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Silicon is Deposited in Leaves of New Guinea Impatiens

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Abstract
Although silicon (Si) is not considered to be an essential plant nutrient because most plants can complete their life cycle without it, many investigators have shown a positive growth effect, such as increased disease resistance, if Si is present. The effect of Si on many ornamental and greenhouse crop species has not been extensively studied. In greenhouse culture, plants are not exposed to Si from mineral soil, so Si should be added to the fertilizer solutions with mineral soil or soil amendments if there are benefits of Si. The initial step in determining potential benefits of Si requires the documentation of Si uptake and localization within the plant. We grew New Guinea impatiens for four weeks and exposed them to 2.0 mM Si in the form of K_2SiO_4. Using scanning electron microscopy and energy dispersive X-ray analysis, we detected Si deposits on the leaf margins in unique, Si-filled cells, primarily near hydathodes. We did not detect Si in the xylem. This report is the first documentation of Si uptake or accumulation in any species of the Balsaminaceae family.

Introduction
Silicon (Si) is not considered to be an essential plant nutrient because most plants can be grown from seed to seed without its presence (13). Still, some plants can accumulate Si at concentrations higher than many essential macronutrients (6). Many investigations have shown a positive growth effect if Si is present, including increased dry mass and yield (12), enhanced pollination (10), and most commonly, increased disease resistance (1,7,15,16). Some beneficial effects of Si, such as reduced incidence of micronutrient and metal toxicity (2,3,8,9), may occur even if Si is not taken up in appreciable amounts (19). Si can also alleviate imbalances between zinc and phosphorus supply (14).

Much of the literature on Si focuses on beneficial effects and uptake in grasses such as corn, wheat, sorghum, and especially rice. In a taxonomic review of Si uptake, Ma et al. (11) analyzed the mineral content in 500 species of plants and concluded that Si accumulation (more than 1% dry mass content Si) is not a universal characteristic in all higher plants, but confined to monocots in the families Graminaceae, Cyperaceae, and Erlocaulales. Intermediate Si accumulators have Si content of 0.5 to 1% and a Si:Ca ratio higher than 1, and include Cucurbitaceae and Urticales. All other plants are defined as non-accumulators. Some dicots, including bean (*Phaseolus vulgaris*) and aster (*Aster ericoides*) (10,19), have been reported to take up significant amounts of Si, which then has beneficial effects, in spite of their classification as "non-accumulators." These taxonomic reviews greatly assist in our understanding of the role of Si in plants, but also steer research away from describing the potential benefits of Si in "non-accumulating" species, which may, in some cases, accumulate Si in specific plant tissues.

The effect of Si on many ornamental and greenhouse crop species has not been extensively studied. Black spot infection (*Diplocarpon rosae*) incidence and severity was reduced in *Rosa hybrida* (7) and bract necrosis in poinsettia (15) was reduced when Si was applied either in the nutrient solution or as a foliar spray. Still, the use of Si in the ornamental and greenhouse industry remains limited to only a handful of species, namely cucumber and rose. This industry has largely changed from culture containing soil to soilless media based on peat, perlite, vermiculite, bark, compost, and other minor components. Even culture in rockwool, which is 47% SiO_2, has little bio-available Si (19). In modern plant culture, these plants are no longer exposed to Si from mineral soil, so if there are benefits of Si uptake and root exposure, Si must be added to the fertilizer solutions or mineral soil should be incorporated into the media mix.

The initial step in determining potential benefits of Si requires the documentation of Si uptake and localization within the plant. Herein, we report the results of our investigations into adding Si to New Guinea impatiens (*Impatiens hawkeri* W. Bull, family *Balsaminaceae*) in the fertilizer solution during vegetative growth.

Growth and Analysis of New Guinea Impatiens with Silicon

Rooted cuttings of New Guinea impatiens (cv. 'Pure Beauty Purple') were planted into a soilless media mix containing 70:30 peat perlite (vol:vol). Plants were grown in a greenhouse and established with 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 mM Fe as Fe-DTPA, 4.5 mM $MnCl_2$, 0.75 mM $ZnCl_2$, 0.75 mM $CuCl_2$, 22.5 mM H_3BO_3, and 0.05 mM Na_2MoO_4. Day temperatures ranged from 26 to 30°C, and night temperatures ranged from 16 to 22°C. Relative humidity ranged from 25 to 50%, as measured with a solid-state humidity sensor (model CS500, Campbell Scientific, Logan, UT). After two weeks, plants were fertilized as needed; ten plants received a nutrient solution containing 2.0 mM Si (as K_2SiO_4) and ten plants received a nutrient solution that did not contain Si. K_2SiO_4 was synthesized with fumed silica (SiO_2, 0.007 µm particle size) dissolved in 0.1 *M* KOH. No glassware was used in making the nutrient solution, and 18 mega-ohm purified water was used exclusively during the course of the trial to minimize Si contamination. The pH was adjusted to 5.6 with H_2SO_4 before the nutrient solution was applied to the plants. Plants were irrigated every 3 to 4 days with the nutrient solution for an additional four weeks. Flowers were removed to maintain vegetative growth.

