Characterization of priming, induced resistance, and tolerance to *Spodoptera frugiperda* by silicon fertilization in maize genotypes

Amanda C. G. Sousa · Bruno H. S. Souza · Paulo E. R. Marchiori · Lissa V. V. Bôas

Received: 12 April 2021 / Revised: 26 November 2021 / Accepted: 4 December 2021 / Published online: 18 January 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
Fall armyworm (FAW) *Spodoptera frugiperda* is considered the main defoliating insect pest of maize in many countries. Silicon (Si) applied to plants has been shown to increase the resistance to insects, especially in grasses such as maize. This study characterized the effects of Si fertilization regarding priming, induced resistance, and tolerance to FAW in a landrace variety and hybrid of maize. Si was applied in soil of potted-plants as H4SiO4 at 2 t ha\(^{-1}\) when maize plants were at V2 stage, and when they reached V3 two FAW neonates were placed in the plant’s whorls to cause herbivory. FAW performance was evaluated on excised leaf sections in the laboratory and on plants with larvae infesting V4-stage plants in the greenhouse. Concentrations of H2O2, malondialdehyde, and Si, and the activities of antioxidant enzymes were recorded and correlated to Si-based responses on plant growth, and FAW injury and performance. As main results, there was reduced FAW injury and larval weight gain in Si-treated plants subjected to herbivory. Greater root dry mass was observed in the landrace variety with Si and without herbivory. Landrace plants showed higher shoot weights than the hybrid under FAW infestation. Si-fertilized plants showed higher H2O2 concentrations. The highest peroxidase activities occurred in Si-treated plants without herbivory, and the catalase and superoxide dismutase activities were highest in Si-treated plants without herbivory or herbivory-injured plants without Si. In conclusion, Si-based defense in maize to FAW involve mixed effects of priming and tolerance, and were more pronounced in the landrace variety.

Keywords  Fall armyworm · Primer · Elicitor · Biostimulant

Key message

• Fall armyworm (FAW) is a food security threat in many countries, needing strategic control.
• We evaluated Si-based defense in maize genotypes and the interaction with FAW herbivory.
• FAW performance and plants growth were correlated with biochemical markers of plant defense.
• Si-based defense effects were more prominent in landrace maize plants in the greenhouse assay.

• Mixed Si-based defense involving priming and tolerance may act together in fertilized maize.

Introduction
Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is considered the main insect pest of maize crop in Brazil (Cruz et al. 2008; Lima Júnior 2012; Eghrari et al. 2021). In recent years, it has become a new invasive species in West and Central Africa, where the first outbreaks were recorded in early 2016; the presence of distinct haplotypes collected from maize samples in Nigeria and São Tomé suggests FAW introduction to the African continent (Goergen et al. 2016). This pest has also been reported more recently in Asian countries (Goergen et al. 2016; Shylesha et al. 2018; Jing et al. 2019; Zhang et al. 2019). The losses in crop yields due to FAW herbivory in these countries vary from 8.3 to 20.6 tons per year (Goergen et al. 2016; Day et al. 2017). Thus, FAW has become a pest...
that threatens food security in many developing countries worldwide and requires cost-effective control strategies.

Varying soil abiotic factors can interfere with plant defense responses, triggering bottom-up effects on organisms of higher trophic levels, ultimately impacting plant–insect interactions (Han et al. 2019). Silicon (Si) is an element that is naturally present in soil, mostly in non-assimilable forms, but to a lesser extent found in the form of silicic acid (H₄SiO₄) (Epstein 1994). Although Si is not considered an essential nutrient for plant development, when Si is available in the soil solution as H₄SiO₄ it can be easily absorbed by the roots, playing an important role in plant growth, nutrition, and mechanical resistance to fungal diseases, insect herbivory, and the chemical conditions of the medium (Epstein 1994; Ma and Yamaji 2006; Kvedarasa et al. 2007; Reynolds et al. 2016). Plants fertilized with Si are reported to show enhanced resistance against chewing insects, including FAW larvae (Goussain et al. 2002; Alvarenga et al. 2017; Oliveira et al. 2017; Nascimento et al. 2017), due to silicification (SiO₂) of the plant cell wall, increasing its rigidity and making it difficult for insects to feed on the plants tissues (Datnoff et al. 2001). In addition to this mechanical/morphological effect in fertilized plants, Si can elicit increased activities of enzymes and metabolites involved in plant chemical defense (Dixon et al. 1994; Fawe et al. 2001; Gomes et al. 2005). Due to the negative effects of Si on insects, fertilization of plants with Si can be considered a viable alternative in integrated management of insect pests with economic importance as FAW (Oliveira et al. 2017; Nascimento et al. 2017).

Plant defense mechanisms involve the expression of both resistance and tolerance that act in response to various types of stress to which they are constantly exposed, whether biotic or abiotic (Walters and Heil 2007; Borges et al. 2017). The defense mechanisms are classified as constitutive or induced depending on whether they are constantly expressed in the plant or are triggered only after a stress condition, respectively (Borges et al. 2017). Production of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), due to stress experienced by plants, e.g., insect herbivory, activates a series of defensive physiological processes where antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) are important in maintaining the balance of ROS and plant cell homeostasis. Plants that express high antioxidant enzyme activities can more efficiently eliminate excess ROS, protecting the cellular components from toxic oxidative damage, allowing them to tolerate the stress (Caverzan et al. 2016). Antioxidant enzymes were reported to be more activated in Si-fertilized plants (Yang et al. 2017).

