

Original Research Article

Resistance induction in *Zea mays* L. cultivars against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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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 *S. frugiperda* caterpillars due to the application of Silicon and Calcium sources. Two cultivars of maize, Genotype A simple hybrid and the Creole Genotype B variety were used. These cultivars were submitted to two treatments of foliar fertilization, calcium chloride and potassium silicate, and a 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 predominance of plants that did not present any type of damage. The Creole variety presented the lowest mean of infestation by *S. frugiperda*, regardless of the product applied. No statistical differences were observed 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 for 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 behaviors were observed in the transpiration variable (E) among the cultivars. In the Creole genotype B variety when submitted to silicon application and in the control, a higher transpiration was obtained comparing 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 infestation of *Spodoptera frugiperda* in relation to the Genotype A simple hybrid maize.

Keywords: control method, fall armyworm, corn

21 1. INTRODUCTION

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

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

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

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

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

45 Silicon (Si) is considered a beneficial element for plants [10], it acts on the reinforcement of
46 the cell wall by depositing solid silica, making the plant more rigid, with better growth and
47 yield, for suppressing diseases and conferring characteristics of resistance to insects [11];
48 [12]. Calcium (Ca) is a macronutrient that is involved in a large number of signaling
49 pathways in plants, from symbiotic interactions, defense responses and various hormones,
50 functioning as a secondary messenger in response to biotic and abiotic signals [13]. Calcium
51 participates in lignin biosynthesis, which favors plant self-defense and can be used as an
52 inducer of pest resistance [14].

53 Other biomorphological factors are also associated with plant defense in relation to insect
54 infestation. As a consequence of the stress caused by pest injury, there are reductions in the
55 physiological parameters such as photosynthetic rate, transpiration and stomatal
56 conductance [15]; [16]. For this purpose, the gas exchange and fluorescence analysis of
57 chlorophyll "a" are able to detect, with safety and reliability, stress and injury effects in the
58 photosynthetic process caused by biotic or abiotic factors [17]. In view of the above, the
59 objective of this work was to evaluate induction resistance in corn cultivars due to the
60 application of Silicon and Calcium sources in the infestation of the *Spodoptera frugiperda*
61 caterpillar.

62 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

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65 The experiment was conducted at the Chã de Jardim experimental farm, belonging to the
66 Agricultural Sciences Center (CCA) of the Federal University of Paraíba (UFPB), Areia-PB,

67 Brazil. The climate of the region is classified as tropical humid, presenting average
68 temperature, relative humidity and precipitation of 23°C, 80% and 1,400 mm annually,
69 respectively [18]. The soil is classified as Yellow Latosol [19].

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

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

86 2.1 Evaluation of the damage and infestation of *Spodoptera frugiperda*

87 The evaluations of the damages caused by *S. frugiperda* were carried out weekly, from the
88 seventh day after the first application of the products (Si and Ca), with this done at 7, 14, 21,
89 35 and 42 days, ending at the beginning of flowering. Ten plants per plot were randomly
90 selected, with individual scores varying between 0 and 5 (Table 1) [20].

91 **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

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

95 2.2 Evaluation of physiological characteristics

96 The Fluorescence of Chlorophyll a, was determined from a portable fluorometer, model OS-
97 30p from Opti-Sciences®. Measurements were made on three plants per plot, between 9
98 and 12 hours, at 2 cm from the margin of the leaf, excluding its veins. To that end, one leaf
99 per plant, randomly selected was adapted to the dark with the use of tweezers, for
100 approximately 30 minutes. The maximum photochemical quantum FSMI (Fv/Fm) parameter,
101 often evaluated by the maximum fluorescence rate of chlorophyll "a", was evaluated to
102 compare healthy and damaged tissues due to biotic factors [21].

103 In the maize bolting (VT) phase, gas exchange and photosynthetic efficiency of maize leaves
104 were evaluated using an Infrared Gas Analyzer (IRGA), LCpro-SD model from
105 BioScientific®. Two plants were used per plot in the morning, between 9 am and 11 am,
106 sampling the first leaf opposite and below the ear. The following physiological parameters
107 were evaluated: Transpiration (E), Internal CO₂ concentration in leaf (IC), Stomatal
108 conductance (gs), Photosynthesis (A), Carboxylation efficiency (EC), Pressure Deficit (PD)
109 and Water Use Efficiency of (WUE).

