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Silicon Differentially Influences Copper Toxicity Response in Silicon-accumulator and Non-accumulator Species

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Abstract. The use of copper (Cu) in agriculture is widespread as a pesticide, and it is present in high concentrations in certain types of manures. As the use of Cu continues and manure management is incorporated into sustainable systems, the likelihood of Cu toxicity increases. Supplemental silicon has been used to successfully counteract potential micronutrient toxicity. There is currently considerable debate regarding the value of including silicon (Si) as a nutrient in fertility programs and as such, it is not part of a typical management practice in floriculture crop production in the United States. We investigated the potential for Si to ameliorate the effects of Cu toxicity in both a Si-accumulating [zinnia (*Zinnia elegans*)] and a Si-non-accumulating [snapdragon (*Antirrhinum majus*)] species. Using visible stress indicators and dry weight analysis, it initially appeared that Si was a significant benefit to only zinnia under Cu toxicity. Enzymatic assays and elemental analysis of leaves, stems, and roots revealed that both species responded to supplemental Si, showing evidence of reduced stress and nutrient concentrations more similar to healthy, control plants than plants exposed to Cu toxicity. Although there appear to be differences in the extent of Si-mediated amelioration of Cu toxicity between these two plants, both responded to supplemental Si. This adds to the growing body of evidence that all plants likely have Si-mediated responses to stress, and its inclusion into fertility programs should be more broadly considered than current practices.

Worldwide, some soils are high in Cu leading to the natural occurrence of Cu toxicity (Alonso et al., 2000; Cook et al., 1997). Most reported Cu toxicity is the result of anthropogenic sources including pesticide use (Hoang et al., 2009) and use of young compost or pig manure (Alonso et al., 2000; Brady and Weil, 2001). In protected agriculture, growers have used electrolytically generated Cu (Zheng et al., 2004) for disinfecting irrigation water; it is not clear if U.S. Department of Agriculture (USDA) statistics account for this source of Cu, so the extent of electrolytically generated Cu use is not known. Recommended rates of Cu ionization are from 7.8 to 15.7 μM. Additional Cu use is primarily from copper hydroxide [$Cu(OH)_2$] and copper sulfate ($CuSO_4$) with an average total rate of Cu use of just under 1.9 kg·ha^{-1} in floriculture and nursery alone (USDA, 2007). Typical concentrations (label rates) for different Cu-containing fungicides range from 1,050 to over 19,000 μM.

The exact threshold between Cu sufficiency to toxicity is largely unknown for most floriculture crop species. A few greenhouse crops grown hydroponically have been well characterized and the Cu concentration that leads to plant stress (root discoloration, stunting, leaf chlorosis) is remarkably low: cucumber (*Cucumis sativus*) was ≈20 μM (Zheng et al., 2010), pepper (*Capsicum annuum*) was 3 μM at the seedling stage and between 8 and 16 μM at the fruiting stage (Zheng et al., 2005), chrysanthemum (*Dendranthema ×grandiflorum*) was 5 μM, miniature rose (*Rosa ×hybrida*) was between 2.4 and 4.7 μM, and geranium (*Pelargonium ×hortorum*) was 8 μM (Zheng et al., 2004). Because matured organic substrates can bind significant amounts of Cu making it unavailable (Brady and Weil, 2001), the threshold for Cu supply to cause toxicity in peat-based substrates is thought to be considerably higher. Zheng has observed detrimental growth effects for chrysanthemum, miniature rose, and geranium at 63 μM Cu in the nutrient solution (Y. Zheng, personal communication), whereas Lee et al. (1996) reported leaf chlorosis at 250 μM Cu in the nutrient solution. It should be noted that in the study by Lee et al. (1996), no treatment levels between 5 and 250 μM were examined.

Given the relatively low threshold for Cu toxicity and the greenhouse industry's use of Cu in fungicides as well as the capture and recirculation of water used in the greenhouse industry (Uva et al., 2001) that may carry residual Cu from different sources, there is potential to encounter Cu toxicity. Because early Cu toxicity often manifests as iron (Fe) deficiency and additional chelated Fe above and beyond the supply already found in complete water-soluble fertilizers can eliminate those symptoms (Bucher and Schenk, 2000), undiagnosed Cu toxicity in floriculture production may be more common than currently believed.

Silicon is not considered to be an essential plant nutrient because most plant species can complete their life cycle without it (Marschner, 1995). Still, some plant species can accumulate Si at concentrations higher than many essential macronutrients ["accumulators" herein described as species that accumulate Si at concentrations greater than 1000 mg·kg^{-1} (Epstein, 1999)],

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and even so-called non-accumulating species (also known as "Si excluders" and defined herein as a species that accumulates less than 1000 mg·kg^{-1}) can acquire Si in their leaf tissues greater than micronutrients and some macronutrients (Frantz et al., 2008, 2010; Mattson and Leatherwood, 2010). Beneficial effects provided by Si are well documented for many field crops and a few ornamentals (Bélanger et al., 1995; Datnoff and Rodrigues, 2005; Gillman et al., 2003; Korndörfer and Lepsch, 2001; Ma et al., 1989; McAvoy and Bible, 1996; Rodrigues et al., 2004). One of the debates about Si in plant biology has been in the classification of plant species as Si accumulators or non-accumulators with varying definitions further separating those main groups. The debate has been instrumental in shaping how we perceive and study the role of Si in plant biology with some statements made that describe so-called non-accumulators as plants that do not respond to supplemental Si (Ma et al., 2001; Mitani and Ma, 2005).

Silicon has been shown to alleviate metal toxicity in a variety of plant species. Toxicity of zinc in the metal-tolerant *Cardaminopsis halleri*, a member of the Brassicaceae, is ameliorated in part by binding with Si and precipitating in cytoplasm or by cotransportation of Si and zinc (Zn) into extracellular compartments (Neumann and zur Nieden, 2001). Toxicity of manganese (Mn) in soybean (*Glycine max*) appears to be alleviated by Si in part by enhancing the distribution of Mn throughout the plant thereby preventing it from reaching toxic levels in a specific site (Horst and Marschner, 1978). Cowpea (*Vigna unguiculata*) has been extensively studied with regard to alleviating Mn toxicity with supplemental Si (Iwasaki et al., 2002a, 2002b, 2002c). In those studies, Si acts to detoxify Mn both through increased binding of Mn on cell wells and interaction between Mn and soluble Si in the apoplast. Toxicity of Cu in arabidopsis (*Arabidopsis thaliana*) was shown to be alleviated by Si through active metal transporter regulation (Li et al., 2008), but alleviating Cu toxicity with Si remains less well understood compared with other metals.

