



THE EFFECTS OF SILICA FERTILIZER AS AN ANTI-HERBIVORE DEFENSE IN CUCUMBER

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Received: August 2016; Accepted: March 2017

ABSTRACT

This study aims to improve our understanding of silicon's role in deterring herbivores from *Cucumis sativa*. We hypothesized that silicon's role in plant defense is due to the presence of silica augmenting other physical and/or chemical defenses used by the plant. Using *C. sativa* plants treated with either a silica fertilizer treatment (Si+) or a control solution (Si-), we monitored feeding preferences of two types of herbivores, a chewing herbivore (*Diabrotica balteata*) and a piercing/sucking herbivore (*Bemisia tabaci*). Leaves from treatment plants were visited less and eaten less than leaves from control plants. We then assessed the differences in physical defenses by comparing the leaf structural components, nutrient and water content, and trichome density between treatment and control plants. For chemical plant defenses, we measured leaf carbon and nitrogen levels in, and volatile organic compounds (VOCs) from treatment and control plants. We found no significant difference between treatment and control plants in: lignin content, most elemental plant nutrients, water content, trichome density, and quantity of carbon and nitrogen. We did see an increase in the VOC Indole, known for plant defense priming, an increase in phosphorous levels and a decrease in cellulose levels in silica treated plants.

Key words: silica, cucumber, *Bemisia tabaci*, *Cucumis sativa*, *Diabrotica balteata*, Indole

INTRODUCTION

Elemental silicon (Si) is the second-most abundant element in the earth's crust and often accumulates in plants as silica (SiO₂) phytoliths, which can play an important role in plant defense (Epstein 1999). Much of the work on silicon's role in defense has been restricted to economically important monocots, including corn (Smith et al. 2007), wheat (Goussain et al. 2005; Gomes et al. 2005; Cotterill et al. 2007), sorghum (Carvalho et al. 1999; Moraes & Carvalho 2002) and turf grass (Massey & Hartley 2006; Redmond & Potter 2006). This focus is due to both the potential of silica as a deterrent of agricultural pests and the fact that monocots tend to accumulate more silicon than do herbaceous eudicots (Hodson et al. 2005; Currie &

Perry 2009). Certain species of eudicots, however, accumulate moderate levels of silicon. For example, some species of Cucurbitaceae accumulate upwards of 4% silica (dry mass) (Currie & Perry 2009; Mitani-Ueno et al. 2011). Hence, our understanding of silicon's role in deterrence of herbivory in eudicots can provide new insights about the co-evolution of plants and herbivores and can lead to better options for pest control in agriculture and a greater understanding of its ecological function.

Assessing silicon's role in defense is complicated by the fact that it may directly or indirectly augment other plant palatability characteristics. For example, the accumulation of silica in soft tissue can take up to 20% of the biomass of the dry-plant weight (Hodson et al. 2005) and could therefore dilute the nutrient concentration in the plant tissues

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and in turn decrease the nutrients available to herbivores (Strömberg et al. 2016). Silica-enforced trichomes increase the strength of physical barriers (Rafi et al. 1997), deterring chewing herbivores from being able to take bites of the soft tissue. Silica-laden epidermal plant tissues wear down insect mandibles (Panda et al. 1995; Massey et al. 2006; Massey & Hartley 2009; Reynolds et al. 2009), decreasing the rate of plant tissue consumption.

Silicon may also play a role in the production of volatile organic compounds, also used for plant defense, by potentially inducing plant signaling to herbivore predators (Kvedaras et al. 2010). In certain systems silicon increases induced plant defenses through the jasmonic acid (JA) pathway (Reynolds et al. 2016; Kvedaras et al. 2010; Pare & Tumlinson 1999). This pathway increases both direct defenses through plant secondary metabolites as well as volatile attraction of predators and parasitoids (Pare & Tumlinson 1999).

Finally, the presence of silica in plants is known to mediate drought tolerance (Ma et al. 2004; Ahmad & Haddad 2011; Chen et al. 2011), thus potentially increasing leaf water content and creating a more desirable food and/or water source for herbivores. Taken together, these mechanisms of defense against herbivores, associated with plant-accumulated silicon, underscore the importance of examining a variety of ways in which silica may be part of plants' defense strategies.