There were no visible differences between plants that were exposed to Si in the nutrient solution and the control plants. However, Si-treated plants were stiffer to the touch, and the serrated edge on the leaf margin of the New Guinea impatiens (Fig. 1) felt sharper than the non-Si-treated plants. This led us to believe that Si may have been deposited on the leaf margins.

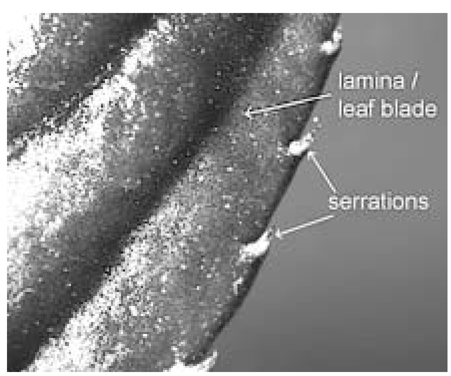

Fig. 1. Edge of a New Guinea impatiens leaf showing the serrations on the margin.

At the end of the growth period, control and treatment leaves were harvested, washed with deionized water, and sampled for analysis with a scanning electron microscope (SEM) (Model S-4700, Hitachi High Technologies America, Inc., Schaumburg, IL). Only mature, fully expanded leaves were used in the analysis. Beam voltage was 10.0 kV at a magnification between 30 and 500×, and raised to 20.0 kV for higher magnification. These voltages were also used for energy dispersive X-ray analysis (EDXA) (Noran, Thermo Electron Corp., Middleton, WI) for the respective images. Fresh cuttings of the edge of the plant leaf including the serrations were mounted on aluminum tabs with carbon stickers and viewed with the SEM. A total of 10 leaf samples were used for each treatment that included about 20 serrations on the edge of the leaf.

Plants not exposed to Si had a uniformly wrinkled surface appearance (Figs. 2A and 2B), which was expected as the plant samples dehydrated within the SEM. Si-treated plants were also wrinkled in the SEM except on certain areas and structures that we named “scales” on the edge of the serration (Fig. 3A). Interestingly, these structures were always found on the adaxial side of the serration. Closer inspection of the New Guinea impatiens scales revealed their attachment to the plant. Sangster et al. (17) also found differing silicon deposition patterns on abaxial and adaxial surfaces of the leaves of *Sieglingia decumbens*. On one leaf sample, the scale developed around a hole or pit in the leaf, which may be a hydathode (Fig. 3B). Note in Figures 2 and 3 the differences in size (see the length scale in the lower right corner of each picture). The presence of Si-scales at this location on the leaves may be due to the location of hydathodes along the edge of the New Guinea impatiens leaf. Guttation was observed on the morning after irrigating these plants. These observations are similar to the findings of Dengler and Lin (5) and Dayanandan and Kaufman (4) who determined Si to be deposited most commonly at sites where water exited from plants or where xylem ended.

Observations of these scales showed that they remained smooth on the surface in the SEM environment indicating the scales either did not dehydrate in the SEM or did not contain water. EDXA maps revealed these scales to have a higher concentration of Si than the surrounding cells (Fig. 4). EDXA measured the Si levels in 8 leaf samples and detected Si above background levels in Si-treated leaf samples but not in the control leaf samples (Figs. 5A and 5B).

Three stem samples from Si-treated plants were also prepared for analysis in the SEM and EDXA, but no Si was detected in either the stem surface or xylem cells (*data not shown*). Xylem cells were specifically targeted because Van der Vorm (18) suggested that, while Si is not necessarily deposited in the xylem, it travels through the xylem and may be deposited where xylem vessels end.

New Guinea impatiens are smooth-surfaced plants and do not contain many trichomes, which is a cell type that often plays a role in defense mechanisms for plants through either mechanical impediments (anti-herbivore) or chemical and salt exudation. Trichomes of a broad variety of species have been noted to accumulate Si at their bases (4,5). Our samples did not contain any trichomes for Si accumulation.