Among the mechanisms of induced resistance, priming consists of the activation of immune memory or a readiness state in stressed plants. Priming is a process in which there is a preparation of plants defense responses, which allows them to act more quickly and efficiently against future threats (Frost et al. 2008). After the first sign of stress, the challenged plant recognizes and stores this information and prepares itself if it is subjected to further stress (Martinez-Medina et al. 2016; Borges et al. 2017). Thus, plant-induced resistance is conditioned upon an initial stress stimulus. They represent a type of immune memory, allowing the plant to use the stored stress information by changing the coding of resistance genes to keep their defense mechanisms activated to deal with upcoming attack (Mauch-Mani et al. 2017).

Tolerance is another plant defense category against insect herbivory (Stenborg and Muola 2017). Tolerance is defined as the ability of plants to withstand injury caused by insects without their growth and production being substantially affected (Peterson et al. 2017). Moreover, tolerance poses no negative effect on either the biology or feeding and oviposition behavior of insects, and overall involves the expression of traits and physiological processes that confer host plants the ability to mitigate the negative impact caused by pest injury (Chen et al. 2015). Such tolerance mechanisms are those that have influence on plant photosynthesis and other physiological processes, such as growth, phenology, realocation, and use of stored nutrients and photoassimilates (Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000). Tolerance has been recognized by some authors as a defense category apart from resistance (Stout 2013; Stenberg and Muola 2017) because of the distinct mechanisms participating in plant defense responses to insect herbivory, and this novel classification is thought to benefit the use of plant resistance in applied research and integrated pest management (Stout 2013).

Si fertilization can also favor plant growth and development (Epstein 1994). Plants fertilized with Si have higher chlorophyll contents and leaves that are more rigid and erect, which favor greater light interception inside the canopy and consequently higher photosynthetic rates (Marschner 1995; Nascimento et al. 2017). The beneficial effects provided by Si on plant growth are more evidenced under stress situations, whereas the effects of fertilization in unstressed plants are reported to be neutral (Coskun et al. 2018), which is inline with the definition and mechanisms of tolerance (Stout 2013; Stenberg and Muola 2017). Recently, Johnson et al. (2019) reported for the first time that Si fertilization to wheat induced tolerance to herbivory by Helicoverpa amirgera (Hubner) (Lepidoptera: Noctuidae) in which plant growth was overcompensated in the Si-fertilized insect-infested condition, in expense of resistance that was not induced.

According to the aforementioned effects of Si fertilization on plant growth and insect performance, there is debate about the actual plant defense responses promoted by Si, raising questions of whether the effects are manifested in the
form of: (1) induced resistance, a condition where fertilized plants have higher level of resistance owing to the application of Si itself; or (2) priming, where induced defense responses are activated at higher levels only after a first attack by an insect in Si-fertilized plants (Walters et al. 2014; Nascimento et al. 2017). Another emerging possibility is that (3) Si fertilization improves plant tolerance, promoting greater vegetative growth under stress by insect herbivory. It is also hypothesized that (4) a mixed defensive response in Si-fertilized plants may occur (Johnson et al. 2019).

Maize is a grass species that has high capacity to accumulate Si (Marschener 1995; Epstein 1999). Although some studies have demonstrated Si-based defense to FAW (Gousain et al. 2002; Alvarenga et al. 2017; Oliveira et al. 2017), none of them dissected whether the defensive responses triggered by Si are related to priming or induction of resistance per se, and whether Si can improve tolerance to insect herbivory in different maize genotypes, which have variable levels of genetic diversity as being more or less improved by conventional breeding, as the landrace varieties and hybrids. A better understanding of the effects of priming, induced resistance, and tolerance may help shed light on the characterization of the specific plant defense responses elicited by Si. This knowledge will certainly aid in the development of strategies of Si application in integrated pest management to mitigate damage caused by FAW in maize crops especially in developing countries where the pest is considered a food security threat. Therefore, this study characterized the effects of Si fertilization on priming, induced resistance, and tolerance to FAW in a hybrid and landrace variety of maize.

Materials and methods

Location of the study

The study was conducted under greenhouse and laboratory conditions in the Laboratory of Plant Resistance and Integrated Pest Management, Department of Entomology, Lavras Federal University (UFLA), in Lavras, Minas Gerais, Brazil. In the laboratory the assays were conducted under controlled environmental conditions (26 ± 2 °C; 70 ± 10% RH; 12L:12D h) and in the greenhouse under controlled temperature (25 ± 2 °C) and ambient light.

FAW larvae

Larvae of FAW used in the study were obtained from a rearing colony established in the laboratory. To establish the colony, larvae were collected from maize fields in an experimental area of UFLA and brought to the laboratory. Larvae of the colony were fed an artificial diet and adults were fed a 10% honey solution, according to the methods of Greene et al. (1976). The insects were kept in a climate-controlled room at 28 ± 2 °C, 70 ± 10% RH, and 12L:12D-h photoperiod.

Treatments and experimental design

The study was set up in a 2 × 2 × 2 factorial scheme (with or without Si × with or without herbivory × hybrid or landrace variety) in a completely randomized design, which formed eight treatments. Twenty plants were prepared for each treatment and maintained in a greenhouse, as described in the next section. Thus, the three factors evaluated in this study (Si fertilization, herbivory, and maize genotype) were combined in the following treatments: T1) landrace variety, with Si, with herbivory; T2) landrace variety, with Si, without herbivory; T3) landrace variety, without Si, with herbivory; T4) landrace variety, without Si, without herbivory; T5) hybrid, with Si, with herbivory; T6) hybrid, with Si, without herbivory; T7) hybrid, without Si, with herbivory; T8) hybrid, without Si, without herbivory.

