Silicon Fertilisation Affects Morphological and Immune Defences of an Insect Pest and Enhances Plant Compensatory Growth

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Abstract

Insect herbivores employ various defences, including morphological, behavioural, and immune responses against their natural enemies (e.g., predators, parasitoids) which can make biocontrol of herbivorous pests challenging. Silicon (Si) accumulation in plants is a potent physical defence against herbivores. However, it remains uncertain how Si affects pest defences to their enemies and plant defences following herbivore attack. We grew the model grass, *Brachypodium distachyon*, hydroponically with (+Si) or without (–Si) Si and investigated the impacts of Si on morphological (integument resistance and thickness), behavioural (flee, headrear, thrash, and regurgitation), and immune defences of the cotton bollworm, *Helicoverpa armigera*. We further examined the effects of Si on plant compensatory growth and leaf trichome production. Larval growth, leaf consumption, and integument resistance were lower when feeding on +Si plants compared to when feeding on –Si plants. Larval integument thickness, defensive behaviours, hemocyte density and lysozyme-like activity in the hemolymph were unaffected by Si. Larvae fed on +Si plants had higher hemolymph phenoloxidase (PO) and total-PO activities than larvae fed on –Si plants, although this did not enhance larval melanisation response. Furthermore, Si supply increased plant compensatory growth and constitutive trichome production whereas herbivory induced trichome production only on –Si plants. We provide the first evidence that Si fertilisation affects insect defences in addition to reducing their growth and feeding. Lower integument resistance might enhance larval vulnerability to parasitoids and pathogens and higher PO activities could impose fitness costs (e.g., delayed development), potentially increasing overall insect susceptibility to enemies.

Introduction

The nutritional quality and physical and chemical defences of plants can substantially impact insect herbivore fitness (i.e., bottom-up effects) (Singer and Stireman 2005; Vidal and Murphy 2018). This can produce cascading effects on natural enemies of insect herbivores such as predators and parasitoids (top-down forces) via changes in host insect defences and vulnerability to attack (Forkner and Hunter 2000; Pekas and Wäckers 2020). For example, feeding on high-quality host plants can enhance insect immune defences such as encapsulation and melanisation of invaders (i.e., parasitoid eggs) (Diamond and Kingsolver 2011; Gherlenda et al. 2016). Moreover, insect herbivores can exploit plant defence chemicals (e.g. secondary metabolites) to self-medicate (Garvey et al. 2021) or to defend against their enemies via sequestration (Winde and Wittstock 2011).

Among insect pests, larval Lepidoptera encounter extensive top-down pressures (Bernays 1997) and are often managed with biological control (Stiling and Cornelissen 2005). As counteradaptations, larvae have evolved an arsenal of defences against enemies, including morphological, behavioural, chemical, and immune defences (Greeney et al. 2012). For instance, thicker integuments or larger body spines can function as morphological defences against parasitoids (Gross 1993). Moreover, larvae can show evasive behaviours including thrashing, twisting, or dropping to avoid enemies, and aggressive behaviours including headrearing, regurgitating, or biting while encountering enemies (Greeney et al. 2012; Gross 1993). However, after successful attacks by pathogens or parasitoids, larvae mostly rely on immune

defence, driven by cellular and humoral mechanisms. Hemocytes, the circulating hemolymph cells in larvae, can cause phagocytosis of microorganisms and encapsulation of foreign entities such as parasitoid eggs or larvae (Lavine and Strand 2002). Humoral responses include the actions of phenoloxidase (PO), a key enzyme that regulates melanisation of invaders and wound healing (Eleftherianos and Revenis 2011), and lysozyme activity that degrades bacteria and fungi (Moreno-García et al. 2013).

Plant defence against herbivores comprises resistance (e.g., physical and chemical barriers to herbivory) and tolerance to attack, including compensatory growth following herbivory (Núñez-Farfán et al. 2007). The physical and chemical defences in plants can be expressed constitutively or induced when challenged by herbivores (Gatehouse 2002). Silicon (Si) accumulation in plants is recognized as an effective physical defence against chewing herbivores (Massey and Hartley 2009; Reynolds et al. 2016), particularly in grasses including cereal crops, as they possess limited secondary metabolite defences and can accumulate relatively higher amounts (up to 10% of dry weight) of Si (Moore and Johnson 2016; Vicari and Bazely 1993). Plants deposit silica (SiO_2) in tissues, including physical defence structures (e.g., trichomes), following active uptake or passive absorption of aqueous orthosilicic acid via roots and transportation via xylem (Ma and Yamaji 2006; Mandlik et al. 2020). Silicification makes plant tissue tougher and abrasive, and hence, less masticable and digestible for chewers, often causing mandibular wear while feeding (Massey and Hartley 2009) and gut damage while passing silicified trichomes through the digestive tract (Andama et al. 2020). Insect herbivores generally feed less on silicified plants and have retarded growth because of reduced nutrient assimilation (Massey and Hartley 2009). However, it remains uncertain whether malnourishment caused by siliceous plants makes insects more vulnerable to their enemies via compromised defences. This question is of interest as Si accumulation by plants has recently been shown to promote the attraction of natural enemies of herbivores (Islam et al. 2021; Liu et al. 2017) and to impact tri-trophic interactions by changing host insects' body size (Hall et al. 2021).

In addition to reducing insect attack, either via direct physical defences or via compromising the defences of insect pests against their own enemies, Si has the potential to improve defences of previously attacked plants against future attack (Johnson et al. 2019). Research on Si-based plant defence against herbivores has mostly focused on plant resistance, with the role of Si in tolerance relatively neglected. Furthermore, herbivory often induces increased Si accumulation in plants (Islam et al. 2020; Massey et al. 2007), however, how Si induction impacts the production of other inducible defence structures such as trichomes remain understudied, though there are reports that Si can enhance constitutive trichome density (Biru et al. 2021; Johnson et al. 2021).

To the best of our knowledge, the impacts of Si on insect defences against their natural enemies have yet to be investigated. We grew the model grass, *Brachypodium distachyon*, hydroponically with (+ Si) or without (− Si) Si and investigated the impacts of Si supply on defences of the cotton bollworm, *Helicoverpa armigera* and plant defences following herbivory. Specifically, our objectives were (i) to elucidate the effects of Si supplementation of plants on larval growth and feeding and consequently on larval morphological (integument resistance and thickness), behavioural (in the absence or presence of a

stimulus) and immune (under immunologically naïve and challenged conditions) defences; and (ii) to determine the impacts of Si supply on plant compensatory growth following herbivory, and constitutive and induced trichome production. *Brachypodium distachyon* is a temperate grass, widely studied because of its genetically tractable genome, short life cycle, and phylogenetic connection to important cereal crops (Scholthof et al. 2018). *Helicoverpa armigera* is a global pest of many high-value agricultural and horticultural crops, costing over US$7 billion annually due to crop losses and management expenses (Jones et al. 2019). We hypothesized that Si negatively affects larval feeding and growth, and thus attenuates larval defences by limiting physiological resources.

