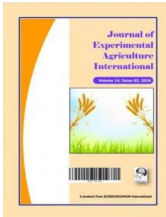

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Resistance Induction in *Zea mays* L. Cultivars against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Kennedy Santos Gonzaga[1*], Gemerson Machado de Oliveira[1], Antonio Carlos Leite Alves[2], Izabela Nunes do Nascimento[1], Fábio Mielezrski[3] and Jacinto de Luna Batista[1]

[1]Departamento de Fitotecnia e Ciências Ambientais, Laboratório de Entomologia, Universidade Federal da Paraíba (UFPB), Paraíba, Brasil.
[2]Departamento de Fitotecnia, Laboratório de Interação Inseto-Planta, Universidade Federal de Viçosa (UFV), Minas Gerais, Brasil.
[3]Departamento de Fitotecnia e Ciências Ambientais, Laboratório de Grandes Culturas, Universidade Federal da Paraíba (UFPB), Paraíba, Brasil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KSG and GMO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ACLA and INN managed the analyses of the study. Authors FM and JLB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The maize crop *Zea mays* L., faces serious limitations in cultivation due to attack by the *Spodoptera frugiperda.* The objective of this work was to evaluate the resistance induction in maize cultivars with *Spodoptera frugiperda* caterpillars due to the application of silicon (Si) and calcium (Ca) sources. Two cultivars of maize viz., Genotype *A* simple hybrid and the Creole Genotype *B* variety were used. These cultivars were submitted to two treatments of foliar fertilisation, calcium

**Corresponding author: E-mail: izabelaufpb@gmail.com;*

chloride and potassium silicate, and treatment without the application of an elicitor. The evaluations of the damages caused by the *S. frugiperda* caterpillar were carried out weekly, from the seventh day after the first application of the products (Si and Ca). In the bolting phase (VT), physiological evaluations of gas exchanges and fluorescence of chlorophyll *a* were carried out. For the Creole variety, there was a pre-dominance of plants that did not present any damage. The Creole variety presented the lowest mean of infestation by *S. frugiperda*, regardless of the product applied. No statistical differences were recorded between the products applied in each cultivar. The maximum quantum efficiency of FSII (F_V/F_M) was not statistically significant for the cultivars nor the cultivars x treatments interaction. For stomatal conductance (gs), a statistical difference between cultivars was found. In the Creole Genotype *B* variety, superior results were obtained in relation to the simple hybrid. The TF variable did not present statistical difference between the evaluated cultivars. Different behaviours were observed in the transpiration variable (E) among the cultivars. In the Creole Genotype *B* variety when submitted to silicon application and in control, higher transpiration was obtained compared to the simple hybrid. The sources of silicon and calcium do not promote the induction of resistance to *S. frugiperda* in the studied variables. The Creole variety Genotype *B* of *Zea mays* suffers minor damage due to an infestation of *S. frugiperda* in relation to the Genotype *A* simple hybrid maize.

Keywords: Control method; fall armyworm; corn.

1. INTRODUCTION

Zea mays L. (maize) is one of the world's largest cereals grown in the United States, China and Brazil as the world's largest producers. In the export ranking, Brazil occupies the second position [1], the Central West and South being the main producing regions of this cereal [2].

Although the country is prominent in world production, it still presents limitations in the cultivation, which implies low productivity mainly due to insect pest attacks. Among the insect complexes that infest the maize crop, the *Spodoptera frugiperda* (J. E. Smith, 1797) caterpillar is the main cause of damage and represents a high cost of control annually [3].

The fall armyqorm becomes an important pest for this crop, both by reducing productivity and decreasing the quality of the final product, besides the difficulty of control [4]. The considerable economic losses caused by this pest cause farmers to intensify the use of chemical insecticides and consequently lead to the development of resistance to many insecticide groups, making their control even more difficult [5].

Different control methods are used to reduce populations of *S. frugiperda*, such as cultural, chemical and biological strategies [6]. Plant resistance is also easily incorporated into integrated pest management because it maintains the pest population below the level of economic damage without unbalancing the environment and has a cumulative and persistent effect beyond the lowest cost of production [7].

Due to the different biochemical, morphological and physiological characteristics among the genotypes of the same species, it is possible that some plants suffer a greater or lesser impact of phytophagy [8,9]. Plant resistance to pests can also be obtained by induction, which is a temporary manifestation, where a plant becomes less favourable to insect attack due to a certain condition that affects its physiology [7]. Among the various induction techniques, fertilisers have been widely used, mainly micro and macronutrients.

