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## SILICON APPLICATIONS HAVE MINIMAL EFFECTS ON *SCIRTOTHRIPS DORSALIS* (THYSANOPTERA: THIRIPIDAE) POPULATIONS ON PEPPER PLANT, *CAPSICUM ANNUM* L.

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### ABSTRACT

Silicon (Si) is the second most abundant element in soils, and at elevated concentration levels in plant tissue Si is reported to improve plant resistance to a range of biotic and abiotic stresses. In the present study, Si solutions at 100, 300 or 500 mg L<sup>-1</sup> made from potassium silicate (K<sub>2</sub>SiO<sub>3</sub>) were applied as foliar sprays or soil drenches to pepper (*Capsicum annum* L.) plants, and their effects on chilli thrips (*Scirtothrips dorsalis* Hood) populations were studied. Data from 3 greenhouse tests showed minimal effects of Si treatments on visual leaf damage and numbers of thrips recovered from infested plants. An addition of jasmonic acid (a plant defense elicitor) with or without Si applications also did not alter the proportion of pepper leaves that sustained thrips damage. Tissue analyses showed that soil-drenched plants were able to absorb Si in roots up to ≈ 2.5% (w/w), but the Si was not being translocated to leaf or stem tissues at an equivalent rate. Foliar application of Si resulted in close to 0.5% (w/w) of Si in leaf tissues. Plant biomass was not affected by Si applications. We conclude that pepper plants treated with potassium silicate solutions did not accumulate to sufficient Si levels in leaf tissues to protect against thrips feeding or reproduction. To our knowledge this is the first study of Si application to pepper plants for attempts to control an insect pest.

Key Words: Silicon; induced resistance; chilli thrips; pepper plant

### RESUMEN

El Silicio (Si) es el segundo elemento más abundante del suelo. El incremento de la acumulación del Si en los tejidos de la planta ha sido reportado como mejorador de la resistencia de la planta a un rango estreses bióticos y abióticos. En este presente estudio, soluciones de Si a 100, 300 y 500 mg L<sup>-1</sup> procedente de silicato de potasio (K<sub>2</sub>SiO<sub>3</sub>) fueron aplicadas a plantas de pimienta (*Capsicum annum* L.) a través de aspersión foliar y en solución saturada al suelo (drench) para evaluar los efectos en poblaciones de chilli Thrips (*Scirtothrips dorsalis* Hood). Datos correspondientes a 3 experimentos de invernadero mostraron mínimos efectos de los tratamientos con Si en daño visual de las hojas y en el número de thrips recuperado de las plantas infestadas. La adición de ácido jasmónico (un inductor de defensa de la planta) con o sin aplicación de Si, tampoco alteró la proporción de hojas de la planta de pimienta que sufrió daños por thrips. Los análisis de los tejidos mostraron que a través de la solución saturada al suelo (drench), las plantas fueron capaces de absorber Si en la raíces hasta ≈ 2.5% (w/w), pero esta no fue trasladada a los tejidos de las hojas o al tallo a una tasa equivalente. La aplicación foliar de Si presentó cerca de 0.5% (w/w) de Si en los tejidos de las hojas. La biomasa de la planta no fue afectada por las aplicaciones. Nosotros concluimos que las plantas de pimienta tratadas con soluciones de silicato de potasio, no acumulan suficiente niveles de Si en los tejidos para protegerlas contra la alimentación y la reproducción de los thrips. Para nuestro conocimiento, este es el primer estudio de aplicaciones de Si en plantas de pimienta con el propósito de intentar controlar un insecto plaga.

Palabras claves: Silicio, resistencia inducida, chilli Thrips, planta de pimienta.

Host plant resistance is an alternative strategy to reduce pesticide selection pressure and the possibility of deleterious environmental effects of pesticides, and therefore is considered an important component of integrated pest management (IPM) (Thomas & Waage 1996). There is a substantial body of research reporting silicon (Si)-mediated plant resistance to a wide range of bi-

otic and abiotic stresses (Epstein 1994; Ma 2004; Ma & Yamaji 2006; Richmond & Sussman 2003). After oxygen, Si is the most abundant element in soils, with concentrations ranging from 0.1 to 0.6 mM in the form of monosilicic acid (H<sub>4</sub>SiO<sub>4</sub>), the soluble hydrated form absorbed by plants (Epstein 2001). Although Si has not been recognized as an essential element for plant growth, H<sub>4</sub>SiO<sub>4</sub>

is absorbed by many plants via the roots, and accumulates in tissues at concentrations ranging from 0.1-10% of dry weight in the form of polymers of hydrated amorphous silica (Epstein 1999; Richmond & Sussman 2003).