Most of these examples of metal tolerance from Si were performed on plants with significant Si accumulation. A few studies have begun to cast doubt on the view that Si must be accumulated in large amounts to provide a plant benefit. Zellner et al. (2011) documented reduced viral symptoms in non-accumulating tobacco (*Nicotiana tabacum*); tomato (*Solanum lycopersicum*) sensitivity to salinity and blossom end rot symptoms were diminished when Si was supplied (Stamatakis et al., 2003); poinsettia (*Euphorbia pulcherrima*) had a longer shelf life and exhibited faster recovery from wilt when fed with Si during pre-production finishing (N.S. Mattson, unpublished data); tomato had induced resistance to *Ralstonia solanacearum* when supplemental Si was provided before and during inoculation (Ghareed et al., 2011). It should be noted that there is evidence that the extent of response and/or beneficial effects from Si may be correlated to the extent of Si uptake (Bélanger et al., 2010).

The purpose of this article is to compare the response of two species, a Si accumulator zinnia and a Si non-accumulator snapdragon, to Cu toxicity with and without supplemental Si grown in hydroponics. We examined the a priori hypothesis that supplemental Si would alleviate Cu toxicity symptoms in zinnia, but supplemental Si would have no effect on snapdragon as a result of differences in their ability to accumulate Si. Assessment of stress was done by documenting visible stress symptoms, dry weight, stress enzyme activity, and elemental tissue analysis.

Fig. 1. Visual symptoms of zinnia and snapdragon exposed to copper (Cu) toxicity. Control zinnia plants (**A**) have no yellowing in the meristem, whereas zinnia treated for 3 weeks with 50 µM Cu (**B**) exhibits interveinal chlorosis in this area, which are similar symptoms as iron deficiency. Similarly, control snapdragon plants (**C**) 4 weeks after transplanting have no yellowing, whereas snapdragon treated for 2 weeks with 100 µM Cu (**D**) exhibits interveinal chlorosis. Supplemental silicon did not alleviate these symptoms in snapdragon, but did in zinnia.

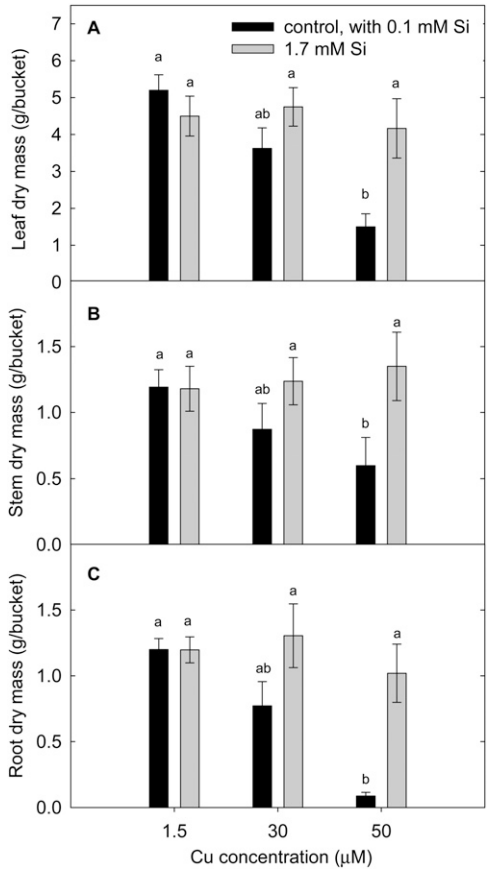

Fig. 2. Average zinnia leaf (**A**), stem (**B**), and root (**C**) dry mass. Plants were grown hydroponically at three different copper supplies (1.5, 30, and 50 µM) and two different silicon supplies (0.1 and 1.7 mM). Values are means of four replicate hydroponic buckets with each bucket containing two plants. Error bars are ± 1 SE. Different letters above the bars within a panel indicate statistically different means based on Tukey's honest significant difference test at $P < 0.05$.

Materials and Methods

Plant growth conditions. Seedlings of zinnia ('Oklahoma White') and snapdragon ('Bedding Rocket White') were initially germinated using foam cubes (15 × 15 × 30 mm each, LC1-type; Smithers-Oasis North America, Kent, OH). When the seedlings developed roots at the edge of the cube (time varied depending on species), they were transplanted into the lids of opaque plastic 4.5-L buckets containing aerated hydroponic solution at a planting density of three plants per tub. The solution was a modified Hoagland's solution containing 2.5 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 1.0 mM $MgSO_4$, 70 μM Fe as Fe-DTPA, 4.5 μM $MnCl_2$, 0.75 μM $ZnCl_2$, 0.75 μM $CuCl_2$, 22.5 μM H_3BO_3, 0.05 μM Na_2MoO_4, and 0.1 mM K_2SiO_3. Silicon treatments contained an additional 1.6 or 3.3 mM K_2SiO_3, with the Si treatments beginning on transplanting seedlings into the hydroponic system. K_2SiO_3 was synthesized with fumed silica (SiO_2, 0.007 μm particle size) dissolved in 0.2 M KOH. No glassware was used in making the nutrient solution, and 18 megaohm purified water was used exclusively during the course of the trial to minimize Si contamination. The low-level (0.1 mM) Si supplied in the control solution was done to represent a more realistic situation with some Si present from a variety of sources such as soil, peat, perlite, lime, fertilizers, etc. found in commercial production. The pH of the hydroponic solution was adjusted to 5.7 with H_2SO_4 or KOH before nutrient solutions were added to the hydroponic containers. Solutions in the hydroponic tubs were supplemented with fresh solution daily as needed and completely replaced weekly.

Cu toxicity treatments began 2 weeks after transplanting into the tubs to allow for sufficient root and shoot growth for subsequent sampling. Nevertheless, insufficient root tissue was occasionally available for both zinnia and snapdragon for both total Si and Cu analysis, so Cu analysis was only performed in those cases. Cu treatments were determined based on preliminary response curves with each species. For zinnia, six different nutrient solution combinations were used including control (1.5 μM Cu and 0.10 mM Si), +Si (elevated Si, 1.5 μM Cu, and 1.7 mM Si), +Cu-1 (elevated Cu-1, 30 μM Cu, and 0.10 mM Si) and +Cu-2 (elevated Cu-2, 50 μM Cu, and 0.1 mM Si), +Cu-1 + Si (elevated Cu-1 with Si, 30 μM Cu, and 1.7 mM Si), and +Cu-2 + Si (elevated Cu-2 with Si, 50 μM Cu, and 1.7 mM Si) with four replications. Each replicate consisted of one hydroponic container that held three plants. The zinnia experiment was conducted twice with one set used for tissue analysis and the other set used for enzymatic assays.

