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THE EFFECTS OF SILICON NUTRITION ON HYDROPONICALLY GROWN LETTUCE, BOK CHOY AND BASIL

By

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ABSTRACT OF THE DISSERTATION

The effects of Silicon nutrition on hydroponically grown lettuce, bok choy and basil

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Considered a “stress nutrient”, silicon has been reported to provide beneficial effects to plants grown under biotic and abiotic stresses. The mechanisms considered are either an accumulation of absorbed silicon in the epidermal tissue, or an expression of metabolic or pathogenesis-mediated host defense responses. In the case of silicon nutrition, plants are considered silicon accumulators or non-accumulators. It is commonly accepted that accumulators can benefit from silicon, but studies have shown that non-accumulators can sometimes benefit from silicon nutrition when under stress on a case-by-case basis. The objective of this dissertation study was to reveal the potential beneficial effects of silicon nutrition on three hydroponically grown silicon non-accumulator vegetable species, lettuce, bok choy, and basil, representing the common leafy green families of *Asteraceae*, *Brassicaceae*, and *Lamiaceae*. None, low and high levels of silicon (0, 25 and 75 ppm)

were added to the hydroponic nutrient solution. The plants were grown under temperature stresses (heat stress to lettuce and bok choy, and cold stress to basil), cut-and-grow-back stresses (lettuce, bok choy and basil), and biotic stresses (lettuce powdery mildew, simulated insect chewing on bok choy, and basil downy mildew). Plant growth, stress responses, and tissue nutrient analysis (including silicon) were evaluated. When grown under heat stress, silicon treatments failed to provide any beneficial effects for lettuce and bok choy. Basil grown under cold stress benefited from silicon treatments resulting in increased cold hardiness and improved survival rates after rates after a single frost event. Lettuce, bok choy, and basil grown under temperature stresses absorbed silicon in small quantities. The cut and grow back treatment did not result in silicon accumulation in lettuce, bok choy, and basil. The lettuce powdery mildew experiments failed due to the inability to establish sufficient disease pressure. The mechanical wounding treatment (representing insect chewing damage) in bok choy did not result in enhanced Si accumulation. For basil exposed to downy mildew, silicon treatments marginally increased the disease resistance without enhancing silicon accumulation. For most of the experiments, Si nutrition did not alter the content of other macro- and micro nutrients in both shoots and roots of lettuce, bok choy and basil. Future experiments are needed to evaluate the feasibility of using silicon as an effective BDM control agent for commercial growers. This dissertation research provided valuable information for understanding the physiology of silicon in non-accumulator plant species, and its potential beneficial effects for non-accumulator crops.

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Introduction

1.1 Topic overview and literature review

Silicon (Si) can bring beneficial effects to many Si-accumulator crops. Often not listed as an essential element for plants, studies have shown that Si is beneficial to the yield and quality of many crops, including rice, barley, wheat, sugarcane, cucumber, pumpkin, melon, strawberry, and soybean (Datnoff et al., 2001; Liang et al., 2015). Studies have shown that Si does not positively affect plants under optimal growing conditions. When the plant is under stress, either biotic or abiotic, Si can strengthen the plant's defense system, thus increasing the productivity of the crop (Datnoff et al., 2001). Currently, Si as a beneficial micronutrient for plant growth is in the process of being recognized by the green industry. Plant growth media companies such as SunGro™ have started to include Si in many of their products. The patented "RESiLIENCE" growing mix from SunGro™ has shown beneficial effects on plant growth for several flower and vegetable species (http://www.sungro.com/insight-innovation/resilience/).

The effect of Si on disease resistance is considered to be due to either an accumulation of absorbed Si in the epidermal tissue, or an expression of metabolic or

pathogenesis-mediated host defense responses (Datnoff, 2014). In natural soil, monosilicic acid, polysilicic acid, organo-silicon compounds, and complex Si compounds are the mobile forms of Si (Matichenkov and Bocharnikova, 2001). Plants absorb Si in the form of the soluble monosilicic acid anion (SiO_3^{2-}) through the roots via passive and active Si transporters (Datnoff et al., 2001; Zellner and Leisner, 2013). After transport to the leaves, and water loss through evapotranspiration, Si accumulates under the cuticle of leaves and stems in the form of polymerized silica gel ($(SiO_2 \bullet H_2O)_n$) (Yoshida et al., 1962). The distribution of Si in the shoot is controlled by transpiration rates. Silica gel becomes immobile in the plants, and it tends to accumulate in older shoot tissues (Ma and Yamaji, 2006). The major function of this deposited layer is to increase shoot rigidity resulting in stronger structures for light interception, strengthen the plant structures against climate factors (Raven, 1983), and increased shearing resistance during root elongation (Hansen et al., 1976). Thus this layer provides structural enhancement and protective functions, including but not limited to abiotic stress resistance, pathogen resistance, pest resistance, and herbivore resistance (Bakhat et al., 2018; Datnoff et al., 2001; Epstein, 1994; Epstein, 2009; Etesami and Jeong, 2018; Liang et al., 2006; Liang et al., 2015). Crops, especially Si accumulators, often show increased productivity as well as increased disease or pest resistance when treated with Si.