Silicon (Si) is considered a beneficial element for plants [10], it acts on the reinforcement of the cell wall by depositing solid silica, making the plant more rigid, with better growth and yield, for suppressing diseases and conferring characteristics of resistance to insects [11,12]. Calcium (Ca) is a macronutrient that is involved in a large number of signalling pathways in plants, from symbiotic interactions, defence responses and various hormones, functioning as a secondary messenger in response to biotic and abiotic signals [13]. Calcium participates in lignin biosynthesis, which favours plant self-defence and can be used as an inducer of pest resistance [14].

Other biomorphological factors are also associated with plant defence in relation to insect infestation. As a consequence of the stress caused by pest injury, there are reductions in the physiological parameters such as photosynthetic rate, transpiration and stomatal conductance [15,16]. For this purpose, the gas exchange and fluorescence analysis of chlorophyll *a* can detect, with safety and reliability, stress and injury effects in the photosynthetic process caused by biotic or abiotic factors [17]. In view of the above, the study aimed to evaluate the induction resistance in corn cultivars due to the application of silicon and calcium sources in the infestation of the *Spodoptera frugiperda* caterpillar.

2. MATERIALS AND METHODS

The experiment was conducted at the Chã de Jardim experimental farm, belonging to the Agricultural Sciences Center (CCA) of the Federal University of Paraíba (UFPB), Areia-PB, Brazil. The climate of the region is classified as tropical humid, presenting average temperature, relative humidity and precipitation of 23°C, 80% and 1,400 mm annually, respectively [18]. The soil is classified as Yellow Latosol [19].

The simple hybrid corn denominated Genotype *A* and the Creole variety denominated Genotype *B* were used. These materials were submitted to two treatments of foliar fertilisations, being applied commercial sources of Calcium Chloride and Potassium Silicate, and a control treatment. Three applications of the products were carried out in the vegetative growth phase of the maize cultivars, specifically in the V3, V6 and V12 stages, following the recommended dosage for culture suggested by the manufacturer of 2 L ha^{-1}. The products were always applied from 4 pm, this being the most recommended time for loss reduction, and manually by a costal spray with 20-litre capacity.

The plots were constituted by four rows of 5 m in length, where after the mechanical preparation of the soil with a harrowing, furrows were opened and manual sowing was done, allowing a spacing of 0.50 m between rows and 0.20 m between plants, totalling of 100 plants per plot and a density of 100,000 plants/ha. The useful area of the plot was constituted by the two central rows, scattering 0.5 m of the border. The nutritional correction was carried out with the fertilisation of macronutrients (NPK) in the foundation and cover, as well as soil acidity, with dolomitic limestone application, according to results of soil chemical analysis. Weed control was performed with manual weeding.

2.1 Evaluation of the Damage and Infestation of *Spodoptera frugiperda*

The evaluations of the damages caused by *S. frugiperda* were carried out weekly, from the seventh day after the first application of the products (Si and Ca), and done at 7, 14, 21, 35 and 42 days, ending at the beginning of flowering. Ten plants per plot were randomly selected, with individual scores varying between 0 and 5 (Table 1) [20].

Table 1. Scale of damages caused by *Spodoptera frugiperda* in *Zea mays* plants [20]

Score	Damage description
0	No damage
1	Scraped leaves
2	Bored leaves
3	Ripped leaves
4	Stem damage
5	Stem destroyed

To evaluate the level of infestation of *S. frugiperda*, two central rows of each plot were used, relating the number of plants attacked by the total number of plants in the crop line.

2.2 Evaluation of Physiological Characteristics

The fluorescence of chlorophyll *a*, was determined from a portable fluorometer(model OS-30p from Opti-Sciences®). Measurements were made on three plants per plot, between 9 and 12 hours, at 2 cm from the margin of the leaf, excluding its veins. To that end, one leaf per plant, randomly selected was adapted to the dark with the use of tweezers, for approximately 30 minutes. The maximum photochemical quantum FSMI (Fv/Fm) parameter, often evaluated by the maximum fluorescence rate of chlorophyll *a*, was evaluated to compare the healthy and damaged tissues due to biotic factors [21].