For snapdragon, nine different nutrient solution combinations were used including the control (1.5 μM Cu and 0.10 mM Si), +Si-1 (elevated Si-1, 1.5 μM Cu, and 1.7 mM Si), +Si-2

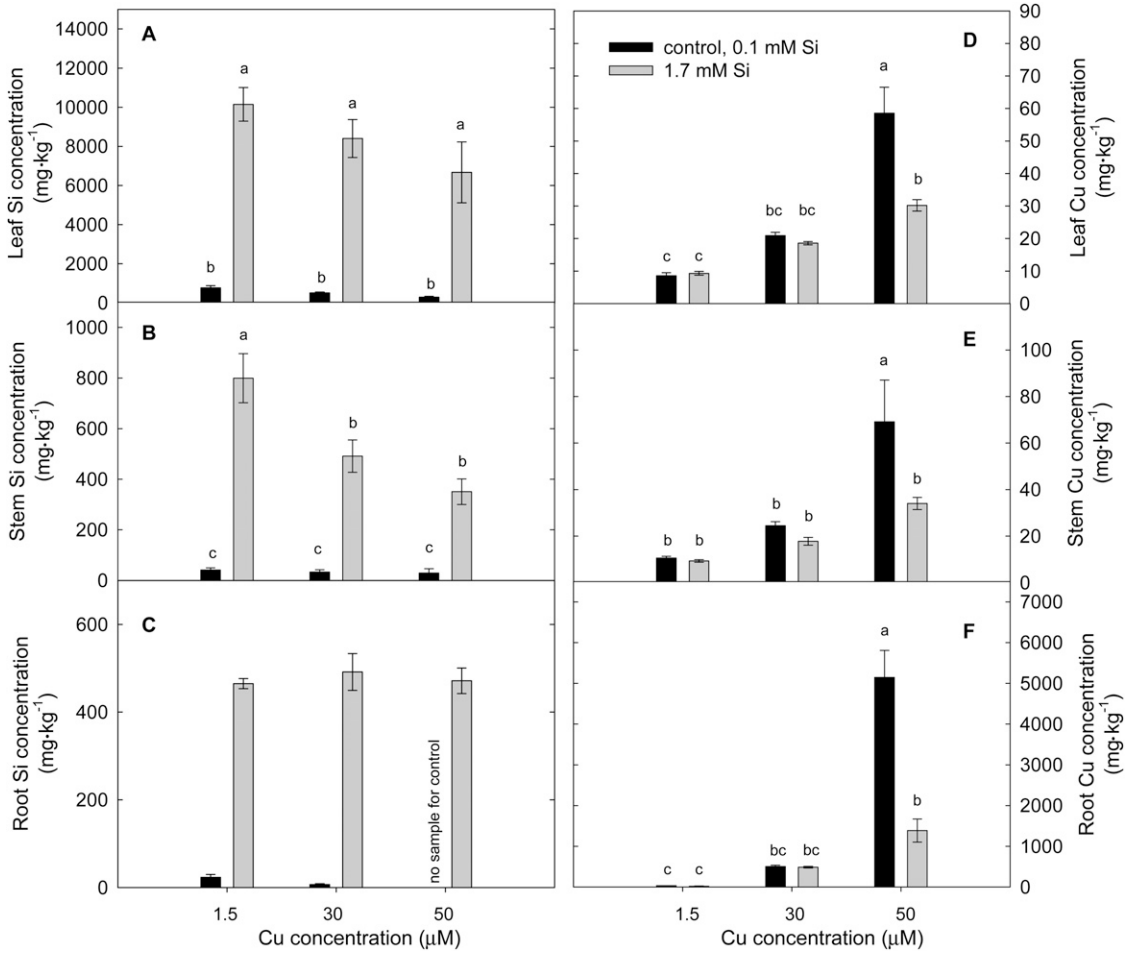

Fig. 3. Silicon concentration in zinnia leaf (**A**), stem (**B**), and root (**C**) and copper (Cu) concentration of zinnia leaf (**D**), stem (**E**), and root (**F**) grown hydroponically at three different Cu supplies (1.5, 30, and 50 μM) and two different silicon supplies (0.1 and 1.7 mM). Values are means of four replicate plant groups; two plants were pooled from a single treatment hydroponic bucket and separated into leaves, stems, and roots. Error bars are ± 1 SE. Different lowercase letters above the buckets within a panel indicate statistically different means based on Tukey's honest significant difference test at $P < 0.05$.

(elevated Si-2, 1.5 μM Cu, and 3.4 mM Si), +Cu-1 (elevated Cu-1, 100 μM Cu, and 0.10 mM Si) and +Cu-2 (elevated Cu-2, 150 μM Cu, and 0.1 mM Si), +Cu-1 + Si-1 (elevated Cu-1 with Si-1, 100 μM Cu, and 1.7 mM Si), +Cu-1 + Si-2 (elevated Cu-1 with Si-2, 100 μM Cu, and 3.4 mM Si), +Cu-2 + Si-1 (elevated Cu with Si-1, 150 μM Cu, and 1.7 mM Si), and +Cu-2 + Si-2 (elevated Cu-2

Table 1. Nutrient concentrations of zinnia leaves exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	9.5 a[y]	76.2 a	18.7 a	6.8 a	3.3 ab	70.9 a	106.7 a	366.6 ab	85.1 a
	30	8.2 ab	73.6 a	19.5 a	7.0 a	3.6 a	83.9 a	105.9 a	423.1 a	74.8 ab
	50	3.8 c	36.3 c	11.9 b	4.3 b	2.8 b	71.6 a	46.1 b	87.9 c	17.5 c
1.7	1.5	10.2 a	73.4 a	19.5 a	5.9 a	3.5 ab	70.3 a	83.0 ab	369.7 ab	67.0 ab
	30	8.9 ab	77.4 a	20.2 a	6.3 a	3.6 a	74.1 a	96.2 ab	385.4 a	76.0 ab
	50	6.5 b	59.2 b	21.3 a	5.7 ab	3.7 a	87.0 a	77.0 ab	256.9 b	49.5 bc
P value	Cu	0.0001	0.0001	0.0558	0.0001	0.1648	0.3021	0.0073	0.0001	0.0001
	Si	0.0059	0.0045	0.0025	0.7368	0.0057	0.7530	0.9311	0.0537	0.4021
	Cu × Si	0.1249	0.0012	0.0039	0.0031	0.0425	0.1619	0.0771	0.0027	0.0081