Materials And Methods

Plants and insect herbivores

Brachypodium distachyon (accession Bd21-3) seeds were obtained from the French National Institute for Agricultural Research (INRA, Versailles, France). Seeds were softened by soaking in water for 2 hrs and dehusked manually using forceps. Seeds were then sterilised in a solution of 0.9% NaOCl and 0.1% Triton-X for 30 min and subsequently, washed in water several times. Sterilised seeds were sown in wet perlite in germination trays and kept refrigerated at 4°C for 7 days for cold stratification and further grown for 12 days in a naturally lit glasshouse at 22/18°C day/night temperatures on a cycle of 14L:10D and 50% (± 6%) relative humidity. Uniform seedlings were transplanted to non-aerated hydroponic vessels, each comprised of two nested disposable plastic cups (480 ml) with a fitted foam disc at the top as per Hall et al. (2020). Foam discs were slot cut to accommodate a seedling in each vessel. Cups were weekly filled with 370 ml of freshly prepared nutrient solution with (+ Si) or without (− Si) Si supplementation as per Hall et al. (2020). Plants (N = 150) were grown in the same glasshouse environment and cups were rotated weekly to avoid any position bias. *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae were supplied by CSIRO Agriculture and Food, Narrabri, NSW, Australia and were reared on an artificial diet modified from Teakle and Jensen (1985) before inoculating on plants.

Experimental design and treatments

Liquid potassium silicate (K_2SiO_3) (21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) was added to the nutrient solution to create + Si treatment. The concentration of silicic acid was maintained at 2 mM (SiO_2 equivalent) in + Si solution as polymerisation occurs above this concentration (Ma and Yamaji 2006). The added K^+ ions in the + Si treatment were balanced in the control treatment (−Si) by adding KCl (Sigma-Aldrich, MO, USA). Both + Si and −Si solutions were adjusted to pH 5.5 with HCl. Plants were assigned to herbivore treatments (insect or no insect) after being grown hydroponically for five weeks. Eighty plants (40 + Si and 40 −Si) were randomly exposed to a second instar *H. armigera* larva for seven days. Larvae were starved for 24 hrs in Petri dishes before being weighed and placed onto plants. The rest of the plants were kept insect-free. All plants were kept caged for seven days in transparent acrylic cylinders (see Johnson et al. (2020) for cage specifications). Larvae were starved for 24 hrs following removal from plants to allow all frass to be expelled, and subsequently weighed to

estimate larval relative growth rates (RGRs). Insect RGR is a combined estimate for plant antixenotic (i.e. effects on growth due to starvation) and antibiotic effects and was calculated as larval mass gain relative to initial body mass per unit of time [mass gained (mg)/initial mass (mg)/time (days)] (Massey and Hartley 2009). We collected larval frass randomly from 40 hydroponic vessels (20 holding + Si plants and 20 holding –Si plants) and matching Petri dishes following larval starvation and oven-dried it at 60°C for three days before weighing (i.e., as a proxy for larval leaf consumption (Murray et al. 2013)). Further, 40 larvae (20 fed on + Si plants and 20 fed on –Si plants) were used for assessing behavioural and morphological defences and the remaining 40 larvae were used for measuring immune defence. Thirty insect-fed plants (15 of each Si treatment) were randomly harvested immediately after insect herbivory, as were 30 insect-free plants (15 of each Si treatment). Plants were oven-dried at 60°C for seven days before measuring shoot dry biomass to assess insect damage on + Si and –Si plants. Eighty other plants (N = 20 of each treatment) were grown for a further two weeks to elucidate the effects of Si supply on plant compensatory growth and leaf trichome density.

Measurements of larval behavioural and morphological defences

We measured larval escape response (i.e., flee) in the absence of any stimulus using 40 larvae as per Vogelweith et al. (2014). Each larva was placed singly on a gridded plastic sheet (86 × 112 cm) and acclimated for 20 s under a glass jar. Following the jar removal, the number of squares crossed by a larva within 60 s (minimum time to exit the sheet as observed in the preliminary experiment) was recorded. As no stimulus was involved, some larvae (seven fed on + Si plants and four fed on − Si plants) did not make any movements and were omitted from the analysis. Following flee measurements, each larva was placed on a white filter paper, acclimated for 30 s under a glass jar and then gently pinched three times at 20 s intervals using bracket-placing tweezers at the abdominal end to imitate a predator attack (Cornell et al. 1987). The behaviours of larvae were video recorded (Logitech c270 webcam) and three distinct larval behaviours were characterized and logged for each larva: (a) ‘headrearing’ as characterized by the backward movement of the head and the anterior body portion to the posterior part, (b) ‘thrashing’ as characterized by the side-to-side swing movement of the head and the anterior body portion, and (c) ‘regurgitating’ on the filter paper (Cornell et al. 1987).

We further measured larval integument resistance and integument thickness following the protocol of Vogelweith et al. (2014) with minor modifications. Larvae were placed at − 20°C for 20 min and then thawed for 10 min before starting measurements. Integument resistance was measured by using a drill press equipped with a hypodermic needle (Terumo, 0.5 × 16 mm). A precision scale (± 0.1 mg) was positioned on the drill press table and each larva mounted on a cardboard sheet was placed over the scale. The needle was slowly lowered down by rotating the lever until it breached the dorsal integument. The scale reading (mg) when the needle breached the integument was recorded. Two measurements were taken from each larva, one on the thoracic region and another on the abdomen, and the average value was counted. Larvae were further dissected and all internal organs and fat bodies were removed

before measuring integument thickness using a thickness gauge (Teclock SM-112, Japan, precision ± 0.01 mm) (Iltis et al. 2018).