We set out to examine all of these parameters in Cucurbitaceae. The first goal of this study was to test whether silicon is an effective deterrent against insect herbivory in a eudicot by exposing plants grown with and without added silica to different types of herbivores. The second goal was to explore potential mechanisms by which silicon may help plants defend themselves against herbivores by quantifying how plant chemical and physical attributes, including water content, may co-vary with silica concentration. In a series of experiments, we manipulated silicon contents of cucumber plants (*Cucumis sativus*) and quantified damage by two types of herbivores: banded cucumber beetles (Chrysomelidae, *Diabrotica balteata* LeConte) and whiteflies (Aleyrodidae, *Bemisia tabaci* Gennadius). The banded cucumber beetle is a chewing herbivore that

feeds on more than 50 species of plants, including *Cucumis sativus*. Whiteflies used in experiments naturally occurred in an infested greenhouse that housed a variety of experimental plants. Whiteflies use their stylets to penetrate leaf epidermis of many vegetables and herbaceous ornamental plants to feed from the phloem (Massey et al. 2006).

MATERIALS AND METHODS

Plants and silica treatment

Cucumber plants 'Burpless' (Atlee Burpee & Co, Warminster, PA) were grown in a greenhouse with a 12 h light-dark cycle and temperature range of 27–32 °C. Plants were grown in a 50–50 mix of fine "play" sand and vermiculite with a slow release fertilizer (Osmocote N : P : K, 18 : 5 : 13). When at least three fully expanded leaves were present (about 5 weeks after sprouting), plants were randomly assigned to Si⁺ and Si⁻ groups. Treatment plants (Si⁺) were provided 30 ml of 2 mM potassium silicate solution (Parrella et al. 2007) daily for a week prior to the start of herbivory trials, and then given weekly silica treatments to provide silica treatment for any new leaves that developed during the experiment. Si⁻ plants were provided equivalent volumes of 2 mM potassium chloride at the same intervals, which balanced the amount of potassium applied to plants in the Si⁺ group. All plants were watered with deionized water as needed. To avoid release of hormones and defensive compounds (Pichersky et al. 2006), plants were protected from herbivory prior to experiments.

Herbivores

Banded cucumber beetles (Saba 1970) were reared in an incubator at 25 °C and a 14 : 10 h light : dark cycle. They were fed lima bean leaves and sweet potatoes. Eggs were collected weekly and hatched onto corn sprouts; larvae fed on the roots until the third and final instar stage. Third instar larvae were allowed to crawl into a container of sterilized soil for pupation. Emerged adults were transferred back into the colony.

Beetle preference on leaf disks

Banded cucumber beetles were exposed to leaf disks of Si⁺ and Si⁻ plants to determine preference

by comparing consumption. A cork borer was used to cut 4 mm diameter disks from Si⁺ and Si⁻ leaves. Fifty, 100-mm petri dishes were lined with moist filter paper to provide water for beetles and to limit desiccation of the disks. Each dish contained six Si⁺ and six Si⁻ disks, placed in rows on opposite sides of the dish, so that at any given point in the dish, the beetle was exposed to both Si⁻ and Si⁺ leaf disks. Dishes were marked on the outside to identify Si⁺ and Si⁻ disks, which were randomized for location. Adult beetles were randomly selected from a laboratory colony. One beetle was placed in the center of each dish and allowed to feed ad libitum for 14 h. To quantify leaf disk water loss, five dishes were set up at the same time with the same distribution of leaf disks but without a beetle. The size of leaf disks in these plates was used to correct for evaporative shrinkage of disks fed upon by beetles. Combined leaf area of treatment/control disks in a given dish was measured with a leaf area meter (LI-3050A, Licor, Lincoln, Nebraska) and subtracted from initial leaf area, corrected for shrinkage, to determine the area of leaf tissue consumed.