In the absence of any biotic or abiotic stresses, Si nutrition has shown to increase the yield of several edible crops, including rice (Houssain et al., 2001), wheat (Sarto et al., 2015), edible sugarcane (Fox et al., 1967), cucumber (Samuels et al., 1993) and

pumpkin (Heckman et al., 2003; Li et al., 2019). Prior studies indicate that Si amendments have altered the pattern and increased the physical strength of the epidermis tissues of both shoots and roots (Samuels et al., 1993; Yoshida et al., 1962).

More importantly, Si is beneficial to plants under biotic stresses. Several experiments have been conducted on Si accumulator crops to investigate the efficacy of Si nutrition against fungal diseases. The deposition of Si is thought to create a physical barrier that slows the fungal penetration and hyphae growth (Araujo et al., 2016; Bakhat et al., 2018; Guerriero et al., 2018; Hayasaka et al., 2008). For example, Si has been shown to reduce rice blast caused by the fungus *Mangaporthe grisea* (Sun et al., 2010; Winslow et al., 1997), wheat powdery mildew caused by *Blumeria graminis* (Belanger et al., 2002; Provance-Bowley et al., 2010) and tomato powdery mildew caused by *Oidium neolycopersici* (Gilardi et al., 2011). Si has also shown beneficial effects against pest and herbivore stresses. Si is involved in toughening plant tissues, acting indirectly by delaying insect penetration of host tissues (Keeping et al., 2009). The abrasiveness of silicified leaves and other plant tissues increases the irreversible wear of mouthparts of pest insects (Massey and Hartley, 2009). Interestingly, the Si deposition layer is more effective against leaf chewing insects than sap-sucking insects (Teixeira et al., 2017). The arrangement and distribution of silicified microstructures in the plant tissue also play important roles in herbivore and insect defenses (Alhousari and Greger, 2018; Araujo et al., 2015). Plants can benefit from Si against root diseases and pests as well, such as Pythium in cucumber (Cherif

et al., 1994) and sugarcane grey back canegrub (Frew et al., 2017).

Si can also help plants against abiotic stresses such as metal toxicity, nutritional imbalance, drought and salt stresses, temperature stresses and physical stresses (Datnoff et al., 2001; Debona et al., 2017; Etsami and Jeong, 2018; Hernandez-Apaolaza, 2014; Liang et al., 2007; Liang et al., 2015; Wu et al., 2013). Si is beneficial to plants under physical stresses such as lodging. In rice, the deposition of Si can enhance the thickness of the culm wall and the size of the vascular bundle and thus increase the stalk's physical stability and strength (Ma et al., 2001).

Si can decrease the availability of phytotoxic metals such as Lead, Arsenic, Chromium, and Cadmium in soil. The mechanism is thought to be due to the formation of precipitative silica complexes. The immobilization takes place through a Si mediated metal precipitation or bond to the plant cell walls (Neumann and zur Nieden, 2001; Liang et al., 2007). This mechanism is beneficial to plants under both micronutrient toxicity and deficiency. The immobilized metal in a non-toxic form inside plant tissue can also serve as a pool for micronutrients, and remobilize under deficient conditions (Bienfait et al., 1985; Briat et al., 1995). Si applied to the soil has been shown to alleviate the deficiency of Iron, Zinc, Copper, and Manganese (Hernandez-Apaolaza, 2014). Si also decreases the reactive oxygen species (ROS) production and enhances the antioxidant system in plants when under heavy metal stress (Adrees et al., 2015, Gu et al., 2011). When under drought and salt stress, Si has been shown to be beneficial by modifying gas exchange, osmotic adjustments and regulating compatible solute distribution in the plant. As a result, the plant gains

an increase in water use efficiency (WUE) and leaf relative water content (RWC) (Debona et al., 2017). For example, photosynthesis, transpiration and leaf water content under water stress were preserved in leaves of tomato plants when supplied with Si nutrition (Shi et al., 2016). When plants are under stress, Si also has shown to regulate the stress-related phytohormone synthesis, such as the production of abscisic acid (ABA), glycine betaine, indole-acetic acid (IAA), jasmonic acid (JA), polyamines (PA), proline and salicylic acid (SA) (Debona et al., 2017; Etsami and Jeong, 2018).