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

Table 2. Nutrient concentrations of zinnia roots exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	9.0 a[y]	121.5 a	6.0 bc	3.5 ab	8.7 a	25.2 a	2356.3 b	944.8 ab	45.5 b
	30	8.4 a	99.2 a	6.4 bc	2.9 b	5.9 ab	24.2 ab	2393.7 b	853.0 bc	73.8 ab
	50	9.1 a	38.6 c	7.2 ab	2.3 b	5.0 b	16.2 b	9042.8 a	73.2 d	99.3 a
1.7	1.5	8.8 a	112.5 a	5.8 c	4.9 a	7.9 ab	26.9 a	1483.9 b	1313.1 a	40.8 b
	30	8.4 a	114.5 a	7.2 ab	3.2 b	8.7 a	28.2 a	1735.9 b	827.3 bc	83.6 a
	50	9.5 a	68.9 b	8.6 a	3.2 b	6.4 ab	23.2 ab	4311.8 b	522.2 c	107.6 a
P value	Cu	0.4012	0.0001	0.0001	0.0007	0.0026	0.0023	0.0002	0.0001	0.0001
	Si	0.9113	0.0227	0.0105	0.0036	0.0470	0.0074	0.0211	0.0031	0.5356
	Cu × Si	0.8986	0.0123	0.0432	0.2569	0.0310	0.3252	0.1091	0.0520	0.6586

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

Table 3. Nutrient concentrations of zinnia stem exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	8.7 ab[y]	73.8 a	9.1 b	3.3 a	2.0 a	20.4 b	80.2 ab	82.8 ab	52.2 a
	30	7.6 bc	71.6 a	8.8 b	3.7 a	2.4 a	22.5 b	57.3 b	155.5 a	48.3 a
	50	3.4 d	33.5 b	9.2 b	3.5 a	2.0 a	30.1 a	69.4 ab	60.8 b	36.6 a
1.7	1.5	10.2 a	74.8 a	10.4 a	3.5 a	2.3 a	24.9 ab	102.5 a	133.9 ab	57.6 a
	30	7.7 abc	71.6 a	10.2 a	3.1 a	2.2 a	23.5 b	64.2 b	123.1 ab	50.6 a
	50	5.4 cd	59.9 a	11.2 a	3.3 a	2.4 a	23.5 b	76.5 ab	80.6 ab	59.0 a
P value	Cu	0.0001	0.0001	0.6559	0.9999	0.7032	0.0119	0.0333	0.0035	0.5324
	Si	0.0149	0.0134	0.0182	0.3296	0.2525	0.7633	0.1908	0.3735	0.0790
	Cu × Si	0.2746	0.0068	0.9073	0.3711	0.1428	0.0018	0.7172	0.0719	0.2892

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

with Si-2, 150 μM Cu, and 3.4 mM Si). The snapdragon experiment was conducted four times. Although all four experiments showed similar responses, data from only one representative study are shown for simplicity. In this study, a single plant in each bucket was harvested for enzymatic assays, whereas the remaining two plants in each pot were harvested for elemental and dry weight analysis. The plants collected from a single bucket were considered to be a single replicate for enzymatic assays and a single replicate for dry weight and elemental analysis.

Harvesting and assays. After 2 weeks (zinnia) or 3 weeks (snapdragon) of treatment, leaves, stems (combined as "shoots" for enzymatic assays), and roots were harvested, rinsed with distilled water, blotted dry, and fresh weight was determined. For elemental and dry weight analysis, tissue was dried in a forced-air oven at 55 °C for 3 d and used for tissue analysis as described subsequently. For other tests, tissue was immediately frozen in liquid nitrogen and stored at –80 °C for subsequent use. Phenylalanine ammonia lyase [PAL (EC 4.3.1.5)] activity was measured by the method described in Liang et al. (2005) with some modifications described in Li et al. (2008). Peroxidase [POD (EC 1.11.1.7)] activity was measured by the method described in Liang et al. (2005).

Elemental analyses were performed according Frantz et al. (2008) and are briefly described here. For total tissue Si quantification, 0.15 g ground tissue or substrate material was digested in 7.5 M KOH in a programmable microwave (MARS Express; CEM Corp., Matthews, NC). One milliliter of the digested solution was diluted with 9 mL deionized water (18 megaohm purity) and injected into the inductively coupled plasma optical emission spectroscopy [ICP-OES (Model IRIS Intrepid II; Thermo Electron Corp., Waltham, MA)]. Every 20 samples, a rice (*Oryza sativa*) standard containing 0.44% Si was run that had been digested in a similar manner as the test species. For other elemental analysis, the same quantity of tissue was digested as according to Li et al. (2008) and briefly described here. A modified U.S. Environmental Protection Agency [EPA method 3051 (Nelson, 1988); HNO_3 digestion at 200 °C with an additional peroxide digestion step] was used for total nutrient concentrations for phosphorus, potassium, calcium, sulfur, magnesium, boron, Cu, Fe, Mn, and Zn from ICP-OES. Total nitrogen was not analyzed. In the set of samples for zinnia root tissue receiving no supplemental Si at the highest Cu rate, not enough tissue was available for Si analysis.

Statistics. Data were subjected to two-way analysis of variance using the statistical software (Statistix Version 9.0; Analytical Software, Tallahassee, FL) with Cu and Si supply as the main effects and a Cu × Si interaction. If significance was determined ($P < 0.05$), Tukey's honest significant difference (HSD) test of means was performed to determine potential differences among group means. No statistical comparisons were made between species, but observations of each species were used to speculate about mechanism of action for Si alleviating Cu toxicity stress symptoms.

