Influence of Silicon on Resistance of *Zinnia elegans* to *Myzus persicae* (Hemiptera: Aphididae)

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ABSTRACT Studies were conducted to examine the effect of treating *Zinnia elegans* Jacq. with soluble silicon on the performance of the green peach aphid, *Myzus persicae* (Sulzer). *Z. elegans* plants were irrigated every 2 d throughout the duration of the experiment with a nutrient solution amended with potassium silicate (K_2SiO_2), or a nutrient solution without K_2SiO_2. Length of the prereproductive period and survivorship of *M. persicae* were not affected by K_2SiO_2 treatment, but total cumulative fecundity and the intrinsic rate of increase (r_m) were slightly reduced on *Z. elegans* plants receiving soluble silicon. Quantification of silicon content in leaf tissues using inductively coupled plasma optical emission spectroscopy (ICP-OES) confirmed significantly higher silicon concentrations in plants treated with K_2SiO_2 compared with control plants. High performance liquid chromatography–mass spectrometry (HPLC-MS) analysis was used to identify and quantify phenolic acids and flavonols in leaf tissue of *Z. elegans*. Compared with untreated control plants, significant elevations in 5-caffeoylquinic acid, *p*-coumaroylquinic acid, and rutin were detected in leaves of *Z. elegans* plants treated with K_2SiO_2, but none of seven other phenolics were significantly affected. Similarly, a slight elevation in guaiacol peroxidase activity was detected in plants treated with K_2SiO_2. Overall, these results indicate treatment of *Z. elegans* with soluble silicon provides a modest increase in resistance levels to *M. persicae*, which may be caused in part by defense-related compounds.

KEY WORDS aphid population fitness, phenolics, peroxidase, green peach aphid, *Myzus persicae*

Silicon is the second most abundant element in soil and is readily absorbed by plant roots in the form of silicic acid [$Si(OH)_4$] (Ma and Yamaji 2006). After transportation through the xylem to vegetative tissues, silicic acid is concentrated through transpiration, polymerized, and deposited in intra- and intercellular spaces (Ma and Takahashi 2002). Although not recognized as an essential element, silicon has been reported to benefit plant growth, development, and yield (Ma and Yamaji 2006). Several studies have also documented the ability of silicon to alleviate abiotic and biotic stress through physical and/or chemical mechanisms (Ma 2004, Fauteux et al. 2006).

Conducting vessels and intra- and intercellular spaces are strengthened by the deposition of silica polymers, which imparts a physical barrier against a variety of folivores and stem borers (Peterson et al. 1988, Goussain et al. 2002, Keeping and Meyer 2002, Kevedaras and Keeping 2007). For example, mandibles of the fall armyworm, *Spodoptera frugiperda* (Smith), erode after consumption of maize, *Zea mays* L., tissue with high silicon content, in addition to increased mortality and cannibalism (Goussain et al. 2002). Similarly, silicon treatment of sugarcane, *Saccharum* sp., reduces stem penetration by the African stalk borer, *Eldana saccharina* Walker, along with reducing borer mass (Keeping and Meyer 2002). Increased tissue hardness and reduced digestibility seem to be the primary mechanisms for resistance of silicon-treated plants to mandibulate insects (Keeping and Meyer 2002).

Because phloem-feeding insects typically probe intercellularly while attempting to locate sieve elements, stylet movement and penetration may be disrupted by the intercellular localization of silicon (Lanning 1963, Ma and Takahashi 2002). Stylet penetration of sieve elements may also be disrupted by the presence of silica polymers in vascular bundles (Hayward and Parry 1973). However, a recent study of the probing behavior of the greenbug, *Schizaphis graminum* (Rondani), using an AC electrical penetration graph system, did not find stylet penetration to be disrupted by silicon-treated wheat, *Triticum aestivum*

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L. (Goussain et al. 2005). Instead, *S. graminum* withdrew its stylets more frequently before ingestion on silicon-treated plants, possibly because of silicon-induced chemical changes in the epidermis and/or mesophyll (Goussain et al. 2005). An increasing body of evidence supports an active role of silicon in the induction of chemical defenses against insect herbivores (Keeping and Kvedaras 2008).

