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ARTIGO

Biology and behavior of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and resistance induction in *Azospirillum brasilense* inoculated maize and silicon application

Biologia e comportamento de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) e indução de resisitência em milho inoculado com *Azospirillum brasilense* e aplicação de silício

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Abstract

The effect of *Azospirillum brasilense* and silicon on maize on the biology and behavior of *Spodoptera frugiperda,* the resistance induction, and the silicon quantification in the leaves were studied. The inoculation of corn seeds was carried out before planting, and the silicon applications (soil and foliar) were performed 16 and 26 days after planting. The bioassay of food preference and survival of 2nd instar larvae was carried out on days 2 and 4 after the applications. The effect of treatments on the immature phase was also evaluated, including the duration of the total larval period and the pupal phase, total mortality, sex ratio, pupal weight, and adult deformation. Bioassays were also carried out to quantify silicon in the leaves and the induced resistance was determined by the peroxidase and phenylalanine ammonia-lyase activities. Larvae were not preferred in the leaves subjected to the silicon treatments. Regarding the survival of 2nd instar larvae, higher mortality rates and cannibalism were found in the treatment containing inoculant + foliar silicon, and foliar silicon. A longer duration of the total larval phase, higher mortality, lower pupal weight, and a higher number of deformed adults were observed for the treatments containing silicon. No significant effects of the treatments were observed for the silicon quantitative bioassays and resistance induction. However, old leaves exhibited a higher silicon concentration. Thus, silicon may be an effective alternative to control *S. frugiperda*, with no isolated effects of the inoculant *A. brasilense*, on the *S. frugiperda* behavior.

Keywords: Lepidopteran behavior. Plant resistance. Peroxidase. Phenylalanine ammonia-lyase.

Resumo

Estudou-se o efeito do *Azospirillum brasilense* e do silício no milho sobre a biologia e comportamento de *Spodoptera frugiperda* e a indução de resistência e quantificação do silício nas folhas. A inoculação das sementes de milho foi realizada antes do plantio e as aplicações de silício foliar e solo, 16 e 26 dias após o plantio. Os bioensaios de preferência alimentar e

sobrevivência de larvas de 2º ínstar foram realizados em dois momentos, 4 e 2 dias após as aplicações. Também foi avaliado o efeito dos tratamentos na fase imatura, onde foram avaliados: duração no período larval total e na fase pupal, mortalidade total, razão sexual, peso pupal e deformação do adulto. Também foram realizados bioensaios para quantificar o silício nas folhas e induzir resistência por meio da atividade da peroxidase e da fenilalanima amônia liase. As larvas não foram preferidas em tratamentos contendo silício. Em relação à sobrevivência das larvas de 2º ínstar, maiores taxas de mortalidade e canibalismo foram encontradas no tratamento contendo inoculante + silício foliar e silício foliar. Houve maior duração da fase larval total, maior mortalidade, menor peso pupal e maior média de adultos deformados nos tratamentos contendo silício. Quanto aos bioensaios quantitativos de silício e indução de resistência, não houve efeito significativo para os tratamentos em nenhum dos bioensaios. Porém, observou-se que as folhas velhas apresentam maior porcentagem de silício. Assim, conclui-se que o silício é uma alternativa para o controle de *S. frugiperda*, em relação ao uso do inoculante A. brasilense, não houve efeitos isolados sobre o comportamento de *S. frugiperda*.

Palavras-chave: Comportamento de lepidópteros. Resistência de plantas. Peroxidase. Fenilalanina amônia-liase.

Introduction

Spodoptera frugiperda (Smith, 1797) (Lepidoptera: Noctuidae) is a polyphagous pest, which occurs in several countries such as Brazil, Argentina, and the USA (Prowell et al., 2004 & Clark et al., 2007), causing economic losses in different crops such as maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merrill), cotton (*Gossypium hirsutum* L.), and beans (*Phaseolus vulgaris* L.) (Pogue, 2002; Nagoshi 2009; Bueno et al., 2011). The attack of this insect pest has increased in recent years, probably due to the large supply of hosts throughout the year, thus impairing the management strategies against *S. frugiperda* (Barros; Torres & Bueno, 2010). The control of this pest is usually performed with the use of insecticides and/or resistant hybrids, which are relatively expensive and do not include ecologically-based production systems.

Therefore, plant resistance to insects is an excellent method of pest control, presenting economic, biological, and environmental advantages (Wisemam & Widstron, 1986). Several studies have shown the effect of silicon application on pest control and induction of resistance of grasses, once these plants are silicon accumulators (Epstein, 2001; Fawe et al. 2001; Feng, 2004; Goussain et al. 2002; Gomes et al., 2005; Gomes et al., 2008, & Nogueira, 2018).

The induced resistance corresponds to the increased defense capacity of the plant against pathogens and insect pests (Dixon; Harrison & Lamb, 1994). The expression of induced resistance may be local or systemic when it is expressed in locations not directly exposed to the inducing agent and may occur after exposure to biotic and/or abiotic agents,

with an emphasis on silicon (Van Loon, Bakker & Pieterse, 1998 & Stadinik, 2000). Plant-induced defense using silicon occurs due to the formation of mechanical barriers and/or alteration of plant biochemical responses to the herbivorous attack, increasing the synthesis of toxins that can act as inhibitors or repellents (Epstein, 1994; Marschner, 1995; Dannon, & Wydra, 2004), besides increasing the defense mechanisms, including accumulation of lignin and phenolic compounds.

Plant resistance involves the activation of latent mechanisms against external inducers with no change in the plant genome (Baysal et al., 2003). Changes in the activities of key enzymes allow monitoring the resistance induction state in plants exposed to the inducing agent (Macagnan et al., 2008), and peroxidases and phenylalanine ammonia-lyase (FAL) stand out for this purpose (Baysal et al., 2003).

Peroxidases oxidize organic substrates by eliminating hydrogen peroxide, reactive oxygen species, and electron acceptors. In addition, these enzymes participate in plant growth and development, cell detoxification, and defense mechanisms such as lignification, wound healing, and oxidation of phenolic compounds (Baysal et al., 2003). Phenylalanine ammonia-lyase (FAL) is fundamental in phenylpropanol biosynthesis and participates in the synthesis of monomers of lignin, salicylic acid, phytoalexins, and flavonoids (Gerasimova et al., 2005).

The biological inoculant *Azospirillum brasilense* has been used in grasses for improving water and nutrient absorption, besides promoting biological nitrogen fixation (Hungria et al., 2010). However, there are few studies on increased plant resistance and pest control. Therefore, the use of *A. brasilense* may increase silicon absorption in plants and aims to determine its effect on the control of pests such as *S. frugiperda*, as well as its profile as a resistance inducing agent in plants.

The determination of the silicon concentration in the plant tissue is performed by the molybdenum yellow method (Hallmark et al., 1982; Korndörfer, Pereira & Nolla, 2004). This method consists in measuring the yellow color formed by the reaction between silicon and ammonium molybdate in an acidic medium, and the yellower the solution, the higher the silicon concentration in the plant material.

Therefore, the objective of this study was to evaluate the effect of the use of silicon and *A. brasilense* on maize plants on the biology and behavior of *S. frugiperda,* and the induction of plant resistance through the expression of peroxidase and phenylalanine ammonia-lyase (FAL) activities. The silicon concentration in the plant tissue by the molybdenum yellow method was also determined.

Methodology

The experiments were carried out at the Biotechnology and Entomology, and Crop Breeding laboratory of Embrapa-CNPSo in Londrina/PR, and the Soil Physics and Phytopathology at the Federal University of Fronteira Sul, Campus Laranjeiras do Sul/PR.

Rearing of *Spodoptera frugiperda*

The initial *S. frugiperda* population was collected from corn crop, in the city of Londrina/PR, and the larvae from the 30th to 38th generations were used, created on an artificial diet adapted by Bowling (1967), at the Crop Breeding Laboratory of Embrapa-CNPSo.

The diet was stored under refrigeration in 50 mL plastic cups containing ¼ artificial diet, which were removed from refrigeration before use until reaching room temperature. The eggs were kept in 200 mL cups sealed with thin paper and covered with thin cardboard until larval hatching. Subsequently, the diet was added to the larvae feeding cups until reaching the third instar stage and then subcultured by placing two larvae in a plastic cup, which was sealed with a thin cardboard lid. The diet was replaced upon dehydration, and the development was monitored until reaching the pupal phase, followed by the identification of the sex of pupae (Butt & Cantu, 1962).