Si was shown to be effective against stress caused by low temperatures in some accumulator species. The mechanism is thought to be a combination of structural support from the Si deposition and alteration in plant physiology (Debona et al., 2017). In cucumber, Si amendments increased the plant's tolerance against chilling condition (15/8°C), and an increase in antioxidant enzyme and lipid peroxidation activity were observed (Liu et al., 2009). An experiment using a freezing susceptible wheat cultivar with Si nutrition showed that Si enhanced the freezing tolerance. The leaf water content was significantly higher in Si supplied wheat under freezing conditions (-5°C). High concentration of tissue H_2O_2, free proline and malondialdehyde (MDA) from freezing stress were suppressed by Si nutrition. Antioxidant enzyme activity and non-enzymatic antioxidants were stimulated significantly in Si treated wheat plants. Overall the freezing susceptible wheat cultivar benefited greatly from exogenous Si application and the shoot biomass was significantly increased (Liang et al., 2008).

In addition to the protection resulting from an improved physical barrier, Si also affects the plant's defenses at the molecular level. Research on the model plant Arabidopsis, a Si non-accumulator species, revealed the effects of Si on gene expression under powdery mildew disease pressure (Fauteux et al., 2005). For plants grown under optimal conditions, Si amendments had no significant effect on any but two of the approximately 40,000 gene transcripts. When inoculated with powdery mildew (*E. cichoracearum*), strong Si absorption and deposition were observed. However, Si did not effectively decrease disease progression. Si amended Arabidopsis plants were still substantially infected, to a lesser extent than plants without Si amendments. Comparing Si treated with non-treated plants exposed to powdery mildew, gene expression analysis indicated down-regulation of primary metabolic pathways, including photosynthesis and energy pathways, amino acid, carbohydrate, lipid metabolism, and protein synthesis. Moreover, the stress responses in plants were up-regulated, such as resistance genes (R-genes), stress-related transcription factors, genes involved in signal transduction, biosynthesis of stress hormones (salicylic acid, jasmonic acid, and ethylene), metabolism of reactive oxygen species, and biosynthesis of antimicrobial compounds. This research concluded that Si can induce a more efficient synchronized defense response by alleviating the disease stress on primary metabolic pathways (Fauteux et al., 2005).

Similar experiments were conducted in Si accumulator species of rice and wheat (Brunings et al., 2009; Chain et al., 2009). Recall that in Arabidopsis, the Si amendment did not affect the plant's general metabolism other than the regulation

of two genes under healthy growing conditions. In rice, Si amendment was shown to affect the expression of 221 genes, including many metabolism and housekeeping genes, without the presence of pathogens. This suggests the possibility that Si is essential for rice. When rice plants were inoculated with rice blast, many defense and stress-related genes were differentially regulated comparing Si versus non-Si treated plants, including ethylene signaling pathway genes, pathogenesis-related proteins, peroxidase genes, several transcriptional factors and protein kinases that were not differentially expressed in the control treatment. When comparing the plant response at the gene level, the infection with rice blast resulted in much less of differentially expressed genes in Si-amended plants than non-amended plants (298 vs. 738). This result indicates the possibility that the Si amendment can "pre-condition" the rice plant to react to stresses (Brunings et al., 2009).

In wheat, which is also considered a Si accumulator, Si amendments without disease presence only altered the regulation of 47 genes of diverse functions, suggesting Si does not strongly affect any specific metabolism. When wheat plants were inoculated with powdery mildew (*Blumeria graminis* f. sp. *tritici*), plants reacted by altering the expression of several hundred genes, such as up-regulating genes linked to stress and metabolic processes, and down-regulating photosynthesis genes. Supplying the plants with Si before disease inoculation not only strongly protected the plants, it also reversed the trends to the gene regulations due to disease attacks. Most of the genes that were up-regulated in Si-/diseased plants were down-regulated in Si+/disease plants, and the opposite occurred for down-regulated

genes. As a result, when comparing Si treated wheat plants under disease vs. no disease, very few genes were regulated. These results suggest that a Si accumulator such as wheat absorbs and accumulates high levels of Si to gain disease resistance. Physiologically, wheat behaves differently than rice, but more similar to the non-accumulator Arabidopsis. The disease resistance appears largely due to the mechanical barrier effect of Si deposition, rather than the differential gene expression from Si nutrition (Chain et al., 2009).

For plants to benefit from Si nutrition, a soluble source of Si needs to be absorbed by the root, transported and deposited in the shoot in order to be effective against stresses. Large variations of shoot Si concentration are present across plant phyla (Hodson et al., 2005). In general, ferns, gymnosperms, and angiosperms accumulate less Si in their shoots than non-vascular species and horsetails. In monocots, species in the orders Poales and Arecales accumulate substantially more Si than other clades in monocots. Some Graminaceous plants such as rice, wheat, ryegrass, and barley take up Si actively as a specific transporter-mediated process (Liang et al., 2006; Tamai and Ma, 2003). Within the non-monocot angiosperms, high relative shoot Si concentrations were observed in the orders of Saxifragalis, Fagales, Rosales, and Asterales. Brassicales and Fabales showed relatively low shoot Si concentration. Most dicotyledonous plants absorb Si passively by diffusion (Ma et al., 2001). But overall, the variation of Si concentration can be substantial among species even within the same groups (Figure 1; Hodson et al., 2005). This is mainly due to the mechanism of Si uptake among different plants. The absorption and accumulation of

Si in plant tissue depend on the regulation and expression of Si transporters, which differ between plant species. Si transporter proteins are localized to the plasma membrane, but, in different plant species, show different expression patterns, polarization patterns, and tissue or cellular localizations that are associated with different levels of Si accumulation, such as uptake, xylem loading, and the distribution of Si. (Ma and Yamaji, 2015).