Results and Discussion

Visible symptoms. In both species, as plants received more Cu with low (0.1 mM) Si, root tips changed from white to tan, brown, or deep orange (root photographs not shown). Leaves and shoots of Cu-toxicity treatment plants not receiving supplemental Si were often stunted and displayed interveinal chlorosis reminiscent of Fe deficiency (Fig. 1A–D). Bucher and Schenk (2000) reported that Cu toxicity symptoms can be alleviated, at least temporarily, with Fe additions, which would

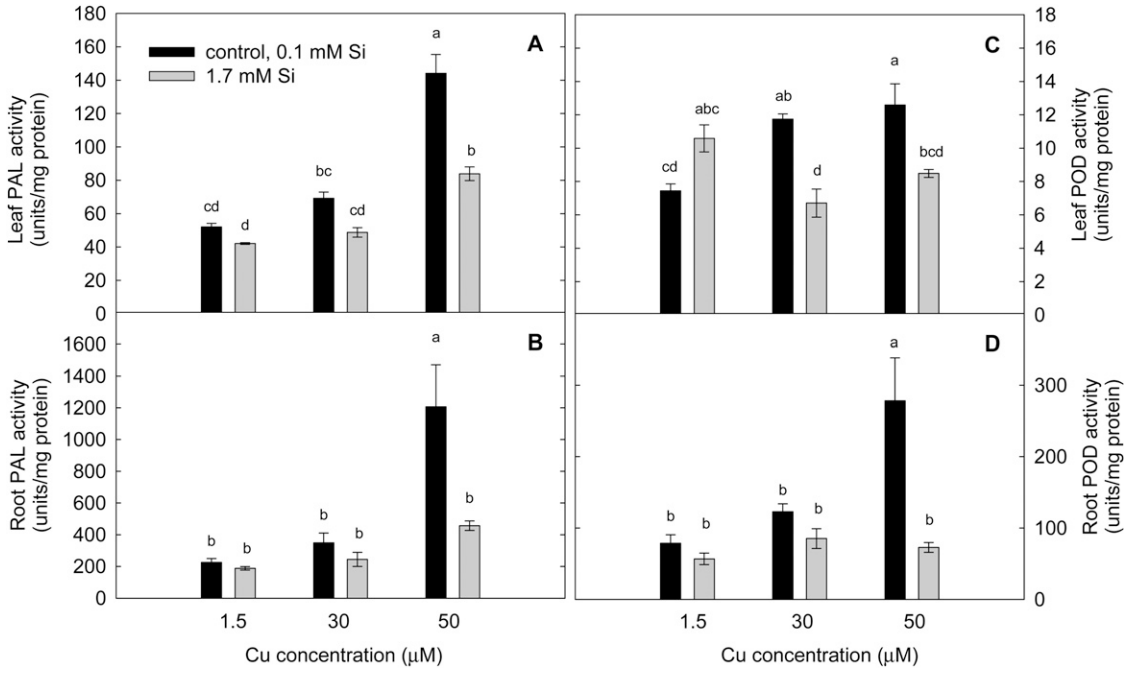

Fig. 4. Average phenylalanine ammonia lyase (PAL) activity of zinnia shoots (**A**) and roots (**B**) and average peroxidase (POD) activity of zinnia shoots (**C**) and roots (**D**) with ± 1 SE. Plants were grown hydroponically at three different copper supplies (1.5, 30, and 50 μM) and two different silicon supplies (0.1 and 1.7 mM). A representative plant was harvested from each hydroponic bucket so values are averages of four plants in each treatment. Different lowercase letters above the bars within a panel indicate statistically different means based on Tukey's honest significant difference test at $P < 0.05$.

certainly confuse the diagnosis of Cu toxicity problems. It is this set of symptoms and the response to Fe supplementation that leads us to believe that Cu toxicity may be more widely encountered than currently reported. In control Cu conditions, root appearance was unaffected by the addition of Si.

For zinnia, as Si was added to treatments also exposed to Cu toxicity, the occurrence of discolored roots diminished, suggesting at least partial alleviation of Cu toxicity symptoms. This response was similar to that observed in arabidopsis (Li et al., 2008). For snapdragon, supplemental Si had no effect on the reduction of visible stress symptoms on any part of the plant.

Zinnia. Dry weight of leaves, stems, and roots diminished as Cu concentration increased in non-Si-supplemented treatments (Fig. 2A–C). Dry weight was significantly greater in leaves, stems, and roots of plants receiving supplemental Si when Cu was 50 μM. The dry weight of the leaves, stems, and roots of high Cu treatments that also received supplemental Si was not statistically different from control tissue.

Leaf, stem, and root Si concentration was significantly greater when supplemental Si was provided to plants (Fig. 3A–C). Cu concentrations of those tissues was also influenced by Cu and Si supply (Fig. 3D–F). As expected, Cu increased with increasing Cu supply; however, at the highest Cu supply, concentration of the metal did not increase as much as when Si was also present. This indicates that Si prevented Cu from accumulating in the tissue at the same extent as unsupplemented plants. In arabidopsis, Si was shown to alter the expression pattern of genes related to metal uptake and distribution (Li et al., 2008). Interestingly, Cu concentration was unaffected in shoots and roots of arabidopsis despite those mechanistic changes. The different response of arabidopsis and zinnia to Cu concentration in response to Cu toxicity with and without Si supplementation suggests that the Si-mediated mechanism is different among species.

Concentrations of other nutrients were also influenced by Si and Cu (Tables 1–3). All nutrient concentrations in one or more tissue types were significantly influenced, either as a main effect or an interactive effect, by Si. The concentrations of potassium (K) and calcium (Ca) were the only nutrients to be significantly influenced in all three tissues by Si. Some effects can be explained by the additional K present in Si-supplemented treatments; K was higher in Si-supplemented treatments, and the effect was more pronounced in the highest Cu treatment. One would expect for Ca to be suppressed when high levels of K are present as a result of competitive, antagonistic effects from positively charged ions. This was not the case because Ca concentrations were occasionally higher (as in stem) in Si-supplemented treatments. In general, nutrient concentration differences were most likely because plants that received Si supplementation were generally healthier (less stressed) when grown at the highest Cu treatment compared with unsupplemented, Cu-toxic treatments.

Leaf PAL activity of both control (1.5 μM Cu) and plants treated with elevated Si (1.5 μM Cu and 1.7 mM Si) was nearly the same (Fig. 4A–B). The same was true for roots of those treatments. Zinnia treated with 30 μM Cu showed a slight increase, although not statistically significant, in both leaf and root PAL activity from average levels in control treatments. Both leaves and roots of zinnia treated with 50 μM Cu showed more dramatic increases in PAL activity that was reduced by addition of extra Si. Hence, Si application results in a reduction in Cu-induced PAL activity in zinnia roots and shoots. Such data probably indicate that the leaves in plants supplemented with Si grown under elevated Cu conditions are under less stress than unsupplemented plants. Perhaps Si helps generate additional apoplastic Cu-binding sites, sequestering the metal and thereby reducing its toxic effects as has been suggested for Mn (Iwasaki et al., 2002a, 2002b, 2002c; Rogalla and Römheld, 2002).