Zinnia elegans Jacq. is a dicotyledon angiosperm belonging to the Compositae family that accumulates silicon in relatively high concentrations compared with other dicots (Frantz et al. 2008). Treatment of *Z. elegans* with soluble silicon delays the incidence and reduces the intensity of powdery mildew infection (Locke et al. 2006). Because plant secondary defenses effective against pathogens can also provide resistance to insect herbivores (Karban et al. 1987), it was hypothesized that treating *Z. elegans* with silicon would reduce aphid performance. Therefore, this study examined the ability of silicon treatment to increase resistance of *Z. elegans* to *M. persicae.* The objectives were to determine the effects of treating *Z. elegans* with K_2SiO_2 on the performance and population fitness of *M. persicae*, and quantify defense-related phenolics and peroxidase activity.

Materials and Methods

Plant Material and Experimental Treatments. *Zinnia elegans* cultivar Oklahoma White was chosen because of its comparatively high deposition of silicon within vegetative tissues after treatment with potassium silicate (Frantz et al. 2008). Seeds of *Z. elegans* were germinated in Oasis Root Cubes (15 by 15 by 30 mm; Smithers-Oasis Co., Kent, OH) and incubated in the laboratory at 24°C for ≈1 wk under a combination of cool-white and aquarium fluorescent bulbs (16:8 L:D). Seedlings were transplanted into pots (7.6 cm diameter) containing Fafard lightweight mix no. 2 (Fafard, Anderson, SC) and placed under the previously described growing conditions.

The experimental design consisted of plants being treated with two different irrigation regimes (i.e., treatments). Plants were arranged in a completely randomized design and treated every 2 d with (1) half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) amended with potassium silicate (K_2SiO_2) or (2) nutrient solution not amended with K_2SiO_2. The base nutrient solution (−Si) consisted of 7.5 mM N (100% as NO_3^-), 0.5 mM P, 3 mM K, 2.5 mM Ca, 1.0 mM Mg, 1 mM S, 71 M μFe (as Fe-DTPA), 9 μM Mn, 1.5 μM Cu, 1.5 μM Zn, 45 μM B, 0.1 μM Mo, 0.2 μM Na, and 24 μM Cl. The +Si nutrient solution also contained 2.0 mM K_2SiO_2 (20 ml/liter of nutrient solution), which was prepared by dissolving fumed silica SiO_2 (0.007 μm) in 0.1 M KOH. The +Si nutrient solution was adjusted accordingly to compensate for the increased K input associated with the addition of K_2SiO_2. The pH of the nutrient solutions was adjusted to 5.6 with H_2SO_4 or KOH.

A new group of plants was used for each of the three experiments to assess aphid performance. A separate group of uninfested plants treated with the two irrigation regimens was used for quantifying silicon, phenolics, and peroxidase activity. Using uninfested plants prevented aphid herbivory from confounding the effects of each irrigation treatment.

Aphid Performance. To assess the effects of the two nutrient regimens on aphid performance, a colony of *M. persicae* was first established by transferring several apterous virginoparae from an existing colony onto *Z. elegans* plants grown in an organdy fabric cage under greenhouse conditions. *M. persicae* was allowed to pass through several generations on *Z. elegans* before being used in experiments. Three adult apterous *M. persicae* were collected from the colony and transferred to dialysis-tube cages (4 cm in diameter, 13 cm in length; Ward's National Science, Rochester, NY) that were placed on leaves and contained ≈12 cm^2 of *Z. elegans* leaf tissue. Plants were treated every 2 d for 2 wk with the aforementioned nutrient solutions before being used in experiments, and irrigation regimens were maintained throughout the course of the experiment. Foam plugs were used to seal both ends of the cage, with one end of the plug being partially split to seal a single leaf within each cage (Tingey 1986). Cages were positioned on leaves at the third most basal internode 24 h before their use in experiments.