In the maize bolting (VT) phase, gas exchange and photosynthetic efficiency of maize leaves were evaluated by using an Infrared Gas Analyzer (IRGA), LCpro-SD model from BioScientific®. Two plants were used per plot in the morning (between 9 am and 11 am), sampling was done in the first leaf opposite and below the ear. The following physiological parameters were evaluated: Transpiration (E), Internal CO_2 Concentration in leaf (IC), Stomatal conductance (gs), Photosynthesis (A), Carboxylation Efficiency (EC), Pressure Deficit (PD) and Water Use Efficiency of (WUE).

2.3 Statistical Analysis

The experimental design was a Randomized Complete Block Design (RCBD), in a factorial scheme (2x3), two maize cultivars (genotype *A* and genotype *B*), two sources of resistance induction (Si and Ca) and one control (without application), with four replicates per treatment, totalising 24 experimental plots.

Damage probability data were submitted to multinomial distribution analysis. The percentage of infestation, data related to gas exchange and chlorophyll *a* fluorescence were subjected to variance analysis, and the means of the treatments were compared by the Tukey test at 5% probability level, to evaluate both the effect of the treatments and the sensitivity differences between the cultivars to said treatments. All the various factors were processed by SAS [22].

3. RESULTS AND DISCUSSION

For leaf damage caused by *S. frugiperda*, it was verified according to the evaluation periods (Fig. 1A) that there was a statistical difference between the simple hybrid and Creole variety (Fig. 1B). It was recorded that in the interval of 14 to 42 days, the simple hybrid presented the highest probability of occurrence of score 4, characterised by the stem of the partially destroyed plant, followed by score 3 that is the plant with symptoms of ripped leaf. In contrast, it was observed that from the second evaluation (14 days), the probability of occurrence of score 0, plants without symptoms of attack was decreased as a function of time, this characterises an increase in the intensity of the attack of the pest.

For the cultivar Creole Genotype *B* in all the evaluation periods, there was a pre-dominance of plants that did not present any damage, i.e., a higher probability of occurrence of score 0 was observed, showing that this variety in the study conditions was a larger degree of resistance than the simple hybrid. Little improved plants may present high levels of resistance but tend to be less productive, which may justify the fact that the Creole variety has presented more plants with no damages when compared to the simple hybrid. Improved plants, such as the hybrid, invest little of their resources in the synthesis of defences against the attack of pest insects because most of the times they are manipulated with the intention of achieving high productivity [23].

When evaluating 15 maize genotypes, 9 of which were Creole and 6 were hybrids (1 Bt hybrid), it was found that 4 Creole genotypes presented moderate resistance to *S. frugiperda* in compared to the other evaluated genotypes, based on visual observations [8].

There are different performances among maize cultivars in relation to *S. frugiperda* attacks, verified that higher damage scores were observed in hybrids when compared to their transgenic isogenic version [24]. When studying different genotypes of maize against *S. frugiperda* attacks, found similar results that corroborate to this research, where the hybrids suffered a higher level of attack in compared to the transgenic cultivar [25].

Table 2. Percentage of infestation of *Spodoptera frugiperda* in *Zea mays* submitted to treatments with Potassium Silicate and Calcium Chloride in five evaluation periods, Areia, 2017

Cultivars	Treatments	*S. frugiperda* infestation (%) in five evaluation periods (days)					µ
		14	21	28	35	42	
Simple hybrid	Si	79.8 Aa	74.6 Bc	76.0 Bb	69.7 Bd	54.9 Be	**71.0 A**
Genotype *A*	Ca	77.8 Bb	76.5 Ad	83.4 Aa	76.9 Ac	60.0 Ae	**74.9 A**
	Control	67.07Cb	59.7 Cc	68.7 Ca	46.0 Cd	43.0 Gs	**56.9 A**
µ		**74.87 A**	**70.3 A**	**76.0 A**	**64.2 A**	**52.6 A**	**-**
Creole variety	Si	16.31 Fd	15.2 Fc	23.8 Fb	40.2 Fa	17.8 Fe	**22.7 A**
Genotype *B*	Ca	19.20 Ed	21.5 Ec	29.1 Ea	21.8 Eb	18.0 Ee	**21.9 A**
	Control	24.30 Dd	32.6 Db	33.3 Da	32.6 Dc	23.6 De	**29.3 A**
µ		**19.93 B**	**23.1 B**	**28.7 B**	**31.5 B**	**19.8 B**	**-**

[1]Averages followed by the same capital letter in the column and lowercase in the row do not differ statistically from each other by Tukey's test at p = 0.05.; µ= Infestation average