Enhanced resistance to insect pests following artificial Si applications has been observed in a wide variety of plant species. Deposition of Si on epidermis of leaves reduced the whitefly [*Bemisia tabaci* (Gennadius); Hemiptera: Aleyrodidae] populations through increased development time and nymph mortality in both cucumber (*Cucumis sativus* L.; Cucurbitales: Cucurbitaceae) (Correa et al. 2005) and soybean [*Glycine max* (L.) Merr.: Fabales: Fabaceae] (Ferrira et al. 2011). Several important rice pests, including the Asiatic rice borer [*Chilo suppressalis* (Walker); Lepidoptera: Crambidae], the yellow rice borer [*Scirpophaga incertulas* (Walker); Lepidoptera: Pyralidae], the rice green leafhopper [*Nephotettix cincticeps* (Umler); Hemiptera: Cicadellidae], white backed planthopper [*Sogetella furcifera* Horvath; Hemiptera: Cicadellidae], and brown planthopper [*Nilaparvata lugens* (Stål); Hemiptera: Delphacidae], have been controlled or suppressed by increasing the silicon concentration in the plants (Savant et al. 1996). In sugarcane, silicon applications also reduced damage from the African sugarcane borer [*Eldana saccharina* (Walker); Lepidoptera: Pyralidae] (Keeping & Meyer 2002) and the shoot borer [*Chilo infuscatellus* Snellen; Lepidoptera: Crambidae] (Rao 1967). In addition, enhanced uptake of Si has been associated with plant growth, quality and yield, and increased protection against certain bacterial and fungal diseases [see reviews by Epstein (1999), Ma & Yamaji (2006), Richmond & Sussman (2003) and Savant et al. (1997)]. These beneficial effects are mostly hypothesized to be due to the hardening of plant cells through accumulation of silica on tissue surfaces; although the ability of Si to function as a signal to induce the production of antimicrobial compounds (phytoalexins) has also been proposed (Ma 2004).

In general, plants can be categorized as 'silicon accumulators', 'silicon neutral' or 'silicon-rejecters' (Ma & Yamaji 2006). In the former category are plants in Gramineae family, such as rice (*Oryza sativa* L.; Poales: Poaceae), which accumulates Si at rates up to 10% of dry weight (Takahashi et al. 1990). Most dicots are unable to accumulate high levels of Si in their shoots, although some, such as cucumber can accumulate moderate levels in leaves. In one study, Takahashi et al. (1990) reported that rice, cucumber, and tomato contained 7.3, 2.3, and 0.2% Si in the shoot dry weight, respectively, when grown under similar conditions. Plants that contain Si at rates between 0.5 and 1.5% w/w of dry weight (such as cucumber) are categorized as 'silicon neutral' plants. Plants that contain Si < 0.5% of dry weight are considered 'silicon-rejecters' because they are either not capable

of absorbing Si through their roots or exclude Si from plant tissues (Ma & Yamaji 2006).

Traditionally Si is missing from commonly used plant nutrient solutions; however Si in the form of potassium silicate ( $K_2SiO_3$ ) is now available for greenhouse use. Although not registered as pesticides, the use of such materials may conceivably confer significant resistance against some insect pests. In the present study, applications of potassium silicate to pepper plants were evaluated to determine to what extent Si accumulates in plant tissues and whether this approach has an impact against chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), an important herbivorous pest in tropical and sub-tropical regions (Arthurs et al. 2009).

## MATERIAL AND METHODS

### Thrips Colony

Chilli thrips were obtained from wild populations collected in 2008 on rose plants, *Rosa* sp.; Rosales: Rosaceae) in Winter Park, Florida. The colony was maintained and reared on cotton, *Gossypium* sp., 'Deltapine 493 Conventional' (Malvales: Malvaceae) in square cages (60 × 60 × 60 cm) under greenhouse conditions. Health of the colony was maintained by periodic introgression of wild thrips from naturally infested plants.