Experiments conduction

Maize seeds were sown in 3-L pots filled with a mixture of soil (dark red latosol), cattle manure, and Tropstrato substrate (4:1:1). Soil analysis was performed and fertilization calculations were done following the recommendations for the use of correctives and fertilizers (Ribeiro et al. 1999). Thus, the soil was fertilized with 180 g of dolomitic limestone and 732 g of NPK (8–28–16), which were incorporated into the soil preparation. A solution of micronutrients was prepared with 1.95 g of magnesium sulfate, 0.65 g of potassium chloride, 1.77 g of urea, 0.012 g of boric acid, 0.017 g of zinc sulfate, and 1.5 g of monoammonium phosphate (MAP) fertilizer, which was applied at 30 mL per pot immediately after sowing. Every 15 days, the plants were fertilized with 30 mL of Hoagland and Arnon nutrient solution. Two maize seeds were sown per pot, and after 10 days of emergence of plants, they were thinned to one plant per pot. The maize seeds were of the landrace variety Amareloa and the conventional non-Bt hybrid SHS4070. The plants were irrigated whenever necessary.

The dose of SiO2 (1% silicic acid solution) (Vetec Fine Chemical, Duque de Caxias, Brazil) used was proportional to 2 t ha⁻¹ (Alvarenga et al. 2017)—equivalent to 2.76 g of diluted SiO2 in 276 mL of distilled water—which was applied to each pot in the treatments assigned to receive Si application. The application of silicic acid solution was made in the pot around the plants at V2 stage (Magalhães et al. 2002), simulating a drench application.

The study consisted of two experiments (“Experiment 1” and “Experiment 2”) that were conducted under the same environmental conditions to test for the proposed...
hypotheses. When the plants in the greenhouse reached V3 stage, they were infested in the whorl with two FAW neonates obtained from the rearing colony with the aid of a fine paintbrush in the set of plants subject to herbivory. These neonates were used as “inducers” in both experiments. In “Experiment 1”, after 7 days of larvae infestation, plants at V4 stage of each treatment were evaluated by recording the aforementioned insect and plant parameters in both experiments, it was possible to correlate the occurrence of resistance (evaluations 1 and 4), tolerance (evaluations 2 and 5) and both defense categories (evaluation 3). The steps and parameters undertaken in “Experiment 1” and “Experiment 2” are described in detail in the following sections.

Experiment 1

Evaluation of FAW larval performance in the laboratory To evaluate and dissect the effects of priming from that of induced resistance in maize by Si application on FAW larval performance (hypothesis i and ii), the two youngest leaves of each treated plant were detached from the sheath and taken to the laboratory where leaf Sects. (5 × 3 cm) were prepared with the aid of scissors. Two leaf sections were prepared on each side of the midrib of maize leaf; one leaf section was transferred to a Petri dish (5 cm diameter) to perform the bioassay, and the other leaf section was used as a control aliquot (i.e., uninjured leaf section). One neonate larva (<24 h old) was confined to each dish, which was allowed to feed on the leaf section for 5 days. In this laboratory bioassay the neonates were evaluated as “challengers”. The dishes were lined with paper towels moistened with distilled water and kept in a climatized room with at 25 ± 2 °C, relative humidity of 60 ± 10%, and a 12L:12D-h photoperiod. For each treatment, five plants were used as replicates, and each replicate consisted of four dishes with leaf sections of the same plant and containing one larva per dish. In total, 20 experimental units (dishes) were used for each treatment.

Five days after setting up the bioassay, FAW larvae weight and leaf consumption were evaluated. For this purpose, we inspected whether the larvae were alive or dead by gently poking them with a fine paintbrush. Live larvae were removed from the Petri dishes and were starved for ~3 h to ensure that midgut of each larva was void. The larvae were dried at 60 °C for 48 h in an oven and then weighed with the aid of a precision analytical balance (AG200, GEHAKA, São Paulo, Brazil). To measure leaf consumption, the remaining leaf sections and the respective aliquots were dried in an oven at 60 °C for 48 h (TE-394/2, TECNAL, Piracicaba, Brazil) and then weighed. Leaf consumption by larvae was calculated by subtracting the dry weight of remaining leaf sections after larvae feeding from the dry weight of intact aliquot leaf sections.

Evaluation of oxidative stress and antioxidant enzyme activities Analysis of the activities of the enzymes SOD, CAT, and APX were performed to verify the antioxidant effect of Si application in the presence and absence of FAW herbivory in maize plants of the landrace variety and hybrid. The enzymatic analyses were performed to give greater support to the improved tolerance caused by Si application (hypothesis iii and iv) and to correlate with these potential underlying tolerance mechanisms. Additionally, the concentrations of H2O2 and MDA were evaluated as indicators of injury and oxidative stress in the plants. Five plants from each treatment were used as replicates, and the two youngest leaves of the plants were taken for chemical analyses. The leaves were carefully detached from plants at the sheath, stored in aluminum foil envelopes, wrapped in a plastic bag, and placed in a Styrofoam box with liquid nitrogen. Samples were taken to the Plant Physiology Sector, Department of Biology of UFLA, and stored in a freezer at −80 °C until analysis.

The extraction method for the analyses of SOD, CAT, and APX activities was based on the protocol of Biemelt et al. (1998). For the enzymatic analyses, SOD activity was evaluated as described by Giannopolitis and Ries (1977); CAT activity was evaluated as described by Havir and McHale (1987); and APX activity was determined according to the protocol of Nakano and Asada (1981). The H2O2 content was quantified as proposed by Velikova et al. (2000), and the lipid peroxidation level was measured in terms of the MDA concentration using the thiobarbituric acid reaction (Buege and Aust 1978). The H2O2 and MDA analyses were performed in duplicates, and the enzymatic analyses were performed in triplicates, according to those protocols.