Whitefly preference on whole plants

To examine the feeding preferences of whiteflies, 16 plants (eight Si⁺ and eight Si⁻), each with four or five leaves (four weeks old), were placed 25 cm apart in a greenhouse infested with whiteflies for two weeks. The greenhouse allowed for air circulation and free movement of white flies and better simulated natural environments than more traditional plant-in-bag designs. For silica treatments and duration, see above. Plants were randomly ordered on a raised bench. Every two days for the duration of the study, all adult whiteflies were collected via aspirator from three randomly selected Si⁺ and Si⁻ plants. Care was taken during aspiration to not disturb the plant or leaf, limiting the impact of movement on the whiteflies. This set-up was replicated three times.

Volatiles

Five Si⁺ and four Si⁻ four-week-old plants – due to a loss of one Si⁻ plant during harvesting – were harvested and placed into glass volatile collection chambers (40 cm long × 5 cm diameter). The entire aboveground portion of each plant was cut at the base of the stem and immediately placed into the

chamber; previous research in the lab has shown that damage to plants from excising does not register for the first 30 minutes, the duration of the volatiles collected. Airflow into chambers was regulated by flow meters (Aalborg, Orangeburg, NY) to maintain a constant rate of 60 ml·min⁻¹. Flexible Teflon tubing (Cole-Parmer, Vernon Hills, IL) was used upstream of the chamber to supply filtered, humidified air; this was balanced with a vacuum and a bubbler to create a slight overpressure. An inline filter packed with 30 mg Super Q adsorbent polymer (Alltech, Nicholasville, KY) was attached between the end of the chamber and the start of Tygon tubing (Saint-Gobain Corp, Paris, France) to capture volatile chemicals in the air stream. Volatiles were collected for the initial 30 min post-harvest. Volatiles were extracted from filters with 200 µl of methylene chloride (Sigma-Aldrich, St. Louis, MO), after 5 µl of an internal standard (80 ng·µl⁻¹ of nonyl acetate (Sigma-Aldrich) in methylene chloride) had been added to the top of the adsorbent polymer material. Volatiles were preliminarily identified from their GC-MS fragmentation patterns. The GC-MS used was a Hewlett Packard HP6890 with HP5973 MS in EI mode with a 30 m × 0.25 mm inside diameter HP1 capillary column and a 0.25 µm film of methyl silicone. The injector inlet temperature was held at 240 °C with a He carrier gas moving through the column at 30 cm·sec⁻¹. The oven held an initial temperature of 35 °C for 1 min followed by a 10 °C·min⁻¹ temperature ramp to 230 °C, held for 5 min. The transfer line to the MS was kept at 240 °C. Headspace volatiles were quantified by analysis with GC-FID (HP 6890 with the same column and parameters as above, except the FID detector was held at 250 °C) and chemical identities were confirmed by analyzing synthetic standards (Sigma-Aldrich).

Plant chemical composition

To evaluate the chemical composition of Si⁺ and Si⁻ plants, dry leaf samples from seven Si⁺ plants and seven Si⁻ plants were analyzed for two macro- and six micronutrients. A half-gram of each oven-dried sample was digested and run on an Inductively Coupled Plasma (ICP)-Atomic Spectrometer using EPA method 200.7 at the University of Florida-IFAS Analytical Research Laboratory. The

nutrients analyzed were potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe). Silicon content was analyzed separately to confirm Si⁺ and Si⁻ treatments created a difference in silicon concentration.

Lignin and cellulose

For fiber content analysis, leaves were ground and dried, then analyzed with a fiber analyzer system (ANKOM Technology, NY, USA), following the protocol described by Alvarez-Clare and Kitajima (2007). Half-gram samples were sealed into a chemical-resistant bag (ANKOM F57 filter bags) and sequentially treated with neutral detergent fiber solution to determine % NDF (including hemicellulose, cellulose, lignin and insoluble ash), acid detergent fiber solution to determine % ADF (including cellulose, lignin and insoluble ash), and 72% sulfuric acid to determine % lignin and insoluble ash. Digestions were followed by % ash determination with combustion at 550 °C (Ryan et al. 1990). From these digestions, % hemicellulose, % cellulose and % lignin were calculated by subtraction, assuming that the masses of non-fiber contents (including ash), hemicellulose, cellulose and lignin summed to the original dry mass of the sample. The first treatment determined ADF-lignin (lignin) and α -cellulose (cellulose) in Si⁺ and Si⁻ plants.