After differentiating males and females, the pupae were placed in Gerbox® and later in acrylic cages until the emergence of the adults. As adults, they were kept in rectangular-shaped acrylic cages, 50x30x30 cm (length, width, and height), covered by an A3 paper sheet. The moths were fed with cotton pads soaked in a 10% honey solution. The cages were kept until the third day of oviposition, and the eggs were removed daily and placed in 200 mL cups for use in experiments and/or rearing continuity.

Seed collection and plant cultivation

Seeds from the cultivar AL Bandeirante were used. The fertilization was carried out using 30g per pot of formulated fertilizer 08-28-16 (N-P-K), which was incorporated into the soil at the time of planting. Six seeds were sown in 12 L pots. Thinning of plants was performed after the expansion of the first leaf (phenological stage V1), leaving two plants per

pot. A drip irrigation system was performed using drippers. The temperature in the greenhouse during the experiment period (January to February) ranged from 25 to 28ºC.

Plant preparation

A completely randomized experimental design was used, with six treatments and 12 repetitions (n = 1). The treatments (T) and their respective doses were: T1: Control with absence of application; T2: Inoculant *A. brasilense* (GrapNod a®) dose of 100 mL for 25 kg/seed applied 30 minutes before planting; T3: Inoculant *A. brasilense* (GrapNod a®) + Soil silicon (Diaflow®) dose of 100 mL for 25 kg/seed applied 30 minutes before planting and 4 g of Diaflow® + 400 mL distilled water applied to the soil 16 and 26 days after planting; T4: Inoculant *A. brasilense* (GrapNod®) + Foliar silicon (Sifol®) dose of 100 mL for 25 kg/seed applied 30 minutes before planting and 10 mL of Sifol® + 1000 mL distilled water applied to leaves 16 and 26 days after planting until draining of the syrup; T5: Soil silicon (Diaflow®) dose of 4g of Diaflow® + 400 mL distilled water applied to the soil 16 and 26 days after planting; T6: Foliar silicon (Sifol®) dose of 10 mL of Sifol® + 1000 mL distilled water applied to leaves 16 and 26 days after planting until draining of the syrup.

For the bioassays 4 and 5, new and old leaves were collected 96 and 48 h after the first and second silicon application. New leaves were considered those completely open and attached to the cartridge (cartridge leaves), while old leaves were those completely open and outside the cartridge (expanded leaves).

Bioassay 1: Food preference through the free choice test

Larvae of the second instar stage and leaf sections of 2 cm in length from the 6 treatments were randomly arranged and equidistant in 15 cm-diameter Petri plates with the bottom coated with moist filter paper. Ten larvae were placed in the center of each plate. After 24, 48, and 72 h, the larvae from each treatment were counted. The experiment was maintained in a climate chamber at 25 ± 2 ºC, 12 h photoperiod, and 80 ± 10% relative humidity. This bioassay was carried out in two moments, in the first and second silicon application, which was performed at 16 and 26 days after planting, respectively. The first trial was performed 4 days after the first application, while the second trial was performed 2 days after the second application. A completely randomized experimental design was used, with six treatments and 20 replications.

Bioassay 2: Survival of second instar larvae on test with no choice

The bioassay was carried out in two moments, in the first and second silicon application, which was performed at 16 and 26 days after planting, respectively. Thus, the first trial was conducted 4 days after the first application, while the second trial was performed 2 days after the second application. A completely randomized experimental design was used, with 6 treatments and 20 replications. Each plot consisted of a 10 cm diameter Petri plate with the bottom coated with moist filter paper, containing a 9 cm long leaf section. Ten newly hatched larvae (up to 24 h) were placed on the leaf. The larvae remained in this set until reaching the third instar stage, and the leaf section was changed daily. The experiment was maintained in a climate-controlled chamber at 25 ± 2ºC, 12 h photoperiod, and 80 ± 10% relative humidity.

Mortality and cannibalism were evaluated at the end of the second instar stage. Larvae that were immobile after stimulation and without body mutilation were considered dead larvae, while mutilated larvae or those with only the cephalic capsules characterized cannibalism.

Bioassay 3: Effect of silicon application on immature phases

The bioassay was performed 4 days after the first silicon application, and the second silicon application was performed on the sixth day of evaluation. A randomized experimental design was used, with 6 treatments and 4 replications. Each repetition consisted of five 100 mL plastic cups with a lid, and the bottom covered with moistened filter paper. A piece of leaf of approximately 4 cm^2 and a newly hatched larva (up to 24 h) were placed in each cup. The leaf sections were changed daily, offering food ad libitum. The cups were kept in a climate-controlled chamber at 25 ± 2ºC, 12 h photoperiod, and 80 ± 10% relative humidity.

The parameters larval phase duration (days); sex ratio; pupal weight (24 h after transformation); pupal phase duration (days); adult deformation (number of individuals) and total mortality and at each instar (%) were evaluated.

Bioassay 4: Silicon concentration of the leaf using the molybdenum yellow method

The methodology used for these procedures was adapted by Korndörfer, Pereira, and Nolla (2004). For that, a pretreatment was performed as follows: 1) previous drying of the material in the open air to remove excess moisture; 2) washing the leaves in a detergent solution; 3) passing the leaves in distilled water to remove the detergent; 4) drying the leaves in a forced circulation oven at 65º C until constant weight; 5) drying the material for a further 30 minutes at 60 °C; 6) milling the material in a 2.5 mm sieve Willey mill; and 7) packaging of the ground material in plastic bags or tubes until use, according to the steps described below.

Extraction/digestion steps: All procedures were performed in a gas exhaustion hood, and the operators wore plastic gloves. For that, 0.1000 g of the ground material was placed in 100 mL polypropylene tubes, and 2 mL of H_2O_2 (300 or 500 g L^{-1}) was added and stirred (magnetic stirrer) for a few seconds, and 3 mL of NaOH (500 g L^{-1}) was added. The tubes were vortexed and placed in a water bath (85ºC) for approximately 1 hour. After the extracts/samples were no longer releasing gases, the tubes were capped and autoclaved for 1h at 123 ºC and 1.5 atm (20 psig), with the addition of 45 mL of distilled water. Subsequently, the extract was transferred to a plastic vial and remained at rest until the residues were deposited at the bottom of the tube.

Sample preparation: A 1 mL aliquot of the extract supernatant was placed in a 50 mL plastic beaker and 19 mL of distilled water was added.

Standard preparation (0, 2, 4, 6, and 8 mg L^{-1}Si): aliquots containing 0; 2; 4; 6, and 8 mL of Si standard solution (50 ppm) were placed in 50 mL flasks, and the volume was completed with distilled water. Subsequently, a 20 mL aliquot of each standard (0, 2, 4, 6, and 8 mg L^{-1} Si) was placed in a 50 mL plastic beaker, and 1 mL HCl (1: 1 or 500 g L^{-1}) + 2 mL ammonium molybdate was added to the beakers containing the standards and the sample (digested extracts) and gently stirred. The more intense the yellow color, the higher the concentration of silicon in the sample. After 5 to 10 minutes, 2 mL of oxalic acid was added and gently stirred. After 2 minutes, readings were performed at 410 ηm% T in a UV-Visible Spectrophotometer. The silicon concentration (ppm) was calculated using the following equation: y = -6.9775x + 96.724 (R2 = 0.9802).

Bioassay 5: Induction of defense enzymes

The collected leaves were placed in a 50 mL Falcon tube, and stored in a Styrofoam box with ice until frozen at -20 ºC. Then, the material was freeze-dried at -50ºC for 30 hours. For the preparation of the extracts, 1.0 g of freeze-dried leaves from each treatment was used. The freeze-dried material was macerated in a crucible containing 0.04g of polyvinylpyrrolidone (PVP) and 4 mL of 0.01 M sodium phosphate buffer pH 6.8. It was then placed in Eppendorf tubes (2 mL) and centrifuged for 20 minutes (14,500 rpm) at 4 ° C. After this procedure, the supernatant was collected and immediately frozen until analysis. The peroxidase and phenylalanine ammonia-lyase (FAL) levels were determined.