Evaluation of leaf Si concentration For the analyses of leaf Si concentrations, before receiving a 4th-instar larva (“challengers”) plants were cut near the soil surface, separating them into shoot and root fractions. These samples were packaged in paper bags and dried in a forced-circula-
tion oven at 60 °C for 72 h. Next, three of the plants from each treatment fractions were ground to a fine powder in a blender, packed in paper bags, and sent for determination of Si concentrations in the Department of Soils of UFLA. The leaf analyses of Si were performed as described by Korndörfer et al. (2004).

Experiment 2

Evaluation of FAW larval performance and injury on maize plants in the greenhouse The same procedures of Si and larvae herbivory (“inducers”) treatments were applied to maize landrace and hybrid plants in the greenhouse as previously described. This part of the experiment evaluated the effects of priming and induced resistance to FAW (hypotheses i and ii). Although these hypotheses were tested in the first experiment as well, we wanted to validate the Si-based defense responses using another methodology that could allow maize plants to respond in a more dynamic way. For this, to determine the injury caused by FAW on five potted-maize plants per treatment, V4-stage plants were re-infested with a fourth-instar larva (“challengers”). The larvae were weighed before infestation and remained feeding on the plants for 7 days. Next, the plants at V5 stage were carefully inspected for the injury, and larvae were removed from the plants, individually placed in containers and taken to the laboratory, where they were weighed on a precision scale. The plants were visually rated for FAW injury, receiving scores from 1 to 9 according to the Davis scale (Davis et al. 1992).

Evaluation of plant growth Five plants of each treatment were used as replicates for assessment of growth parameters of maize and correlate the effects of improved tolerance promoted by Si (hypotheses iii and iv). Plant height was measured before and after infestation with a fourth-instar larva (“challengers”), that is, when the plants were at V4 and V5 stages, respectively. Plant height was measured with the aid of a ruler, from the soil surface to the insertion of the uppermost leaf in the sheath of plants. The relative chlorophyll content was read using a SPAD-502 portable chlorophyll meter (Konica Minolta Sensing, TECNAL, Piracicaba, Brazil). This reading was taken on each of the two youngest leaves of the plants, and the mean of the readings was used for statistical analysis. To determine the shoot and root dry weights, the V5-stage maize plants were carefully removed from the pots with moist soil and cut near the soil surface so that they were separated into the shoot and root fractions. Each part of the plants was packed in paper bags, dried in a forced-circulation oven at 60 °C for 72 h, and then weighed on a balance.

Statistical analysis

Data obtained in the experiments were subjected to exploratory analysis to check for normality of residuals and homogeneity of variances and were then subjected to three-way ANOVA to verify the main effects of Si application, herbivory, maize genotype, and all their interactions. Means of the treatments were compared by Tukey’s test (α = 0.05) when the main or corresponding interactive effects of ANOVA was significant. The analyses were performed in R software (R Core Team, 2014). The values and significance of all statistical analyses are shown in Supplementary 3.

Results

Experiment 1

Evaluation of FAW larval performance in the laboratory

There were no significant (P > 0.05) effects of treatments on the biomass of FAW larvae (data not shown) in the laboratory bioassay. The relative consumption rate (RCR) of the larvae was significantly affected by the maize genotype × Si interaction (F1, 32  = 5.88; P = 0.0212). Larvae RCR was higher in the hybrid when there was no Si application (Fig. 1). When Si was applied, there was no difference in larvae RCR between maize genotypes. The efficiency of conversion of ingested food (ECI) of the larvae was not significantly affected (P > 0.05) by treatments (data not shown).

Fig. 1 Effects of maize genotype × Si interaction on the relative consumption rate (RCR) of Spodoptera frugiperda larvae. Different uppercase letters for the effect between genotypes and lowercase letters for the effect between Si treatments denote significant differences (P < 0.05)
Evaluation of oxidative stress and antioxidant enzyme activities

The concentrations of H$_2$O$_2$ were higher when Si was applied to plants in the absence of herbivory (Fig. 2; $F_{1,32} = 9.99; P = 0.0034$). This demonstrates that applied Si itself is recognized by the plant as a stress condition, leading to increased concentrations of H$_2$O$_2$ in the leaf tissues. When there was no Si application, the H$_2$O$_2$ concentrations were similar between the conditions with and without herbivory, although there was some variation in the data likely due to the intensity of injury by FAW larvae, which tended to be greater in infested plants.

There was a significant effect of the maize genotype × Si × herbivory interaction ($F_{1,32} = 4.68; P = 0.0380$) on lipid peroxidation, as measured by the MDA concentrations. The highest MDA concentrations were observed in the maize hybrid without Si application and without herbivory (Table 1). MDA concentrations were significantly different in the hybrid with vs. without Si; the effect was greater when there was no Si application in the absence of herbivory or when there were both Si application and herbivory.

In the maize landrace variety, APX activity was higher in the Si-without herbivory condition (Table 2; $F_{1,32} = 6.36; P = 0.0169$). This is similar to what have occurred with H$_2$O$_2$, where Si application itself increased the H$_2$O$_2$ concentrations (Fig. 2). The enzymatic activity of APX was higher in the hybrid when there was no Si application and no herbivory (Table 2).

The enzymatic activity of CAT was higher in the hybrid when there was no Si application and no herbivory (Table 3; $F_{1,32} = 11.03; P = 0.0022$). In the presence of herbivory, CAT activity in the hybrid was higher with Si application. In the landrace variety, higher CAT activity occurred when there was no Si application in the presence of herbivory or when there was Si application in the absence of herbivory. In this maize genotype, both herbivory and Si treatments signaled stress conditions to the plants, increasing the enzymatic activity of CAT.