Leaf trichomes

Leaf trichome density was assessed on leaves of three age cohorts of Si⁺ and Si⁻ plants. As leaves emerged from ten Si⁺ and ten Si⁻ plants, they were marked with colored twist ties to record leaf age. Leaves were categorized into three age groups: young (< 2 weeks old), middle (2–6 weeks old), and old (7–10 weeks old). Because leaf age was a variable of interest, plants were allowed to grow longer than in the previous experiments to provide a wider distribution of leaf ages. When at least 10 fully expanded leaves were present on the plant, all leaves were harvested. Using a 1 mm² grid and a dissecting light microscope with camera, leaf trichomes were counted at four 1 cm² randomized locations on each leaf. These values were averaged to obtain the average density of leaf trichomes per 1 mm². Also the number of broken leaf trichomes was recorded to assess whether leaf trichomes were more brittle on Si⁺ or Si⁻ plants.

Silica extraction

After trials, all plants were harvested. Leaf wet mass, dry mass, and area were determined. We calculated percent water as (wet leaf mass – dry leaf mass)/wet leaf mass.

To verify that potassium silicate treatments effectively increased the amount of silicon in Si⁺ plants, silicon was extracted using an alkaline extraction of biogenic silica with a sodium carbonate solution (Conley et al. 1993). For all samples, 25 mg of dried and ground leaf tissue was mixed with 20 ml of 1% sodium carbonate solution in a 60 ml polycarbonate Nalgene bottle, and left to shake overnight in a 85 °C water bath. Si concentration in the extract was determined by the molybdate blue method (Sauer et al. 2006), with 1-amino-2-naphthol-4-sulfonic acid (Lab. Chem. Inc., Pittsburgh, PA. Cat. LC10890). Absorbance at 650 nm was measured with a R Spectronic 401 spectrophotometer and compared to a standard curve established with a sodium silicate solution. Results were expressed as percent of silica.

All statistics were performed using the statistical software JMP 9 (SAS, Raleigh, NC 2011). Beetle preference on leaf disk was determined using a paired t-test on the total amount of leaf area eaten of Si⁺ and Si⁻ disks in each dish. The number of whiteflies per leaf for each plant was recorded and a one-way ANOVA, blocked by day, was performed on the total number of whiteflies on Si⁺ and Si⁻ leaves. A Principal Components Analysis (PCA) was performed using the quantity of each detected volatile to detect clustering of Si⁺ and Si⁻ plants. A t-test was performed separately for each micro- or macro- nutrient on Si⁺ versus Si⁻ plants. Because results of lignin and cellulose are expressed as percent per dry leaf mass and were skewed, data were arcsine square-root transformed for normality. Even after transformation, however, data for cellulose and lignin were not normally distributed. Thus, a generalized linear model (GLM) with a binomial distribution, using an identity link was used to compare those constituents between Si⁺ and Si⁻ plants. Because there was no significant difference in leaf trichome density among leaf cohorts, all age groups were combined for the final analysis. A one-factor ANOVA was used to compare leaf trichome density

between Si⁻ and Si⁺ leaves. Additionally, leaf trichome breakage was assessed using a one-way ANOVA on the old leaf cohort, since the young and middle leaf cohorts had little if any leaf trichome breakage. We analyzed the leaf area consumed using a logit transformation and GLM with a normal distribution and a log link function. To test for overall silica concentration differences between Si⁺ and Si⁻ plants, percent silica was logit converted and compared using a GLM with a normal distribution and an identity link function.

RESULTS

Watering with potassium silicate did increase silica content of plants; Si⁺ plants accumulated approximately 33% more silica than did Si⁻ plants ($p = 0.008$, Fig. 1A). There was no significant difference in % water content of the Si⁺ ($87.4\% \pm 4.9$) and Si⁻ plants ($88.8\% \pm 2.2$; $p = 0.33$).