Plants (+Si or −Si) inoculated with aphids were arranged in a completely randomized design under the aforementioned light and temperature conditions. Cages were inspected daily until nymphiposition began, at which point all individuals were removed except for two first instars. Nymphs were allowed to develop until one reached reproductive maturity, after which the second nymph was removed. Fecundity was subsequently measured daily until 18 d after birth. This procedure allowed for calculating the length of time from birth to reproductive maturity, total offspring production, and survivorship over an 18-d period. Measurements of aphid performance were repeated on three different occasions with 8 replicates per treatment per experiment, which resulted in a total of 24 replicates per treatment.

The intrinsic rate of natural increase (r_m) was estimated using the equation of Wyatt and White (1977): $r_m = 0.74(\ln M_d)/TTR$, where 0.74 is a correcting constant, *TTR* is the time from birth to reproduction, and M_d is the number of young produced in the first period of reproduction equal to *TTR* (Wyatt and White 1977). Doubling time ($T_d = \ln 2/r_m$), net reproductive rate ($R_o = \sum l_x m_x$), and generation time ($T = \ln R_o/r_m$) were also calculated, whereby l_x = age-specific survivorship and m_x = age-specific fecundity (Andrewartha and Birch 1954, DeLoach 1974).

Silicon Quantification. Plants used for Si and phenolic analyses were arranged in a completely randomized design and maintained under the aforementioned growing conditions. After 2 wk of treatment with the +Si and −Si nutrient solutions, an individual leaf was collected from the third basal internode from +Si and −Si plants and placed in a drying oven (60 ± 5°C) for 3 d. An individual leaf was removed from three separate plants per treatment. Total silicon concentration

in leaf tissue was quantified according to Frantz et al. (2008). In short, dried, ground tissue (0.1 g) was combined in a 55-ml Teflon vessel with 3.0 ml of 7.5 M KOH. The solution was heated in a programmable microwave (MARS Express; CEM, Matthews, NC) by increasing to 200°C over a period of 15 min and held for an additional 15 min. After the digested material cooled to room temperature, 2 ml of H_2O_2 was added, and the samples were heated again by ramping to 200°C over 15 min and holding at 200°C for an additional 5 min. After cooling, 10 ml of 18-M-ohm purity water was added, and the solution was filtered (Whatman's No 2); 1 ml of the filtrate was diluted further with 9 ml deionized water (18-M-ohm purity) and analyzed by inductively coupled plasma optical emission spectroscopy, ICP-OES (model IRIS Intrepid II; Thermo Electron, Waltham, MA). A total of three replicates were prepared for each treatment.

Settings for the ICP-OES were as follows: flush and analysis pump rate, 130 rpm; RF power, 1,150 W; nebulizer pressure, 32.1 PSI; auxillary gas, 1.0 liters/min. The ICP-OES was calibrated every 10 samples with a blank and high standard solution of 9.985 mg/liter with a background matrix of 0.75 M KOH. Every 20 samples, a laboratory-grown standard of rice straw containing 9.6 g/kg (dry weight) was analyzed as a reference.

Extraction, Identification, and Quantification of Phenolics. Leaves were excised 2 wk after initiating the irrigation regimens (i.e., nutrient solution +Si or −Si) from the third most basal internode of *Z. elegans*, frozen at −80°C for 24 h, and freeze-dried for 3 d. Freeze-dried leaves were coarsely chopped using a razor blade, and 30 mg was placed in 2.0-ml Eppendorf tubes with 1 ml of 1% acetic acid in 80% MeOH. Tissues were ground with a microhomogenizer (Omni International, Marietta, GA) and placed on a benchtop shaker for 2 h. Samples were centrifuged at 13,000 rpm for 10 min, and the supernatant was filtered using Spin-X microcentrifuge filters at 5,000 rpm for 0.5 min. Filtered extracts were flash frozen using liquid N_2 and stored at −40°C until analysis. A total of five replicates were prepared for each treatment, with each replicate analyzed in duplicate.