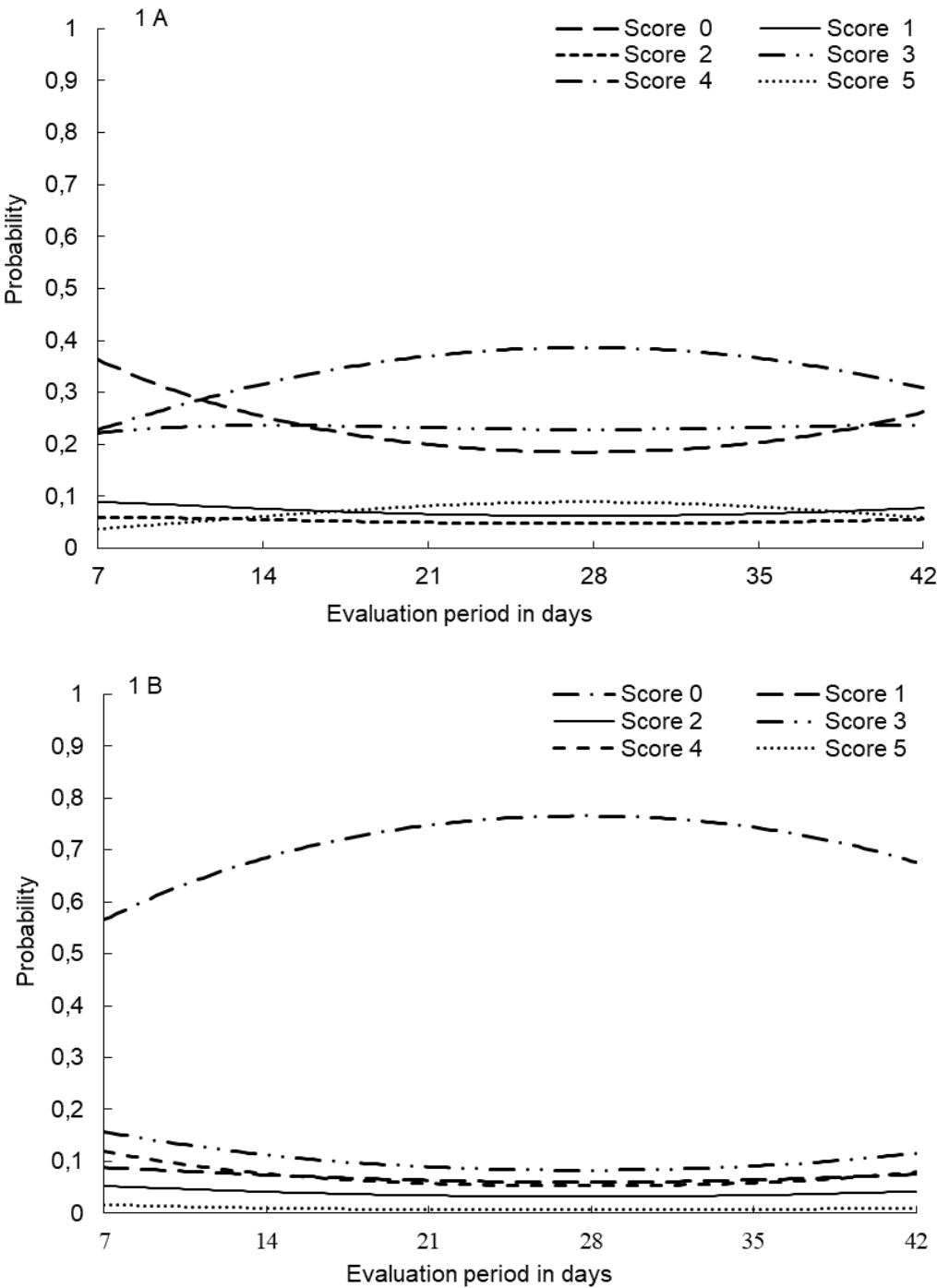
110 **2.3 Statistical analysis**

111 The experimental design was a randomized complete block (DBC), in a factorial scheme
112 (2x3), two maize cultivars (genotype A and genotype B), two sources of resistance induction
113 (Si and Ca) plus one control (without application), with four replicates per treatment,
114 totaling 24 experimental plots.

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

121 **3. RESULTS AND DISCUSSION**

122 For leaf damage caused by *Spodoptera frugiperda*, it was verified according to the
123 evaluation periods (Figure 1 A) that there was a statistical difference between the simple
124 hybrid and Creole variety (Figure 1 B). It was observed that in the interval of 14 to 42 days,
125 the simple hybrid was the one that presented the highest probability of occurrence of score
126 4, characterized by the stem of the partially destroyed plant, followed by score 3 that is the
127 plant with symptoms of ripped leaf. In contrast, it was observed that from the second
128 evaluation (14 days), the probability of occurrence of score 0, plants without symptoms of
129 attack, was decreasing as a function of time, this characterizes an increase in the intensity of
130 the attack of the pest.