Measurements of larval immune defences

We measured larval innate immunity (10 larvae fed on + Si plants and 10 larvae fed on − Si plants) in terms of hemocyte density, phenoloxidase (PO) activity, total-PO activity (combined estimate of PO and its precursor, prophenoloxidase), and lysozyme-like enzyme activity in the hemolymph. For this, immunologically naïve larvae were chilled on ice for 20 min and a proleg was removed from each larva using a sterile micro scissor and 3 µl of hemolymph was collected immediately using a sterile micropipette. Of this, 2 µl was flushed into a pre-chilled microcentrifuge tube containing 20 µl of cold phosphate buffer saline (PBS, pH 7.4), and 10 µl of this hemolymph-PBS mixture was immediately spread over a Neubauer Improved Haemocytometer and hemocytes were counted under a phase-contrast microscope at 400x magnification (Axio Vert.A1, Zeiss, Australia). We measured total hemocyte density as well as the density of individual hemocytes, classified according to Vogelweith et al. (2016) and Ribeiro and Brehélin (2006) into prohemocytes, granulocytes, plasmatocytes, spherulocytes, and oenocytoids. The rest of the hemolymph-PBS mixtures was stored at − 20°C for the later measurements of PO and total-PO activities following the protocol of Vogelweith et al. (2011). Briefly, samples were thawed slowly on ice and centrifuged (3000 g, 4ºC) for 15 min. Further, 5 µl of supernatant from each sample was transferred to a microplate well containing 20 µl of PBS with either 140 µl of distilled water for measuring PO activity or 140 µl of chymotrypsin solution (Sigma-Aldrich, 0.98 mg/14 ml of distilled water) for measuring total-PO activity. Finally, 20 µl of L-DOPA solution (Sigma-Aldrich, 8 mg/2 ml of distilled water) was added as a substrate to each microplate well and the absorbance readings were taken for samples and negative controls at 490 nm in a microplate reader (CLARIOstar Plus, BMG Labtech) for 40 min at 60 s intervals. The enzyme activity was calculated from the slope of the reaction curve (i.e., change in optical density per min) at the linear phase and are reported as the activity per microliter of pure hemolymph.

An additional 1 µl of pure hemolymph was flushed into a pre-chilled microcentrifuge tube containing 10 µl of reaction buffer (pH 6.24, Sigma-Aldrich) and stored at − 20°C for the subsequent measurements of lysozyme-like activity. Lysozyme-like activity was measured by a turbidity assay using *Micrococcus lysodeikticus* (Sigma-Aldrich) as a substrate following the method modified from Adamo et al. (2016). In short, 10 µl of hemolymph-PBS mixture from each sample was transferred to a microplate well containing 180 µl of a suspension of *M. lysodeikticus* cells (1 mg/10 ml) in reaction buffer (pH 6.24, Sigma-Aldrich). Lysozyme standards (Sigma-Aldrich) within the linear range of assays were run concurrently as positive controls along with the test samples and negative controls, and the kinetic decrease in absorbance was recorded in the microplate reader at 450 nm wavelength for 10 min at 50 s intervals. Lysozyme-like activities are presented in ‘unit’ where one unit represents 0.001 change in optical density per minute.

Furthermore, we measured encapsulation and melanisation responses of larvae (10 larvae fed on + Si plants and 10 larvae fed on − Si plants) when immunologically challenged, simulating a solitary

endoparasitoid attack (Moreno-García et al. 2013). For this, a nylon monofilament (0.2 mm diameter) was implanted 2 mm deep in the hemocoel through the dorsal abdominal part of each larva according to Gherlenda et al. (2016). After 24 hrs, we collected 3 µl of hemolymph from each larva and measured hemolymph PO, total-PO and lysozyme-like activities as per the procedures described previously. The nylon implants were removed and photographed using a stereo microscope (Olympus, SZ61) mounted camera (Infinity1, Teledyne Lumenera) along with a control filament (i.e., not implanted in hemocoel). The photos were further processed in ImageJ (National Institutes of Health, Maryland; Version 1.52) and the mean grey values were estimated on a scale of 0 (light) to 255 (dark). The melanisation scores were calculated using the formula: 1 – (mean grey value of the treatment filament/mean grey value of the control filament) (Garvey et al. 2021).

Measurements of leaf trichome density, plant biomass, and Si concentrations

We measured the density of non-glandular trichomes (or macrohairs) on leaves of all 80 plants (N = 20 of each treatment) grown for an additional two weeks following herbivory or no herbivory. Non-glandular trichomes can defend plants against insect herbivores, often more effectively than plant secondary metabolites (Carmona et al. 2011), and herbivory can induce increased trichome density on newly emerged leaves (Dalin et al. 2008). We sampled the newly emerged, fully expanded leaves from plants and counted trichomes on abaxial and adaxial surfaces (4 mm × 4 mm area in the middle) under a stereo microscope (Olympus, SZX10). Plants were further harvested, oven-dried at 60ºC for 7 days and shoot and root biomass were recorded. Of these plants, we measured Si concentrations (% dry mass) in leaves and roots of all + Si plants (20 insect-fed plants and 20 insect-free plants). For this, ca. 80 mg oven-dried, ball-milled samples were analysed in an X-ray fluorescence spectrometer (Epsilon 3^x; PANalytical, EA Almelo, The Netherlands) according to Reidinger et al. (2012). Measurements were standardized using a certified plant material of known Si concentration (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel).

Statistical analyses

All data were analysed in the statistical software environment R, version 3.6.1 (R Core Team 2019). The distributions of dependent and independent variable datasets were compared using quantile-quantile plots and homogeneity of variance was assessed using ‘residuals versus fits’ plots. Larval frass and morphological and behavioural defence parameters were analysed using Wilcoxon’s rank-sum tests as the assumptions of normality of residuals or homoscedasticity were violated. Larval RGR, hemocyte density, PO, total-PO, lysozyme-like activity, and leaf and root Si concentrations were analysed using Student’s *t*-tests. The effects of Si on larval integument resistance and PO activity were further analysed using multiple linear regressions, incorporating larval weight gain or final weight as a covariate. The relationships between larval integument resistance and weight gain and final weight were explored using Spearman’s correlations. Leaf trichome density and plant shoot and root biomass were analysed using two-way analysis of variance (ANOVA) tests, considering Si and insect herbivore as fixed factors. In case of significant effects, differences between group means were determined by Tukey’s HSD tests.

Results

Insect growth, feeding, and defensive traits

Larval RGR and frass production were significantly lower (− 300% and − 85%, respectively) when feeding on + Si plants compared to when feeding on −Si plants (Fig. 1a-1b; Table 1). However, none of the larval defensive behaviours (flee, headrear, thrash, and regurgitation) were affected by Si (Table S1). Larvae fed on + Si plants had significantly lower integument resistance than larvae fed on −Si plants (Fig. 2a; Table 1) despite no changes in integument thickness (Fig. 2b; Table 1). Integument resistance was strongly and positively correlated with larval weight gain ($\rho = 0.83$, $p < 0.001$) and final weight ($\rho = 0.98$, $p < 0.001$) (Fig. S1). Multiple linear regression analyses show that Si had a significant effect on integument resistance after adjusting for the effect of larval weight gain as a covariate (Table 3). However, Si had no significant effect on integument resistance when the effect of larval final weight was controlled (Table 3). The density of individual and total hemocytes in larval hemolymph was unaffected by Si (Fig. S2; Table S2). Larvae fed on + Si plants had significantly higher PO and total-PO activities in the hemolymph compared to larvae fed on −Si plants under both immunologically naïve (unchallenged) (+ 71% and + 86%, respectively) and challenged conditions (+ 33% for both) (Fig. 3a-3b; Table 1). Accordingly, Si had a significant effect on hemolymph PO activity after controlling for the effect of larval weight gain or final weight as a covariate (Table 3). However, the melanisation responses of larvae fed on + Si or −Si plants were similar (Fig. 4, Table 1). Lysozyme-like activity in the hemolymph was unaffected by Si, regardless of immunity challenge (Fig. S3; Table S2).