Peroxidase

Peroxidase activity was determined by measuring the conversion of guaiacol to tetraguaiacol in a spectrophotometer at 470 nm (Lusso & Pascholati, 1999). For that, 0.2 mL of protein extract and 2.8 mL of paraenzyme substrate (306 µL of hydrogen peroxide PA, 12.5 mL of 2% guaiacol, and 87.5 0.01 M phosphate buffer, pH 6.0) were mixed in a 3mL cuvette and allowed to react for one minute at 30 ° C. The enzyme activity was determined using the extreme values of the linear increment range, and the results were expressed in absorbance units at 470 nm min^{-1}mg $protein^{-1}$.

Phenylalanine ammonia-lyase (FAL)

Phenylalanine ammonia-lyase activity was determined as described by Umesha (2006). For that, 100 µL of the enzyme extract was mixed with 400 µL of 0.025 M Tris-HCl buffer pH 8.8, and 500 µL of 0.05 M L-phenylalanine (825.9 mg diluted in 100 mL of 0.025 M Tris-HCL buffer, pH 8.8). The mixture was incubated at 40 ° C for 2 h. Then, 60 µL of 5 M HCl was added to stop the reaction, and spectrophotometer readings were performed at 290 nm. The phenylalanine ammonia-lyase activity consisted of the difference between the absorbance of the mixture containing the sample and the control (100 µL of enzyme extract and 900 µL of 0.025 M Tris-HCl buffer pH 8.8). The results were plotted on a standard curve of trans-cinnamic acid and expressed as mg of trans cinnamic acid h^{-1} mg $protein^{-1}$.

Statistical analysis

All data were subjected to analysis of variance and means were compared by Tukey test at 5% probability, using the Sisvar statistical program (Ferreira, 2014).

Results

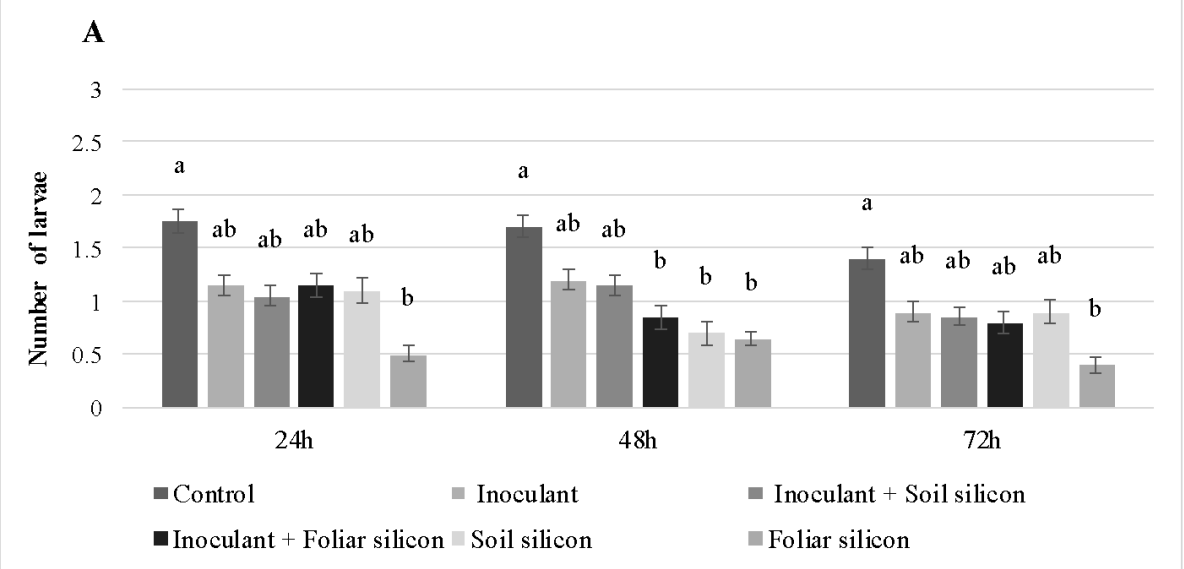
Food preference through the free choice test

The food preference behavior was determined by comparing the treatments with the control regardless of the number of silicon applications (Figures 1A and 1B). In addition, there was no food preference for silicon-containing treatments, mainly for the samples with the application directly on the leaf (Figures 1A and 1B).

Regarding the experiment with only one silicon application, a higher number of larvae was observed in the control for all treatments, at 24, 48, and 72 hours after the beginning of the experiment, and the treatment with foliar silicon application showed the lowest preference (Figure 1A). In the second evaluation, 48 hours after the beginning of the experiment, the treatments containing inoculant + foliar silicon, silicon applied to the soil, or foliar silicon (T4, T5, and T6) showed the lowest number of larvae (Figure 1A).

Figure 1A

Number of larvae per treatment at different times (24h, 48h, and 72h) in the feed preference test (bioassay 1) with one application (16 days after planting). Bioassay performed 4 days after application. Controlled conditions of 25 ± 2ºC, 12h photoperiod, and 80 ± 10% RH



Note. Means ± SD, followed by the same letter within the evaluation time did not differ statistically (Tukey,

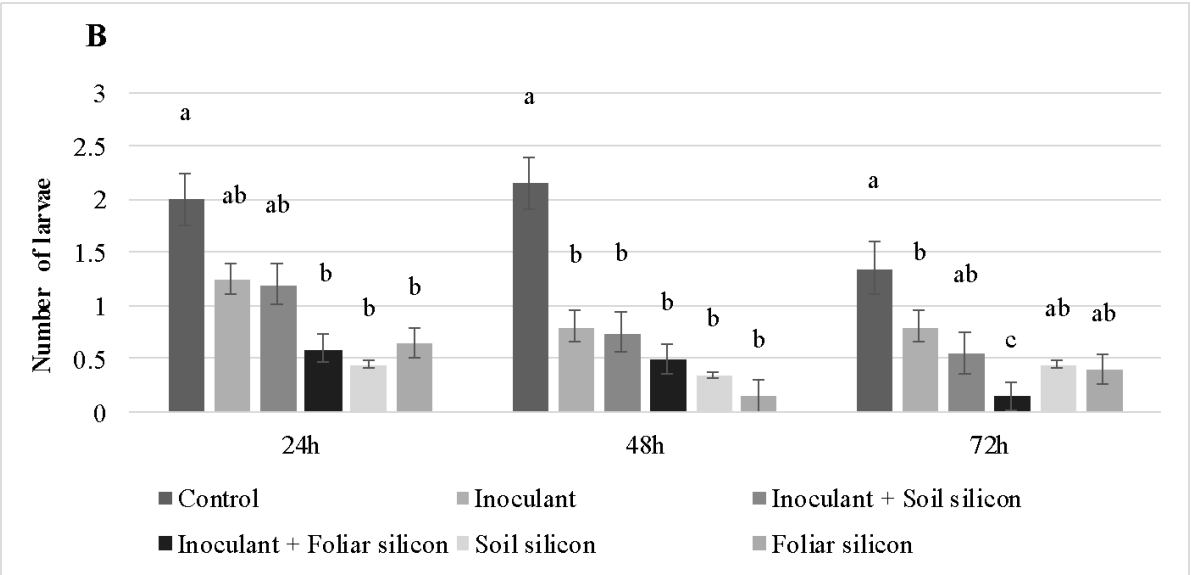
$p \geq 0.05$). Data expressed as $\sqrt{X+1}\ \sqrt{X+1}$.

Source: Elaborate by the author.

In the second experiment, with two silicon applications, a higher number of larvae was found in the control at 24, 48, and 72 hours after the beginning of the experiment (Figure 1B). In contrast, a lower feeding preference of larvae was observed 24 h after the beginning of the experiment in the treatments inoculant + foliar silicon, silicon applied to the soil, and foliar silicon (Figure 1B). In the second evaluation, 48 hours after the beginning of the experiment, a lower food preference was observed for all treatments except for the control. After 72 hours, the food with inoculant and foliar silicon application showed a lower feeding preference (Figure 1B).

