

Original Research Article

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

ABSTRACT

The maize crop (*Zea mays* L.) faces serious limitations in cultivation due to attack by the *Spodoptera frugiperda* insect-pest. 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 FII (F_v/F_m) was not statistically significant for the cultivars nor for the cultivars x treatments interaction. For 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 among 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 simple hybrid maize Genotype A.

Keywords: control method, fall armyworm, corn

1. INTRODUCTION

Zea mays L. maize is one of the world's largest cereals grown with 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 the 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* caterpillar (J. E. Smith, 1797) is the main cause of damage and represents a high cost of control annually [3].

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



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



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

36 Silicon (Si) is considered a beneficial element for plants [10], it acts on the reinforcement of
37 the cell wall by depositing solid silica, making the plant more rigid, with better growth and
38 yield, for suppressing diseases and conferring characteristics of resistance to insects [11];
39 [12]. Calcium (Ca) is a macronutrient that is involved in a large number of signaling
40 pathways in plants, from symbiotic interactions, defense responses and various hormones,
41 functioning as a secondary messenger in response to biotic and abiotic signals [13]. Calcium
42 participates in lignin biosynthesis, which favors plant self-defense and can be used as an
43 inducer of pest resistance [14].

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

53 54 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

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

61 The simple hybrid corn denominated Genotype A and the Creole variety denominated
62 Genotype B were used. These materials were submitted to two treatments of foliar
63 fertilizations, being applied commercial sources of Calcium Chloride and Potassium Silicate,
64 and a control treatment. Three applications of the products were carried out in the vegetative
65 growth phase of the maize cultivars, specifically in the V3, V6 and V12 stages, following the
66 recommended dosages for culture suggested by the manufacturer of 2 L ha⁻¹. The products

67 were always applied from 4pm, this being the most recommended time for loss reduction,
68 and manually by a costal spray with a 20-liter capacity.

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

77 2.1 Evaluation of the damage and infestation of *Spodoptera frugiperda*

78 The evaluations of the damages caused by *S. frugiperda* were carried out weekly, from the
79 seventh day after the first application of the products (Si and Ca), with this done at 7, 14, 21,
80 35 and 42 days, ending at the beginning of flowering. Ten plants per plot were randomly
81 selected, with individual scores varying between 0 and 5 (Table 1) [20].

82 **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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84 To evaluate the level of infestation of *S. frugiperda*, the two central rows of each plot were
85 used, relating the number of plants attacked by the total number of plants in the crop line.

86 2.2 Evaluation of physiological characteristics

87 The Fluorescence of Chlorophyll a, was determined from a portable fluorometer, model OS-
88 30p from Opti-Sciences®. Measurements were made on three plants per plot, between 9
89 and 12 hours, at 2 cm from the margin of the leaf, excluding its veins. To that end, one leaf
90 per plant, randomly selected was adapted to the dark with the use of tweezers, for
91 approximately 30 minutes. The maximum FSMI photochemical quantum (Fv/Fm) parameter,
92 often evaluated by the maximum fluorescence rate of chlorophyll "a", was evaluated to
93 compare healthy and damaged tissues due to biotic factors [21].

94 In the maize bolting (VT) phase, gas exchange and photosynthetic efficiency of maize leaves
95 were evaluated using an Infrared Gas Analyzer (IRGA), LCpro-SD model from
96 BioScientific®. Two plants were used per plot in the morning, between 9am and 11am,

sampling the first leaf opposite and below the ear. The following physiological parameters were evaluated: Transpiration (E), Internal CO₂ concentration in leaf (Ci), Stomatal conductance (gs), Photosynthesis (A), Carboxylation efficiency (EC), Pressure Deficit (DP) and Efficiency of use of water (EFUA).