Leaf trichomes, plant compensatory growth, and silicon concentrations

Silicon in interaction with herbivory significantly affected leaf trichome density, whereby + Si plants had higher (+ 51%) trichome density on abaxial leaf surfaces than − Si plants in the absence of insect herbivory (Fig. 5a; Table 2). However, following herbivory, trichome density on abaxial leaf surfaces of − Si plants increased by 46% and became statistically similar to insect-fed + Si plants. Likewise, trichome density on adaxial leaf surfaces of − Si plants significantly increased (+ 62%) following herbivory (Fig. 5b; Table 2). In terms of plant biomass, insect herbivory significantly reduced shoot biomass of both − Si and + Si plants (− 16% and − 14%, respectively) harvested immediately after herbivory (Fig S4, Table S3). There was a significant interaction between Si and herbivory on shoot biomass of plants harvested two weeks afterwards, whereby insect-fed + Si plants produced similar shoot biomass to insect-free, +Si or −Si plants (Fig. 6a; Table 2), indicating a higher compensatory growth with the provision of Si. However, root biomass was unaffected by Si or herbivory (Fig. 6b; Table 2). Besides, herbivory significantly increased leaf Si accumulation by ca. 29% but had no effect on root Si concentrations (Fig. 7; Table 1).

Discussion

We report for the first time, to our knowledge, how Si supplementation of plants impacts the morphological and behavioural defences of an insect herbivore. Silicon reduced larval growth and

feeding, thereby lowering integument resistance which was associated with compensatory production of immunity proteins in the hemolymph. Moreover, Si supply enhanced constitutive trichome production on leaves and augmented plant compensatory growth whereas insect herbivory induced trichome production on plants that had no access to Si.

Silicon weakened larval morphological defences but did not affect defensive behaviours

Larvae fed less on + Si plants (i.e., as evident from the lower levels of frass production) and displayed retarded growth rates which are in line with the existing literature on the effects of Si against chewing herbivores, including folivores (Biru et al. 2021; Islam et al. 2020; Johnson et al. 2020), borers (Kvedaras et al. 2009; Nikpay et al. 2015) and root feeders (Frew et al. 2017). The three-fold decrease in larval growth rates observed here due to malnutrition could potentially impact biocontrol by natural enemies. For example, Remmel et al. (2011) estimated that a two-fold rise in the linear size of folivorous insect larvae increases 3.6-fold avian predation rates while reducing 4.9-fold arthropod predation rates.

We found a strong positive correlation between larval final weight and integument resistance. In accordance with this, Iltis et al. (2018) reported a positive correlation between larval body size and integument resistance of the lepidopteran grape pest, *Lobesia botrana*, despite no changes in integument thickness. Although it is not evident how larger larvae showed higher integument resistance despite the same integument thickness, we speculate that there might have some variations in the integument ultrastructures of larvae fed on –Si plants compared to larvae fed on + Si plants, including the extent of sclerotization and tanning. This finding suggests that feeding on + Si plants could make larvae more vulnerable to parasitoid attacks as parasitoid oviposition is more successful in smaller larvae with a lower integument resistance (Beckage and Riddiford 1978; Gross 1993). We observed consistent defensive behaviours in *H. armigera* larvae, irrespective of lower leaf consumption and growth rates. This concurs well with the findings of Zhou et al. (2017) who found that evasive (escape) and aggressive (thrashing and dropping) behaviours of the oriental armyworm, *Mythimna separata*, were unaffected by body size and weight.

Interactions between larval morphological and immune defences

Our results indicate a potential trade-off between insect morphological and immune defences; larvae fed on + Si plants had lower integument resistance and higher hemolymph PO and total-PO activities. Such trade-offs between different defensive traits have been demonstrated in other lepidopteran (Vogelweith et al. 2014) and hymenopteran (Boevé and Schaffner 2003) larvae, including trade-offs due to feeding on different host plants (Vogelweith et al. 2011). For instance, in several sawfly species, a lower integument resistance and propensity for bleeding have been linked to a higher hemolymph deterrence against predators such as ants and wasps (Boevé and Schaffner 2003). Our results differ from the previous study by Frew et al. (2017) who found no effects of Si supplementation of sugarcane on PO activity of the root-

feeding grub, *Dermolepida albohirtum*. Even though the underlying mechanisms of how Si enhanced PO activity in the hemolymph are not clear, previous research has shown that consumption of plant toxins (e.g., nicotine) or starvation could be immunotherapeutic for insects and could enhance PO activity (Garvey et al. 2021; Yang et al. 2007). We suggest that Si accumulation in plants forced larvae into starvation and consumption of silicified tissues and trichomes might cause larval gut damage and exert physiological stress on them, contributing to higher hemolymph PO activities. This high level of PO can impose fitness costs on larvae as production and maintenance of PO is very costly (González-Santoyo and Córdoba-Aguilar 2012) and resource allocation to PO can constrain resources for other physiological functions, including larval development (Cotter et al. 2008) and expression of sexual traits (Siva–Jothy 2000).

Higher PO activity did not enhance melanisation

Notably, we found that higher PO activities in the hemolymph did not enhance the melanisation response when larvae were immunologically challenged. Since PO functions against pathogens and parasitoids via melanogenesis (i.e., a process whereby PO oxidises phenols to quinones which further polymerises to melanin) (Eleftherianos and Revenis 2011), this finding suggests that high PO activity does not imply high immune defences in larvae (González-Santoyo and Córdoba-Aguilar 2012). The possible explanations for similar melanisation responses in larvae are twofold. First, melanisation depends on both PO activity and hemocyte density as invaders need to be first coated (i.e., encapsulation) by adhesive hemocyte cells (Lavine and Strand 2002). Given that the density of hemocytes in larvae fed on + Si or –Si plants was similar, larvae might produce an identical encapsulation of the nylon implants. Second, both the activation of PO and the production of melanin (a nitrogen-rich compound) require substantial investments of nitrogen (González-Santoyo and Córdoba-Aguilar 2012), which can limit nitrogen for melanogenesis in larvae fed on + Si plants, especially considering the fact that Si and nitrogen accumulation in plants can interact antagonistically (Wu et al. 2017).

Post-attack plant defences

We found that Si increased constitutive trichome density on leaves, which substantiates previous studies on grasses (Biru et al. 2021; Johnson et al. 2021) and other crops (Acevedo et al. 2021). Interestingly, herbivory induced trichome production only on –Si plants, because + Si plants were already better defended with constitutively-produced silicified trichomes, which were found to be essential for defence against chewing herbivores in rice (Andama et al. 2020). We suggest that investment in induced defence by –Si plants might compromise, in part, their capacity for compensatory growth as there could be a potential trade-off between plant growth and defence (Herms and Mattson 1992; but see Koricheva 2002). Conversely, with the provision of Si, insect-fed plants produced similar biomass to insect-free plants within two weeks of the recovery period, despite initial biomass losses, substantiating the previous finding that Si can support plant compensatory growth (Johnson et al. 2019).