Leaf phenolics were identified by high performance liquid chromatography coupled with mass spectrometry (HPLC–MS–MS). The HPLC system (10VP Series; Shimadzu, Columbia, MD) used a Hypersil Gold C_{18} (3-μm particle size; 150 mm length by 3.0 mm ID; Thermo Electron). Five microliters was injected onto the column, and a gradient elution was used for separations. Solvent A consisted of 10% MeOH in H_2O adjusted to pH 3.5 with formic acid. Solvent B consisted of 20% H_2O (pH 3.5), 20% MeOH, and 60% acetonitrile. At a flow rate of 0.3 ml/min, the following gradient was used: 0 min, 100% A; 10 min 20% A; 20 min, 40% A; 40 min, 0% A; held at 0% A for 15 min. Five minutes of equilibration at 100% A was performed before and after each injection. Effluent from the column was introduced into a triple-quadrupole mass spectrometer (Micromass, Beverly, MA) equipped with a pneumatically assisted electrospray ionization source (ESI). Negative ionization mode was used under the following parameters: capillary voltage, 3 kV; source block temperature, 120°C; desolvation gas temperature, 400°C. Nitrogen was used as the drying and nebulizing gas at flow rates of ≈50 and 450 liters/h. For full-scan HPLC–ESI–MS analysis, spectra were scanned in the range of 50–1,200 m/z. Data acquisition and processing were performed using a Mass-Lynx NT 3.5 data system (Micromass). Identifications of phenolic acids and flavonols were made by comparing retention times, UV spectral patterns, and ESI-MS fragmentation patterns with authentic standards (Indofine Chemical, Somerville, NJ) and published data.

Identified phenolics were quantified using an HPLC (Waters, Milford, MA) equipped with a Waters 996 Photodiode Array Detector (PDA). Quantification of phenolic acids was based on a standard curve prepared with 5-caffeoylquinic acid. Quercetin derivatives were quantified using the corresponding authentic standard for each compound. Luteolin-3-galactoside and isorhamnetin-3-galactoside were quantified using quercetin-3-rhamnoside as a standard. For both the standards and extracts, 5 μl was injected and separated using the aforementioned column and solvent system, but at a flow rate of 1 ml/min. Scanning by the PDA occurred at 325 and 366 nm, whereas data acquisition and processing were performed by Waters Empower Chromatography Software (Waters).

Protein and Peroxidase Assay. Leaves were excised 2 wk after initiating the irrigation regimens (i.e., nutrient solution +Si or −Si) from the third most basal internode of *Z. elegans* and immediately placed in a chilled mortar and pestle and ground to a fine powder in the presence of liquid N_2. Two milliliters of cold potassium phosphate buffer (0.1 M, pH 7.0) containing 1% polyvinylpyrrolidone was added to the chilled mortar and held for 2 min at 4°C. A 1.5-ml aliquot of the extract was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was immediately flash frozen using liquid N_2 and held at −40°C for no longer than 24 h before protein and peroxidase analyses. A total of four replicates were prepared for each treatment, with each replicate analyzed in duplicate.

Total protein concentration was quantified in duplicate in the *Z. elegans* leaf extracts using a modification of the DC Protein Assay (Bio-Rad, Hercules, CA) (Alam 1992, Cipollini 1998). Serial dilutions were prepared using Bio-Rad bovine serum albumin, with 5 μl of each standard dilution or *Z. elegans* plant extracts being added to individual wells in a thoroughly chilled microplate. A multichannel pipette was used to add 195 μl of a Bio-Rad dye solution (6 ml of Bio-Rad Protein Assay Dye Reagent Concentrate + 15 ml H_2O) to each microplate well. Absorbance values were measured in each well after 10 min at 595 nm, and protein concentrations were determined as mg/ml in 15 μl of sample extracts.

Guaiacol peroxidase activity in *Z. elegans* leaf tissue was assayed by first preparing a guaiacol substrate solution (pH 6.0) consisting of 1 ml of guaiacol (8.7 M; Fluka), 5 ml of 30% hydrogen peroxide, and 394 ml of

Table 1. Performance and population fitness parameters of *M. persicae* reared on *Z. elegans* irrigated with a nutrient solution amended with K_2SiO_2 (+Si) or a nutrient solution without K_2SiO_2 (−Si)

Treatment	Prereproductive period (d)[a] (mean ± SD)	Survivorship (%) 18 d after birth[b]	Intrinsic rate of increase (r_m) (±95% CI)[c]	Net reproductive rate (R_o)	Generation time (T)	Doubling time (T_d)
+Si	9.39 ± 0.24a	100a	0.249 (0.237–0.262)	30.40	13.67	2.77
−Si	9.38 ± 0.25a	100a	0.293 (0.280–0.305)	36.27	12.37	2.39