Figure 1B

Number of larvae per treatment at different times (24h, 48h, and 72h) in the feed preference test (bioassay 1) with two applications (16 and 26 days after planting). Bioassay performed 2 days after second application. Controlled conditions of 25 ± 2ºC, 12h photoperiod, and 80 ± 10% RH



Note. Means ± SD, followed by the same letter within the evaluation time did not differ statistically (Tukey, $p \geq 0.05$). Data calculated as $\sqrt{X+1}\ \sqrt{X+1}$.

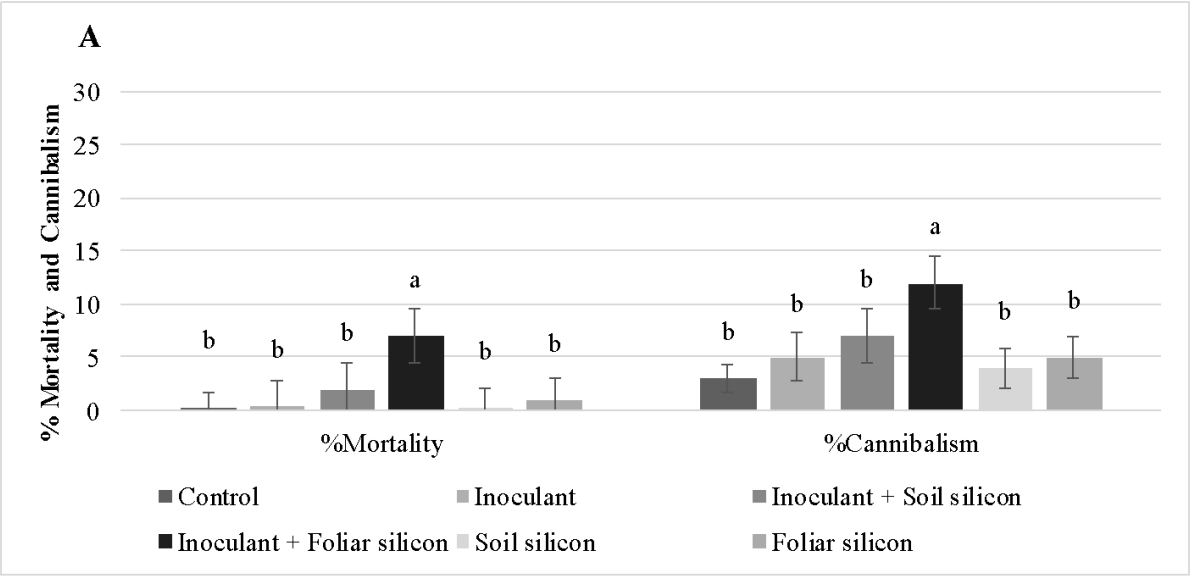
Source: Elaborate by the author.

Survival of second instar larvae in the test with no food choice

Higher mortality and cannibalism rates of *S. frugiperda* at the end of the second instar were observed for larvae fed with leaves treated with inoculant and foliar silicon application after 16 days of planting (Figure 2A).

Figure 2A

Percentage of mortality and cannibalism of S. frugiperda larvae at the end of the second instar fed with corn leaves, with or without the addition of inoculant and silicon (bioassay 2) after one silicon application (16 days after planting). The bioassay was performed 4 days after application. Controlled conditions of 25 ± 2ºC, 12h photoperiod, and 80 ± 10% RH



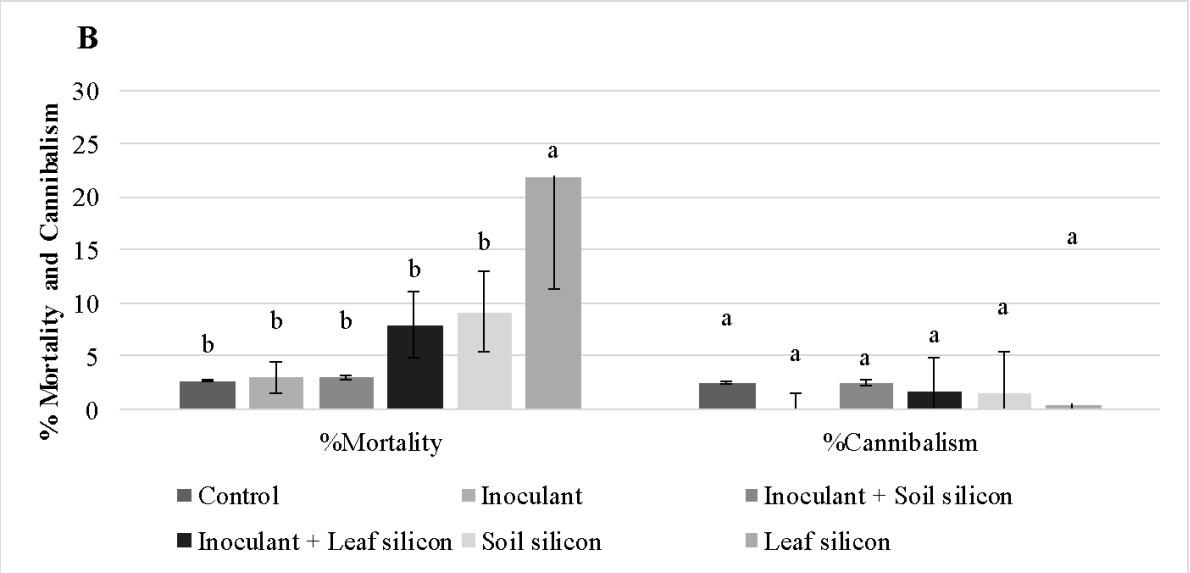
Note. Means ± SD, followed by the same letter within the evaluation time did not differ statistically (Tukey, $p \geq 0.05$). Data calculated as $\sqrt{X + 0.5}\ \sqrt{X + 0.5}$.

Source: Elaborate by the author.

Concerning the mortality, the treatment with foliar silicon applications at 16 and 26 days after planting showed the highest average (Figure 2B). Although the presence of cannibalism was observed, there was no difference between treatments (Figure 2B).

Figure 2B

Percentage of mortality and cannibalism of S. frugiperda larvae at the end of the 2nd instar fed with corn leaves, with or without the addition of inoculant and silicon (bioassay 2) after two applications (16 and 26 days after planting). The bioassay was carried out 2 days after the second silicon application. Controlled conditions of 25 ± 2ºC, 12h photoperiod, and 80 ± 10% RH



Note. Means ± SD, followed by the same letter within the evaluation time did not differ statistically (Tukey, $p \geq 0.05$). Data calculated as $\sqrt{X + 0.5}\,\sqrt{X + 0.5}$.

Source: Elaborate by the author.

Effect of silicon on immature phases

Regarding the duration of the larval period, an increase in the total larval period was observed for the treatments with inoculant + foliar silicon, soil silicon, and foliar silicon, while the shortest larval period was observed for the control (Table 1). The sex ratio did not differ among treatments (Table 1).

Concerning the total larval mortality, the treatments with inoculant and inoculant + soil silicon had the highest mortality rates, with values of 16% and 15%, respectively. The treatments inoculant + foliar Si and soil Si had a mortality rate of 12% (Table 1).

Table 1

Biological characteristics of S. frugiperda (bioassay 3) fed with inoculated maize and silicon-treated leaves (25 ± 2°C, 80 ± 10% RH and photoperiod of 12:12[L:D])

Treatments	Larvae-adult duration (days)	Mortality total larvae-adult (%)	Sex ratio[1]
Control	13.03±0.48 b	0.01±0.16 c	0.15±0.61[ns]
Inoculant	13.32±0.55 ab	0.03±0.17 bc	0.05±0.49
Inoculant + Soil silicon	13.63±0.72 ab	0.15±0.20 a	0.10±0.68
Inoculant + Foliar silicon	13.96±0.73 a	0.12±0.22 ab	0.10±0.64
Soil silicon	13.97±0.62 a	0.12±0.22 ab	0.10±0.64
Foliar silicon	14.09±0.50 a	0.16±0.22 a	0.10±0.70
CV (%)	2.83	43.65	12.19

Note. Means ± SD followed by the same letter in the columns for each species did not differ statistically (Tukey test, $p \leq 0.05$). [ns]ANOVA Not significant. [1]Data calculated as $\sqrt{X + 0,5}$.