Conclusions

Our study establishes that Si supplementation of plants can affect defensive traits of insect herbivores, which might generate cascading effects on higher trophic levels. Moreover, Si supply can underpin constitutive trichome production on leaves and plant compensatory growth following herbivory. We found a potential trade-off between larval morphological and immune defences when fed on siliceous plants; a lower integument resistance was associated with higher PO and total-PO activities in the hemolymph although it did not enhance the melanisation response of larvae when challenged. We presume that the lower integument resistance of larvae could enhance their vulnerability to some natural enemies and higher PO activities could impose physiological costs, potentially impeding other physiological functions and possibly expanding overall insect susceptibility to enemies. Further research is needed to understand the consequences of such changes in host insect defences and nutritional quality for predators and parasitoids. Nonetheless, our study provides evidence for the bottom-up effects of siliceous host plants on insect defensive traits and presents a framework for studying the impacts of Si on top-down control via changes in host insect defences.

Declarations

Contribution of authors

TI and SNJ conceived and designed the study. TI conducted experimental work, collected and analysed data, and drafted the manuscript. SNJ and BDM supervised TI. All authors critically revised the draft and approved the final manuscript for publication.

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Data availability

The data that supports the findings of this study are available in the figshare repository, https://doi.org/10.6084/m9.figshare.15085815.v1

Conflicts of interest

The authors declare that there is no conflict of interest.

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Tables

Table 1 Summary output of Student's *t*-tests for comparing larval RGR, larval immune defence parameters, and plant Si concentrations and of Wilcoxon's rank-sum tests for comparing larval frass weight and morphological defence parameters. Statistically significant effects ($p < 0.05$) are indicated in bold

Response variable	Fig.	Statistical analysis		
		df	Test statistic (t^a/W^b)	*p* value
Larval RGR [a]	1a	78	11.18	**<0.001**
Frass dry weight [b]	1b	-	381.5	**<0.001**
Larval morphological defences [b]				
Integument resistance	2a	-	385.5	**<0.001**
Integument thickness	2b	-	227.5	0.442
Immune defences of naïve (unchallenged) larvae [a]				
PO activity	3a	18	−2.94	**0.009**
Total-PO activity	3b	18	−2.74	**0.014**
Immune defences of challenged larvae [a]				
PO activity	3a	18	−2.28	**0.035**
Total-PO activity	3b	18	−2.82	**0.011**
Melanisation score	4	18	1.27	0.222
Si concentrations in plants [a]				
Leaf Si	5	38	−4.85	**<0.001**
Root Si		38	−0.06	0.949

[a]Analysed using a Student's *t*-test.

[b]Analysed using a Wilcoxon's rank-sum test.

Table 2 Summary output of two-way ANOVA tests for the effects of Si and insect herbivory on leaf trichome density and plant biomass. Statistically significant effects ($p < 0.05$) are indicated in bold

Response variable	Fig.	*df*	Si		Herbivore		Si × Herbivore	
			F	*p* value	*F*	*p* value	*F*	*p* value
Abaxial trichome density	6a	1,76	7.49	**0.008**	5.08	**0.027**	9.69	**0.003**
Adaxial trichome density	6b	1,76	0.02	0.888	24.62	**<0.001**	8.11	**0.006**
Shoot biomass	7a	1,76	63.13	**<0.001**	5.00	**0.028**	13.42	**<0.001**
Root biomass	7b	1,76	0.001	0.982	0.95	0.333	0.01	0.908

Table 3 Summary output of multiple linear regression analyses for the effects of Si on larval integument resistance and hemolymph PO activity after controlling for the effect of larval weight gain or final weight as a covariate. Statistically significant effects ($p < 0.05$) are indicated in bold

Predictor	Integument resistance				PO activity			
	β	*t*	*df*	*p* value	*β*	*t*	*df*	*p* value
(Regression model 1)								
Larval weight gain	11.09	1.37	37	0.179	−0.0003	−0.70	37	0.491
Si	−188.77	−2.25	37	**0.031**	0.03	2.96	37	**0.005**
(Regression model 2)								
Larval final weight	2.19	19.50	37	**<0.001**	−0.0003	−0.80	37	0.431
Si	−15.98	−0.87	37	0.393	0.03	3.13	37	**0.003**

Figures

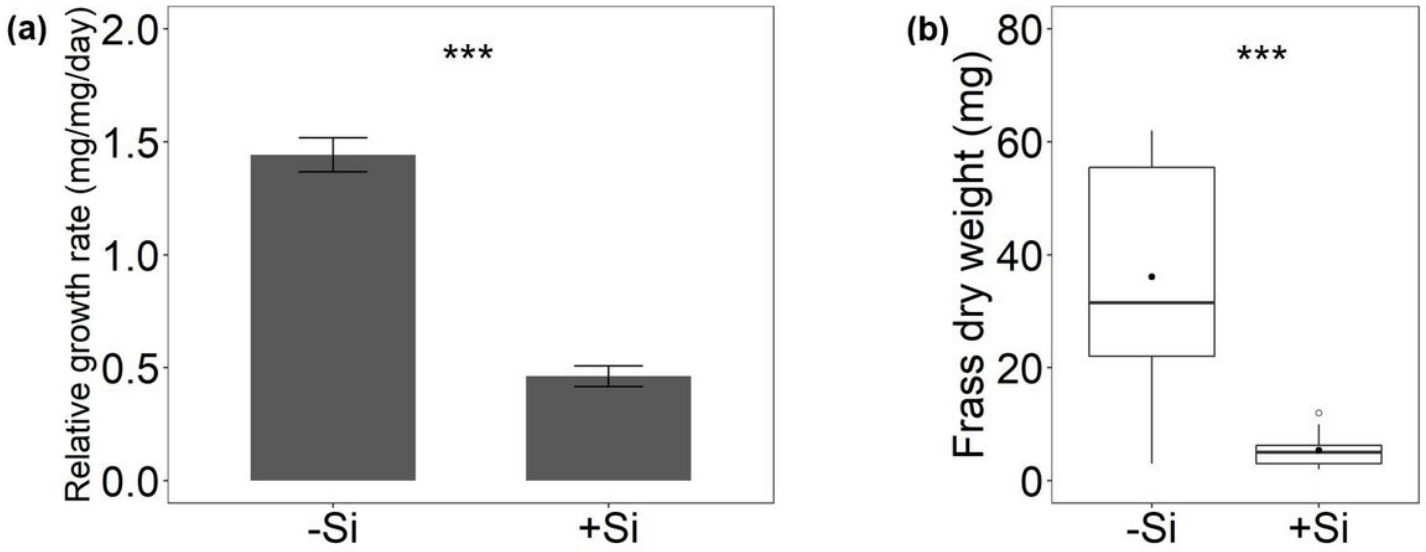

Figure 1

(a) Larval relative growth rate (mg/mg/day) and (b) leaf consumption (mg) on –Si and +Si plants. Mean ± SE shown (N = 40). Group means were compared using Student's t-tests. Asterisks indicate the level of statistical significance (*** $p < 0.001$) at 95% confidence intervals