SOD activity was higher in the landrace variety without Si application and with FAW herbivory (Table 4; $F_{1,32} = 5.14; P = 0.0303$). The enzymatic activity was also higher in the landrace variety when the plants were Si-fertilized in the absence of herbivory.

Evaluation of leaf Si concentration

The concentrations of Si that were absorbed by the roots and accumulated in the shoots of maize plants did not differ between infested and non-infested plants with Si fertilization. However, when the plants were not fertilized, the highest leaf Si content was observed when FAW herbivory occurred. In the absence of herbivory, maize plants

| Genotype | + Herbivory | − Herbivory |
|----------|-------------|-------------|
| + Si     | − Si        | + Si        | − Si        |
| Landrace | 132.69 ± 27.53 Aa | 55.00 ± 50.84 Aa | 100.77 ± 67.44 Aa | 69.61 ± 27.77 Ba |
| Hybrid   | 262.31 ± 36.38 Aa* | 159.61 ± 35.14 Aa | 72.31 ± 45.40 Aa | 391.92 ± 129.86 Aa* |

Means followed by different uppercase letters in columns to compare between maize genotypes, lowercase letters in rows to compare between Si treatments, and * to compare between herbivory events are significantly different by Tukey’s test ($P < 0.05$).
accumulated higher Si contents when fertilized (Fig. 3; \( F_{1,32} = 7.45; P = 0.0149 \)).

**Experiment 2**

**Evaluation of FAW larval performance and injury on maize plants in the greenhouse**

There was a significant effect of the maize genotype \( \times \) Si \( \times \) herbivory interaction on the larval weight gain of FAW infesting potted-plants in the greenhouse (Table 5; \( F_{1,32} = 26.71; P < 0.0001 \)). The larval weight gain was lower in plants of the landrace variety treated with Si and subjected to herbivory. In the absence of herbivory, the greatest larval weight gain of FAW occurred in the hybrid without Si. The effect of herbivory in the hybrid was very different under the two Si conditions: the weights of larvae were higher with Si application in the presence of herbivory or without Si application and without herbivory.

For the FAW injury on the plants, there were significant effects of the Si \( \times \) herbivory interaction (Fig. 4a; \( F_{1,32} = 7.45; P = 0.0149 \)).

![Fig. 3 Effects of Si \( \times \) herbivory interaction on Si concentration in the shoot of plants. Different uppercase letters for the effect between Si treatments and lowercase letters for the effect between herbivory treatments denote significant differences (\( P < 0.05 \))](image-url)

**Table 3** Effects of maize genotype \( \times \) Si \( \times \) herbivory interaction on CAT activity (\( \mu \text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ FW} \))

| Genotype | + Herbivory | − Herbivory |
|----------|-------------|-------------|
|          | + Si        | − Si        | + Si        | − Si        |
| Landrace | 0.54 ± 0.05 Bb | 1.22 ± 0.42 Aa | 1.00 ± 0.07 Aa | 0.76 ± 0.18 Ba |
| Hybrid   | 1.29 ± 0.16 Aa | 1.07 ± 0.12 Aa | 1.36 ± 0.16 Ab | 2.00 ± 0.09 Aa* |

Means followed by different uppercase letters in columns to compare between maize genotypes, lowercase letters in rows to compare between Si treatments, and * to compare between herbivory events are significantly different by Tukey’s test (\( P < 0.05 \)).

**Table 4** Effects of maize genotype \( \times \) Si \( \times \) herbivory interaction on SOD activity (U SOD min\(^{-1}\) mg\(^{-1}\) FW)

| Genotype | + Herbivory | − Herbivory |
|----------|-------------|-------------|
|          | + Si        | − Si        | + Si        | − Si        |
| Landrace | 0.26 ± 0.00 Ab | 0.29 ± 0.00 Aa | 0.29 ± 0.00 Aa* | 0.28 ± 0.00 Aa |
| Hybrid   | 0.28 ± 0.00 Aa | 0.27 ± 0.00 Ba | 0.28 ± 0.00 Aa | 0.27 ± 0.00 Aa |

Means followed by different uppercase letters in columns to compare between maize genotypes, lowercase letters in rows to compare between Si treatments, and * to compare between herbivory events are significantly different by Tukey’s test (\( P < 0.05 \)).
The injury scores of FAW were lower in Si-fertilized plants subjected to herbivory than in plants without Si application and with herbivory. Plants that did not experience herbivory showed lower injury scores than those subjected to herbivory under both Si conditions. The landrace variety exhibited significantly lower injury than the hybrid (Fig. 4b; $F_{1,32}=8.04; P=0.0080$).

**Evaluation of plant growth**

The root dry weight showed a significant interaction of maize genotype × Si × herbivory ($F_{1,32}=4.27; P=0.0469$). The greatest root mass was observed in the landrace variety with Si application and without herbivory, and it was significantly lower when this maize genotype was under Si application and herbivory conditions (Table 6). In the hybrid, the root dry mass was slightly greater when there was no Si application and no herbivory than under the condition without Si and with herbivory. For the shoot dry mass, there was a significant effect of the maize genotype × herbivory interaction ($F_{1,32}=10.26; P=0.0031$). Plants of the landrace variety had a greater shoot dry mass than the hybrid only when the plants were infested with FAW (Fig. 5a).

The relative chlorophyll index measured before larvae infestation was significantly affected by the interaction of maize genotype × Si ($F_{1,32}=5.73; P=0.0227$). The chlorophyll index was higher in the hybrid without Si application than in the fertilized treatment. This effect was not observed in the landrace variety (Fig. 5b). The chlorophyll index measured after larvae infestation showed no significant effects ($P>0.05$) of the treatments (data not shown).

