SHORT COMMUNICATION

Silicon enhances natural enemy attraction and biological control through induced plant defences

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Abstract

Silicon (Si) is known to have a role in constitutive plant defence against arthropod pests, and recent work has illustrated involvement in induced plant defences. The present tri-trophic study tested the hypothesis that Si increases natural enemy attraction to pest-infested plants and improves biological control. Cucumber plants treated with potassium silicate (Si^+) and untreated control plants (Si^-) were maintained in separately vented glasshouse compartments. Y-tube olfactometer studies showed that adult *Dicranolaius bellulus* were significantly more attracted to Si^+ plants upon which *Helicoverpa armigera* larvae had fed compared with Si^-, pest-infested plants. Predators were not significantly more attracted to Si^+ plants when comparing uninfested cucumbers. In a field experiment, we placed *H. armigera*-infested and uninfested Si^+ and Si^- cucumber plants in a lucerne stand. Removal rates of *H. armigera* egg baits showed predation was greater for Si^+ infested plants than for other treatments. Results suggest that Si applied to plants with a subsequent pest infestation increases the plants' attractiveness to natural enemies; an effect that was reflected in elevated biological control in the field.

Keywords: trophic interactions, herbivore-induced plant volatiles, natural enemies, olfactometer

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Introduction

Silicon is not generally considered an essential plant nutrient; however, its role in conferring plant resistance to a range of abiotic and biotic stresses is now beyond doubt (Ma & Yamaji, 2006; Kvedaras *et al.*, 2007a). Silicon is taken up by plants in the form of monosilicic acid ($Si(OH)_4$) and transported from the root to the shoot (Jones & Handreck, 1967) where it polymerises into biogenic opal (amorphous $SiO_2 \cdot H_2O$). It has long been recognised that Si has a role in constitutive plant defences (i.e. those that are expressed continually even in the absence of biotic or abiotic stressors) (Djamin & Pathak, 1967; Goussain *et al.*, 2002; Kvedaras & Keeping, 2007; Kvedaras *et al.*, 2007a,b, 2009a). There is growing evidence for a role of Si in the induced defence of plants attacked by fungal pathogens (Menzies *et al.*, 1992; Chérif *et al.*, 1994; Bélanger *et al.*, 2003; Rodrigues *et al.*, 2004; Savvas *et al.*, 2009). Silicon has also been shown to act as an inducer of aphid resistance in wheat (Gomes *et al.*, 2005) and potato (Gomes *et al.*, 2008). There has been considerable debate around Si-based constitutive defence against fungal pathogens, with some authors arguing that they may have little or no role at all, and that the responses may be entirely physiological (Fauteux *et al.*, 2005). This stands in quite marked contrast to the insect work, where there is good evidence that Si-based constitutive defences are important, but can be enhanced by induced Si-based mechanical and physiological responses under high and/or sustained herbivore pressure (Gomes *et al.*, 2005; Kvedaras & Keeping, 2007; Massey & Hartley, 2009).

Despite such two-trophic level work on the direct effects of Si in induced defence against plant pathogens and pests, very little work has been conducted on tri-trophic level interactions involving natural enemies of insect pests. This is an important gap because of the wealth of literature on plant attractiveness to predators and parasitoids by herbivore-induced plant volatiles (HIPVs). The arthropods responding to these chemical cues benefit from the prey or host insects available, and the reduced herbivory benefits plant productivity (Sabelis *et al.*, 1999). Only one study has tested for the effect of Si on natural enemies (Moraes *et al.*, 2004). That work demonstrated detrimental effects of Si on the pest *Schizaphis graminum* (Rondani) but no effect on natural enemies. A methodological limitation of that study was that non-choice conditions were used, and parasitoid wasps, *Aphidius colemani* Viereck, were restricted to individual wheat plants that were narrowly spaced. Further, experiments conducted with the predator *Chrysoperla externa* (Hagen) were still less favourable to detection of induced plant defences involving volatile cues because *S. graminum* were removed from the test plants and fed to predators that had never been exposed to plants (Moraes *et al.*, 2004).