The POD data are similar to those for PAL (Fig. 4C–D). POD activity in leaves and roots was elevated by increased Cu and this was reduced by an additional high level of Si. For both leaves and roots, elevated Cu caused an increase in POD activity that was depressed by the additional high level of Si. Cu toxicity increases POD activity in maize (*Zea mays*) seedlings (Mocquot et al., 1996). These researchers proposed that Cu toxicity induces the formation of active oxygen species, which induces POD production to detoxify these highly reactive molecules. If this is also the case for other plants, then Si may cause a reduction in active oxygen species production, thereby reducing POD expression. If Si induces more Cu-binding sites in the cell wall, it is possible that the metal is sequestered away from the plant cell interior, reducing the formation of reactive oxygen species, again leading to a reduction in POD activity. Alternatively, Si may have an effect on

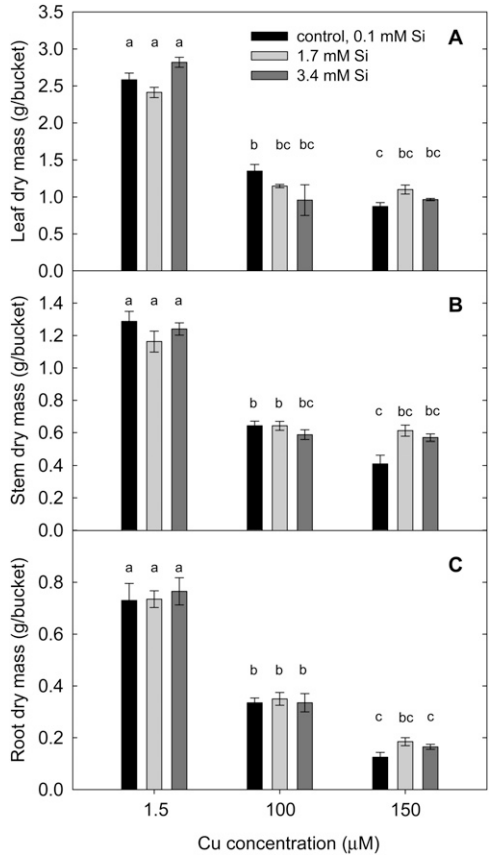

Fig. 5. Average snapdragon leaf (**A**), stem (**B**), and root (**C**) dry mass. Treatments consisted of low (0.1 mM), medium (1.7 mM), and high (3.4 mM) supplemental silicon and 3 weeks after exposure to control (1.5 μM), medium (100 μM), and high (150 μM) copper. Values are means of four replicate hydroponic buckets with each bucket containing two plants. Error bars are ± 1 SE. Different lowercase letters above the bars within a panel indicate statistically different means based on Tukey's honest significant difference at $P < 0.05$.

POD gene expression or enzyme activity independent of reducing the production of active oxygen species.

Snapdragon. Cu toxicity significantly decreased leaf, stem, and root mass (Fig. 5A–C). The extent of dry matter decrease was slightly, yet statistically significant, different as Cu concentration increased with and without Si supplementation. This led to a significant interaction between Cu and Si, although Tukey's test of means for a given Cu concentration resulted in no differences in mean tissue mass. So although statistically significant, the visible symptoms combined with dry matter analysis revealed little, if any, biological benefit provided by supplemental Si.

The concentration of Si increased with Si supplementation in snapdragon in leaves, but to a far lower extent than zinnia (Fig. 6A). Interestingly, Si concentration in leaves increased as Cu supply increased. A similar observation was made by Zellner et al. (2011), where it was hypothesized that tobacco (also a non-accumulator) leaves can regulate Si uptake in response to biotic stress. That is, in some plants, Si uptake may be stimulated by stress, thereby enabling the plant to make use of the element in stress alleviation. Stem analysis revealed mostly non-detectable Si concentrations regardless of treatment (data not shown). Additionally, there was insufficient root biomass for both total Si and all other elemental analysis for many of the replicate plants, so total Si data are not shown for roots.

Cu increased in all tissues as Cu supply increased (Fig. 6B–D). The extent of that increase was modulated by Si supply and was different for leaves, stems, and roots. In both leaves and stems, there was a significant interaction between Cu and Si with Si suppressing slightly the extent of increased Cu accumulation; the Tukey's HSD test did not indicate any significant differences within a Cu supply. Root Cu concentrations were significantly influenced by Si both as an interaction with Cu and as a main effect. At the highest Cu supply (150 µM), Si increasingly suppressed tissue Cu concentration with the 3.4 mM Si concentration resulting in significantly lower Cu in the root than the unsupplemented (0.1 mM Si supply), high Cu treatment. As a main effect, both Si supplies (1.7 and 3.4 mM Si) resulted in significantly lower Cu concentrations than unsupplemented plants (0.1 mM Si). Although the extent of suppressing Cu uptake was not as great as that observed in zinnia, the mechanism of Si potentially influencing Cu toxicity appears more like zinnia than arabidopsis (Li et al., 2008) based solely on tissue analysis data.

All other nutrients tested were influenced by Si either as a direct or interactive effect (Tables 4–6). In general, the greater the Si supply during Cu toxicity, the more similar that tissue's nutrient concentrations were to the concentrations of control plants. This is evidence that overall nutrient uptake was less impacted by Cu toxicity when Si was also present in sufficient quantities than when Si was lacking. Interestingly, root tissue concentrations were less influenced by the presence of Si than either shoot tissue despite being washed with Si; the shoot tissue concentrations are indications that uptake was functioning better in Si-amended solutions than unamended solutions when Cu was at toxic levels. Iron levels in snapdragon leaves were significantly decreased at both Cu concentrations and they were not significantly affected by Si supplementation. This would agree with the symptom data in which Cu-treated plants showed leaf chlorosis similar to Fe deficiency that was not reversed by Si application.