Beetle preference on leaf disks

Overall, beetles consumed 24.3% more total leaf area of Si⁻ leaf disks than Si⁺ leaf disks ($t_{47} = -2.25$, $P = 0.029$, Fig. 1B). Within each dish, beetles consumed more Si⁻ than Si⁺ tissue in 32 dishes, compared to 15 in which they consumed more Si⁺ than Si⁻ tissue. In the three remaining dishes, leaf consumption was negligible because beetles in those dishes died soon after the experiment started – a rate of mortality similar to that of our larger colony for newly emerged beetles, such as these.

Whitefly preference on whole plants

The number of whiteflies on Si⁻ plants was 44.5% higher than on Si⁺ plants ($F_{1,17} = 16.5$, $P = 0.0012$, Fig. 1C), indicating a strong preference for plants with less silica. Si⁻ plants had a daily average of 71.7 ± 10.7 whiteflies/leaf, whereas Si⁺ plants had 45.5 ± 4.9 whiteflies/leaf.

Volatiles

A total of 48 compounds were detected and compared between Si⁻ and Si⁺ plants. Overlaying the chromatographs of Si⁺ and Si⁻ plants revealed little consistent difference in volatile signature, due to high variation in peak size among individual plants. Comparison of the amount (ng) of each compound revealed significant differences in just one peak between the Si⁺ plants and Si⁻ plants. Indole

was approximately 64% lower in Si⁻ than Si⁺ plants ($df = 4.89$, $t = -4.02$, $P = 0.0051$). The PCA of all volatile compounds showed no pattern of separation or clustering of Si⁺ and Si⁻ plants. For a full list of volatiles identified from the plants, see Table 1.