The aim of the present study was to test whether Si enhanced the attractiveness of natural enemies to plants under attack by arthropod herbivores. When attacked, Si-supplemented plants produce systemic stress signals, such as salicylic acid and jasmonic acid (Fauteux *et al.*, 2005), that are key to plant induced defences (Gatehouse, 2002). Jasmonic acid is particularly important because this compound can trigger production of HIPVs by the attacked plant, which aid location by predators and parasitoids. Thus, there is good reason for believing that Si may have unrecognised importance in resistance induced by arthropod feeding and subsequent attraction of natural enemies. This paper presents results from studies of the effects of soil-applied Si on cucumber (*Cucumis sativus L.*). Silicon-treated and control plants was analysed using Y-tube olfactometer bioassays to compare attractiveness of plants with and without the arthropod pest *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to a generalist predator, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae). Finally, a free-choice field experiment compared 'wild' predator activity on *H. armigera* larvae infested and uninfested Si^- and Si^+ cucumber plants using bait cards bearing *H. armigera* eggs. Our overall hypothesis was that silicon treatment would amplify the defence response of cucumber plants when attacked by arthropod herbivores, with consequent elevation in attractiveness to natural enemies and levels of biological control achieved.

Materials and methods

Plant growth and treatments

Cucumber seeds cv. Long Green (Mr Fothergill's, Windsor, Australia) were germinated on moist paper towels for two days in an incubator at 25°C day/20°C night. Vigorous germinated seeds were placed singly in peat pellets in a glasshouse with natural lighting at 25 ± 3°C. After one week, peat pellets with young plants were placed singly into 25 cm diameter plastic plant pots filled with cocopeat medium (Galuku, Sydney, Australia). Plants were irrigated every 2–3 days with water from a deionising unit fitted with D0813 and D0809 cartridges (Barnstead International, Boston, USA). At the end of the experimental period, a sample of the water analysed by a commercial, National Association of Testing Authorities (NATA) accredited laboratory (SGS Australia, Newbridge, Australia) contained $2 \, mg \, l^{-1}$ Si. On two occasions in the first week after potting, a Si-free compound fertiliser (Miracle-Gro Water Soluble Plant Food®, Scotts, Baulkham Hills, Australia) was added to the deionised water. Silicon-treated plants were each supplied with 300 ml of 1.7 mM Si ($48 \, gl^{-1}$ Si) as potassium silicate (AGSIL27®, PQ Australia, Dandenong South, Australia) at four weeks and again seven weeks from potting. Control plants received no supplementary Si but were treated with potassium chloride to balance nutrition to plants across treatments. Each plant pot stood in a separate plastic tray to catch and allow subsequent uptake of leachate. A total of 60 plants were grown for each of the two Si treatments. To avoid possible 'cross-talk' by plants, the treatments were accommodated within four separate polythene enclosures from which air was drawn to the exterior of the glasshouse with a motorised extraction fan; therefore, they were not laid out in a randomised pattern in the greenhouse. Immediately after experimental work, 12 plants from each treatment were oven dried to constant weight. In order to attain sample dry weights exceeding the minimum (3 g) required for a Si assay, plants were pooled into four samples, each comprising three plants. Silicon assays of the plant tissue were conducted by SGS Australia (Newbridge, Australia) and data analysed by ANOVA.

Insect bioassay

One week after the second application of Si and control treatments, when plants were eight weeks old, the third youngest true leaf of plants was inoculated with ten second