### Pepper Plants

Seeds of *Capsicum annum* L. cv. 'California Wonder' (Solanales: Solanaceae) (Tomato Growers Supply Company, Ft. Myers, Florida) were germinated in 9-cm Petri dishes containing moist filter papers. Germinated seeds were transferred to seedling trays and seedlings at the 6-8 true leaf stage were planted into 15 cm pots using Fafard Growing Mix 2 (Conrad Fafard, Inc., Agawam, Massachusetts). Potted plants were placed in cages (91 × 91 × 91 cm) in a greenhouse under natural photoperiod and initially fertilized weekly with a solution made from Peters professional 20-20-20 NPK (The Scotts Co., Marysville, Ohio) at a nitrogen concentration of 238 mg kg<sup>-1</sup>. Temperatures in the greenhouse ranged from 25 to 35 °C, and RH varied from 50-100%.

### Silicon Treatments

Pepper plants were treated with Si prior to their use in insect bioassays described below. Aqueous solutions were prepared from  $K_2SiO_3$  (Pro-TeKt® 0-0-3, Dyna-Grow™, Richmond, California) which contained 7.8%  $SiO_2$  and 3.7%  $K_2O$ , i.e. 3.6% elemental Si and 2.6% K (% v/v). Due to the presence of  $K_2O$  in the Si solutions, corresponding K concentrations were prepared as a K control using KCl to check that Si alone

was responsible for any observed effects. The pH was checked and, if needed, adjusted to 5.8-6 with phosphoric acid. An additional control was water. Pepper plants were treated through either soil-drenching or foliar-spraying to run off with solutions of Si, K and water, respectively. Foliar treatments were applied using a 1 L spray bottle (Sprayco, Livonia, MI) with the base of plants covered with plastic to prevent treatment of soil. Drenches were applied to the soil until saturation (approx. 200 ml per pot). All plants were maintained inside insect-proof cages during this time to prevent pest infestation. Applications of Si were made at 4-7 d intervals, as detailed below. A controlled release fertilizer (15-9-12 NPK Osmocote Plus, the Scotts Co., Marysville, Ohio) was applied at 5 g per pot prior to use in bioassays.

#### Greenhouse Bioassays

Insect bioassays were conducted on silicon treated plants. The first experiment used pepper plants previously treated 7 times (4 day intervals) with 4 treatments: i.e., 2 concentrations (100 and 300 mg L<sup>-1</sup> Si) as well as K<sub>2</sub>O alone (83 and 248 mg L<sup>-1</sup> K), each applied as either a foliar spray or soil drench, along with untreated controls. Plants (30-40 cm high) were arranged as a randomized block design, placed inside cages (91 × 91 × 91 cm) covered with nylon mesh (28 threads/cm) and each plant infested with 50 adult *S. dorsalis* using previously described methods (Doğramaci et al. 2011). Plants were watered every third day, and no additional silicon applications were made. Plants were examined weekly for 3 weeks and thrips activity was determined by assessing the number of leaves with thrips scarring damage. Fruits were removed from plants since we wanted to assess foliar damage. Each treatment was replicated 5 times. One month following thrips infestation, all plants were harvested, dried at 80 °C, and dry weights were compared. All roots, stems, and leaves of 3 randomly selected plants per treatment were harvested, dried, and ground in a steel Wiley mill. Silicon and K concentrations in the roots, stem, and leaves were analyzed using X-ray fluorescence spectroscopy and ICP-AES (Midwest Laboratories, Inc., Omaha, Nebraska) (Reidinger & Ramsey 2012).

The study was repeated twice with some modifications. In the second experiment, plants were treated 8 times (4 day intervals) with Si (300 mg L<sup>-1</sup>) as either foliar spray or soil drench. In addition, after the last application, a known plant defense elicitor [0.5 mM solution jasmonic acid (JA) dissolved in ethyl alcohol] was sprayed onto half of the Si-treated plants. JA and alcohol only controls were included. Each plant was infested with 50 adult *S. dorsalis* 7 days after the final treatment, and foliar damage and dry weight were as-

sessed as described earlier. Each treatment was replicated 7 times.