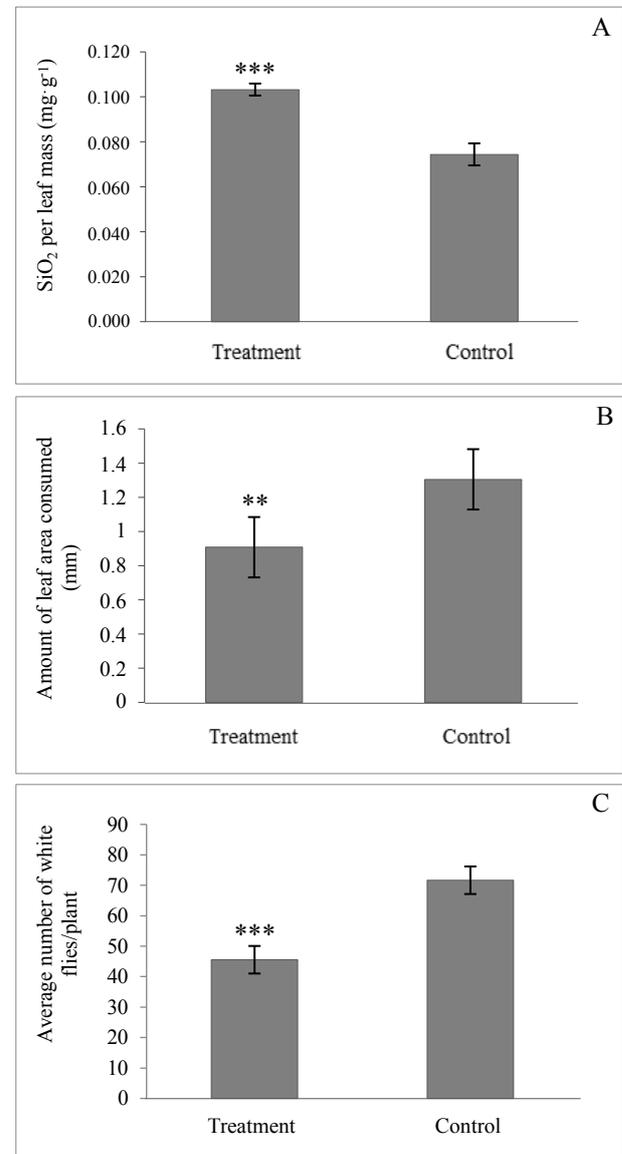


Fig. 1. A) Average mg SiO₂ per g of dry leaf material for Si⁺ and Si⁻ plants (N = 15 of each). B) Average leaf area consumed from 12.5 mm² leaf disks of each treatment. C) Average number of whiteflies (*Bemisia tabaci*) per cucumber plant for treatment (Si⁺) and control (Si⁻) plants. Si⁺ plants received nutrient solution containing 2 mM of potassium silicate, while Si⁻ plants received solution without added silicon. Error bars represent standard errors. Asterisk indicate significance (with more asterisk indicating lower p values).

Table 1. Volatile organic compounds extracted from leaves and stems of control (Si-) and silica treated (Si+) *C. sativus* plants 30 min post-harvest. "Unknown" indicates volatile peak of an unknown or unidentifiable compound. An asterisk indicates a significant difference between the level of volatiles in control and treatment plants. Values are the mean concentration of the compound plus/minus the standard error of the samples. Negative numbers indicate concentrations lower than the blank cuvette

Compound	Si- ± SE		Si+ ± SE	
	N-octane	0.2±	0.21	0.5±
E-2-hexenal	0.7±	0.35	1.0±	0.62
Z-3-hexen-1-ol	0.4±	0.09	0.4±	0.13
Alpha-pinene	18.0±	13.58	17.6±	17.93
Beta-pinene	3.0±	1.25	9.5±	10.15
Myrcene	0.5±	0.41	0.6±	0.70
Z-3-hexenyl acetate	7.8±	5.86	1.8±	1.31
Limonene	0.0±	0.09	0.1±	0.32
E-beta ocimene	1.6±	0.57	20.0±	21.02
Linalool	0.6±	0.62	0.7±	0.74
Nonatriene	0.6±	0.27	20.3±	21.84
Z-3-hexenyl isobutyrate	-2.0±	1.33	-1.5±	2.14
MSA	1.0±	0.47	0.8±	0.29
E-2-hexenyl butyrate	7.8±	5.86	1.8±	1.31
Indole*	0.8±	0.22	2.1±	0.42
Z-jasmone	0.3±	0.15	0.3±	0.20
Caryophyllene	11.3±	5.51	10.6±	2.68
Bergamotene	1.4±	1.10	2.0±	1.53
Alpha-humulene	-0.1±	0.46	0.7±	0.56
Beta-farnescene	-0.5±	0.50	0.7±	0.56
Alpha-farnescene	0.2±	0.10	0.3±	0.18
Nerolidol	0.6±	0.43	0.0±	0.23
Tridecatetraene	0.5±	0.28	0.8±	0.34
Unknown 1	0.5±	0.47	9.3±	9.73
Unknown 2	0.5±	0.12	0.5±	0.48
Unknown 3	0.8±	0.46	4.5±	4.03
Unknown 4	31.6±	28.55	35.5±	25.96
Unknown 5	4.2±	4.04	8.1±	5.12
Unknown 6	-4.4±	2.39	-0.5±	3.27
Unknown 7	2.4±	2.24	5.6±	3.87
Unknown 8	18.0±	13.58	17.6±	17.93
Unknown 9	19.8±	13.01	23.6±	27.11
Unknown 10	3.6±	2.20	4.3±	3.29
Unknown 11	2.7±	1.15	13.4±	16.50
Unknown 12	2.1±	1.22	8.3±	10.25
Unknown 13	0.8±	0.36	1.0±	0.58
Unknown 14	7.8±	5.86	1.8±	1.31
Unknown 15	7.1±	4.39	7.9±	6.16
Unknown 16	1.6±	0.57	20.0±	21.02
Unknown 17	0.2±	0.49	1.8±	0.84
Unknown 18	0.6±	0.27	20.3±	21.84
Unknown 19	11.3±	5.51	10.6±	2.68
Unknown 20	0.6±	0.48	0.7±	0.73
Unknown 21	0.5±	0.50	2.9±	2.27
Unknown 22	0.3±	0.37	0.6±	0.65
Unknown 23	0.9±	0.83	1.7±	0.87
Unknown 24	3.8±	2.93	9.8±	2.63

Table 2. Plant nutrients and structural components of cucumber plants grown with and without silica (Si+ and Si-, respectively). Values indicate means and standard deviations. N indicates sample size (e.g. N = 13 indicates 7 Si+ plants and 6 Si- plants) and asterisks indicate a significant difference between Si+ and Si- plants.