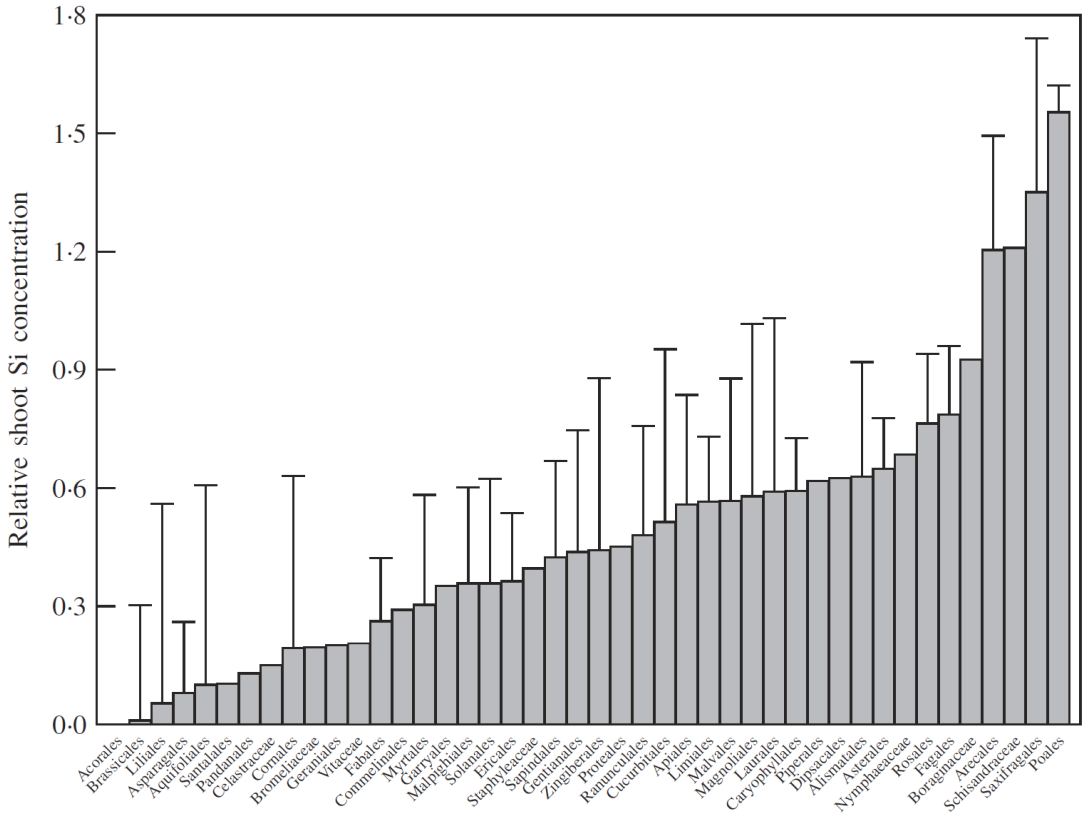


Fig. 1. Mean (±standard error of differences of means) relative shoot Si concentration in 44 angiosperm clades. Species Si concentrations were considered as relative values on a linear scale. Figure from Hodson et al. (2005).

Si transporter genes were first identified in rice (Ma et al., 2006). Two polar transporter genes are *OsLsi1*, a passive, channel-type transporter that facilitates passive transport of Si across the plasma membrane, and *OsLsi2*, an active efflux transporter that is responsible for the transport of Si out of the plant cell. In contrast to the passive transport mechanism of *OsLsi1*, the efflux transport of Si by *OsLsi2* is an active process that is driven by the proton gradient across the plasma membrane. The distribution of the two transporters in plant tissue is thought to determine the flow and accumulation of Si (Ma et al., 2004). The transporter genes or homologs have been identified in other species such as wheat (Montpetit et al., 2012), barley (Yamaji et al., 2012), maize (Mitani et al., 2009), soybean (Deshmukh et al., 2013), pumpkin (Mitani et al., 2011) and cucumber (Sun et al., 2017). Although many land plants, including those in Gramineae, Arecaceae, Solanaceae, Rosaceae, Cucurbitaceae, and Leguminoceae contain Si transporter-like genes or homologs, most plant species cannot benefit from Si due to the lack of an efficient Si transport system (Ma and Yamaji, 2015). Research has shown that transgenic manipulation of Si transporters in non-accumulator species can alter the Si accumulation in the plant, and thus lead to certain effects. For example, petunia with heterologous expression of rice Si transporters *OsLsi1* gained drought tolerance (Yang et al., 2014). Overall, the molecular mechanism of Si transport and accumulation in plants is still poorly understood (Figure 2; Ma and Yamaji, 2015).