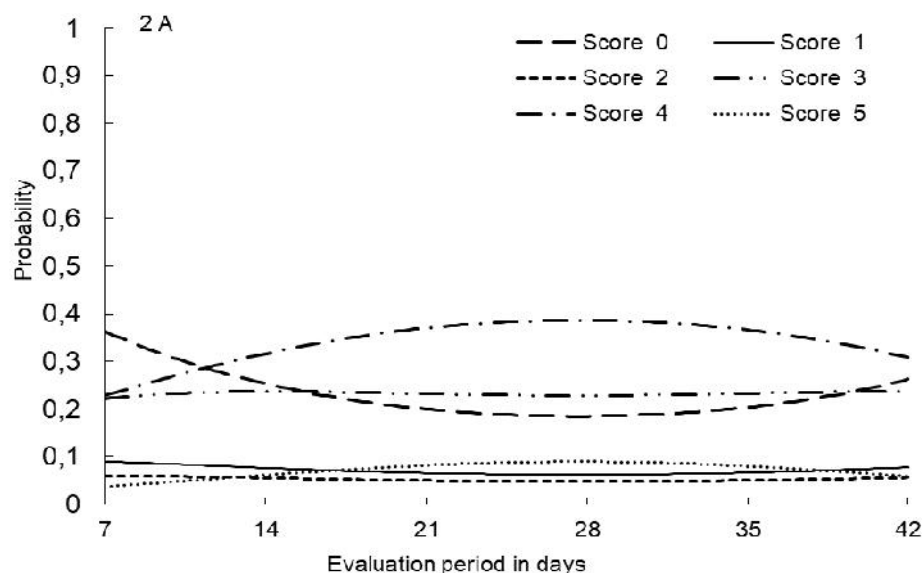
2.3 Statistical analysis

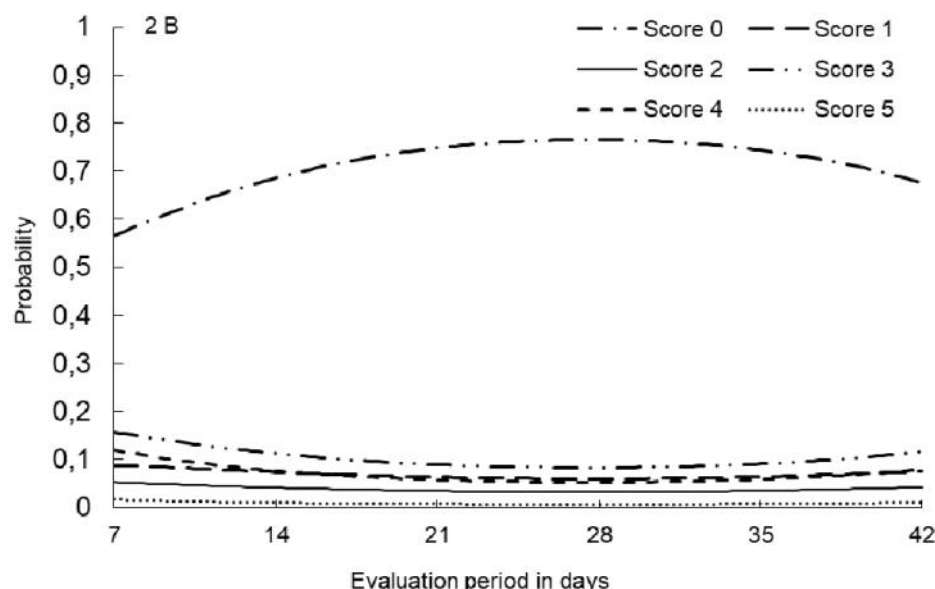
The experimental design was a randomized complete block (DBC), in a factorial scheme (2x3x4), two maize cultivars (genotype A and genotype B), two sources of resistance induction (Si and Ca) plus one control (without application), with four replicates per treatment, totaling 24 experimental plots.

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, to evaluate both the effect of the treatments and the sensitivity differences between the cultivars to said treatments, all the variation factors were processed by SAS [22].

3. RESULTS AND DISCUSSION

For leaf damage caused by *Spodoptera frugiperda*, it was verified according to the evaluation periods (Figure 1A) that there was a statistical difference between the simple hybrid and Creole variety (Figure 1B). It was observed that in the interval of 14 to 42 days, the simple hybrid was the one that presented the highest probability of occurrence of score 4, characterized 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 decreasing as a function of time, this characterizes an increase in the intensity of the attack of the pest.