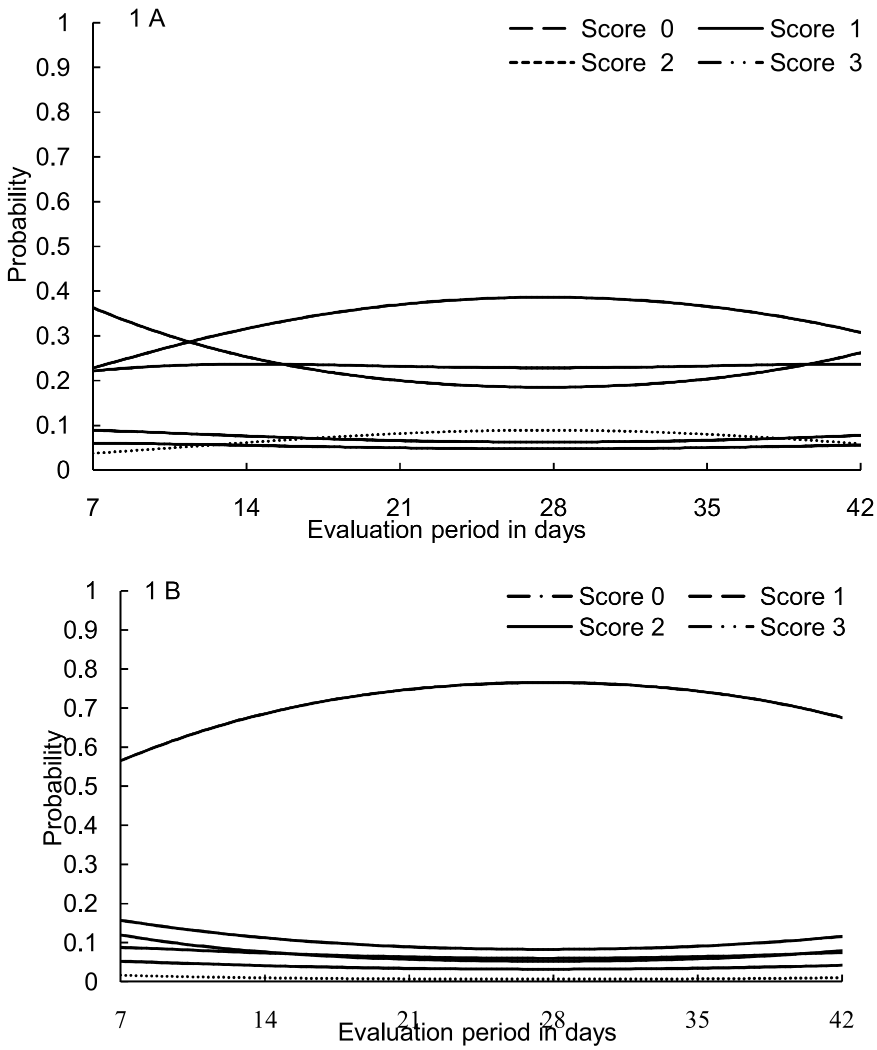

Fig. 1. Damage caused by *Spodoptera frugiperda* on the simple hybrid Genotype *A* (1 A) and Creole variety Genotype *B* (1 B) in the evaluation period

Regarding perennial infestation, there was a statistical difference between the evaluated cultivars (Table 2). The Creole Genotype *B* variety showed the lowest mean of *S. frugiperda* infestation, independent of the applied product in relation to the simple hybrid Genotype *A* in all the evaluation periods. The highest percentage of infestation (76.0%) occurred at 28 days after application of the products for Genotype *A* while Genotype *B* at the same time presented an average percentage of infestation of 28.7%, which means a difference of 37.7% in the percentage of infestation between the two cultivars. This difference between the materials is also reported in a study that evaluated the fertilisation responses in different maize genotypes and the effect on *S. frugiperda* infestation [26].

No statistical differences were recorded in the mean percentage of infestation of *S. frugiperda* in plants where the treatments (Si and Ca) were applied (Table 2). This result differed from another work with maize fertilisation, which indicates that the types of fertilisation possibly altered the response of the plant to the defence. In the present study, the sources of leaf fertilisation did not alter any of the genotypes tested in this aspect [27].

In relation to the evaluation periods, there was a tendency to reduce the percentage of infestation over time in all the evaluated treatments. This observation may be related to the insect's biology, which after its larval phase around 15 days, leaves the plant to complete its cycle. Phenology of the plant may be another factor that influences the pest preference for younger tissues.