Since final pest damage was relatively high in the first 2 experiments, a third looked for possible interactions with Si treatments and pest pressure. In this study, a reduced pest density (5 thrips per plant) was used. Additionally, to increase the chance of finding significant effects of Si applications on thrips damage, an additional concentration (500 mg L<sup>-1</sup> Si) was included. Three solutions (100, 300 and 500 mg L<sup>-1</sup> Si) were applied as drenches only (no foliar spray) weekly for 4 wk before infestation and (unlike the first 2 studies) also continued weekly after thrips infestation, for a total of 7 applications. In addition to visual damage rating, 3 terminal leaves per plant (where thrips were concentrated) were examined *in situ* at 10X for numbers of adults and nymphs. Each treatment was replicated 8 times.

#### Data Analysis

The effects of Si treatments on dry weights and Si levels detected in plant tissues were assessed through univariate ANOVA, with significant factors or interactions examined through Tukey's HSD tests at  $P < 0.05$  (SPSS for Windows v. 17). The effects of Si treatments on thrips feeding, i.e., the proportion of damaged leaves and numbers of thrips life stages, were also compared using one and two-way repeated measures ANOVA with 3 or 4 time intervals (wk). Where needed, data were normalized using arcsine (proportion) or log n+1 (count) prior to analysis.

## RESULTS

#### Silicon Accumulation

Plant tissue analyses revealed significant accumulations of Si in roots and leaves, but not stems. Highest accumulation (up to 2.5% dry weight) was observed in roots with less than 0.5% w/w in leaves and less than 0.02% w/w in stems (Fig. 1). However, there were differences according to treatment. Drench, but not foliar treatments, resulted in significant Si accumulation in roots (Fig. 1A). Foliar applications of Si at 300 mg L<sup>-1</sup> increased Si levels in leaves approximately 2-fold, while other Si treatments did not increase Si levels in leaves significantly (Fig. 1B). No treatments accumulated Si in stems (Fig. 1C). A 3-way ANOVA revealed 1, 2- and a 3-way interaction for Si accumulation according to application method (foliar or drench), Si concentration (100 or 300 mg L<sup>-1</sup>) and plant tissue (root, leaves and stems) (Table 1). Tissue analysis also revealed some accumulation of K (from the KCl and K<sub>2</sub>SiO<sub>3</sub> treatments), especially in roots (i.e. average of 6.7% w/w compared with 5.5% in controls), but no significant accumulation was observed in leaves where thrips were feeding.

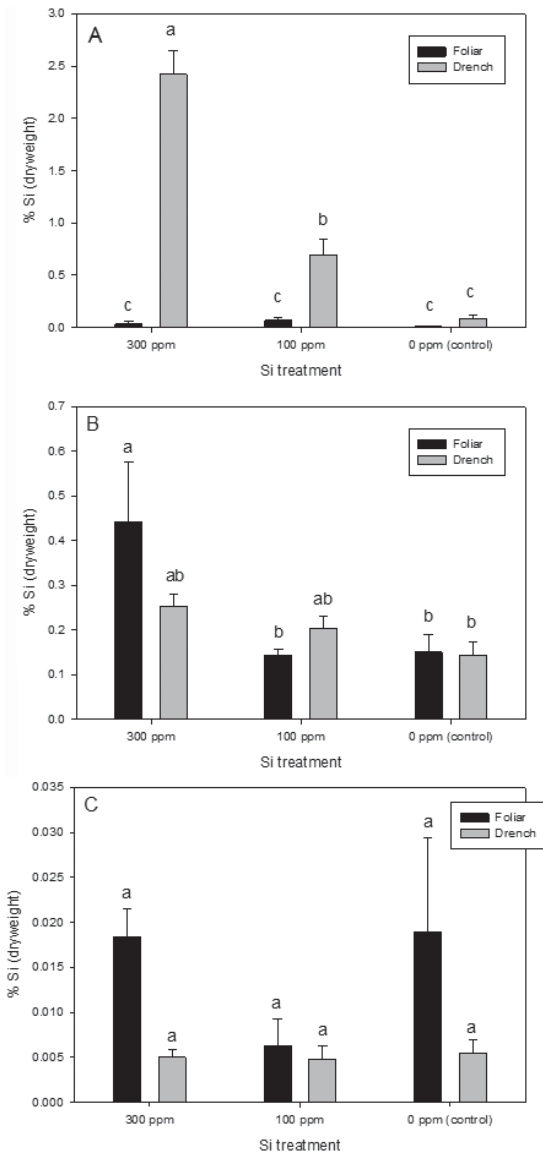


Fig. 1. Accumulated silicon (Si) element in tissues of *Capsicum annum* pepper plants treated with fertilizers containing 7.8 % SiO<sub>2</sub> and 3.7% K<sub>2</sub>O by foliar or drench applications; (A) root, (B) leaf and (C) stem. Data are mean ± SEM from 3 tissue samples and letters are Tukey's comparisons at  $P < 0.05$ ; note different axis scales between tissues (experiment 1).