Source: Elaborate by the author.

The average pupal weight was higher in the control and lower in the treatments containing foliar silicon (Table 2). Regarding the duration of the pupal phase, a difference was observed between the control and the other treatments, with a shorter and longer duration, respectively (Table 2). A significant difference was observed for wing deformation of adults among treatments, and the highest number of deformed individuals was observed for those fed with leaves treated with foliar silicon (Table 2). The treatments with *A. brasilense* inoculant and the control showed lower deformation of adults (Table 2).

Table 2

Biological characteristics of S. frugiperda (bioassay 3) fed with inoculated maize and silicon-treated leaves (25 ± 2°C, 80 ± 10% RH and photoperiod of 12:12[L:D]

Treatments	Pupal weight (g)	Pupae (days)	Deformed adults[1]
Control	0.32±0.15 a	10.25±0.44 b	0.25±0.71 b
Inoculant	0.28±0.16 ab	11.19±0.74 a	0.25±0.71 b
Inoculant + Soil silicon	0.27±0.17 ab	11.67±0.69 a	0.50±0.76 ab
Inoculant + Foliar silicon	0.25±0.12 b	11.67±0.49 a	1.25±0.71 ab
Soil silicon	0.27±0.18 ab	11.33±0.49 a	1.25±1.12 ab
Foliar silicon	0.26±0.13 b	11.33±0.69 a	2.00±0.90 a
CV (%)	9.21	3.42	18.74

Note. Means ± SD followed by the same letter in the columns for each species did not differ statistically (Tukey test, $p \leq 0.05$). [ns]ANOVA Not significant. [1]Data calculated as $\sqrt{X+1}\,\sqrt{X+1}$.

Source: Elaborate by the author.

Silicon concentration in the leaf determined by the molybdenum yellow method

There were no differences between the treatments for the amount of silicon in the leaves by the yellow method, both in young and old leaves submitted to Si application (Table 3). However, a difference was observed for young and old leaves in the treatment containing foliar Si, with a higher average for the old leaves (Table 3).

Table 3

Silicon concentration (%) in new leaves (cartridge leaves) and old leaves (expanded leaves) using the yellow method (bioassay 4) with silicon application at 16 days after planting

	Application (16 DAP)	
Treatments	New leaves (%)[1]	Old leaves (%)
Control	0.61±0.51 aA	0.88±0.47 aA
Inoculant	0.82±0.30 aA	1.17±0.66 aA
Inoculant + Soil silicon	0.83±0.60 aA	1.19±0.44 aA
Inoculant + Foliar silicon	0.77±0.64 aA	1.23±0.68 aA

Soil silicon	1.02±0.63 aA	1.28±0.44 aA
Foliar silicon	0.59±0.47 aB	1.32±0.51 aA
CV (%)	8.87	27.27

Note. Means ± SD followed by the same letter do not differ statistically, lowercase letters in the column and uppercase letters in the row, by the Tukey test at 5% probability. [1]Data calculated as $\sqrt{X+1}\,\sqrt{X+1}$.
Source: Elaborate by the author.

After two silicon applications, there was no difference between the treatments for both young and old leaves (Table 4). However, the old leaves of the treatments with *A. brasilense* + foliar Si, and soil Si showed higher Si concentrations when compared to the young leaves (Table 4).

Table 4

Silicon concentration (%) in new leaves (cartridge leaves) and old leaves (expanded leaves) using the yellow method (bioassay 4) with silicon application at 16 and 26 days after planting

	Application (26 DAP)	
Treatments	New leaves (%)	Old leaves (%)
Control	0.32 ± 0.44 aA	0.69 ± 0.58 aA
Inoculant	0.26 ± 0.32 aA	0.52 ± 0.51 aA
Inoculant + Soil silicon	0.75 ± 0.70 aA	1.14 ± 0.59 aA
Inoculant + Foliar silicon	0.66 ± 0.41 aB	0.99 ± 0.17 aA
Soil silicon	0.50 ± 0.39 aB	1.26 ± 0.58 aA
Foliar silicon	0.53 ± 0.61 aA	1.37 ± 0.61 aA
CV (%)	9.21	7.73

Note. Means ± SD followed by the same letter do not differ statistically, lowercase letters in the column and uppercase letters in the row, by the Tukey test at 5% probability. Data calculated as $\sqrt{X+1}\,\sqrt{X+1}$.
Source: Elaborate by the author.

Peroxidase activity

No differences between the treatments were observed for the peroxidase activity of new leaves and old leaves after silicon application at 16 DAP (Table 5), which remained after two silicon applications (Table 6).

Table 5

Peroxidase activity (Abs min^{-1}.mg.protein^{-1}) of new leaves (cartridge leaves) and old leaves (expanded leaves) (bioassay 5) with silicon application at 16 days after planting

	Application (16 DAP)	
Treatments	New leaves	Old leaves
Control	0.04±0.24^{ns}	1.06±0.99^{ns}
Inoculant	0.24±0.68	0.20±0.39
Inoculant + Soil silicon	0.05±0.14	0.28±0.57
Inoculant + Foliar silicon	0.14±0.44	1.10±0.98
Soil silicon	0.02±0.07	0.36±0.62
Foliar silicon	0.25±0.67	0.37±0.52
CV (%)	18.39	28.28

Note. Means ± SD without significant differences by Tukey test at 5% significance. ^{ns}ANOVA Not significant.

Data calculated as $\sqrt{X+0.5}\ \sqrt{X+0.5}$.

Source: Elaborate by the author.

Table 6

Peroxidase activity (Abs min^{-1}.mg.protein^{-1}) of new leaves (cartridge leaves) and old leaves (expanded leaves) (bioassay 5) with silicon application at 16 and 26 days after planting

	Application (16 and 26 DAP)	
Treatments	New leaves	Old leaves
Control	0.03±0.12^{ns}	0.38±0.64^{ns}
Inoculant	0.15±0.41	0.51±0.68
Inoculant + Soil silicon	0.12±0.44	0.46±0.61
Inoculant + Foliar silicon	0.12±0.19	0.33±0.42
Soil silicon	0.13±0.29	0.31±0.41
Foliar silicon	0.12±0.31	0.36±0.51
CV (%)	9.63	18.66

Note. Means ± SD without significant differences by Tukey test at 5% significance. [ns]ANOVA Not significant.

Data calculated as $\sqrt{X+0.5}\ \sqrt{X+0.5}$.

Source: Elaborate by the author.

Phenylalanine ammonia-lyase (FAL) activity

There was no difference in phenylalanine ammonia-lyase (PAA) activity for all treatments, both for young and old leaves submitted to only one silicon application (Table 7), which was also not observed after two silicon applications (PAF) (Table 8).

Table 7

Phenylalanine ammonia-lyase (FAL) activity (Abs min^{-1}.mg.protein^{-1}) of new leaves (cartridge leaves) and old leaves (expanded leaves) (bioassay 5) with silicon application at 16 days after planting

	Application (16 DAP)	
Treatments	New leaves[1]	Old leaves[2]
Control	1.46±1.14[ns]	0.27±0.50[ns]
Inoculant	2.36±1.41	0.26±0.61
Inoculant + Soil silicon	2.04±1.27	0.44±0.51
Inoculant + Foliar silicon	1.60±1.30	0.23±0.37
Soil silicon	1.49±0.91	0.20±0.35
Foliar silicon	1.30±0.95	0.28±0.56
CV (%)	25.31	17.64

Note. Means ± SD with significant differences by Tukey test at 5% significance. [ns]ANOVA Not significant.

[1]Data calculated as $\sqrt{X+1}\ \sqrt{X+1}$. [2]Data calculated as $\sqrt{X+0.5}\ \sqrt{X+0.5}$.

Source: Elaborate by the author.

Table 8

Phenylalanine ammonia-lyase (FAL) activity (Abs. min^{-1}.mg.protein^{-1}) of new leaves (cartridge leaves) and old leaves (expanded leaves) (bioassay 5) with silicon applications at 16 and 26 days after planting

Treatments	Application (16 and 26 DAP)	
	New leaves	Old leaves
Control	0.48±0.40^{ns}	0.27±0.48^{ns}
Inoculant	0.59±0.63	0.68±0.63
Inoculant + Soil silicon	0.90±0.64	0.87±0.65
Inoculant + Foliar silicon	0.78±0.94	0.20±0.39
Soil silicon	0.72±0.92	0.20±0.41
Foliar silicon	0.70±0.47	0.42±0.56
CV (%)	23.01	15.60

Note. Means ± SD with significant differences by Tukey test at 5% significance. ^{ns}ANOVA Not significant. Data calculated as $\sqrt{X+0.5}\,\sqrt{X+0.5}$.