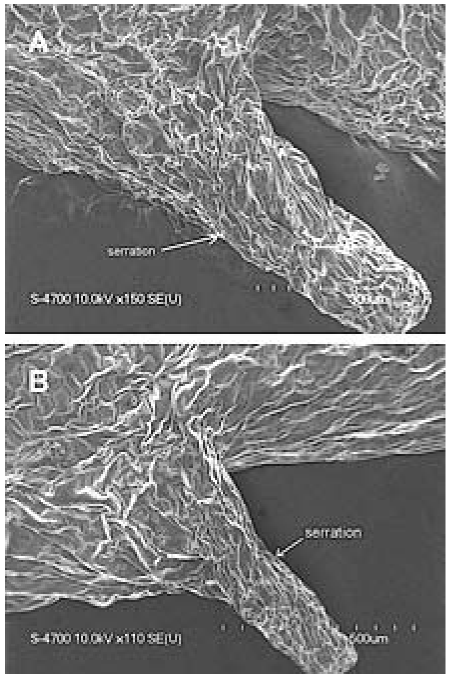

Fig. 2. Scanning electron micrographs (A and B) of control plants (no Si). Cells are wrinkled due to the fresh samples rapidly becoming dehydrated under the vacuum of the SEM.

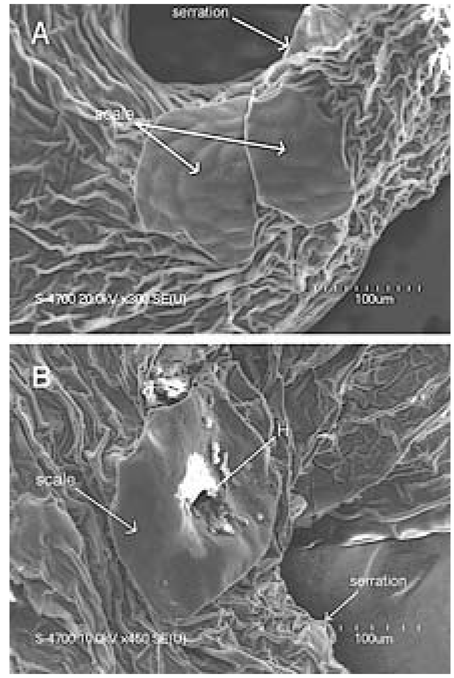

Fig. 3. Scanning electron micrographs of treated plants (2.0 mM Si added to nutrient solution). Note the "scales" on the serrations of the New Guinea impatiens leaves. The scales are clearly attached, as evidenced by the right side of the right-most scale (A) as well as the top and base of the scale in B. There is a hole in the middle of the lower scale (B), which may be the outline of a hydathode (H). Cells surrounding the scales are wrinkled due to the fresh samples rapidly becoming dehydrated under the vacuum of the SEM, but the scales themselves do not dehydrate, indicating some rigidity, perhaps from Si deposition.

Fig. 4. A scale on a serration of a New Guinea impatiens with the corresponding X-ray map of the Si deposition overlaid on the photograph. There is a stronger concentration of Si, as indicated by the dot map, in the area occupied by the scale indicating Si is localized in the scales.

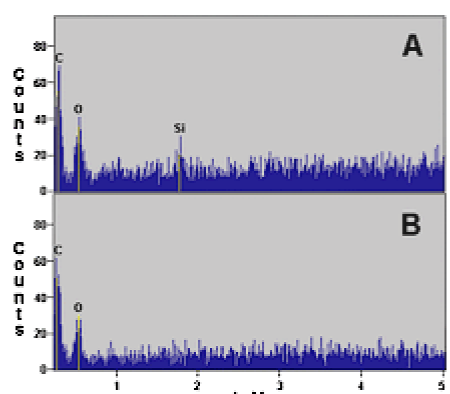

Fig. 5. Spectrographs of Si-treated (A) and not treated (B, control) leaf margins. The spectrograph was able to detect Si in significant amounts in the Si-treated samples (above background levels), but was not able to detect Si in the control plants.

Summary and Conclusions

This report is the first documentation of Si uptake or accumulation in any species of the Balsaminaceae family. The data presented here casts doubt on earlier phylogenetic studies that have suggested that Si uptake is limited to few genera of monocot plant species. Consequently, there may be potential for using Si in greenhouse ornamental plant production that were previously believed to not have Si uptake and accumulation. Demonstration of a potential benefit from Si fertilization could benefit greenhouse plant producers because most production is currently using soilless media that are devoid of Si. This information, and other growth characteristics of plants grown when they were exposed to Si, is a significant first step in determining the potential for using Si as a beneficial element in greenhouse fertilizer solutions to produce higher quality bedding plants with fewer agrochemical inputs.

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