The plant height of maize before infestation showed a significant effect of the maize genotype × Si interaction ($F_{1,32}=3.07; P=0.0313$). Plant height before infestation was higher in the landrace variety than the hybrid only in the condition without Si (Fig. 5c). The greatest plants height after herbivory was observed in the landrace variety (Fig. 5d; $F_{1,32}=8.38; P=0.0068$), as previously expected since landrace varieties have agronomic characteristics genetically different from those of hybrids.

**Discussion**

Our results showed that Si-based defense responses to FAW were manifested in fertilized maize plants, which partially interacted with herbivory and were more specific to the landrace variety. Greater root growth was found in Si-fertilized landrace plants in the absence of herbivory, while in the hybrid greater root growth occurred in the absence of both

| Genotype | + Herbivory | − Herbivory |
|----------|-------------|-------------|
|          | + Si        | − Si        | + Si        | − Si        |
| Landrace | 3.46±0.15 Aa | 3.65±0.26 Aa | 6.57±0.10 Aa* | 3.26±0.39 Ab |
| Hybrid   | 4.06±0.58 Aa | 4.39±0.54 Aa | 4.69±0.64 Ba  | 4.71±0.33 Aa* |

Means followed by different uppercase letters in columns to compare between maize genotypes, lowercase letters in rows to compare between Si treatments, and * to compare between herbivory events are significantly different by Tukey’s test ($P<0.05$)
Si and herbivory, being correlated with higher chlorophyll index. Si-fertilized landrace plants subjected to herbivory provided lower FAW larvae weight and showed less injury in exchange for a lower plant height. Such effects indicate there was a Si-priming effect in landrace maize, and resistance was later induced by FAW herbivory, leading to lower weight gain because the larvae fed less on Si-fertilized landrace plants due to increased deterrence, resulting in reduced injury to the plants. The landrace variety were naturally more resistant and tolerant than the hybrid of maize, as evidenced by the lower injury and higher shoot biomass and plant height after infestation. It is possible that landrace variety has specific resistance mechanisms, such as genes that encode Si transporters in the roots since the effects were observed only in this genotype, which has broader genetic variability due to less intensified breeding for agronomic traits. The Si transporters Lsi1 and Lsi2 were found in the roots of rice, maize, wheat, and pumpkin, while Lsi6 is an intervascular transport protein functioning in Si discharge from the xylem to leaf tissues (Yamaji and Ma, 2009; Reynolds et al. 2016).

Analyses of leaf Si concentrations indicated that Si applied in soil led to greater accumulation in the shoots of maize plants, with no effect of herbivory on mineral concentration; in the absence of fertilization though, plants accumulated more Si when FAW herbivory occurred relative to unfested plants. This suggests that Si applied to soil was absorbed by maize roots, transported through the plants, and deposited in the leaves; upon herbivory, even without Si supplementation, the plants absorbed Si to aid in inducing resistance to insect attack, since the element is naturally present in soil (Epstein 1994). Previous studies have shown increased leaf Si concentrations in maize and other grass species fertilized with Si, contributing with insect-resistance (Gossain et al. 2002; Vilela et al. 2014; Alvarenga et al. 2017; Nascimento et al. 2017; Nogueira et al. 2018; Pereira 2018). In turn, information is very scarce on Si interaction with insect herbivory in improving plant tolerance. Johnson et al. (2019) showed increased shoot biomass of Si-fertilized wheat plants under herbivory compared to fertilized plants without herbivory, suggesting that insect attack could not reduce the shoot biomass of fertilized plants. In the present study, greater shoot dry mass was found in the landrace variety when there was herbivory, indicating that landrace maize activated tolerance mechanisms to compensate for the injury, benefiting plant growth.

Si application itself increased H$_2$O$_2$ concentrations in the leaves of maize plants, signaling a stress condition (Fester and Hause 2005). Hydrogen peroxide is one of the main ROS produced by plants under stress, which stimulates reactions leading to expression of defense genes, protecting plants from upcoming biotic stress (Torres
Fertilized landrace maize plants in the absence of herbivory showed higher APX activity, supporting the hypothesis that Si played a role in ROS scavenging by increasing antioxidant metabolism against biotic and abiotic stress (Gong et al. 2005; Torabi et al. 2015; Shi et al. 2014; 2016). The highest CAT and SOD activities in the landrace variety occurred in contrasting conditions where only one type of treatment was applied, i.e., when there was no Si application and the presence of herbivory or when there was Si application without herbivory. Greater enzyme activities may have resulted in higher plant shoot biomass, so the increased activities in response to FAW herbivory contributed to ROS scavenging, and hence greater tolerance in landrace plants. Lipid peroxidation caused by ROS results in increased concentrations of MDA, one of the main products of lipid peroxidation of cell membranes that is used as proxy of oxidative damage (Corbineau et al. 2002; Yang et al. 2017). Therefore, our results indicate that Si fertilization reduces MDA concentrations in maize under herbivory, reducing oxidative damage to plant cells, improving tolerance to FAW. Ma et al. (2016) also concluded that Si decreased lipid peroxidation in fertilized plants, and Yang et al. (2017) reported there were increased CAT and SOD activities in Si-fertilized rice plants under insect herbivory. The highest CAT and APX activities in the maize hybrid occurred in the condition without both Si and herbivory, as there were higher MDA concentrations and greater FAW larval weights. Because the effects were genotype-dependent, higher enzyme activities served to remove excess ROS in hybrid plants likely due to abiotic stress imposed by the experimental conditions, e.g., the lesser nutrient availability to potted-plants may have been an influential factor, since maize hybrids are bred to respond to higher levels of macronutrients for high yields (Amorim and Souza 2005).