Source: Elaborate by the author.

Discussion

The feeding preference of second instar larvae for the untreated leaves was probably due to the deposition of amorphous silica in the epidermal cell wall of plant tissues, forming a physical barrier, increasing the hardness of plant tissues, and reducing digestibility and access to nitrogen and carbon during digestion (Keeping, Kvedaras, & Bruton, 2009; Dias et al., 2014).

Similar results were reported by Nascimento et al. (2014), who found no feeding preference in silicon-treated rice plants when compared to the control, and concluded that silicon application in rice affects the feeding preference of *S. frugiperda*. Moreover, more effective results were observed for foliar application, with greater practicality of application, as also reported by Reis et al. (2007).

Given the results of this study and its correlation with similar studies in crops of the Poacea family (Reis et al., 2007), it is possible to assume that the feeding preference of caterpillars is directly correlated to the silicon deposition on corn leaves. The use of the biological inoculant *A. brasilense* also interfered in the feeding preference of second instar

larvae. Probably, the inoculant bacteria acted on plant growth through the production of growth-promoting substances, providing better root growth, improving water and nutrient absorption, thus resulting in a more vigorous plant (Correa et al., 2008; Hungria et al., 2010), which makes the plant less attractive to insects (Dourado Neto & Severino, 2001).

The mortality and cannibalism observed for the treatments with foliar silicon are due to the increase in leaf tissue stiffness. Similar results were observed by Goussain et al. (2002), who verified mortality of 2nd instar *S. frugiperda* larvae treated with silicon. Thus, it is possible to correlate these results with the hypothesis that Si increases the resistance of plant tissues, increasing the thickness of the epidermis, which makes it difficult for pest insects, such as larvae, to chew (Datnoff, Snyder & Korndörfer, 2001), leading to death.

However, the inoculant *A. brasilense* also affected the cannibalism index, when used together with foliar silicon application. This result may have been due to *A. brasilense* enables biological nitrogen fixation and increased nutrient absorption (Hungria et al., 2010), which may have increased the silicon absorption, leading to this type of behavior, favored by the food stress of *S. frugiperda*.

Although studies have shown increased nutrient absorption using this bacterium in seed inoculation, few studies have correlated *A. brasilense* with pest control. Thus, it is possible to suggest the hypothesis that this bacterium can favor the silicon absorption by the plant, allowing the hardening of the tissues, thus making it difficult to feed the larvae. However, although cannibalism was verified after two silicon applications, no difference was observed between the treatments. In contrast, Goussain et al. (2002) observed a higher cannibalism rate rather than mortality of *S. frugiperda* larvae confined with silicon-treated leaves. However, it is noteworthy that in this study, larval mortality increased exponentially after two foliar silicon applications, which may explain the low cannibalism rate.

The longer total larval period in silicon-containing treatments may be correlated with lower palatability and digestibility and lower food acceptance in the first instars, thus increasing the larval period. Moreover, these results may be due to the quality and quantity of food consumed, which may affect the development time, body weight, and survival of lepidopterans, including *S. frugiperda* (Nation, 2002; Golizadeh et al., 2009; Silva et al., 2017).

In the pupal phase, a lower duration was observed for the control, with the emergence of adults before the other treatments, accelerating the reproduction cycle of these pests. In contrast, Nogueira et al. (2018) found no differences in the pupal phase when feeding larvae with treated and untreated silicon rice plants.

Regarding the pupal weight, a higher average was observed for the control, suggesting that these larvae were fed without limiting factors, such as stiffness of plant tissue towards silicon. These results once again are correlated with the quality and quantity of food consumed, thus affecting pupal weight (Silva et al., 2017). Thus, it is important to correlate the pupal weight with the size of the emerged adult since the heavier the pupae, the larger the emerged adult and, consequently, the greater the acceptance and access to the copulation, increasing the reproduction of this species (Panizzi & Parra, 2009).

The lowest pupal weight was observed for the treatments with foliar silicon application, with advantages over the drench method due to the ease of application. The inoculant *A. brasilense* also affected pupal weight when combined with foliar silicon, leading to the hypothesis of improved plant nutrition and increased silicon absorption.

The treatments did not affect the sex ratio, thus they did not act in determining the sexes of the species studied. In contrast, Nogueira et al. (2018) found differences in sex ratio when comparing *S. frugiperda* larvae fed on rice plants without and with the application of silicon. The sex ratio may be affected by the quality and quantity of food consumed in the larval phase, as well as other species parameters (growth rate, development time, final weight, dispersion, and survival) and, in certain cases, fertility and dispersion of adults (Nation, 2002; Golizadeh et al., 2009; Silva et al., 2017).

The higher rate of individuals with deformed wings from the silicon-containing treatments may also be due to the nutritional inadequacy (quality and quantity of foods offered in the larval phase), causing this abnormality, highlighting its impact on the development of *S. frugiperda*.

Silicon affected the behavior and biology of *S. frugiperda,* including mortality. It is worth emphasizing that the highest mortality was observed in the pre-pupal phase, thus suggesting that the silicon treatments reduced the larvae feeding, not allowing them to accumulate enough energy for the metamorphosis process. This result may be probably due to the deposition of silica on the leaf tissues, preventing the larvae from feeding properly, thus not meeting the nutritional requirements. It is noteworthy that insect nutrition is classified into qualitative and quantitative aspects. Qualitative aspects refer to the basic nutritional requirements of essential and non-essential nutrients (Parra, Panizzi, & Haddad, 2009), which can be correlated with the present results. Those authors have reported that the quantitative nutrition aspects refer to the amount of food ingested, digested, assimilated, and converted into growth tissues. However, it cannot be stated that larvae have ingested smaller amounts of food once the leaf consumption was not investigated. The effect of silicon may also be

associated with hormonal changes in larvae, interfering with ecdysis, affecting their metamorphosis (Bogorni & Vendramim, 2005).

In general, the highest mortality rate observed for the silicon-containing treatments may be due to the physical barrier of silicon, making the plant tissues more rigid (Fawe et al., 2001), which can lead to food stress and a higher mortality rate.

Studies on silicon application concerning the behavioral aspects and biology of *S. frugiperda* have shown a physical barrier formation in plants (Jones & Handreck, 1967; Malavolta, 1980; Marschner, 1995). According to Malavolta (1980), the uptake and accumulation in plant cells vary from species to species. Therefore, it is important to highlight that grasses have a greater capacity to accumulate silicon, leading to the suppression of larvae feeding and changes in their development and behavior.

The higher silicon concentration observed in the old leaves when compared to the young leaves may be due to the mobility of silicon in the plant, suggesting a greater accumulation in the old leaves, which is not redistributed in young leaves. According to Wise, Nikolic, and Römheld (2007), silicon deposits on old plant sections may not be redistributed to the new section, with higher silica concentrations in the shoot rather than the root, and higher concentration in the old leaves and the basal part of the grass leaves, once these plants are silicon accumulators. This behavior is due to the type of silicon deposition on the plants (amorphous silica SiO_2nH_2O), in which silicon is poorly mobile or immobile since it combines with cellular organic compounds such as cellulose and hemicellulose, which impairs the mobilization process (Balastra et al., 1989 & Jarvis, 1987).

Although few studies have reported the effect of the use of *A. brasilense* inoculant together with silicon application, a higher silicon concentration was observed in old leaves, once it can improve root growth and increase the absorption of water and nutrients (Hungria et al., 2010). Therefore, it is suggested that the use of *A. brasilense* may have increased silicon absorption.

When accumulated in the leaf epidermis, silicon can activate genes involved in the production of secondary metabolites and plant defense-related enzymes (Gratão et al., 2005), such as peroxidase and phenylalanine ammonia-lyase (FAL) (Gomes et al., 2005). Gomes et al. (2005) have reported that the enzymes peroxidase and phenylalanine ammonia-lyase (FAL) are involved in the lignin synthesis route from phenolic compounds in the phenylpropanoid pathway.