#### Thrips Damage

In the first experiment, the proportion of leaves with thrips damage remained high throughout the study in all treatments (Fig. 2). Overall, damage was not significantly affected by different Si treatments ( $F_{4,40} = 0.55, P = 0.70$ ). However, when untreated controls were excluded, thrips damage was marginally affected by Si application method

TABLE 1. UNIVARIATE ANOVA DESCRIBING EFFECTS OF APPLICATION METHOD (FOLIAR OR DRENCH), CONCENTRATION (100 OR 300 mg L<sup>-1</sup>), AND PLANT TISSUE (ROOT, STEM OR LEAVES) ON ACCUMULATION OF SILICON (Si) IN PEPPER PLANTS (PERCENT W/W).

Source	Type III SS	df	F	Sig.
Plant tissue	1.24	2	99.5	.001
Application method	.60	1	96.4	.001
Si concentration	.22	1	35.8	.001
Plant * app.	1.43	2	115.2	.001
Plant * Si conc.	.17	2	13.3	.001
App. * Si conc.	.08	1	12.1	.002
Plant * App.* Si conc.	.36	2	29.2	.001
Error	.15	24		

used, i.e. drench versus foliar treatments ( $F_{3,32} = 4.65, P = 0.039$ ), with a non-significant interaction ( $F_{3,32} = 0.57, P = 0.64$ ). Comparisons suggested this was due to increased damage with drenches compared with corresponding foliar treatments

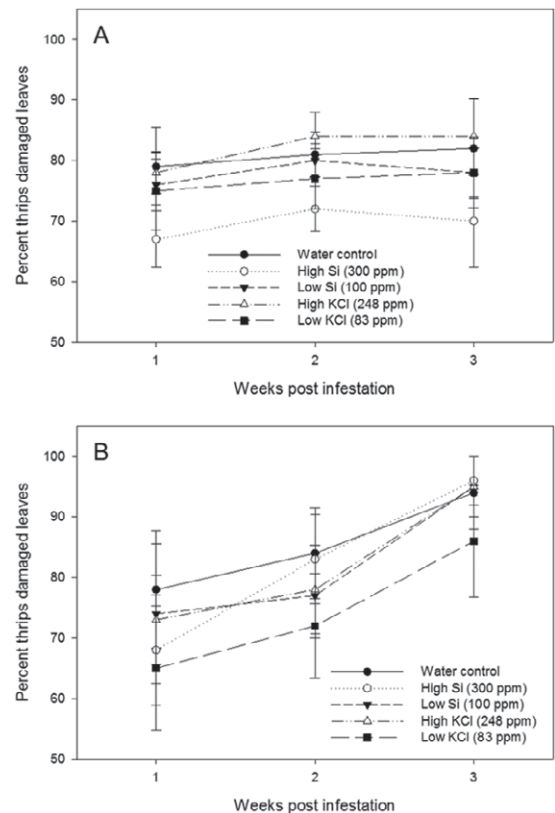


Fig. 2. Proportional leaf damage (mean ± SEM) due to *Scirtothrips dorsalis* feeding on pepper plants treated with different concentrations of silicon suspensions and KCl applied via (A) foliar sprays and (B) drenches, along with untreated controls (experiment 1).

(Fig. 2A versus Fig. 2B). Broadly similar results were observed in the second experiment, i.e. the main effect of Si-treatments did not explain variation in thrips damage ( $F_{6,42} = 0.55, P = 0.77$ ). In this case when the 4 Si treatments were analyzed separately (two-way ANOVA in repeated measures model), neither application method (drench versus foliar) ( $F_{1,24} = 0.14, P = 0.72$ ), the addition of JA ( $F_{1,24} = 2.97, P = 0.10$ ) nor their interaction ( $F_{1,24} = 0.04, P = 0.85$ ) were significant variables (Fig. 3). In the third experiment, damage was slightly lower in Si-treated plants (Fig. 4A) and fewer thrips were generally recovered from Si-treated plants (Fig. 4B). However, Si-treatment was again not a significant factor in the repeated measures model for either leaf damage ( $F_{3,28} = 1.25, P = 0.31$ ), or numbers of thrips recovered (i.e., adult thrips  $F_{3,28} = 1.01, P = 0.37$ , nymphs  $F_{3,28} = 2.10, P = 0.12$ , or life-stages combined  $F_{3,28} = 1.59, P = 0.21$ ).