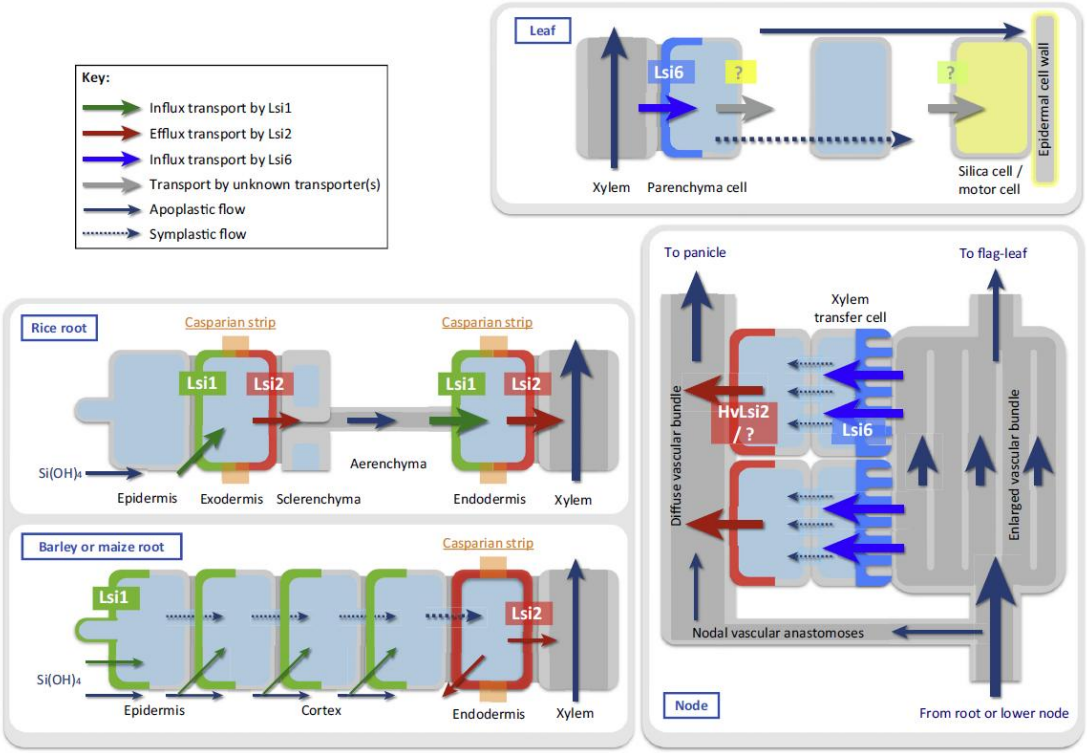


Fig. 2. Schematic representation of the current understanding of the combined system of Si transport including uptake, xylem unloading, and distribution in plants. Arrows with different colors indicate transport processes mediated by different transporters, apoplastic flow, and symplastic flow of Si. Figure from Ma and Yamaji (2015).

In the case of Si nutrition for common edible crops, plants fall into two categories: Si accumulators and non-accumulators. Under normal, non-stressed growing conditions, Si accumulators (such as Poales species and cucurbits) absorb Si readily and can have more than 5% of total dry weight as Si. Si non-accumulators (most leafy green vegetables are non-accumulators) usually contain less than 0.5% Si by dry weight (Datnoff et al., 2001). Both Si accumulators and non-accumulators deposit most of the Si in leaf tissue rather than stem, root or flower (Boldt et al., 2018).

Intuitively, people may think Si non-accumulator plants cannot benefit from Si nutrition. A review of the literature showed that most previous research has neglected to study the effects of Si on stress defenses in non-accumulator crops. Recent studies have indicated that Si non-accumulators can benefit from Si nutrition when under stress. It has been reported (Zellner et al., 2011) that for tobacco, a Si non-accumulator, when inoculated with tobacco ringspot virus (TRSV), the plants showed increased Si transport from roots and deposits of Si in leaves that resulted in a delay of the onset of disease. This phenomenon is disease specific. When repeated with the tobacco mosaic virus (TMV), the tobacco plants did not show increased Si uptake and deposition. The mechanism of this disease selectivity is unclear, but studies have shown that foliar Si level is significantly reduced when exogenous abscisic acid (ABA) was applied to plant leaves. This effect was not seen for other plant hormones. Since some pathogens are capable of producing or inducing ABA to enhance disease development, this could explain why Si did not enhance disease

resistance in all pathosystems (Zellner, 2012).