Higher peroxidase and phenylalanine ammonia-lyase (FAL) activities are directly related to the increase in plant resistance against adversity, which may cause deficits in plant

development, such as pest and disease attacks (Janas et al., 2000).

However, this event was not verified in this study, probably due to the temperature at the time of collection, once higher temperatures were observed during the experiment period in the northern region of Parana, with averages between 25.1 ºC and 24 ºC in January and February, respectively (Agrometereology Laboratory - EMBRAPA SOJA, 2019). This inference is corroborated by Gobbo and Lopes (2007), who reported that high temperatures led to excessive loss of secondary metabolites due to the degradation of the leaf tissue (Duarte, 2010).

Opposite results were observed by Gomes et al. (2005), who found an increase in peroxidase activity in wheat plants with silicate fertilization. Also, Gomes et al. (2008) found an increase in peroxidase activity in potato plants subjected to foliar and soil silicon applications, when compared to plants not subjected to silicon application.

The enzyme peroxidase stands out in the biotechnological scenario once it is found in various natural sources, it does not depend on cofactors and acts on a large number of substrates (Mohamed et al., 2011). This enzyme belongs to the group of reducing oxides and participates in several physiological processes, such as lignification (Gomes et al., 2005).

Regarding the phenylalanine ammonia-lyase (FAL), Gomes et al. (2005) found no increase in FAL activity in wheat leaves when using silicon, thus corroborating the present study. Gomes et al. (2008) also observed that silicate fertilization of potato plants did not affect FAL activity, while Guerra et al. (2013) reported that silicate fertilization of cotton increased the FAL activity, differing from this study.

The enzyme phenylalanine ammonia-lyase (FAL) arouses much interest among researchers due to its importance in the secondary metabolism of plants. It stands out as a key and regulatory enzyme in the biosynthesis pathway of phenylpropanoids and their derivatives (Cheng et al., 2001). According to those authors, FAL is responsible for the deamination of amino acid L-phenylalanine, changing into trans-cinnamic acid and ammonia, and can be incorporated in several phenolic compounds, which are present in the formation of esters, coumarins, flavonoids, and lignins. Importantly, it is stimulated and regulated by environmental factors such as plant nutritional level, light, among other factors (Barros et al., 2010).

According to the results of the present study, the low mobility of silicon impaired its redistribution from the old parts to the new parts of the plant. It is worth noting that although studies have shown a relationship between silicon and increased enzymatic activities such as peroxidase and FAL, this behavior was not observed in this study. Therefore, it is assumed

that silicon may have conferred only a physical barrier, once it affected the behavioral parameters and biology of *S. frugiperda*. However, further studies are needed for this statement. It is also important to mention that few studies have reported the correlation between the use of *A. brasilense* inoculant and enzymatic activities, which was not observed in the present study, suggesting that the inoculant acts in other physiological areas such as higher root growth, plant height, and chlorophyll contents rather than the enzymatic activity associated with resistance induction of plants.

Conclusion

The present results showed that silicon can be considered as an alternative in reducing injuries caused by *S. frugiperda*. In addition, the foliar application was more effective when compared to the drench route, considering the practicality at the time of application, which can be an alternative to the ecologically-based production systems, besides reducing the use of synthetic insecticides. Further studies are needed to understand the impact of leaf consumption, once a higher mortality rate was observed in the pre-pupal phase.

Also, it was observed that silicon was not redistributed from old to new leaves, with no effect on the resistance induction through the peroxidase and phenylalanine ammonia-lyase (FAL) activities for both old and leaves. However, the effect of silicon on the resistance induction of plants deserves attention.

Regarding the use of the inoculant *A. brasilense,* no direct effect was observed for the parameters studied, suggesting an effect on the physiological parameters of the plant, which was not evaluated in this study.

References

Balastra, M. L. F., Perez, C. M., Juliano, B. O., & Villreal, P. (1989). Effects of silica level on some properties of *Oryza sativa* straw and hult. *Canadian Journal of Botany*, *67*(8), 2356-2363.

Barros, E. M., Torres, J. B., & Bueno, A. F. (2010). Oviposição, desenvolvimento e reprodução de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) em diferentes hospedeiros de importância econômica. *Neotropical Entomology*, *39*(6), 996-1001.

Barros, F. C., Sagata, E, Ferreira, L. C. C., & Juliatti, F. C. (2010). Indução de resistência em plantas contra Fitopatógenos. *Bioscience Journal*, *26*(2), 231-239.

Baysal, O., Soylu, E. M., & Soylu, S. (2003). Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis ssp. Michiganensis*. *Plant Pathology*, *52*, 747753.

Bogorni, P. C., & Vendramim, J. D. (2005). Efeito subletal de extratos aquosos de *Trichilia* spp. sobre o desenvolvimento de *Spodoptera frugiperda* (J.E.Smith) (Lepidoptera: Noctuidae) em milho. *Neotropical Entomology*, *34*, 311-317.

Bowling, C. C. (1967). Rearing of two lepidopterous pests of rice on common artificial diet. *Annals of the Entomological Society of America*, *60*(6), 1215-1216.

Bueno, R. C. O. F., Bueno, A. F., Moscardi, F., Parra, J. R., & Hoffmann-Campo, C. B. (2011). Lepidopteran larvae consumption of soybean foliage: basis for developing multiple-species economic thresholds for pest management decisions. *Pest Management Science*, *67*,170-174.

Butt, B. A., & Cantu, E. (1962). *Sex determination of lepidopterous pupae*. Washington, DC: USDA.

Cheng, S. H., Sheen, J., Gerrish, C., & Bolwell, G. P. (2001). Molecular identification of phenylalanine ammonia-lyase as a substrate of a specific constitutively active Arabdopsis CDPK expressed in maize protoplasts. *FEBS Letters*, *503*(2/3), 185-8.

Clark, P. L., Molina-Ochoa, J., Martinelli, S., Skoda, S. R., Isenhour, D. J.; Lee, D. J., Krumn, J. T., & Foster, J. E. (2007). Population variation of *Spodoptera frugiperda* (J. E. Smith) in the Western Hemisphere. *Journal of Insect Science*, *7*,1–10.

Correa, O. S., Romero A. M., Soria, M. A., & Estrada, M. de. (2008). *Azospirillum brasilense*-plant genotype interactions modify tomato response to bacterial diseases, and root and foliar microbial communities. In: Fabricio Dario Cassan & Ines Garcia de Salamone. (Eds.) *Azospirillum sp.: cell physiology, plant interactions and agronomic research in Argentina* (p. 87-95). Buenos Aires: Asociación Argentina de Microbiologia.

Dannon, E. A., & Wydra, K. (2004, May). Interaction between silicon amendment, bacterial wilt development and phenotype of *Ralstonia solanacearum* in tomato genotypes. *Physiological and Molecular Plant Pathology*, *64*(5), 233-243.

Datnoff, L. E., Snyder, G. H., & Korndörfer, G. H. (2001). *Silicon on agriculture* (403 p.). Amsterdam: Elsevier Science.

Dias, P. A. S., Sampaio, M. V., Rodrigues, M. P., Korndörfer, A. P., Oliveira, R. S., Ferreira, S. E., & Korndörfer, G. H. (2014, Ago). Induction of resistanceby silicon in wheat plants to alate and apterous morphs of *Sitobionavenae* (Hemiptera: Aphididae). *Environmental Entomolgy, 43*(4), 949-956. Doi: 10.1603/EN13234.

Dixon R. A., Harrison, M. J., & Lamb, C. J. (1994). Early events in the activation of plant defense responses. *Annual Review of Phytopathology*, *32*, 479-501.

Dourado Neto, D., & Severino, F. J. (2001). *A teoria da Trofobiose*. Piracicaba: Universidade de São Paulo.

Duarte Filho, P. (2010). *Cinética Enzimática*. Bagé – RS: Universidade Federal do Pampa.

Epstein, E. (2001). Silicon in plants: facts vs concepts. In: L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (eds.). *Silicon in agriculture* (403 p.). The Netherlands: Elsevier Science.