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Figure 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.**

For the cultivar Creole Genotype **B** in all the evaluation periods, there was a predominance of plants that did not present any type of damage, that is, a higher probability of occurrence of score 0 was observed, showing that this variety in the study conditions was 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 synthesis of defenses 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 hybrids (1 Bt hybrid), found that 4 Creole genotypes presented moderate resistance to *S. frugiperda* in comparison 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, when compared to the transgenic cultivar [25].

In terms of 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 found in a study that evaluated the fertilization responses in different maize genotypes and the effect on *S. frugiperda* infestation [26].

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.

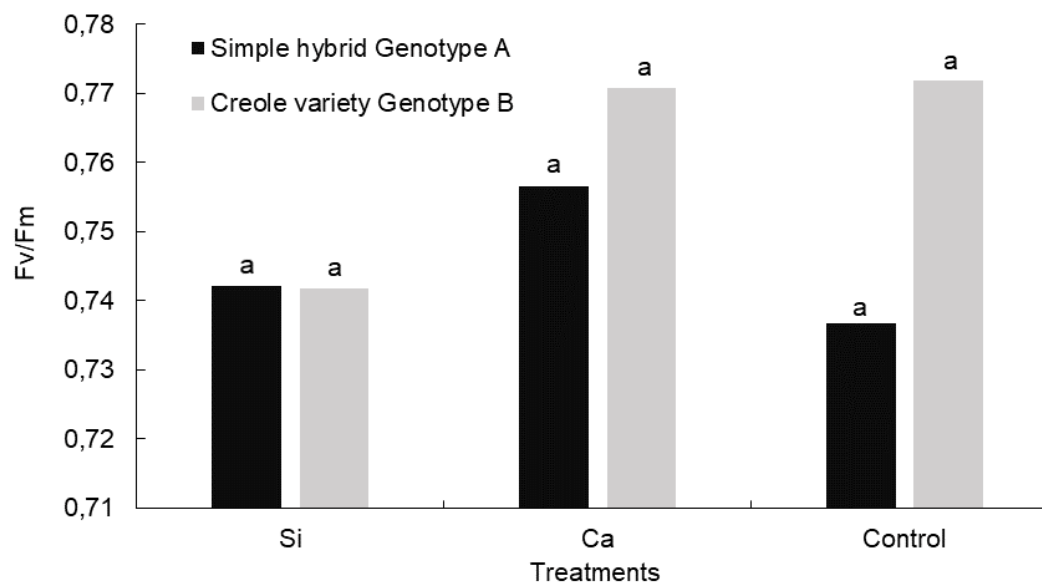
Cultivars	Treatments	<i>S. frugiperda</i> infestation (%) in five evaluation periods (days)					μ
		14	21	28	35	42	
Simple hybrid Genotype A	Si	79.8 Aa	74.6 Bc	76.0 Bb	69.7 Bd	54.9 Be	71.0 A
	Ca	77.8 Bb	76.5 Ad	83.4 Aa	76.9 Ac	60.0 Ae	74.9 A
	Control	67.07Cb	59.7 Cc	68.7 Ca	46.0 Cd	43.0 Gs	56.9 A
μ		74.87 A	70.3 A	76.0 A	64.2 A	52.6 A	-
Creole variety Genotype B	Si	16.31 Fd	15.2 Fc	23.8 Fb	40.2 Fa	17.8 Fe	22.7 A
	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	-

¹Averages 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

No statistical differences were observed 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 that found in another work with maize fertilization, which indicates that the types of fertilization possibly altered the response of the plant to the defense, in this work the sources of leaf fertilization 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 evaluated treatments. This observation may be related to the insect's own 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) (Figure 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]



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Figure 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.

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

For the stomatal conductance (gs), a statistical difference was observed 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 (Figure 3A).