Comparing the results between “Experiment 1” and “Experiment 2”, it is possible to note varying effects for Si-based resistance. In the laboratory bioassay, there were no significant differences in leaf consumption and FAW larvae biomass for the landrace as was observed in the greenhouse assay; although not being significant, there was a trend of higher FAW relative consumption rate in Si-fertilized landrace leaf sections than unfertilized, but this did not result in greater efficiency of conversion of food to body mass, inferring that larvae enhanced feeding rates to compensate for the lower nutritional quality of silicified tissues. For the hybrid, there was lower leaf consumption rate in Si-fertilized leaf sections in the laboratory, while in the greenhouse there were specific interactive effects in function of the presence and absence of Si and herbivory. Differences in the effects between experiments were due to the methods used. The presence of older FAW larvae feeding (“challengers”) on plants in the greenhouse was essential for induced defense be expressed in a more effective
way; in turn, in the laboratory bioassay predetermined Si-based resistance due to Si deposition and polymerization (SiO2) in maize tissues (Ma and Yamaji 2006) were most likely observed. Thus, the conduction of in situ greenhouse experiments are more appropriate for evaluating Si-based defense effects in plants to insect herbivory than in vitro laboratory bioassays.

Although Si is not classified as an essential nutrient for plant development, this element can interact with plant defense signaling pathways by regulating various physiological processes under biotic and abiotic stress (Ye et al. 2013). The jasmonic acid (JA) pathway acts in mediating plant defense responses to chewing insects, and it is reported that JA synthesis occurs more rapidly in Si-fertilized plants attacked by herbivores due to Si priming (Hall et al. 2019). The results of our study and others indicate that Si-based defense responses probably cannot be characterized as resistance induction, since Si application per se did not induce substantial levels of resistance to insect herbivory. Thus, we encourage the use of a more appropriate term for Si as “defense elicitor” in integrated pest management literature than “resistance inducer” as it has been employed in previous papers, broadly encompassing Si-based defense effects of priming, induced resistance, and tolerance. Another interesting result was the genotype-dependent biostimulant effect in Si-fertilized landrace plants without herbivory that resulted in greater root growth. “Biostimulant” is a novel term adopted by the scientific community and industry to characterize chemical and microbial compounds that improve nutritional efficiency, tolerance to abiotic stresses, and crop quality traits, regardless of nutrients content (Du Jardin 2015; Yakhin et al. 2017). Vargas-Hernandez et al. (2017) suggest that some compounds play both the roles of resistance elicitor and biostimulant depending on the doses used, resembling effects of hormesis, i.e., positive or stimulatory effects when applied at lower doses and negative or toxic effects at higher doses. Because biostimulant effects of Si have been previously reported (Van Oosten et al. 2017), this is a topic that deserves in-depth investigation with practical implications for Si use as its application suggest to elicit hormetic-like effects (Abreu et al. 2021).

Molecular analyses are also encouraged to better answer important questions, such as which genes are up- and down-regulated upon Si fertilization, and the abundance of transporters in the roots of different plant genotypes, so that desired defense effects could be potentiated in integrated pest management to express higher levels of resistance and tolerance to insect herbivory. The results obtained in our study are important for characterizing the Si-based defense mechanisms provided by Si fertilization in maize genotypes with different genetic variability against FAW, suggesting the occurrence of mixed effects involving priming and tolerance. This novel information will aid in the deployment of more effective strategies for Si use in integrated pest management.

**Author contributions**

All authors conceived and designed the study. LF conducted the experiment, analyzed the data, and wrote the manuscript. KE helped with part of the data collection. BS and KE assisted in writing and revising the manuscript. AB developed and provided the corn hybrids. All authors read and approved the manuscript.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01468-y.

**Acknowledgements** The authors thank the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) for granting scholarship to the first author and the Graduate Program in Entomology of the Lavras Federal University (UFLA) for all scientific support provided.

**Availability of data and material** Data transparency and originality.

**Declarations**

**Conflict of interest** There are no conflict of interest.

**References**

Abreu RAA, Assis FA, Souza BHS, Nascimento AM, Latini AO, Pio LAS (2021) Effects of silicon application on the biochemistry of passion fruit and performance of *Dione junho junho* (Lepidoptera: Noctuidae). Arthropod-Plant Int. https://doi.org/10.1007/s11829-021-09827-3

Alhousari F, Greger M (2018) Silicon and mechanisms of plant resistance to insect pests. Plants 7:33. https://doi.org/10.3390/plants7010033

Alvarenga R, Moraes JC, Auad AM, Coelho M, Nascimento AM, Latini AO, Pio S7020 033

Amorim EP, Souza JC (2005) Híbridos de milho inter e intrapopulações obtidos a partir de populações S0 de híbridos simples comerciais. Bragantia 64:561–567. https://doi.org/10.1590/S0006-87052005000400005

Albiert C, Pastor V, Gamir J, Flors V, Mauch-Mani B (2015) The ‘prime-ome’: towards a holistic approach to priming. Trends Plant Sci 20:443–452. https://doi.org/10.1016/j.tplants.2015.04.002

Borges M, Michereff MFF, Moraes MCB, Magalhães DM, Hassemer MJ, Laumann RA, Birkett, M (2017) Metodologias para o estudo da defesa de memória (Priming) em plantas frente a estresse biótico. Embrapa Recursos Genéticos e Biotecnologia
plant defense priming. Trends Plant Sci 21:818–822. https://doi.org/10.1016/j.tplants.2016.07.009