Epstein, E. (1994). The anomaly of silicon in plant biology. *Proccedings National of Academy Science*, *91*, 11‑17.

Fawe, A., Menzies, J. G., Chérif, M., & Bélanger, R. R. (2001). Silicon and disease resistance in dicotyledons. In: L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.). *Silicon in agriculture* (403 p.). The Netherlands: Elsevier Science.

Feng, M. J. (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, *50*(1), 11‑18.

Ferreira, D. F. (2014). Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia*, *38*(2), 109-112.

Gerasimova, N. G., Pridvorova, S. M., & Ozeretskovskaya, O. L. (2005). Role of L-phenylalanine ammonia-lyase in the induced resistance and susceptibility of potato plants. *Applied Biochemistry and Microbiology*, *41*, 103-105.

Gobbo Neto, L., & Lopes, N. P. (2007). Plantas Medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, *30*(2), 374-81.

Golizadeh, A., Kamali, K., Fathipour, Y., & Abbasipour, H. (2009). Life table of the diamondback moth, *Plutellaxylostella* (L.) (Lepidoptera: Plutellidae) on five cultivated brassicaceous host plants. *Journal of Agricultural Science and Technology*, *11*,115-124.

Gomes, F. B., Moraes, J. C., Santos, C. D., & Goussain, M. M. (2005, Nov./Dez.). Resistance induction in wheat plants by silicon and aphids. *Scientia Agricola*, *62*(6), 547-551.

Gomes, F. B., Moraes, J. C., Santos, C. D., & Antunes, C. S. (2008, mar./abr.). Uso de Silício como indutor de resistência em batata a Myzuspersicae (Sulzer) (Hemiptera: Aphididae). *Neotropical Entomology*, *37*(2), 185-190.

Goussain, M. M., Moraes, J. C., Carvalho, J. G., Nogueira, N. L., & Rossi, M. L. (2002). Efeito da aplicação de silício em plantas de milho no desenvolvimento biológico da lagarta-do-cartucho *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Neotropical Entomology*, *31*(2), 305-310.

Gratão, P. L., Polle, A., Lea, P. J., & Azevedo, R. A. (2005). Making the life of heavy metal-stressed plants a little easier. *Functional Plant Biology*, *32*, 481-494.

Guerra, A. M. N. M., Rodrigues, F. A., Berger, P. G., Barros, A. F., Silva, Y. C. R., & Lima, T. C. (2013). Aspectos bioquímicos da resistência do algodoeiro à ramulose potencializada pelo silício. 2 *Bragantia*, *72*(3), 292-303.

Hallmark, C. T., Wilding, L. P., & Smeck, N. E. Silicon. (1982). Methods of soil analysis, Chemical and Microbiological properties v.2.: Madison. *Agronomy Monograph,* (9), 263-273.

Hungria, M., Campo, R. J., Souza, E. M., & Pedrosa, F. O. (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, *331*, 413-425.

Janas, K., Cvikrová, M., Palagiewicz, A., & Eder, J. (2000). Alterations in phenylpropanoid content in soybean roots during low temperature acclimation. *Plant Physiology and Biochemistry*, *38*(7/ 8), 587-93.

Jarvis, S. C. (1987). The uptake and transport of silicon by perennial ryegrass and wheat. *Plant and Soil*, Dordrecht, *97*(3), 429-437.

Jones, L. H. P., & Handreck, K. A. (1967). Silica in soils, plant and animals. *Advances in Agronomy*, *19*, 107‑149.

Keeping, M. G., Kvedaras, O. L., & Bruton, A. G. (2009). Epidermal silicon on sugarcane: cultivar differences and role in resistance to sugarcane borer *Eldana saccharina*. *Environmental and Experimental Botany*, *60*(1), 54-60. Doi:10.1016/j.envexpbot.2008.12.012.

Korndörfer, G. H., Pereira, H. S., & Nolla, A. (2004). Análise de silício: solo, planta e fertilizante. *Boletim Técnico*, (1), 34.

Lusso, M. F. G., & Pascholati, S. F. (1999). Activity and isoenzymatic pattern of soluble peroxidades in maize tissues after mechanical injury or fungal inoculation. *Summa Phytophatologica*, *25*, 244-249.

Macagnan, D., Romeiro, R. S., Baracat Pereira, M. C., Lanna Filho, R., Batista, G. S., & Pomella, A. W.V. (2008). Atividade de enzimas associadas ao estado de indução em mudas de cacaueiro expostas a dois actinomicetos residentes de filoplano. *Summa Phytopathologica*, *34*, 34-37.

Malavolta, E. (1980). *Elementos de nutrição mineral de plantas* (251p.). São Paulo: Agronômica Ceres.

Marschner, H. (1995). *Mineral nutrition of higher plants* (887p.). New York: *Academic Press*.

Mohamed, S. A., Abulnaja, K. O., Ads, A. S., Khan, J. A., & Kumosani, T. A. (2011). Caracterisation of na anionic peroxidase from horseradish cv. *Food Chemistry*, *128*, 725-730.

Nagoshi, R. N. (2009). Can the amount of corn acreage predict fall armyworm (Lepidoptera: Noctuidae) infestation levels in nearby cotton? *Journal of Economic Entomology, 102*, 210- 218.

Nascimento, A. M., Assis, F. A., Moraes, J. C., & Sakomura, R. (2014). Não preferência a *Spodoptera frugiperda* (Lepidoptera: Noctuidae) induzida em arroz pela aplicação de silício. *Revista Brasileira de Ciências Agrárias, 9*, 215–218.

Nation, J. L. (2002). Insect Physiology and Biochemistry. Boca Raton: *CRC Press*.

Nogueira, A. M., Jesus, K. A., Lins Junior, J. C., & Bezerra, C. E. S. (2018). Efeito do silício no desenvolvimento de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) na cultura do arroz. *Connection Line,* 19.

Panizzi, R. A., & Parra, J. R. P. (2009). Consumo e utilização do alimento para o crescimento da fase larval. In: A. R. Panizzi, & J. R. P. Parra (Eds.). *Bioecologia e nutrição de insetos: base para o manejo integrado de pragas* (Cap.2, p. 65- 90). Brasília: Embrapa Informação Tecnológica.

Parra, J. R. P., Panizzi, A. R., & Haddad, M. L. (2009). Índices nutricionais para medir consumo e utilização de alimentos por insetos. In: A. R. Panizzi, & J. R. R. Parra (Eds.). *Bioecologia e nutrição de insetos: base para o manejo integrado de pragas* (p. 37-90). Brasília: Embrapa Informação Tecnológica.

Pogue, G. M. (2002). A world revision of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae). *Memoirs of the American Entomological Society*, *43*,1-202.

Prowell, D. P., Mcmichael, M., & Silvain, J. F. (2004). Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America 97*,1034-1044.

Reis, T. H. P., Guimarães, P. T. G., Figueiredo, F. C., Pozza, A. A. A., Nogueira, F. D., & Rodrigues, C. R. (2007). *O silício na nutrição e defesa de plantas*. Belo Horizonte: EPAMIG.

Silva, D. M., Bueno, A. F., Andrade. K., Stecca, C. S., Neves, P. O. M. J., & Oliveira, M. C. N. (2017, Jan/Feb). Biology and nutrition of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on different food sources. *Scientia Agricola*, *74*(1), 18-31.

Stadinik, M. J. (2000). Indução de resistência a oídios. *Summa Phytopathologica*, *26*, 175-177.

Umesha, S. (2006). Phenylalanine ammonia lyase activity in tomato seedlings and its relationship to bacterial canker disease resistance. *Phytoparasitica*, *34*(1), 68- 71.

Van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*, *36*, 453-483.

Wisemam, B. R., & Widstron, N. W. (1986). Mechanisms of resistance in "Zapalote Chico" corn silks to fall armyworn (Lepidoptera: Noctuidae) larvae. *Journal of Economic Entomology*, *795*, 1390-1393.

Wise, H., Nikolic, M., & Römheld, V. (2007). Silicon in plant nutrition - Effects on zinc, manganese and boron leaf concentrations and compartmentation. In: Burkhard Sattelmacher, & Walter J. Horst (Eds.). *The apoplast of higher plants: compartment of storage, transport and reactions* (p. 33-470). London: *Springer*.