Some Si non-accumulators can also benefit from Si when under abiotic stresses. A recent study has shown that potato, a Si non-accumulator, when fertilized with sodium silicate under drought stress, resulted in up-regulation of the *StLsi1* gene, a passive channel-type Si transporter homologous to *OsLsi1* in rice (Vijaya et al., 2016). The *StLsi1* transcript was detected in roots and leaves and its level increased two-fold following Si fertilization, and upon drought treatments with added Si, the transcript increased approximately fivefold in leaves. Nevertheless, increased Si accumulation was only detected in the tuber peel. As a result, the tuber peel cell morphology and cell wall composition were altered. The low accumulation of Si in the roots and leaves was considered a result of low Si transport activity.

1.2 Research objectives

Knowing that Si can be beneficial to non-accumulators on a case-by-case basis, it would be interesting to observe the effects of Si on non-accumulator crops under abiotic and biotic stresses. This research aimed to study the application of different levels of Si on several leafy green crops (lettuce, basil, and bok choy, representing the common leafy green vegetable families of Asteraceae, Lamiaceae, and Brassicaceae), and observe the plant's growth under heat, cold, mechanical damage and several typical disease stresses.

The outcome of this research can provide the plant nutrient industry and

growers with insights into the beneficial effects of Si nutrition for Si non-accumulators crops. By studying and comparing the effects of Si on different plant families under different stresses, our understanding of the biological mechanisms of how Si non-accumulator plant species can benefit from Si nutrition might be enhanced.

1.3 General methods used during most of the experiments

1.3.1 Greenhouse and growth chamber location, environmental settings and data acquisition methods

All seed germination was carried out in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) located at Rutgers University New Jersey Agricultural Experiment Station Research Greenhouse facility in New Brunswick, New Jersey (40°28'40" N, 74°26'08" W; elevation 36 m). All post-transplant growth was carried out in a double layer polyethylene greenhouse located at the Rutgers University Vegetable Research Farm III in New Brunswick, New Jersey (40°27'45" N, 74°25'45" W; elevation 21 m, 1.59 km away from the growth chamber). The greenhouse was equipped with one gas-fired heater (120,000 BTU/hr, 35,136 W. PDP150AE0130SBAN, Modine Manufacturing Company, Racine, WI), two ventilation fans (0.75HP, DC36, Windmaster, Muskogee, OK) and an integrated temperature control system (ACC-I, Climate Controller Model II, ACME Engineering &

Manufacturing Corporation, Muskogee, OK).

Greenhouse air and hydroponic solution temperature, relative humidity (RH) and light intensity (Photosynthetic Active Radiation, PAR) were recorded every minute using sensors and a data logger. Details about the sensor set up are illustrated in Table 1. The program code used for the datalogger can be found in Appendix A. At the end of every week, the environmental data was retrieved from the datalogger. Air temperature for outside was also retrieved from the New Jersey weather and climate network (data from New Brunswick, NJ local station, https://www.njweather.org/data). A graph of greenhouse internal and external temperature, hydroponic solution temperature, RH and PAR was made weekly. The daily light integral (DLI) for each day was calculated from the PAR data. A sample of the weekly environmental data can be found in Appendix B.

Table 1. Data acquisition equipment and recording intervals.

	Manufacturer	Model / [Measuring unit]	Recording interval	Quan-tity	Placement in the greenhouse
Datalogger	Campbell Scientific, Logan, UT	CR1000, [N/A]	N/A	1	On the bench, near the hydroponic boxes
Datalogger software	Campbell Scientific, Logan, UT	PC200W, [N/A]	N/A	1	N/A
Air Temp and RH	Vaisala, Helsinki, Finland	HMP60, [Temp: °C RH: %]	Snap-shot, 1 min	1	In an aspirated box equipped with fan, hanging 1 meter above the bench at the center of the greenhouse
Water Temp	Homemade thermo-couples (Type T)	[°C]	Snap-shot, 1 min	4	20 cm submerged into the hydroponic solution (total depth = 25 cm)
PAR	LI-COR, Lincoln, NE	LI-190R, [µmol/(m^2 s)]	Snap-shot, 1 min	2	At plant canopy level, next to the floating hydroponic systems

1.3.2 Description of the nutrient solution and Si amendments used for the hydroponic systems

The nutrient solution used for the experiments was made from lab-grade chemicals. The recipe (Table 2) was a modified Sonneveld solution (recipe derived from the Cornell Lettuce Handbook; Mattson, 2014). For the experiments described in this dissertation, a half-strength Sonneveld solution was used to grow the plants during both seedling and after-transplant stages. The Si treatments were applied to the nutrient solution using a commercially available K_2SiO_3 source (Pro-Tekt® 0-0-3, Dyna-Gro, Richmond, CA). The nutrient solution was amended with either 0 (control), 25 or 75 ppm of Si resulting in three different treatments. Si concentrations beyond 75 ppm were found to result in a Ca_2SiO_4 precipitate and were therefore not used. The nutrient solutions of all Si treatments were titrated to a pH of 5.8 using 6 N nitric acid.