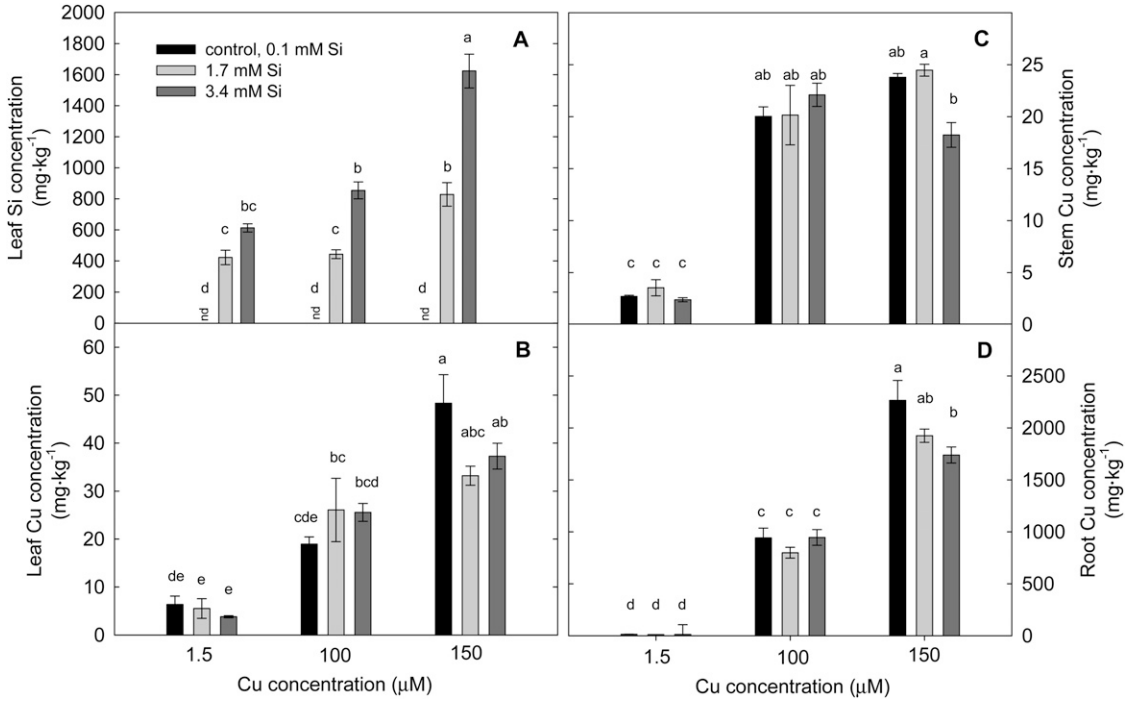

Fig. 6. Leaf silicon (Si) concentration (**A**), and copper (Cu) concentration in leaf (**B**), stem (**C**), and root (**D**) of snapdragon harvested 5 weeks after transplanting seedlings into a hydroponic system containing low (0.1 mM), medium (1.7 mM), and high (3.4 mM) supplemental Si and 3 weeks after exposure to control (1.5 µM), medium (100 µM), and high (150 µM) Cu. Error bars are ± 1 SE, whereas different lowercase letters above the bars within a panel indicate statistically different means based on Tukey's honest significant difference test at $P < 0.05$. Leaf Si concentration was not measured above background levels, so nd = not detectable in those treatments. A value of 0.1 mg·kg^{-1} was inserted in those replicates for statistical purposes.

PAL activity was higher in both roots and shoots as copper supply increased (Fig. 7A–B). This indicated the plant experienced greater levels of stress and increased stress response accordingly. Root tissue was unaffected by Si addition either directly or as an interactive effect. This was similar to the minimal effect that Si had on nutrient concentrations in the root tissue. Leaf PAL activity was significantly influenced by Si. As Si levels increased, PAL activity decreased indicating the stress was minimized with supplemental Si treatment. The extent of the minimization depended on Cu supply with both Cu toxicity levels responding with less PAL activity at higher Si supply rates than control levels of Cu, in which PAL activity was unchanged across all Si supply rates.

Accumulators versus non-accumulators. Our a priori hypothesis that supplemental Si does nothing in snapdragon plants experiencing Cu toxicity because it is not a Si accumulator and is not supported by our experimental data. The observed response could best be described as muted in snapdragon, but Si clearly had a measurable effect on overall stress response and plant health as measured by stress enzyme activity and nutrient status. With observations on poinsettia (N.S. Mattson, unpublished data), tomato (Stamatakis et al., 2003), tobacco (Zellner et al., 2011), and now snapdragon (all so-called non-accumulators) clearly being influenced by the presence of Si during specific stress events, we should instead focus on the extent of response from Si, not if Si can induce a response.

These data on Si uptake potential for various crops suggest that plants use different mechanisms to accomplish specific tasks. In the case of these two plants, Si-accumulating zinnia is more effective at using this element, perhaps by generating

Table 4. Nutrient concentrations of snapdragon leaves exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	11.9 a[y]	72.2 ab	19.1 ab	14.5 a	4.6 b	32.8 abc	97.4 a	173.4 b	25.3 bcd
	100	11.4 a	58.3 cd	21.0 a	12.6 b	5.4 a	37.1 ab	38.5 c	394.8 a	31.1 abc
	150	5.3 c	27.8 g	11.4 d	5.5 e	2.0 d	23.2 d	55.6 c	66.2 c	23.8 cd
1.7	1.5	11.5 a	73.1 a	20.1 ab	12.4 bc	4.6 b	32.0 bc	99.8 a	212.9 b	29.3 abcd
	100	12.1 a	54.3 d	22.9 a	11.0 cd	5.4 a	40.1 a	66.5 bc	380.1 a	34.7 a
	150	6.4 bc	35.6 f	14.1 cd	4.6 e	2.5 d	28.6 cd	40.0 c	72.0 c	22.5 d
3.4	1.5	11.2 a	75.6 a	16.9 bc	11.4 bcd	4.3 b	27.8 cd	86.7 ab	243.7 b	25.2 bcd
	100	11.9 a	65.2 bc	19.6 ab	10.5 d	5.8 a	36.7 ab	48.0 c	366.3 a	33.1 ab
	150	8.3 b	43.7 e	15.1 cd	4.9 e	3.3 c	33.4 abc	46.7 c	75.7 c	29.8 abcd
P value	Cu	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	Si	0.1136	0.0001	0.0098	0.0001	0.0042	0.1626	0.2924	0.4409	0.1686
	Cu × Si	0.0211	0.0015	0.0071	0.0173	0.0005	0.0010	0.0277	0.0775	0.0348

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

Table 5. Nutrient concentrations of snapdragon roots exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	8.8 c[y]	49.6 ab	4.1 a	5.3 c	3.3 c	22.7 bc	341.7 b	1233.3 a	101.7 b
	100	12.9 ab	53.9 ab	3.7 a	9.8 a	4.5 a	17.9 d	4893.1 a	704.8 c	587.4 a
	150	8.4 c	36.8 b	5.0 a	5.2 c	4.1 abc	30.8 a	2465.8 ab	239.4 cd	161.7 b
1.7	1.5	10.3 bc	57.9 a	4.2 a	4.8 c	3.6 bc	22.2 bc	589.2 b	1106.4 ab	108.5 b
	100	13.8 a	55.6 ab	4.4 a	7.9 ab	4.3 ab	20.6 cd	4870.6 a	401.5 cd	569.0 a
	150	7.7 c	38.7 ab	4.3 a	5.4 c	4.0 abc	26.4 ab	1341.8 ab	130.2 d	140.0 b
3.4	1.5	9.5 c	51.4 ab	3.8 a	4.2 c	3.5 bc	21.6 cd	457.9 b	709.2 bc	95.0 b
	100	13.4 a	55.1 ab	4.0 a	9.5 a	4.9 a	19.3 cd	3432.2 ab	444.4 cd	582.4 a
	150	8.7 c	36.4 b	4.2 a	6.4 bc	4.2 abc	19.9 cd	1550.8 ab	112.5 d	146.1 b
P value	Cu	0.0001	0.0002	0.2043	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	Si	0.6010	0.4643	0.3760	0.1305	0.2448	0.0003	0.5239	0.0028	0.9328
	Cu × Si	0.6542	0.8666	0.3270	0.0460	0.2435	0.0001	0.7057	0.0847	0.9924