The results of the maximum quantum efficiency of FSII (F_V/F_M) were not statistically significant for the cultivars nor for the cultivars x treatments interaction (Si, Ca and control) (Fig. 2). However, in this study, the presented results of the F_V/F_M ratio were close to the standard value for a plant with no damage to the photosynthetic apparatus, which is an approximate value of 0.8 [28].

Evaluating the impact of *Coccus hesperidum* (Linnaeus, 1758) infestation on *Citrus limon*, it was found that the F_V/F_M rates were significantly affected when the insect was fed from the plant [29].

For the stomatal conductance (gs), a statistical difference was recorded between the cultivars. In the Creole variety Genotype *B*, superior results were observed in relation to the simple hybrid Genotype *A*, specifically in silicon and control treatments, promoting to the hybrid a greater restriction to the stomatal opening. In addition, the Creole variety was different among the treatments, presenting a lower conductance in the treatment with calcium application (Fig. 3A).

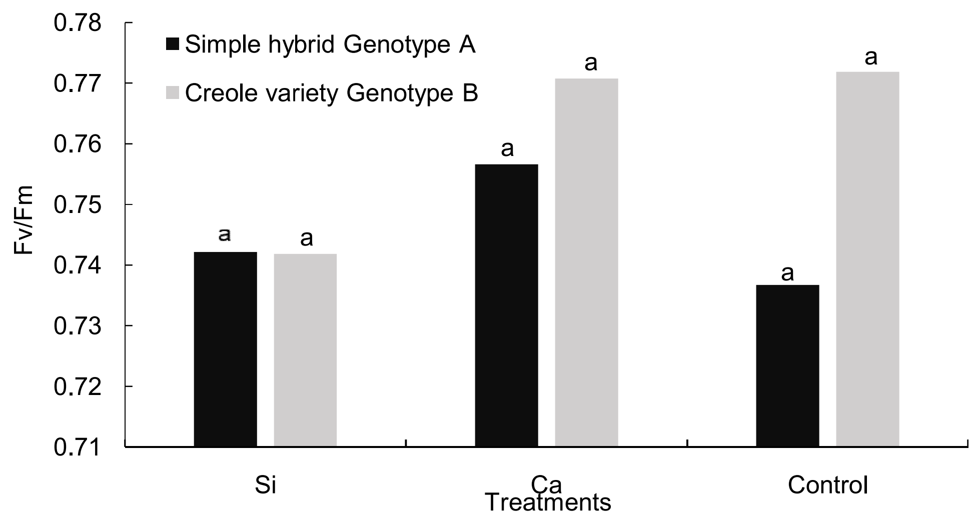

Fig. 2. Maximum quantum efficiency of FSII (F_V/F_M) in *Zea mays* submitted to treatments with Potassium Silicate, Calcium Chloride and control (without application), due to the stress caused by *Spodoptera frugiperda* attack

Table 3. Internal CO_2 Concentration (IC), Pressure Deficit (PD), Water Use Efficiency (WUE) and Carboxylation Efficiency (CE) in maize cultivars under the application of potassium silicate, calcium chloride and control, Areia, 2017

Cultivars	**Parameters evaluated**			
	IC	**PD**	**WUE**	**CE**
Simple hybrid	72.52 a	2.48 a	4.57 a	0.26 a
Creole variety	68.37 a	2.40 a	4.36 a	0.38 a
Treatments				
Silicon	66.37 a	2.327 a	4.57 a	0.329 a
Calcium	76.75 a	2.528 a	4.44 a	0.219 a
Control	68.20 a	2.461 a	4.38 a	0.408 a
C.V. (%)	30.14	10.72	20.29	81.39

[1]*Means followed by the same letter in the column do not differ statistically from each other, by Tukey's test at p = 0.05*