K_2SiO_3 is an alkaline compound. When applied to the nutrient solution, extra nitric acid was added to bring the pH back to 5.8. As a result, Si treated nutrient solutions contain more potassium and nitrogen. Compared with the non-Si treated control, the 25 ppm Si treatment solution contains 18.87% more potassium and 2.92% more nitrogen, and the 75 ppm Si treatment solution contains 40.10% more potassium and 8.76% more nitrogen. When evaluating the effects of the Si treatments on the plant's growth and stress responses, this substantial increase of potassium and nitrogen should be considered when evaluating the effects of Si.

Table 2. Target nutrient concentrations for the nutrient solution (full strength). Adapted from the "Cornell lettuce nutrient solution" (Mattson, 2014). The full-strength solution was diluted once to obtain the half-strength solution that was used to grow the plants. The system utilized two stock tanks to reduce precipitation. Tank A contains calcium and iron, and tank B contains phosphorus, magnesium, sulfur and other micronutrients.

Macro-nutrient	Concentration (mMol/L)	ppm	Micro-nutrient	Concentration (µMol/L)	ppm
N	17.65	247	Fe	33.54	1.87
P	2.00	62	Mn	5.07	0.28
K	10.99	430	B	3.00	0.03
Ca	4.12	165	Cu	0.75	0.05
Mg	2.04	50	Zn	3.83	0.25
S	2.17	70	Mo	4.92	0.47

1.3.3 Protocols for hydroponic system preparations, seeding, transplanting and growing methods

All growth experiments were carried out using the floating hydroponic system (Figure 3). This system was used because:

1) All of the vegetable species investigated could be grown hydroponically. These crops are grown commercially in hydroponic operations.
2) Using a hydroponic system with the half-strength Sonneveld nutrient solution eliminated the natural variability of nutrient availability in soils.
3) It allowed for careful control of nutrient and Si levels available to the plants.
4) It provided easy access to the roots.
5) It was relatively easy to maintain Si levels at 0, 25 and 75 ppm.

Fig. 3. Deep flow hydroponic systems built for this research project. Fourteen of such hydroponic systems were made. Each individual system had a volume of 100 L (61 cm * 61 cm * 25.4 cm, equivalent to 2 ft * 2 ft * 10 inches) with space for 64 plants. The nutrient solution was continuously aerated with an air pump connected to an airstone. The temperature of the nutrient solution was not controlled but recorded. The pH of the solution was checked at least once a week and maintained at 5.8 ± 0.2 by titrating with 6 N nitric acid.

To start a batch of seedlings, individual seeds were placed into a sheet of Oasis hortcubes (Oasis^{TM} HORTCUBES 1" thin-cut growing Medium, 276 cells per sheet, Oasis Grower Solutions, Kent, Ohio). Each sheet of hortcubes was pre-soaked and drained twice to remove any particulate matter and chemicals from the manufacturing process before seeding. After dropping one seed per hole, a toothpick or tweezers was used to push the seed 3 mm down into the cube material. Therefore, the seed germinated inside the cube material and thus avoiding any "pop-up" seedlings. Sheets were placed in individual seeding trays without bottom drain holes. When an experiment required fewer seedlings, only one half or one third of a sheet was used. Trays were placed in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) with a constant temperature set point of 20°C, a 16-hour photoperiod, 60% relative humidity (RH) and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light [10 incandescent (69W, Philips, Amsterdam, The Netherlands) and 22 fluorescent (165W, F72T12CW1500, General Electronics, Boston, MA) lamps combined]. The spectrum of the light source was not measured.

The first irrigation took place after the seeds were sown. Three liters of nutrient solution were irrigated to fill each tray to a depth of 2.5 cm. After the initial irrigation, the trays were irrigated with 1 L of nutrient solution every other day. During most experiments, the seedlings reached optimal sizes for transplanting after 11 or 12 days. At this stage, roots have emerged from the bottom of the hortcube, and the sizes of the first true leaves could be evaluated for seedling selection.

During transplant, seedlings were removed from the growth chamber and

moved to the greenhouse. The sheet of hortcubes was broken into single cubes. About 30% of the seedlings were selected for transplant based on their uniformity in size, developmental stage and healthy appearance. The selected plants were placed into the growing board, which was made of 2.5 cm thick Styrofoam® foam boards (Lowes, New Brunswick, NJ) with drilled holes (Φ = 2.5 cm). The top of the cube (base of the plant shoot) was leveled with the top surface of the growing board. The bottom of the board had a small amount of hortcube sticking out to ensure the cube was in contact with the nutrient solution. The board was floated on top of the nutrient solution. Each hydroponic box had the capacity to grow 64 (8 X 8) plants.