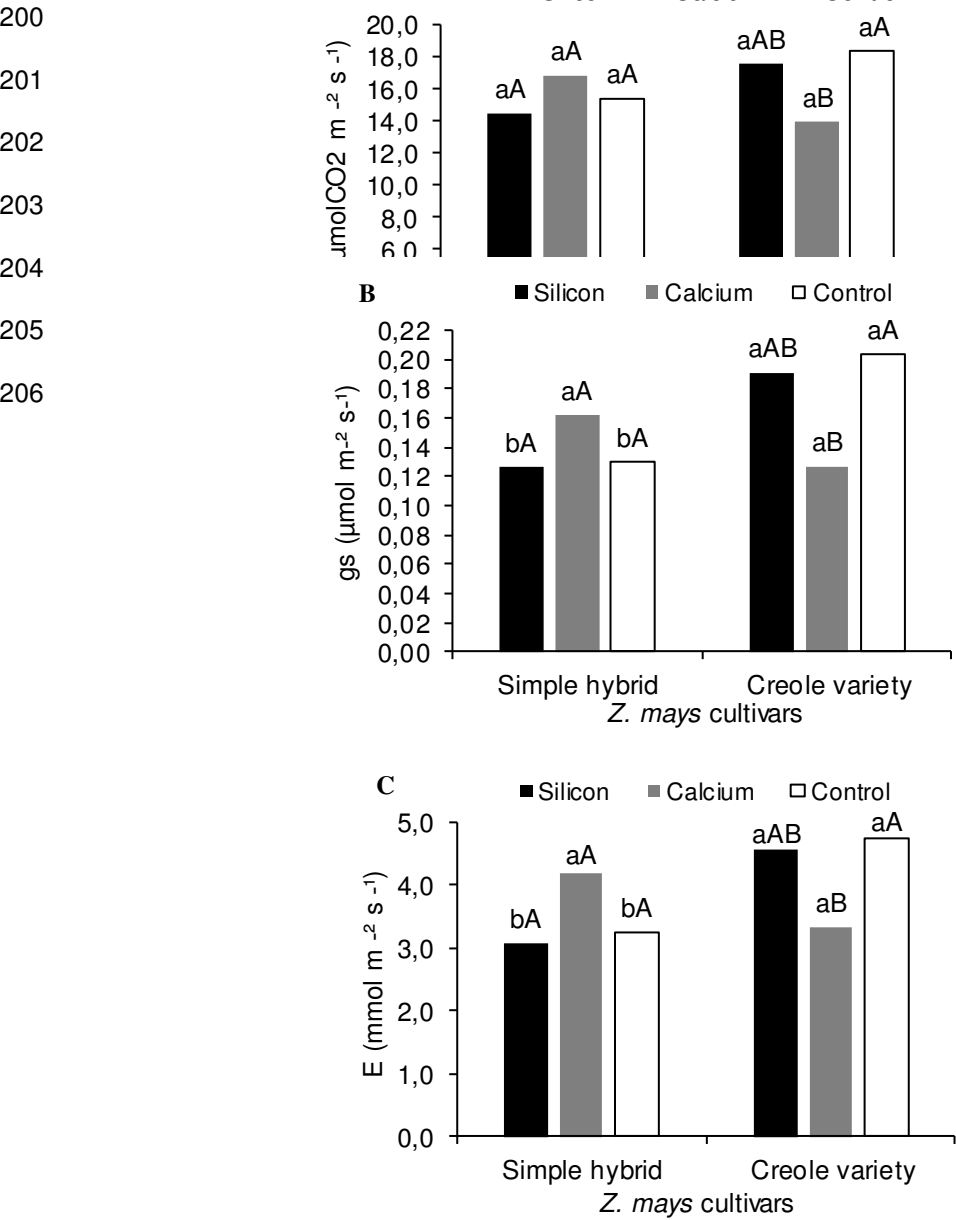


Figure 3. Evaluation of Photosynthesis (A); Stomatal Conductance (gs) (B) and Transpiration (E) (C), in maize under the application of Potassium Silicate, Calcium Chloride and control. Values followed with the same lowercase letter did not differ among cultivars, and with the same capital letter they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

The variable photosynthetic rate (A) did not present statistical difference among the cultivars evaluated. 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 (Figure 3B). This order to compensate for insect attack, in most cases, plant defense responses are associated with the reduction in photosynthetic rate [30].

Different transpiration behaviors (E) were observed among the cultivars, in the Creole variety when submitted to silicon application and in the control, it was obtained a greater transpiration compared to the simple hybrid (Figure 3C). This reduction may be related to the results of stomatal conductance (Figure 3A). In the Creole variety, there was a significant difference between the applied treatments, with lower transpiration when calcium was applied (Figure 3C). These results differ from those found in a study with rice cultivation, where increased transpiration under calcium fertilization 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 observed 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 CO₂ Concentration (IC), Pressure Deficit (PD), Water Use Efficiency (WUE) and Carboxylation Efficiency (CE), no significant differences were observed between the cultivars evaluated nor the treatments evaluated (Table 3). This result may 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.

Table 3. Internal CO₂ 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

Means followed by the same letter in the column do not differ statistically from each other, by Tukey's test at $P = 0.05$.

4. CONCLUSIONS

Silicon and Calcium sources do not promote the resistance induction to *S. frugiperda* attack in the simple hybrid Genotype A and in the Creole variety Genotype B of maize.

243 The Creole variety of *Zea mays* suffers less damage by *Spodoptera frugiperda* infestation
244 than the simple hybrid Genotype A maize.

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