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125 **Figure 1. Damage caused by *Spodoptera frugiperda* on the simple hybrid Genotype A**
126 **(2A) and Creole variety Genotype B (2B) in the evaluation period.**

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

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

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

144 In terms of perennial infestation, there was a statistical difference between the evaluated
145 cultivars (Table 2). The Creole Genotype B variety showed the lowest mean of *S. frugiperda*
146 infestation, independent of the applied product, in relation to the simple hybrid Genotype A in
147 all the evaluation periods. The highest percentage of infestation (76.0%) occurred at 28 days
148 after application of the products for Genotype A while Genotype B at the same time
149 presented an average percentage of infestation of 28.7%, which means a difference of
150 37.7% in the percentage of infestation between the two cultivars. This difference between
151 the materials is also found in a study that evaluated the fertilization responses in different

maize genotypes and the effect on *S. frugiperda* infestation. 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.

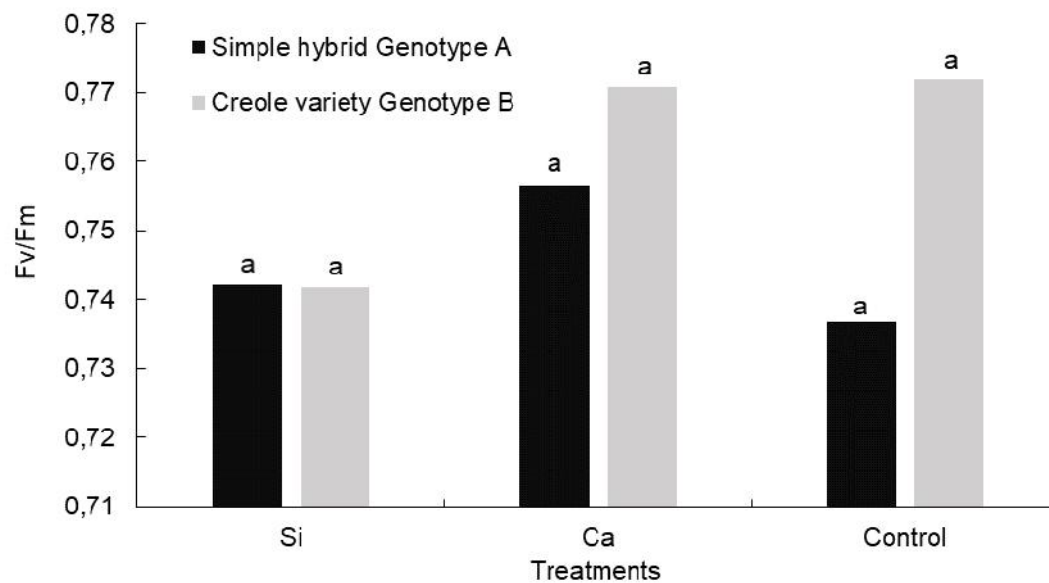
Cultivars	Treatments	<i>S. frugiperda</i> infestation (%) in five evaluation periods (days)					μ
		14	21	28	35	42	
Simple hybrid Genotype A	Si	79.8 Aa	74.6 Bc	76.0 Bb	69.7 Bd	54.9 Be	71.0 A
	Ca	77.8 Bb	76.5 Ad	83.4 Aa	76.9 Ac	60.0 Ae	74.9 A
	Test	67.07Cb	59.7 Cc	68.7 Ca	46.0 Cd	43.0 Gs	56.9 A
μ		74.87 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
	Test	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 $P = 5$.
 μ =Infestation average

No statistical differences were observed in the mean percentage of infestation of *S. frugiperda* in plants where the products (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]