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

Table 6. Nutrient concentrations of snapdragon stems exposed to different Cu and Si concentrations.[z]

Supply		Macronutrients (g·kg^{-1})					Micronutrients (mg·kg^{-1})			
Si (mM)	Cu (μM)	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Boron	Iron	Manganese	Zinc
0.1	1.5	3.9 bc[y]	68.1 ab	9.5 e	7.7 b	1.2 b	19.4 cd	23.2 a	150.4 c	14.2 b
	100	6.7 a	56.2 cd	13.3 bc	12.1 a	2.7 a	22.8 bc	11.6 a	348.5 a	78.4 a
	150	2.4 d	24.6 f	5.7 f	2.2 c	0.6 c	30.2 a	13.1 a	62.0 d	9.1 b
1.7	1.5	4.2 b	73.0 ab	12.8 bcd	7.1 b	1.3 b	20.7 cd	20.1 a	216.5 bc	12.5 b
	100	6.8 a	54.5 d	16.5 a	10.5 a	2.8 a	25.5 b	17.5 a	357.4 a	91.7 a
	150	3.3 c	27.8 ef	10.2 de	2.3 c	0.9 bc	30.0 a	12.5 a	73.5 d	9.8 b
3.4	1.5	4.0 bc	76.2 a	10.5 cde	6.6 b	1.2 b	18.4 d	20.9 a	265.2 b	13.8 b
	100	7.4 a	64.0 bc	14.7 ab	10.3 a	2.9 a	25.6 b	9.2 a	365.0 a	93.3 a
	150	3.4 bc	34.9 e	9.0 e	2.1 c	0.9 bc	32.5 a	13.2 a	69.2 d	8.1 b
P value	Cu	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0090	0.0001	0.0001
	Si	0.0001	0.0001	0.0001	0.0106	0.0491	0.0761	0.7425	0.0033	0.2840
	Cu × Si	0.0255	0.3655	0.4087	0.1892	0.6605	0.0209	0.6179	0.0127	0.1658

[z]Probability values are reported for main effects from Cu and Si supply as well as the interaction between the two. For all treatment groups, n = 4.
[y]Different letters within a column indicates significant differences in means from other treatments for that nutrient based on Tukey's honest significant differences test at $P < 0.05$.
Cu = copper; Si = silicon.

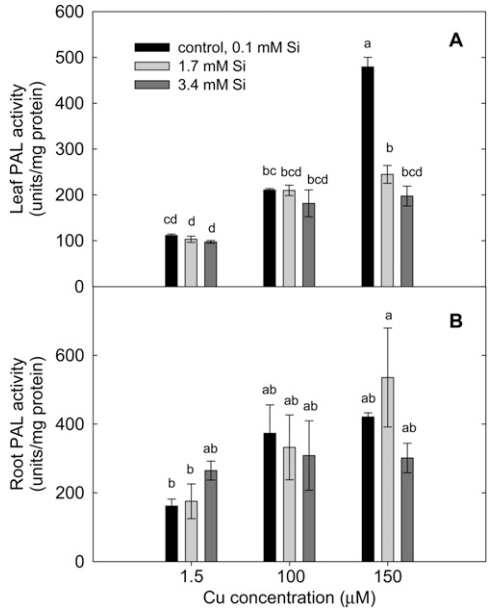

Fig. 7. Average phenylalanine ammonia lyase (PAL) activity of snapdragon shoots (**A**) and roots (**B**) with ± 1 SE. A representative plant was harvested from each hydroponic bucket so values are averages of four plants in each treatment. Treatments consisted of low (0.1 mM), medium (1.7 mM), and high (3.4 mM) supplemental silicon and 3 weeks after exposure to control (1.5 μM), medium (100 μM), and high (150 μM) copper. Different lowercase letters above the bars within a panel indicate statistically different means based on Tukey's honest significant difference test at $P < 0.05$.

more cell wall binding sites for Cu, thus reducing its apoplastic bypass flow as proposed for other metals (Ma and Yamaji, 2006). Because snapdragon is not as effective at accumulating Si, this element may play strictly a more active role (exclusively modulating stress pathways) for the generation of Cu-binding sites. It has been observed that in cucumber, shortly after removal of supplemental Si (days), plants can no longer effectively combat powdery mildew (*Sphaerotheca fuliginea*) stress despite tissue concentrations remaining high (Samuels et al., 1991). This strongly indicates that a soluble fraction of Si is necessary to elicit Si-induced resistance, and because Si is so poorly soluble, a steady, consistent supply is necessary to derive maximum benefit. It is also possible that snapdragon uses additional mechanisms to alleviate Cu stress and perhaps only activates Si-mediated processes once a particular threshold is exceeded. Support for this hypothesis comes from the observation that a much higher level of Cu was needed to elicit a detrimental response in snapdragon as compared with zinnia. It could also be proposed that Si-accumulators already possess the necessary machinery to take advantage of Si, whereas Si-non-accumulators lack some or all of the extensive genetic or enzymatic machinery for such responses.

Noting that Si is omnipresent in the lithosphere and that our understanding of Si's role in plant biology is still under development, Epstein (1999) wrote that fertilizer recommendations and hydroponic solutions that do not consider Si are at worst an artifact of real-world situations. Because more and more species are found to respond to Si in different stress situations, we should consider including Si in fertility programs at least in research situations to account for these yet-to-be-described pathways. It has been observed that detrimental effects can occur with elevated supplemental Si (Kamenidou et al., 2008), but the effect is not widely appreciated. The fact that some subset of species responds with a set of characteristics that we view more favorably than others in our production systems should not cloud the fact that a much broader range of species responds in some measurable way to supplemental Si than previously thought. At best, this response could enhance additional management practices, whereas at worst, Si response may exacerbate them. Not including Si in fertility management practices may inhibit a better understanding of the general plant response to stress.

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