instar *H. armigera* larvae. *Helicoverpa armigera* were supplied as eggs and reared on sweetcorn cobs. A mesh sleeve (Megaview, Taiwan) was placed over each inoculated leaf (and over uninoculated leaves on control plants) to confine larvae. Mesh sleeves were of two sizes, 20×40 cm and 30×70 cm, and these were used non-systematically on all plants (whether treated with Si or not and with or without pests). All sleeves were made from nylon netting, 100×80 mesh. Plants were then held in separate greenhouse enclosures for each of the four treatments: non-pest infested Si$^-$ and Si$^+$ plants and pest-infested Si$^-$ and Si$^+$ plants. Subsequent bioassays compared, either Si$^-$ pest$^-$ with Si$^+$ pest$^-$ or Si$^-$ pest$^+$ with Si$^+$ pest$^+$. Due to the time-consuming nature of the work, bioassays were conducted between 14 and 22 h after inoculation with *H. armigera*. A Y-tube olfactometer (OLFM-YT-2425F; Analytical Research Systems, Gainesville, Florida, USA), internal diameter 24 mm with 25 cm arms, was used to test the response of the generalist predatory red and blue beetle, *D. bellulus*, to volatiles induced by herbivore-infested and non-infested, Si$^-$ and Si$^+$ plants at the Charles Sturt University campus, 4 km north of Orange, New South Wales (33°14′48″S 149°7′2″E). The predators were sweep net-collected from lucerne, *Medicago sativa* L., within 36 h of use and held individually in gelatine capsules under fluorescent lighting at 23.6–29.1°C in the olfactometer laboratory prior to introduction into the olfactometer port. A blank run (no plants within each bell jar) comprising eight *D. bellulus* showed no bias for the left or right arm of the olfactometer. An additional run of nine *D. bellulus* showed a 5:4 result for un-infested, Si$^-$ cucumber plants. Each odour source consisted of a whole potted cucumber plant, placed singly in a 10-l glass bell jar standing in a dish of deionised water to ensure it was air tight. The mesh sleeve was removed from the plant just prior to placement in the bell jar, although the *H. armigera* larvae, frass etc. remained on the pest$^+$ plants. Humidified air was fed into each bell jar at 200 ml min^{-1}, and a separate plastic tube vented the headspace down one of the olfactometer arms. For pest-infested plants, a two-way comparison was made between Si$^+$ and Si$^-$ plants. Equivalent Si comparisons were made for uninfested plants. Adult predators were introduced singly into the olfactometer port and observed for up to 5 min. A choice was recorded when the predator passed a join in the glassware, 4 cm from the olfactometer intersection within this time and did not backtrack for 30 s. It was otherwise recorded as a 'non-choice'. Temperatures during olfactometer experiments ranged from 23.6–29.1°C and were run in darkness, except for a single fluorescent light box positioned centrally behind the olfactometer. Nine predators were tested for each pair of plants and predator individuals which comprised one run. Plants were used only once. After each run, the olfactometer components and bell jars were rinsed with 100% ethanol and oven dried at 50°C. To give a total of four temporal replicates, this procedure was repeated four times for pest-infested plants (Si$^-$ and Si$^+$) and a further four times for un-infested plants (Si$^-$ and Si$^+$). Treatment combinations and placement of plants were non-systematically allocated to olfactometer arms in successive runs. A *G*-test was performed to check for variability between runs within treatments. Since no significant variability was evident ($G < 2.406$, df = 3, $P > 0.493$), data for all runs of a given treatment combination (Si$^-$ pest$^-$/Si$^+$ pest$^-$ or Si$^-$ pest$^+$/ Si$^+$ pest$^+$) were pooled, but beetles recorded as 'non-choice'

were excluded. Chi-square analysis was then used to test for a significant departure from unbiased, 50:50 selection of odour sources by the predatory beetles.

Field experiment

In preparation for a field experiment, six-week-old cucumber plants as described above were infested with *H. armigera*. Ten larvae were added to each of four Si$^+$ and four Si$^-$ plants, and a plastic bag placed over each plant and sealed around the rim of the pot to prevent escape of larvae. Plants allocated to pest$^-$ treatments (four Si$^+$ and four Si$^-$) were similarly bagged. All plants were held in the laboratory overnight in a fully randomised pattern and, next morning, were placed into a stand of lucerne that prior sweep netting had shown to harbour large numbers of predatory arthropods, especially *D. bellulus*. Potted cucumbers were positioned to constitute single-plant plots in a randomised block design with four replicates. Pot spacing was 3 m between plants within blocks and 10 m between blocks with plants at least 3 m from the edge of the plot. As the plastic bag covering each plant was removed, a bait card bearing between 30 and 47 *H. armigera* eggs was immediately stapled to the third youngest leaf. After 24 h, the numbers of eggs remaining was counted to provide an indication of field activity of predators on each plant. The proportion of eggs missing was analysed using a generalised linear model with a binomial distribution with a logit link, as the data set was not normally distributed.