Trait	N	Si+ plants \pm SE	Si- plants \pm SE
P* (mg/kg)	13	1076 \pm 97	838 \pm 72
K (mg/kg)	13	1688 \pm 635	1198 \pm 528
Ca (mg/kg)	13	2127 \pm 622	2316 \pm 927
Mg (mg/kg)	13	1854 \pm 266	25301 \pm 893
Zn (mg/kg)	13	11 \pm 4	8 \pm 1
Mn* (mg/kg)	13	81 \pm 18	44 \pm 31
Cu (mg/kg)	13	0.50 \pm 0.77	0.07 \pm 0.68
Fe (mg/kg)	13	14 \pm 6	15 \pm 5
N (%/mg)	7	2 \pm 1	3 \pm 0.5
C (%/mg)	7	29 \pm 1	31 \pm 4
Lignin (%/mg)	16	3 \pm 2	5 \pm 2
Cellulose* (%/mg)	16	33 \pm 18	67 \pm 16
Leaf trichomes/cm ²	10	105 \pm 5	105 \pm 4

Plant nutrients

Plant nutrient concentrations differed little between Si+ and Si- plants. Concentrations (mg/kg dry mass) of potassium, calcium, magnesium, zinc, copper and iron were not significantly different between Si+ and Si- leaves (Table 2). Phosphorus, however, was approximately 25% higher in Si+ than Si- plants ($p = 0.004$). The percentages of nitrogen and carbon were not significantly different between Si+ and Si- plants.

Lignin, cellulose and leaf trichomes

Although Si+ and Si- plants had similar amounts of lignin, the amount of cellulose was significantly higher in Si- plants than Si+ plants ($p = 0.004$; Table 2).

The density of leaf trichomes did not differ between Si+ and Si- plants ($p = 0.97$, Table 2) and there was no difference in the number of broken leaf trichomes on old leaves between Si+ and Si- plants.

DISCUSSION

The percent of silica accumulated in Si+ plants is similar to that reported in cucumber plants provided with varying concentrations of silica treatments in greenhouses (Liang et al. 2005; Ma & Takahashi 2007), and to other eudicots in natural settings (Hodson et al. 2005). Higher foliar silica concentration in Si+ plants resulted in lower leaf tissue consumption

by chewing beetles and a decrease in the presence of a piercing/sucking herbivore. Contrary to a study that concluded silica had no effect on a phloem feeder (*Sitobion avenae*) on five different grass species (Massey et al. 2006), we found that foliar silica deterred at least one species of piercing/sucking herbivore, more in line with other studies on monocots that demonstrated deterrence of silica on piercing/sucking herbivores (Keeping & Kvedaras 2008).

Changes in leaf tissue nutrient content could alter the desirability of a plant to insect herbivores. We found that plants with high silica content also had higher phosphorus content however we found no differences in other micro-nutrients (K, Ca, Mg, and Na). Higher phosphorus levels in silica treated plants is consistent with a previous study, which reported elevated levels of phosphorus in silica-treated cucumber plants compared to non-silica treated plants (Gorecki & Danielski-Busch 2009).