[a] Means followed by the same letter in a column are not significantly different ($\alpha = 0.05$; Tukey's studentized range [HSD] test).
[b] Percentages followed by the same letter in a column are not significantly different ($\alpha = 0.05$; Fisher's exact test; $n = 24$ for each treatment).
[c] Bootstrap estimate; nonoverlapping 95% CI corresponds to a significant treatment effect ($\alpha = 0.05$).

0.1 M potassium phosphate buffer (Cipollini 1998). After thoroughly chilling a microplate on ice, 15 μl of chilled potassium phosphate buffer (pH 6.0) and 15 μl of recently thawed sample extracts were combined in the microplate wells, in duplicate. Blank wells contained 30 μl of potassium phosphate buffer. A multichannel pipettor was used to quickly add 150 μl of guaiacol substrate solution to the microplate wells. Peroxidase activity was determined by monitoring the change in absorbance every 15 s for 1 min at 470 nm (ELx800 Absorbance Microplate Reader; Bioteck, Winooski, VT) and comparing against blank controls. Guaiacal peroxidase activity was calculated as Δ470 nm/min/mg protein.

Statistical Analyses. Aphid performance parameters ($n = 24$/treatment), silicon ($n = 3$/treatment) and phenolic concentrations ($n = 5$/treatment), and peroxidase activity ($n = 4$/treatment) were analyzed using one-way analysis of variance (ANOVA; PROC GLM; SAS Institute 2001). To help stabilize the variance, data were square root transformed before analysis, but untransformed data are presented. Because no difference at $\alpha = 0.05$ in aphid performance parameters was detected within treatments across the three experiments, treatment data were pooled for analysis. Means were separated using Tukey's studentized range (honestly significant difference [HSD]) test at $\alpha = 0.05$. The percentage of *M. persicae* surviving 18 d after birth on *Z. elegans* subjected to +Si or −Si was compared using an R × C contingency table using the PROC FREQ procedure (SAS Institute 2001), with Fisher exact test (two-tail) being used to test for significant differences between observed and expected frequencies ($\alpha = 0.05$).

The bootstrap technique was used to estimate 95% confidence intervals (CIs) for r_m values associated with each treatment (Meyer et al. 1986, Petitt et al. 1994, R Project for Statistical Computing 2005). Nonoverlapping 95% CI corresponds to the rejection of no treatment effect hypothesis at $\alpha = 0.05$ (Maia et al. 2000). Because T_d and T are functions of r_m, and R_0 represents the population's response, statistical analyses were only performed on r_m; however, values for each are provided for comparative purposes.

Results

Aphid Performance. No difference in the amount of time for *M. persicae* to reach reproductive maturity or survivorship was detected between aphids reared on *Z. elegans* plants treated with and without K_2SiO_2 (Table 1; $P > 0.05$). However, treatment of *Z. elegans* with K_2SiO_2 resulted in a significantly lower total cumulative fecundity (29.3 ± 1.5) by *M. persicae* compared with aphids reared on plants not receiving K_2SiO_2 (38.0 ± 1.7; $F = 15.02$; df = 1,46; $P = 0.0003$; Fig. 1). The duration of time from birth to reproductive maturity and offspring production over an amount of time equivalent to the prereproductive period allowed for the intrinsic rate of increase (r_m) of *M. persicae* to be calculated for the −Si and +Si treatments (Table 1). The highest r_m value of 0.293 was associated with *M. persicae* reared on −Si *Z. elegans* plants, which was significantly higher than +Si plants ($r_m = 0.249$). The net reproductive rate (R_0), generation time (T), and doubling time (T_d) of *M. persicae* were also calculated for −Si and +Si *Z. elegans* plants (Table 1). The lowest R_0 and longest T and T_d were associated with aphids reared on +Si plants. Overall, the population fitness of *M. persicae* was lower on plants treated with a nutrient solution containing K_2SiO_2 compared with plants not receiving K_2SiO_2.