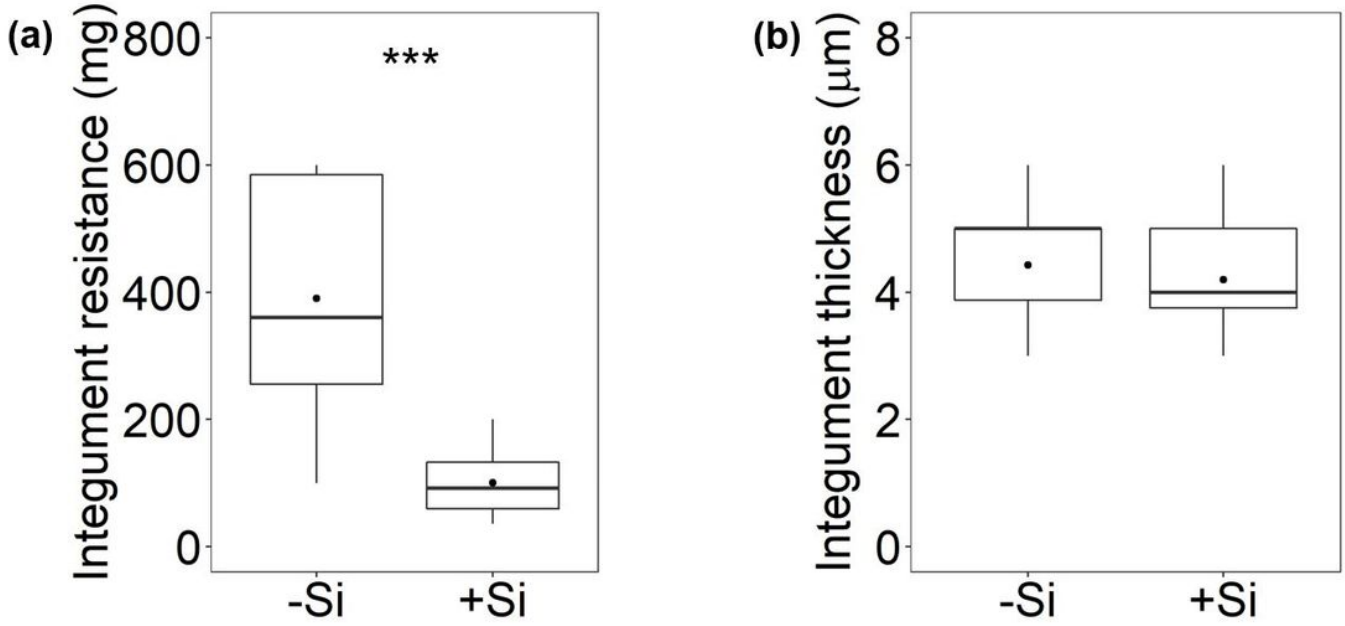

Figure 2

(a) Integument resistance (mg) and (b) integument thickness (µm) of larvae fed on –Si and +Si plants. Median and interquartile range (N = 20) are shown along with the mean (black circle). Differences

between treatments were determined using Wilcoxon's rank-sum tests. Asterisks indicate the level of statistical significance at a 95% confidence interval (*** $p < 0.001$)

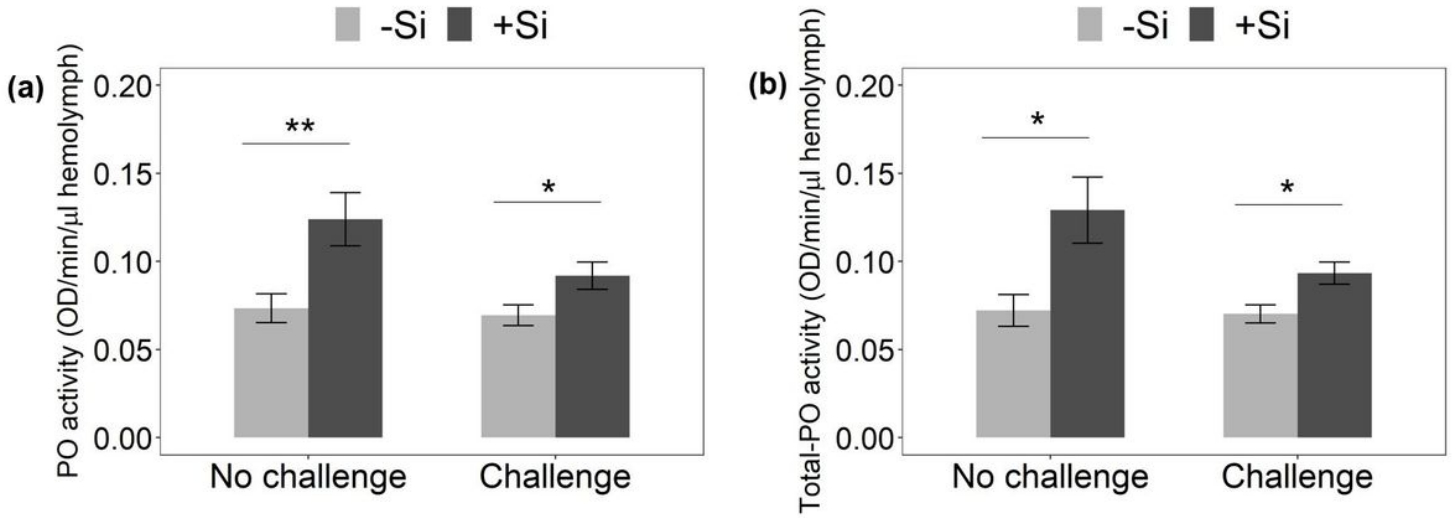

Figure 3

The activity of (a) phenoloxidase (PO) and (b) total-PO per microliter of larval hemolymph. Larvae were either kept unchallenged or challenged by implanting a nylon filament following feeding on –Si or +Si plants. Mean ± SE shown (N = 10). Group means were compared using Student's t-tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$) at 95% confidence intervals