The physiological mechanism of higher phosphorus contents with potassium silicate solution is unknown. Higher phosphorus in the diets of leaf beetles (*Diorhabda sublineata* Lucas) and tobacco hornworms (*Manduca sexta* Linnaeus) leads to higher larval mass, growth rates and adult fecundity (Perkins et al. 2004; Guenther et al. 2011). Additionally, when provided a choice between high and low phosphorus plants, tarnished plant bugs (*Lygus hesperus* Knight) and crickets (*Acheta domesticus* Linnaeus) prefer

plants with high phosphorus, suggesting that phosphorus availability may be limiting in these species (Forbes & Rosenheim 2011; Visanuvimol & Bertram 2011). The phosphorus levels in our Si⁺ plants were similar to the levels found in the plants in the studies by Geunther et al. (2011) and Visanuvimol et al. (2011). These previous studies suggest that insects should show a preference for the plants with higher phosphorous levels. Yet, in our study both cucumber beetles and whiteflies preferred Si⁻ plants, which had 25% less phosphorus, suggesting that silica is a sufficiently strong deterrent to overcome the presumed increase in attractiveness of silica-rich leaves due to high phosphorus content.

Silica is found in combination with cellulose in the epidermal cells of the leaf blade of oats (Jones et al. 1963; Lewin & Reimann 1969), suggesting that there may be a relationship between silica and cellulose. This idea is supported by a comparison study of multiple physical defenses of plants in natural habitats that found higher levels of silica were sometimes associated with higher levels of lignin and cellulose (Schoelynck et al. 2010). However, under controlled environmental conditions we found no difference in lignin and less, not more, cellulose in Si⁺ than Si⁻ plants. The observed association of high levels of lignin, cellulose and silica in Schoelynck et al.'s (2010) study may be correlative or driven by unknown environmental effects. These results require additional study, using controlled silica levels in field conditions, to see if cellulose levels vary consistently with silica levels.

We found that Si⁺ plants did not have a higher density of trichomes than Si⁻ plants, indicating that the presence of silica did not stimulate leaf trichome formation. Silica is deposited in trichomes (Cheng & Horng 1989; Rafi et al. 1997), and therefore Si⁺ plants may have been more deterrent to herbivores than those of Si⁻ plants because of attributes that we did not evaluate, such as increased toughness or hardness. If so, we might have expected a difference in the number of broken trichomes on mature leaves. We found no such difference, suggesting that the toughness or brittleness of leaf trichomes was not different between Si⁺ and Si⁻ plants.

Kvedaras et al. (2010) concluded that plants with high amounts of silica being consumed by herbivores can attract herbivore predatory insects. The authors

from the study hypothesized that it may be the presence of particular volatiles that were responsible for triggering the attraction, and that the observed increase in number of herbivore predators attracted to the silica-treated plants may indicate plant signaling driven by production of particular volatiles. In cucumbers, we found one difference between Si⁺ and Si⁻ plants in production of volatiles, an increase in Indole. Although, our volatile extraction was conducted on harvested plants that had not experienced herbivory, while the Kvedaras et al. (2010) study used plants that were currently undergoing herbivore damage, the constitutive increase in Indole indicates that Si⁺ plants are more primed for herbivore defense, and able to communicate attack to surrounding plants (Erb et al. 2015). This, along with the potential greater sensitivity of the JA pathway through silicon, could indicate that along with increased physical defenses the volatiles also helped deter the mobile piercing sucking herbivores (Pare & Tumlinson 1999, Reynolds et al. 2016).

CONCLUSION

We conclude that the levels of silica present in our treatment plants are adequate to serve as an herbivore deterrent for at least two types of insect herbivore feeding guilds. Because silica is associated with both physical and chemical defenses in cucumbers, cucumbers may benefit from soil augmentation with a silica fertilizer. We show that silica contributes to plant defenses in terms of increasing defense volatile emissions. However, we found no increase in the physical defenses of lignin and leaf-trichomes.

Future studies on volatile release during herbivore feeding and more extensive bioassays to examine herbivore deterrence and predator attraction towards plants with increased silica will provide a better picture of the potential role of silica in plant communication and chemical defense.

Acknowledgments

We would like to thank Amanda Pendleton, Shaji Faisal, and Alyson Bradshaw for their assistance in the lab, Dr. Karou Kitajima (UF) for lab space and equipment for silica extractions and Dr. Eric Schmelz (USDA-ARS, Gainesville) for advice and equipment on volatile extraction. We would also like to thank the funding sources: Howard Hughes Medical Institute: Group Advantaged Training of Research Small Research Grant.

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