DAE	3.82	1.08
60 DAE	3.77	-[2]
Test F_{time}	17.59**	0.65 ns
Hybrids (H)		
AG 1051	4.44a	1.13a
Morgan 20A55	4,11b	1.11a
Morgan 30A91 PRO	2.23c	0.88b
Test $F_{hybrids}$	18.30**	8.76**
Silicate dose (S)		
0	5.04	1.14
300	4.22	0.96
600	3.10	1.00
900	2.02	1.06
Test $F_{silicate\ dise}$	16.78**	2.13ns
Interaction		
Teste $F_{(T\ x\ H)}$	2.7*	1.59ns
Teste $F_{(T\ x\ S)}$	1.15ns	1.45ns
Teste $F_{(S\ x\ H)}$	7.77**	2.27*
Teste $F_{(T\ x\ H\ x\ S)}$	0.72ns	1.12ns
CV (%)	19.57	31.12

[1]Means in the same column followed by the same letter do not differ according to the Tukey test at 5% probability (P ≤ 0.05). **, *, ns: significant at 1 and 5% probability and non-significant. [2] insufficient number of insects in the plot
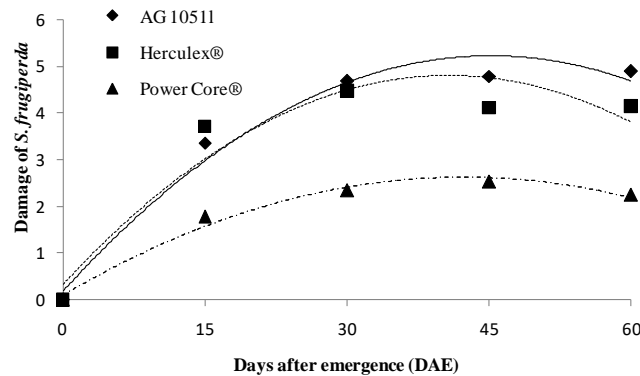
Fig 1. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) damage in corn hybrids at different times. ♦ $y = -0.002x^2 + 0.222x + 0.198$, $R^2 = 0.98$**; ■ $y = 0.002x^2 + 0.22x + 0.338$, $R^2 = 0.919$; ▲ $y = 0.001x^2 + 0.120x + 0.091$, $R^2 = 0.982$.
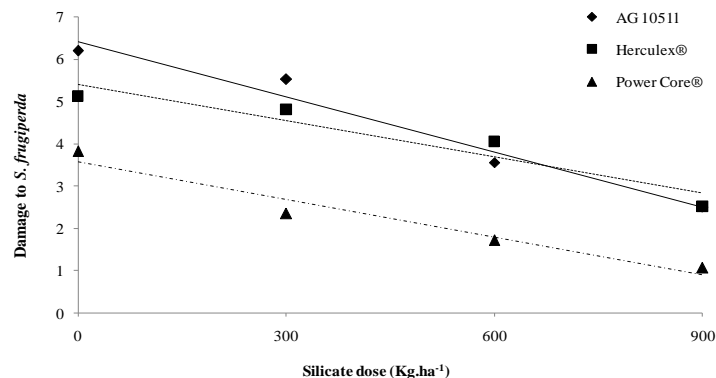
Fig 2. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) damage in corn hybrids with different calcium and magnesium silicate doses according to the Davis scale. ♦ $y = -0.004x = 6.397$, $R^2 = 0.97$**; ■ $y = 0.002x = 5.404 + 0.904$, $R^2 = 0.90$**; ▲ $y = 0.001x^2 + 0.120x + 0.091$, $R^2 = 0.95$**.

Table 2. Ear diameter (ED), ear length (EL), number of rows per ear (NRPE), number of grains per row (NGPR), and 100-grain weight (100 W) in corn hybrids with different calcium and magnesium silicate doses.