Silicon Quantification. ICP-OES successfully quantified silicon concentrations in *Z. elegans* leaves from plants irrigated with nutrient solutions with and without K_2SiO_2 (Fig. 2). The highest mean (±SE) silicon concentration was associated +Si plants (12.4 ± 1.7 g/kg dry weight), which was significantly higher than

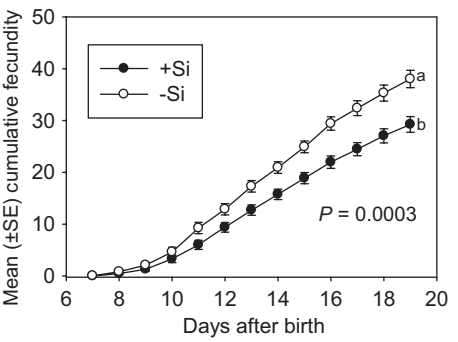

Fig. 1. Mean (±SE) cumulative fecundity of the green peach aphid, *M. persicae*, confined to leaves of *Z. elegans* irrigated with a nutrient solution amended with potassium silicate (+Si) or a nutrient solution without potassium silicate (−Si). Different letters indicate significant differences in cumulative offspring production 18 d after birth ($\alpha = 0.05$; Tukey's studentized range [HSD] test; $n = 24$ for each treatment).

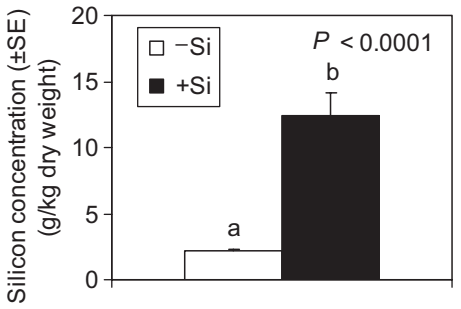

Fig. 2. Mean (±SE) silicon concentration in leaves of *Z. elegans* plants irrigated with a nutrient solution amended with potassium silicate (+Si) or a nutrient solution without potassium silicate (−Si). Different letters indicate significant differences ($\alpha = 0.05$; Tukey's studentized range [HSD] test; $n = 3$ for each treatment).

−Si plants (2.2 ± 0.2 g/kg dry weight; $F = 278.18$; df = 1,6; $P < 0.0001$).

Phenolic Identification and Quantification. HPLC–ESI–MS identified a total of 10 previously known phenolics in leaf extracts from *Z. elegans* (Table 2). Descriptions of mass spectral elucidations are not provided because all of the phenolic compounds identified and quantified from *Z. elegans* have been characterized in previous studies (Schieber et al. 2001, Whitaker and Stommel 2003, Sanchez-Rabaneda et al. 2004, Ranger et al. 2007, Wilson et al. 2008). Four of the 10 compounds were identified as phenolic acids, whereas 6 compounds were classified as flavonols.

Only slight quantitative differences in phenolics were documented in *Z. elegans*, with total concentrations of the 10 compounds identified in this study being 3.3 and 3.9 mg/g of leaf tissue in plants treated with and without K_2SiO_2, respectively (Table 3). The phenolic acids 5-caffeoylquinic acid and *p*-coumaroylquinic acid were significantly higher in +Si plants compared with −Si plants. The flavonol rutin was also slightly higher in +Si plants (Table 3). Significant differences between +Si and −Si plants were not detected for the remaining phenolic acids and flavonols quantified from *Z. elegans* leaf tissue.

Peroxidase Activity. Peroxidase enzymes were successfully extracted from leaf tissue of *Z. elegans* plants treated with and without K_2SiO_2. A slight increase in guaiacol peroxidase activity (Fig. 3) was detected in leaf tissue of +Si plants compared with −Si plants ($F = 24.35$; df = 1,6; $P = 0.003$).