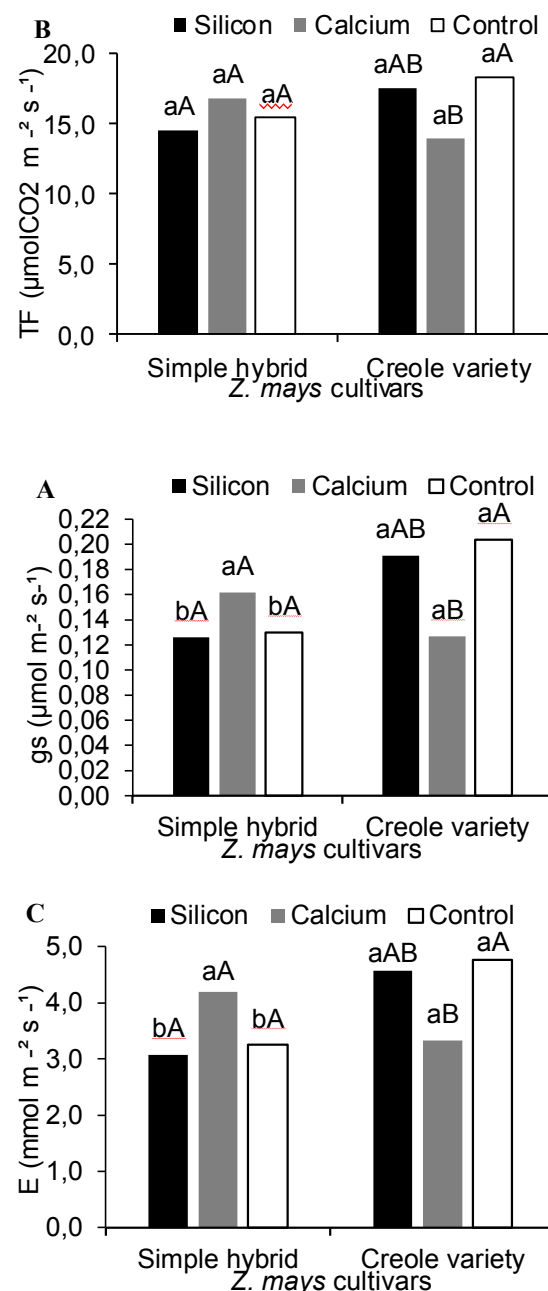


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180 **Figure 2. Maximum quantum efficiency of PSII (F_v/F_m) in *Zea mays* submitted to**
 181 **treatments with Potassium Silicate, Calcium Chloride and control (without**
 182 **application), due to the stress caused by *Spodoptera frugiperda* attack.**

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

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



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Figure 3. Evaluation of Stomatal Conductance (gs) (A); Photosynthesis (A) (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.

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

204 Different transpiration behaviors (E) were observed among the cultivars, in the Creole variety
205 when submitted to silicon application and in the control, it was obtained a greater
206 transpiration compared to the simple hybrid (Figure 3C). This reduction may be related to the
207 results of stomatal conductance (Figure 3A). In the Creole variety, there was a significant
208 difference between the applied treatments, with lower transpiration when calcium was
209 applied (Figure 3C). These results differ from those found in a study with rice cultivation,
210 where increased transpiration under calcium fertilization was observed [31]

211 In this study, the results showed a significant reduction in the number of infestations (Table
212 2) and damage caused by *S. frugiperda*. The insect-plague in this one, causing greater
213 infestation and damage to the plants, being this similar result in work with cabbage and
214 beans that describes that the photosynthetic rate, the stomatal conductance and the
215 perspiration are significantly reduced under attack of plagues [32].

216 Regarding the physiological parameters of Internal CO₂ Concentration (Ci), Pressure Deficit
217 (DP), Water Efficiency (EFUA) and Carboxylation Efficiency (EC), no significant differences
218 were observed between the cultivars evaluated nor the treatments evaluated (Table 3). This
219 result may be related to the same cultural management and availability of water provided to
220 the cultivars, possibly not being these parameters sensitive or affected by the presence of
221 pests.

222 **Table 3. Internal CO₂ Concentration (Ci), Pressure Deficit (DP), Water Use Efficiency**
223 **(EFUA) and Carboxylation Efficiency (EC) in maize cultivars under the application of**
224 **potassium silicate, calcium chloride and control, Areia, 2017.**
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Cultivars	Parameters Evaluated			
	Ci	DP	EFUA	EC
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

226 [†]Means followed by the same lowercase letter in the column do not differ statistically from each other,
227 by Tukey's test at P = .05.

228 4. CONCLUSION

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

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

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