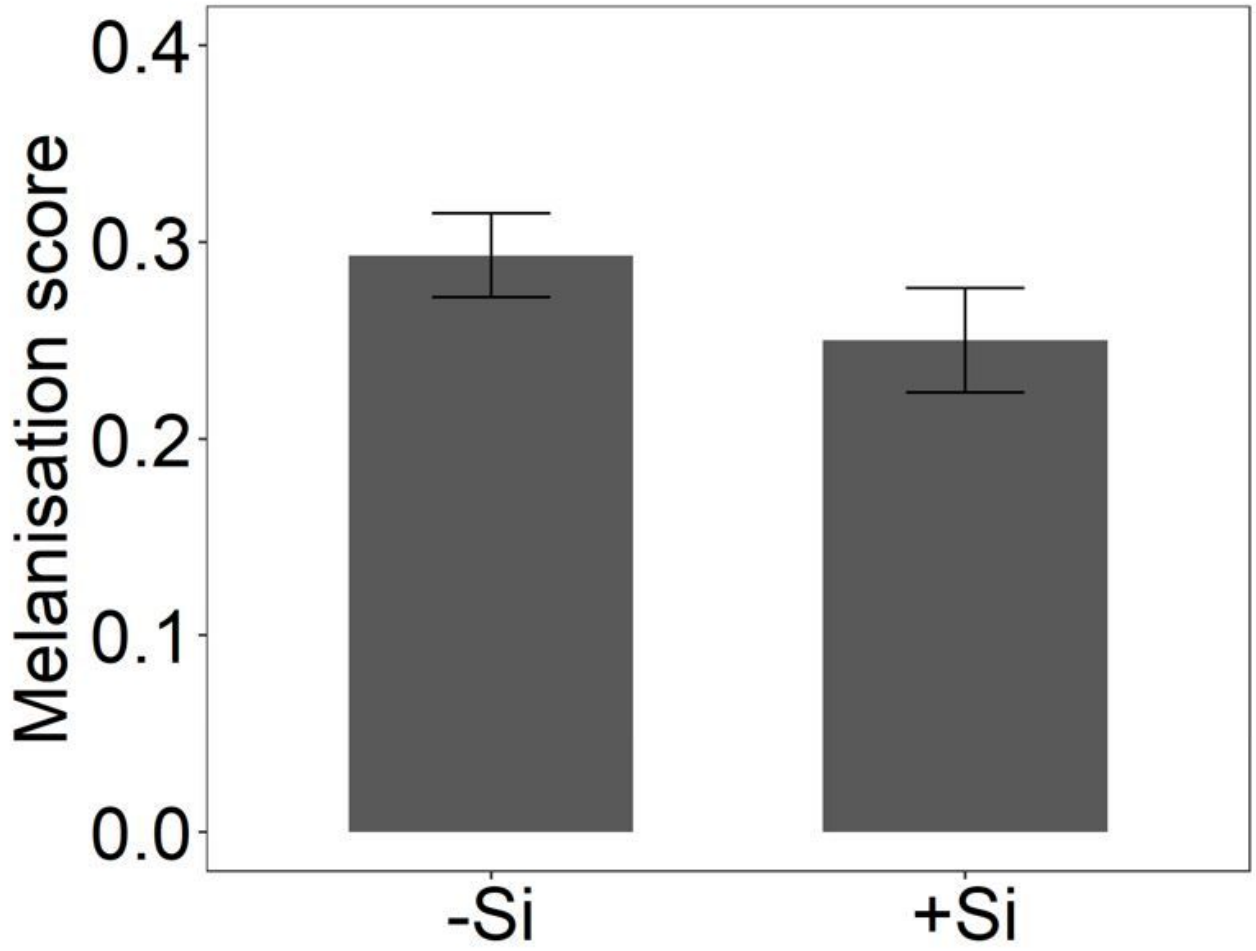

Figure 4

Melanisation responses of larvae fed on –Si and +Si plants. Mean ± SE shown (N = 10). Group means were compared using a Student's t-test

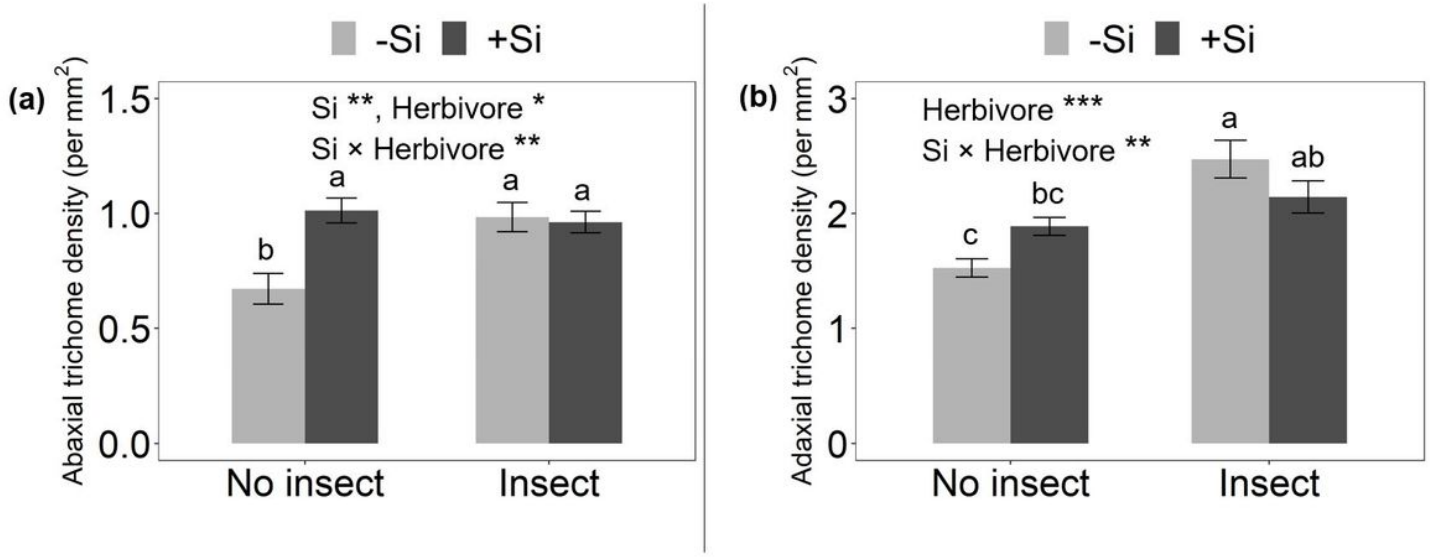

Figure 5

Impacts of Si and herbivory on the density of trichomes (number per mm2) on (a) abaxial and (b) adaxial leaf surfaces. Mean ± SE shown (N = 20). Data were analysed using two-way ANOVA tests and further using Tukey's HSD tests. Asterisks indicate the level of statistical significance (* p < 0.05, ** p < 0.01, *** p < 0.001). Different lowercase letters indicate significant differences between means at 95% confidence intervals