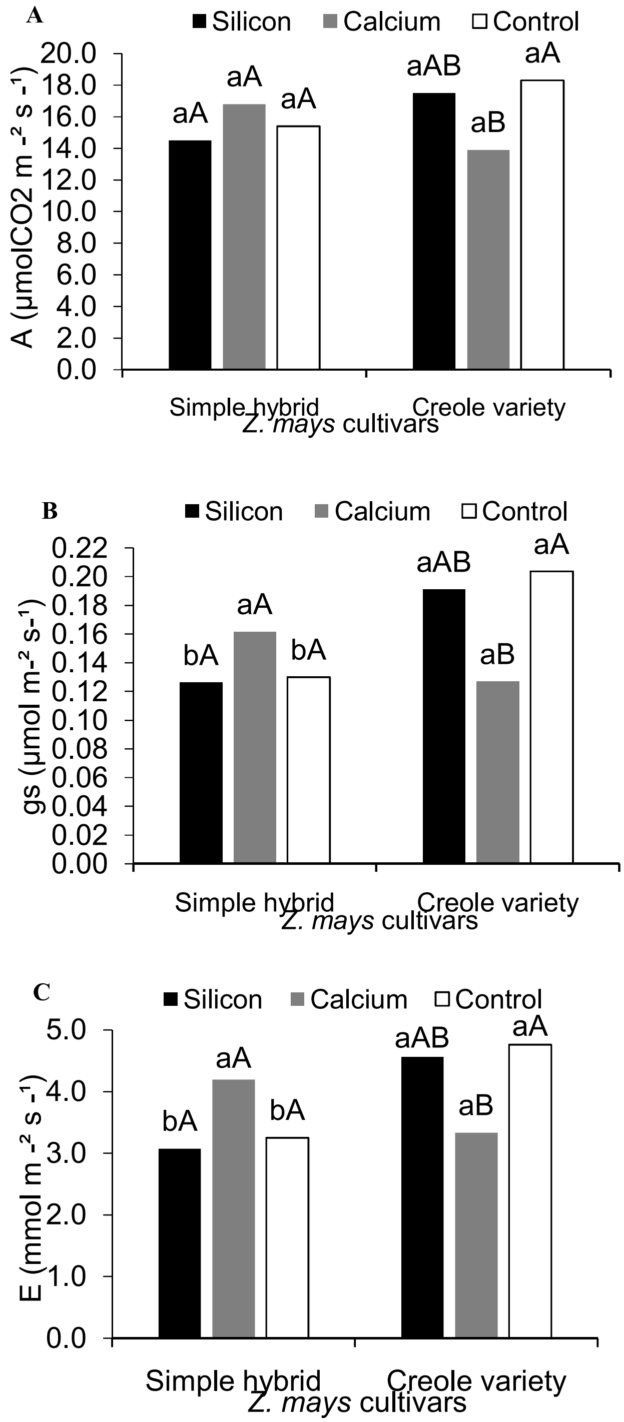

Fig. 3. Evaluation of Photosynthesis (A) (A); Stomatal Conductance (gs) (B) and Transpiration (E) (C), in maize under the application of Potassium Silicate, Calcium Chloride and control. Values followed with the same lowercase letter did not differ among cultivars, and with the same capital letter they did not differ among the treatments of each cultivar by the Tukey test at 5% of probability level

The variable photosynthetic rate (Fig. 3A) did not present statistical difference among the evaluated cultivars. However, the treatment with calcium application was different from the control in the Creole variety, where a lower photosynthetic rate was observed when applied to the calcium source. This order to compensate for insect attack, in most cases, plant defense responses are associated with the reduction in photosynthetic rate [30].

Different transpiration behaviours (Fig. 3C) were observed among the cultivars. Creole variety when submitted to silicon application and in control, it was obtained greater transpiration in compared to the simple hybrid. This reduction might be related to the results of stomatal conductance (Fig. 3B). In the Creole variety, there was a significant difference between the applied treatments, with lower transpiration when calcium was applied (Fig. 3C). These results differ from those found in a study with rice cultivation, where increased transpiration under calcium fertilisation was observed [31].

In this study, the results showed a significant reduction in the percentage of infestations (Table 2) and damages caused by *S. frugiperda*. A similar result was reported in the work with cabbage and beans which describes that the photosynthetic rate, stomatal conductance and transpiration are significantly reduced under pest attack [32].

Regarding the physiological parameters of Internal CO_2 Concentration (IC), Pressure Deficit (PD), Water Use Efficiency (WUE) and Carboxylation Efficiency (CE), no significant differences were recorded between the cultivars evaluated nor the treatments evaluated (Table 3). This result might be related to the same cultural management and availability of water provided to the cultivars, possibly not being these parameters sensitive or affected by the presence of pests.

4. CONCLUSIONS

The study concludes that silicon and calcium sources do not promote or induction the resistance towards *S. frugiperda* attack in the simple hybrid Genotype *A* and in the Creole variety Genotype *B* of maize. The Creole variety of *Zea mays* suffers less damage by *Spodoptera frugiperda* infestation in compared to the simple hybrid Genotype *A* maize.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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