Plant Biomass

Above ground plant biomass (total dry weight) was not influenced by Si treatment in either the first ( $F_{1,28} = 2.30, P = 0.14$ ) or third greenhouse experiment ( $F_{1,32} = 2.11, P = 0.16$ ). In the second experiment, plants receiving Si treatments had significantly decreased biomass ( $F_{1,47} = 12.67, P < 0.001$ ) compared with untreated control plants (i.e.  $6.2 \pm 0.4$  g versus  $8.5 \pm 0.5$  g).

DISCUSSION

Studies on Si-mediated plant resistance to insect pests have largely focused on grasses (known Si accumulators), such as sugarcane (*Saccharum officinarum* L.; Poales: Poaceae), rice (*Oryza*

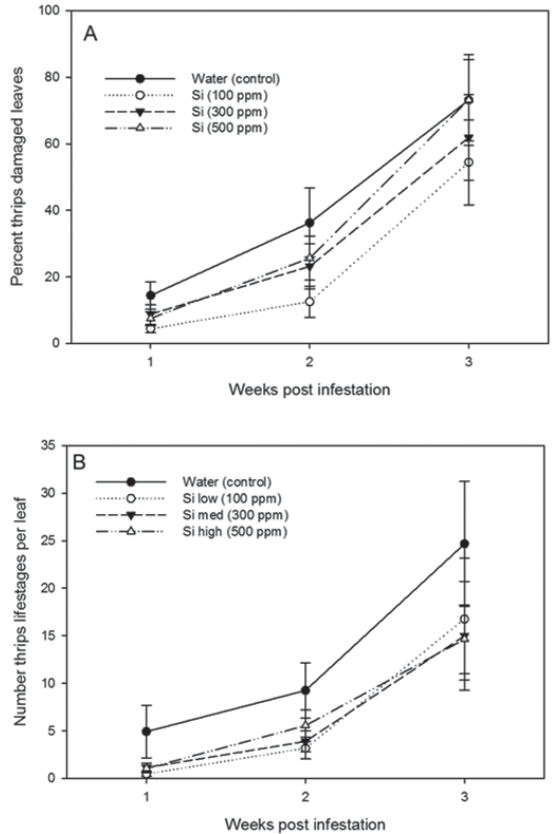


Fig. 4. Effect of silicon treatments (drenches) on subsequent leaf damage (A) and numbers of *Scirtothrips dorsalis* (B) recovered from infested pepper plants. Data are (mean  $\pm$  SEM) from 8 replicate cages (experiment 3).

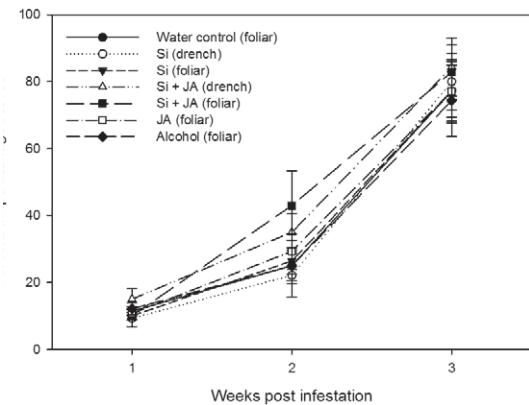


Fig. 3. Proportional leaf damage (mean  $\pm$  SEM) caused by *Scirtothrips dorsalis* feeding on pepper plants treated with aqueous silicon suspensions with and without jasmonic acid, and untreated controls (experiment 2).