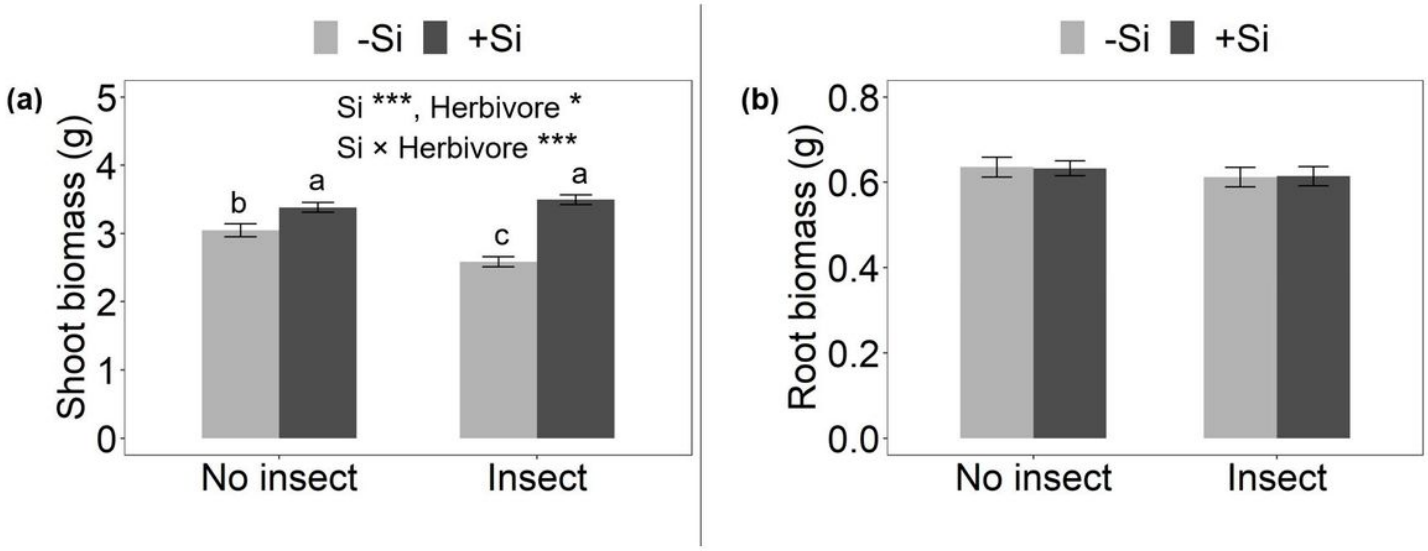

Figure 6

Impacts of Si and herbivory on dry (a) shoot biomass and (b) root biomass of plants. Mean ± SE shown (N = 20). Data were analysed using two-way ANOVA tests and further using Tukey's HSD tests. Asterisks indicate the level of statistical significance (* p < 0.05, ** p < 0.01, *** p < 0.001). Different lowercase letters indicate significant differences between means at 95% confidence intervals

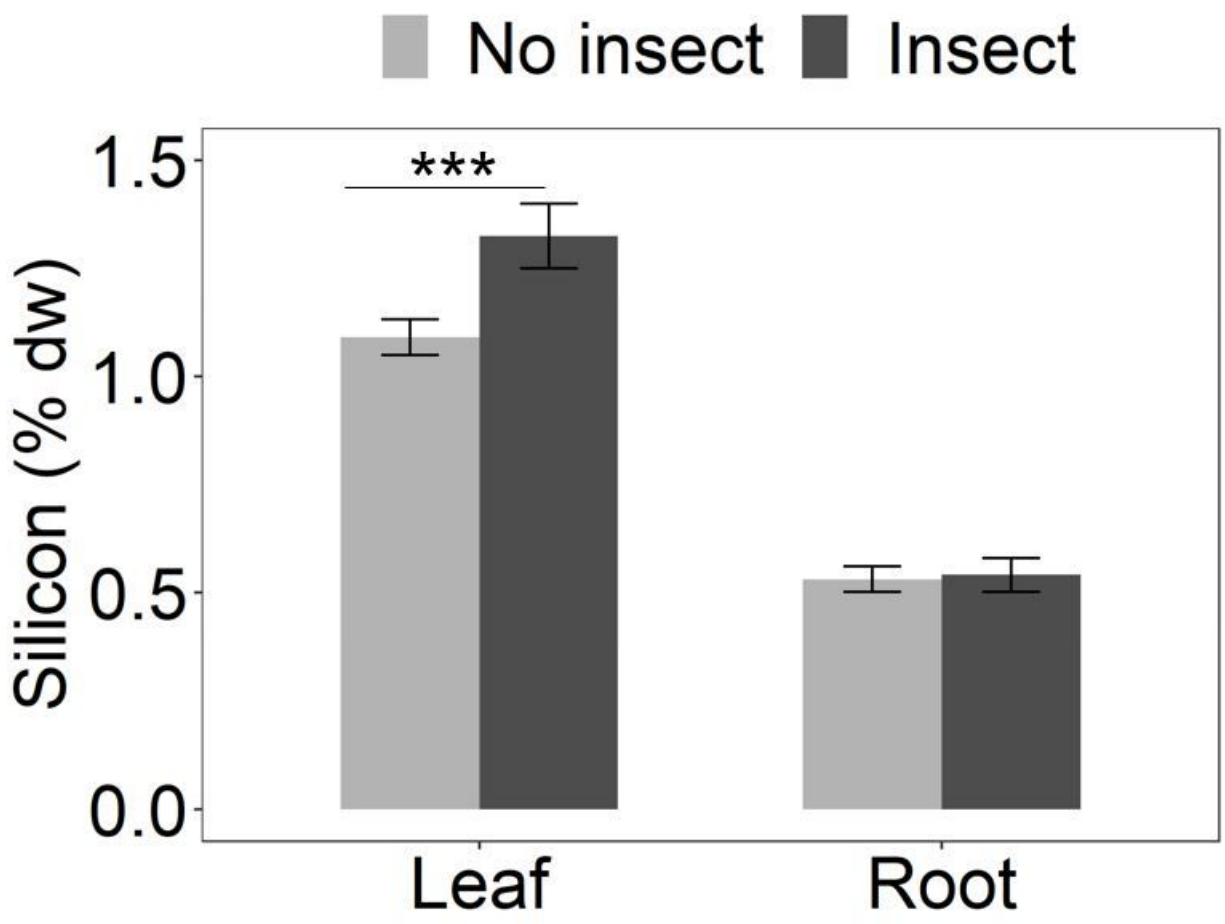

Figure 7

Concentrations (% dry weight) of Si in the leaves and roots of +Si plants in the absence or presence of insect herbivory. Mean ± SE shown (N = 20). Group means were compared using Student's t-tests. Asterisks indicate the level of statistical significance at a 95% confidence interval (*** $p < 0.001$)

Supplementary Files

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