Mauch-Mani B, Baccelli I, Luna E, Flors V (2017) Defense priming: an adaptive part of induced resistance. Annu Rev Plant Biol 68:485–512. https://doi.org/10.1146/annurev-arplant-042916-041132

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Physiol 22:867–880. https://doi.org/10.1093/oxfordjournals.ppc.a076232

Nascimento AM, Assis FA, Moraes JC, Souza BHS (2017) Silicon application promotes rice growth and negatively affects development of Spodoptera frugiperda (JE Smith). J Appl Entomol 142:241–249. https://doi.org/10.1111/jen.12461

Nogueira AM, De Jesus KA, Junior JCL, Bezerra CES (2018) Efeito do silício no desenvolvimento de Spodoptera frugiperda (Lepidoptera: Noctuidae) na cultura do arroz. Connection Line, Revista Eletrônica do Uninav 19:52–62. https://doi.org/10.18312/connectionline.v0i19.1195

Oliveira F, Vilela M, Moraes JC, Mendes S (2017) Silício como estratégia para o manejo de Spodoptera frugiperda. Boletim de Pesquisa e Desenvolvimento (INFOTEC-A). http://www.infotecaembrapa.br/infoteca/handle/doc/1081842. Access in 12 October 2018

Oliveira RS, Peñaflor MFGV, Gonçalves FG, Sampaio MV, Korndorfer AP, Silva WD, Bento JMS (2020) Silicon-induced changes in plant volatiles reduce attractiveness of wheat to the bird cherry-oat aphid Rhopalosiphum padi and attract the parasitoid Lysiphlebus testaceipes. PLOS ONE 15:e0231005. https://doi.org/10.1371/journalpone.0231005

Reynolds OL, Padula MP, Zeng R, Gurr GM (2016) Silicon-augmented silicon amendment does not reduce population growth of Spodoptera frugiperda (Lepidoptera: Noctuidae). Invasive Pest Science: a global perspective. Front Plant Sci 7:2049. https://doi.org/10.3389/fpls.2016.02049

Shylesha AN, Jalali SK, Gupta A, Varshney R, Venkatesan T, Shetty P, Rakshit O, Prabhu C, Omprakash N, Subaharan K, Bakhavatsalam N, Chandish R, Bakhavatsalam N (2018) Studies on new invasive pest Spodoptera frugiperda (JE Smith)(Lepidoptera: Noctuidae) and its natural enemies. J Biol Control 32:145–51. https://doi.org/10.18311/jbc/2018/21707

Stenberg JA, Muola A (2017) How should plant resistance to herbivores be measured. Front Plant Sci 8:663. https://doi.org/10.3389/fpls.2017.00663

Stout MJ (2013) Reevaluating the conceptual framework for applied research on host-plant resistance. Insect Sci 3:263–272. https://doi.org/10.1111/j.1744-7917.2011

Strauss SV, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. Trends Ecol Evol 5(179):185. https://doi.org/10.1016/S0169-5347(98)01576-6

Stowe KA, Marquis RJ, Hochwender CG, Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. Annu Rev Ecol Syst 565:595

Tiffin P (2000) Mechanisms of tolerance to herbivore damage: what do we know. Ecol Evol 4–5:536

Torabif R, Majid A, Enteshari S (2015) The effect of silicon on alleviation of salt stress in borage (Borago officinalis L.). Soil Sci Plant Nut 61:788–798. https://doi.org/10.1080/00380768.2015.1005540

Torres MA (2010) ROS in biotic interactions. Physiol Plant 138:414–429. https://doi.org/10.1111/j.1399-3054.2009.01326.x

Vargas-Hernández M, Macias-Bobadilla I, Guevara-Gonzalez RG, Romero-Gomez SD, Rico-Garcia E, Ocampo-Velazquez RV, Alvarez-Aquinta LL, Torres-Pacheco I (2017) Plant hormesis management with biostimulants of biotic origin in agriculture. Front Plant Sci 8:1762. https://doi.org/10.3389/fpls.2017.01762

Velekova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:81–90. https://doi.org/10.1016/S0168-9452(00)00197-1

Vilela M, Moraes JC, Alves E, Santos-Cividanes TM, Santos FA (2014) Induced resistance to Diatraea saccharalis (Lepidoptera: Crambidae) via silicon application in sugarcane. Rev Colomb Entomol 40:44–48. https://doi.org/10.1007/s1399-017-0085-2

Walters DR, Heil M (2007) Costs and trade-offs associated with plant volatiles reduce attractiveness of wheat to the bird cherry-oat aphid. PLoS ONE 15:e0231005. https://doi.org/10.1371/journalpone.0231005

Ye M, Song Y, Long J, Wang R, Baerson SR, Pan Z, Zhu-Salzman K, Xie J, Cai K, Luo S, Zeng R (2013) Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. P Natl Acad Sci 110(E361):E3639. https://doi.org/10.1073/pnas.1305848110
Zhang L, Liu B, Zheng W, Liu C, Zhang D, Zhao S, Xu P, Wilson K, Withers A, Jones CM, Smith JA, Chipabika G, Kachigamba DL, Nam K, D’Alençon E, Liu B, Liang X, Jin M, Wu C, Chakrabarty S, Yang X, Jiang Y, Liu J, Liu X, Quan W, Wang G, Fan W, Qian W, Wu K, Xiao Y (2019) High-depth resequencing reveals hybrid population and insecticide resistance characteristics of fall armyworm (Spodoptera frugiperda) invading China. BioRxiv. https://doi.org/10.1101/813154

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.