spp.; Poales: Poaceae) and wheat (*Triticum aestivum* L.; Poales: Poaceae) (Moore 1984.; Salim & Saxena 1992; Sétamo et al. 1993; Keeping & Meyer 2002; Gomes et al. 2005), with relatively few tests conducted with dicots. However, Parrilla et al. (2007) observed a significant reduction in leafminer emergence in chrysanthemum plants, *Dendranthema morifolium* L. (Asterales: Asteraceae), treated with potassium silicate 200 ppm and higher. In this study it was hypothesized that application of potassium silicate might similarly provide protection to pepper plants from chilli thrips by accumulating Si in leaf tissues and decreasing thrips feeding and reproductive success. The rationales were that chilli thrips, a recent invasive species, causes severe damage to pepper plants; although there is no information regarding Si accumulation in pepper, but its relative, tomato, has been shown to absorb Si (Takahashi et al., 1990) and even show Si deficiency symptoms if no Si is supplied (Miyake and Takahashi, 1978). However, our data revealed minimal effects of treatments on damage and numbers of

thrips recovered from infested plants. Plant tissue analyses confirmed that while Si was able to accumulate to some extent inside roots (up to  $\approx$  2.5% w/w) following soil drenches, it was not being translocated to leaf or stem tissues at an equivalent rate. Moreover, only limited quantities of Si (i.e.,  $<$  0.5% w/w) accumulated inside leaf tissues from foliar applications. Therefore, pepper plants can be best classified as 'silicon neutral', based on earlier classification (Ma & Yamaji 2006).

Since chilli thrips feed through rasping the epidermis and mesophyll of young leaves, the lack of significant Si treatment effects may relate to insufficient accumulation of silicon at the feeding site. Similar findings have been reported in tests with phloem feeding insects. Hogendorp et al. (2009a, b) tested drenches of potassium silicate against the citrus Mealybug (*Planococcus citri* Risso; Hemiptera: Pseudococcidae) feeding on green coleus (*Solenstemon scutellarioides* L.; Lamiales: Lamiaceae) and fiddleleaf fig (*Ficus lyrata* Warb.; Rosales: Moraceae). Drenches applied up to 1600 ppm did not negatively affect mealybug fecundity or developmental parameters, and the authors concluded that neither plant accumulated sufficient quantities of silicon to impact epidermal toughness (stylet penetration) and mealybug feeding. Hogendorp et al. (2010) also reported that potassium silicate fertilizers applied on poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch; Malpighiales: Euphorbiaceae) at up to 800 ppm did not confer resistance to greenhouse whitefly (*Trialeurodes vaporariorum* Westwood; Hemiptera: Aleyrodidae) developing on leaves. Ranger et al. (2009) also reported that the pre-reproductive period and survivorship of the green peach aphid (*Myzus persicae* Sulzer; Hemiptera: Aphididae) was not affected by potassium silicate solutions on *Zinnia elegans* Jacq. (Asterales: Asteraceae) plants, although cumulative fecundity and intrinsic rate of increase of aphids were slightly reduced on plants receiving soluble silicon.

Several studies have shown that other plant-derived chemicals can serve as natural elicitors, leading to changes in herbivore preference or rates of damage sustained. For example, in many plants, JA and its conjugates are signaling compounds that can up-regulate defensive compounds when plants are attacked by herbivores (Gundlach et al. 1992). Artificial applications of JA have also been shown to induce measurable defense reaction to pests in cotton (Omer et al. 2001), grape (*Vitis vinifera* L.; Vitales: Vitaceae) (Omer et al. 2000), and tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae) (Thaler 1999), as well as stimulate the release of plant volatiles that are attractive to predaceous arthropods (Dicke et al. 1999). In the present study, no effect of JA applications on the proportion of pepper plant leaves that sustained thrips damage was detected. However, the relatively high insect pressure applied

in this test (50 thrips per plant), and short time period after which insects were infested (1 wk) may have masked more subtle effects of plant resistance. Moreover, since the proportional leaf area damaged was not measured, the possibility that reduced rates of feeding may have occurred on JA-treated plants cannot be excluded.

In conclusion, pepper plants did not accumulate sufficient Si levels in pepper leaves to protect adequately against thrips feeding or reproduction. To our knowledge this is the first study of Si accumulation in pepper plants for attempts to mediate resistance to an insect pest. Studies on the effects of Si accumulation in woody dicot tissues over an extended time period (involving multiple or overlapping pest generations) may be warranted. Genetically manipulating the Si uptake capacity of the roots of dicots might also help some plants accumulate more Si and, hence, more able to overcome both biotic and abiotic stresses (Ma & Yamaji 2006).

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