Hybrids (H)[1]	ED	EL	NRPE	NGPR	100 W
AG 1051	36.45[c]	15.47[a]	13.23[c]	26.53[b]	22.90[b]
Morgan 20A55	47.47[a]	15.00[a]	17.73[a]	30.74[a]	26.43[a]
Morgan 30A91 PRO	42.15[b]	14.60[a]	15.45[b]	28.56[ab]	26.89[a]
Teste F$_{tecnologia}$	8.57[**]	0.94[ns]	4.73[**]	5.78[**]	2.93[*]
Silicate doses (S)					
0	42.60	13.66	15.25	25.15	23.89
300	42.75	14.53	16.10	27.92	24.86
600	41.09	16.27	15.78	28.80	26.75
900	41.65	18.63	14.88	30.57	26.99
Test F	0.59[ns]	5.01[**]	1.98[ns]	7.10[**]	0.87[ns]
Test F $_{(G \times S)}$	0.48[ns]	0.68[ns]	1.24[ns]	2.15[ns]	1.76[ns]
CV (%)	8.44	11.92	8.61	12.24	9.45

[1]Means in the same column followed by the same letter do not differ according to the Tukey test at 5% probability (P ≤ 0.05). [**], [*], [ns]: significant at 1 and 5% probability and non-significant.

Table 3. Dry phytomass, yield and stand for different hybrids and silicate doses and interaction between all factors.

Hybrids (H)[1]	Dry phytomass (g)	Yeld (kg.ha^{-1})	Stand/ha
AG 1051	208.53[b]	6310.27[b]	65718.75[a]
Morgan 20A55	243.27[ab]	7982.90[a]	65898.75[a]
Morgan 30A91 PRO	231.96[a]	7645.46[a]	65937.50[a]
Teste F $_{híbridos}$	5.33	11.57	1.24
Silicate doses (S)			
0	212.67	6039.97	66665.50
300	232.89	6376.76	66670.50
600	236.08	8798.08	68083.60
900	241.98	8336.71	65416.67
Test F$_{silicate\ doses}$	1.42[ns]	6.21[**]	2.38[ns]
Test F $_{(H \times S)}$	0.51[ns]	0.75[ns]	2.38[ns]
CV (%)	13.47	13.56	2.44

[1]Means in the same column followed by the same letters do not differ according to the Tukey test at 5% probability (P ≤ 0.05). [**], [*], [ns]: significant at 1 and 5% probability and non-significant.
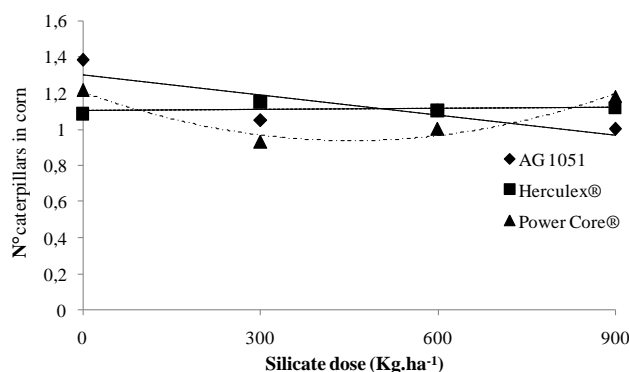
Fig 3. Number of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) caterpillars in corn hybrids with different calcium and magnesium silicate doses. ♦ y = -0.000x + 1.298, R^2 = 0.685[*]; ■ y = 2E-05x + 1.104, R^2 = 0.049[ns]; ▲ y = 1E-06x^2 - 0.001x + 1.203, R^2 = 0.949[*].

The mean number of caterpillars per plant and the damage caused by *S. frugiperda* were assessed (Davis, et. al, 1992). Each damage assessment was performed in triplicate. Grain yield was estimated based on the weight of grains harvested in the useful plot area and corrected to 13% moisture. Plants from 3 linear meters of useful area were collected and dried in a forced air oven (Model SP-102/2000) at 65ºC for 72 hours to determine the phytomass. To assess the production parameters, 10 ears were randomly collected to measure ear length (EL) and diameter (ED), the number of rows per ear (NRPE), and the number of grains per row (NGPR). After threshing, 100 grains of corn were randomly collected to determine the weight (100W).

Statistical analysis

The results were subjected to analysis of variance (ANOVA), and when significant, the means were compared using the Tukey test at 5% probability.

Conclusion

Calcium and magnesium silicate application induces resistance in corn plants, reducing the damage caused by *S. frugiperda*. Corn responds to resistance induction with increasing doses of calcium and magnesium silicate. The transgenic hybrid with three proteins (Morgan 30A91 PRO)

provided the best protection against *S. frugiperda*. The interaction between silicate fertilization and transgenic hybrids resulted in a synergistic effect, which suggests that this combination is a viable alternative for *S. frugiperda* control.

Acknowledgments

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