Discussion

Treating plants with soluble silicon affects the performance of a variety of mandibulate folivores and stem borers (Peterson et al. 1988, Goussain et al. 2002, Keeping and Meyer 2002, Kevedaras and Keeping 2007). Similarly, silicon decreases the food intake, growth, adult longevity, fecundity, and population growth of the xylem feeding whitebacked planthopper, *Sogatella furcifera* (Horvath) (Salim and Saxena 1992). Treatment of monocotyledonous and dicotyledonous plants with soluble silicon also reduces offspring production and population fitness of phloem-feeding insects, namely *M. persicae* on potato, *Solanum tuberosum* L.; *S. graminum* on wheat, *Triticum aestivum* L.; and the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), on cucumber, *Cucumis* sp. (Basagli et al. 2003, Correa et al. 2005, Gomes et al. 2005, 2008, Goussain et al. 2005). Results presented herein document a reduction in fecundity and population fitness of *M. persicae* when reared on *Z. elegans* plants treated with K_2SiO_2. Thus, soluble silicon seems to be associated with plant defenses effective against a variety of insect feeding guilds, which may be based on physical and/or induced compounds (Keeping and Kvedaras 2008).

The deposition of silica in intra- and intercellular spaces may affect the feeding behavior of phloem and xylem feeding insects (Salim and Saxena 1992, Goussain et al. 2005). However, feeding behavior studies of *S. graminum* on wheat and analyses of defense-related compounds indicated silicon-based chemical changes are more important for resistance levels than physical factors (Gomes et al. 2005, Goussain et al. 2005). It is unclear if the slight increase in 5-caffeoylquinic acid, *p*-coumaroylquinic acid, rutin, and/or peroxidase activity contributed to the reduction in offspring production by *M. persicae* on silicon-treated *Z. elegans*. Plant phenolics have been correlated with reducing aphid feeding and population fitness (Leszczynski et al. 1989, Leszczynski et al. 1995). Similarly, peroxidases are involved in the synthesis of phenolics and

Table 2. Retention times, λ_{max}, molecular ions $[M-H]^-$, and fragmentation patterns from HPLC-ESI-MS of polyphenolics from *Z. elegans* leaf extracts

RT	λ_{max} (nm)	$[M-H]^-$ and Fragmentation	Structure	References
14.07	205.3, 221.2, 287.6, 320.1	470, 375, 355, 334, 191, 179, 135	Dihydroxycinnamoyl amide	Whitaker and Stommel (2003)
17.25	217.2, 241.9, 297.7, 326.1	353, 191, 178.9, 127, 111	5-Caffeoylquinic acid	Ranger et al. (2007), Wilson et al. (2008)
23.28	216.1, 242.1, 298.7, 325.1	337, 163	*p*-Coumaroylquinic acid	Sanchez-Rabaneda et al. (2004)
26.79	207.5, 299.6, 357.1	447, 285	Luteolin-3-galactoside	Sanchez-Rabaneda et al. (2004)
28.33	206.3, 255, 355	609, 301	Rutin	Ranger et al. (2007)
34.21	207.5, 299.6, 357.1	447, 301	Quercetin-3-rhamnoside	Wilson et al (2008)
34.60	212.2, 298.6, 326.7	515, 353, 191	3–5-Dicaffeoylquinic acid	Whitaker and Stommel (2003)
35.87	220.7, 247.8, 351.2	477, 315	Isorhamnetin-3-galactoside	Schieber et al. (2001)
38.18	207.8, 282, 371.2	435, 273	Phloridzin	Sanchez-Rabaneda et al. (2004)
41.25	226.55, 256.1, 357.1	301	Quercetin	Ranger et al. (2007), Wilson et al. (2008)

Table 3. Phenolic acid and flavonol derivatives quantified from leaves of *Z. elegans* plants irrigated with a nutrient solution amended with K_2SiO_2 (+Si) or a nutrient solution without K_2SiO_2 (−Si)