Results and discussion

Analysis of the shoots of cucumber plants used for the Y-tube olfactometer and plant volatile studies showed that Si treatment more than doubled the Si tissue concentration of plants from 0.515% to 1.225% ($F_{1,7} = 15.22$, $P = 0.008$).

In Y-tube olfactometer comparisons of plants upon which *H. armigera* larvae had been feeding, a total of 24 *D. bellulus* chose the olfactometer arm connected to Si$^+$ plants compared with the 12 predators attracted to the Si$^-$ treatment ($\chi^2 = 4.000$, df = 1, $P = 0.045$). No significant effect was apparent when Si treatments were compared for cucumber plants that had not been exposed to pests (Si$^+ = 22$, Si$^- = 14$; $\chi^2 = 1.778$, df = 1, $P = 0.182$). That twice as many predators were attracted to Si$^+$ plants in the comparison of pest-infested plants is consistent with the hypothesis that *D. bellulus* were responding to a change in the HIPV blend that was brought about by the application of Si.

In the field experiment, a significantly higher level of removal of *H. armigera* eggs (approx. 1/3) from Si$^+$ pest$^+$ cucumbers than from Si$^-$ pest$^+$ plants, indicated a differential attraction of wild predators from the lucerne onto plants of the former treatment (fig. 1). Average egg removal rates were significantly lower in all three other treatments. These results support the often-cited observation in the Si literature that Si has little effect when plants are not subject to some form of stress (biotic or abiotic) (Kvedaras *et al.*, 2009b).

Collectively, the present results support the hypothesis that Si enhanced the attraction of the predator *D. bellulus* to *H. armigera*-infested plants under laboratory conditions and increased biological control by wild predators in a small
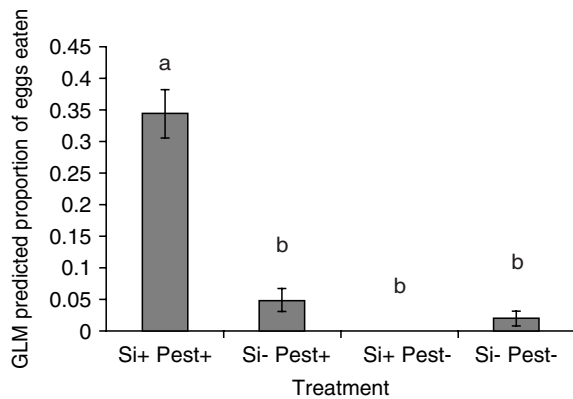
Fig. 1. Proportion of *Helicoverpa armigera* eggs removed over a 24-h period from potted cucumber plants placed in a lucerne (*Medicago sativa*) stand with numerous predatory arthropods; effect of prior treatment with potassium silicate (Si^+) and infestation with ten *H. armigera* larvae/plant ($pest^+$) ($N = 4$). Columns with differing letters differ (LSD test, $P = 0.05$).

scale field experiment. We hypothesise that this is due to a change in the plant volatile profile (HIPVs) produced by cucumber plants when attacked by a herbivore. Further work is warranted to measure and identify the compounds produced by pest-infested cucumber plants, particularly those which may be strongly affected by treatment of the plants with Si. Natural enemies other than Coleoptera, including those from other guilds such as parasitoids, may respond to the induced plant defences of cucumbers as well as those of other crops. For example, field studies in the USA have shown that Encyrtidae and Mymaridae parasitoids were attracted to grapevines to which dispensers of three HIPVS (methyl salicylate, methyl jasmonate or (Z)-3-hexenyl acetate) were applied (James & Grasswitz, 2005). Accordingly, the present work may constitute the opening of a new avenue in biological control whereby inexpensive Si-containing materials could be used to amplify HIPV production by various crops and enhance the contribution of biological control to pest management.

Acknowledgements

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