Compound	Mean ± SE concentration (μg/g of dry weight)[a]		*P*
	+Si	−Si	
5-Caffeoylquinic acid	2436.38 ± 30.92[a]	1944.72 ± 6.93b	0.0001
p-Coumaroylquinic acid	930.16 ± 2.71[a]	835.80 ± 8.64b	0.0005
Dihydroxycinnamoyl amide	330.64 ± 1.69[a]	318.24 ± 6.22[a]	>0.05
Rutin	165.47 ± 9.52a	126.66 ± 4.53b	0.032
Phloridzin	33.19 ± 0.73a	37.03 ± 0.88a	>0.05
Quercetin	22.13 ± 0.98a	22.33 ± 5.60a	>0.05
Quercetin-3-rhamnoside	16.39 ± 2.64a	18.90 ± 5.06a	>0.05
Luteolin-3-galactoside	6.01 ± 2.98a	1.01 ± 1.00a	>0.05
Isorhamnetin-3-galactoside	4.49 ± 0.61a	6.94 ± 0.99a	>0.05
3,5-Dicaffeoylquinic acid	1.93 ± 0.85a	2.48 ± 1.09a	>0.05

[a] Means followed by the same letter in a row are not significantly different ($\alpha = 0.05$; Tukey's studentized range [HSD] test; $n = 5$ for each treatment).

hypersensitive responses and reduce the digestibility and protein availability of plants to insect herbivores (Bowles 1990, Duffey and Stout 1996).

Compared with *Z. elegans* plants not amended with K_2SiO_2, only a slight increase was detected in phenolics and peroxidase activity in healthy, unstressed +Si *Z. elegans* plants. Rodrigues et al. (2004) found momilactone phytoalexins were slightly induced in healthy rice plants treated with soluble silicon. Similarly, Gomes et al. (2005) detected an increase in peroxidase activity in silicon-treated wheat. Lignin concentrations also increase in potato after silicon treatment (Gomes et al. 2008). Other studies have documented minimal to negligible effects on the induction of plant defense-related compounds after treatment with soluble silicon (Chérif et al. 1994, Fawe et al. 1998, Rémus-Borel et al. 2005).

Although not addressed in this study, the ability of soluble silicon to act as an elicitor of defense-related metabolites is more apparent in insect- or pathogen-stressed plants compared with unstressed plants (Chérif et al. 1994, Fawe et al. 1998, Rodrigues et al. 2004, Gomes et al. 2005, Rémus-Borel et al. 2005). For instance, treatment of wheat with soluble silicon enhanced peroxidase and polyphenoloxidase activities, but considerably higher levels were associated with silicon treated plants preinfested with aphids (Gomes et al. 2005). Soluble silicon seems to act as a modulator of induced resistance, whereby plants respond faster or more efficiently to abiotic or biotic stress with an active defense that is not solely based on a mechanical barrier (Fauteux et al. 2006). A greater level of phenolic or peroxidase induction than we observed in silicon-treated *Z. elegans* therefore might occur if plants were prestressed by *M. persicae* feeding. It should also be noted that the results obtained in this study may depend on *Zinnia* genotype.

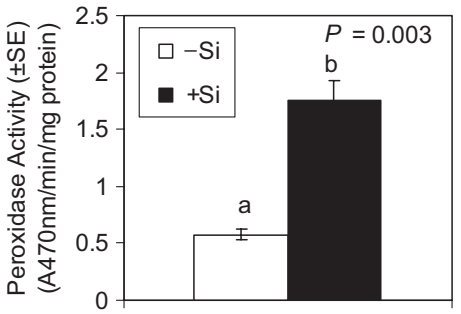

Fig. 3. Mean (±SE) guaiacol peroxidase activity in leaves of *Z. elegans* plants irrigated with a nutrient solution amended with K_2SiO_2 (+Si) or a nutrient solution without K_2SiO_2 (−Si). Different letters indicate significant differences ($\alpha = 0.05$; Tukey's studentized range [HSD] test; $n = 4$ for each treatment).

This study was designed to provide a baseline of information regarding the effect of silicon treatment on resistance levels of unstressed *Z. elegans.* Future studies should delve further into the interactions between silicon treatment and *M. persicae* herbivory on the induction of defense-related compounds. The results presented within support previous findings that soluble silicon enhances plant resistance to piercing-sucking insects, but the modest increase in resistance levels indicates supplementary control tactics would be necessary for controlling *M. persicae* on *Z. elegans.* However, because treating *Z. elegans* with soluble silicon dramatically reduces the incidence and severity of powdery mildew (Locke et al. 2006), silicon amendment might be useful in a multifaceted pest management program.

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