The frame of the hydroponic boxes was made from pine lumber (Home Depot, New Brunswick, NJ). Four pieces of 61 cm * 30.5 cm (equivalent to 2 ft * 1 ft) boards were fixed together with metal angle brackets. The boxes were placed on benches with an expanded metal surface. The bottom of the box was made of a piece of drywall. The inside of the box was lined with a 0.15 mm (equivalent to 6-mil) clear plastic sheet. The plastic sheet was wrapped over the edges of the box and taped down to the wood frame. Before each experiment, the boxes were pre-filled with water to check for leaks and stabilize the water temperature. At the end of each experiment, the plastic liners were replaced after the removal of the nutrient solution.

During the experiments, the nutrient solution inside the hydroponic boxes was aerated using an air pump (GH2716, General Hydroponics, Santa Rosa, CA) and air stones. The pH of the solutions was checked weekly or more often. If the pH deviated

more than ±0.2, adjustments were made by slowly pipetting 6 N nitric acid into the solution and making sure the solution was well mixed afterward.

Chapter 2

Effects of silicon amendments on lettuce, bok choy, and basil grown under temperature stress

2.1 Introduction

Lettuce (*Lactuca sativa*) is an important leafy vegetable crop grown and consumed globally. In the United States, most of the field lettuce production occurs in California and Arizona. In the year of 2015, lettuce was produced on 166,800 acres across the US, with 8,087 million pounds harvested that totaled nearly $1.9 billion, making lettuce the leading vegetable crop in terms of value (Ag Marketing Resource Center, 2018). Lettuce is a cool-season annual crop. The optimal indoor growing temperature varies among different crop varieties, but it is generally suggested to keep the temperature around 24 °C day / 19 °C night. When grown hydroponically, the rootzone temperature should be controlled at 25 °C (Brechner and Both, 2013). Midsummer commercial lettuce produced at higher temperatures often develop physiological disorders such as rib discoloration, bolting and firm texture, causing major losses in quality and yield (Jenni, 2005).

Bok Choy (*Brassica rapa* var. *chinensis*), sometimes known as ‘pak choi’ or ‘pok choi’, is another important leafy vegetable crop consumed globally. Bok choy originated in China and gained popularity in the western world due to its light, sweet

flavor, crispy texture, and high nutritional value (Pan and Sasanatayart, 2016). The optimal growing temperature for Bok choy ranges from 18 °C to 22 °C, and its production is seriously impacted by heat stress in many regions. Heat stress causes growth inhibition of shoot and root, and can be observed from symptoms such as leaf etiolation and bleaching (Wang et al., 2011).

Sweet basil (*Ocimum basilicum*) is a globally important herb crop valued for its unique aroma and flavor (Akbari et al., 2018). Basil is a tropical plant within the mint family (Lamiaceae) grown in many regions around the world (Satpute et al., 2019). Basil originated from moist, tropical rain forests in East Africa and favors a warm climate and long day sunny conditions (Caliskan et al., 2009). Basil is sensitive to cold stress. Temperatures below 10 °C can lead to significant leaf necrosis resulting in brown discoloration (Ribeiro and Simon, 2007). Cold stress can significantly impact the essential oil composition, affecting its taste, flavor, and marketability (Senji et al., 2018; Akbari et al., 2018). The postharvest shelf life of basil is also impacted by cold temperatures (Cantwell and Reid, 1993).

Si has shown to increase the chilling tolerance in multiple plant species (Debona et al., 2017; Liang et al., 2008; Liu et al., 2009). The mechanism is considered a combination of structural support from the Si deposition and changes in plant physiology at the molecular level (Datnoff, 2014). Previous studies mostly focused on Si accumulator species, but studies on non-accumulator species have been limited. The effect of Si on heat stress tolerance of non-accumulator species is also an understudied area. This study aims to reveal the effects of Si amendments on heat

and cold stress tolerance of three non-accumulator leafy green species: lettuce, bok choy, and basil grown in hydroponics.

2.2 Materials and methods

Lettuce and bok choy grown under higher temperature conditions

To evaluate the efficacy of Si amendments on hydroponically grown lettuce and bok choy grown under heat stress, two experiments were conducted. Experiment 1 started with seeding on July 26, 2017. Experiment 2 started with seeding on Aug. 11, 2018. During each experiment, the lettuce and bok choy experiments were conducted simultaneously.

For Experiment 1, the lettuce cultivar Black Seeded Simpson (leaf lettuce, from W. Atlee Burpee & Co, Warminster, PA) and bok choy cultivar Asian Delight (white stem bok choy, from Johnny's Selected Seeds, Fairfield, ME) were used. For Experiment 2, the lettuce cultivar Rex (Boston butterhead lettuce, from Johnny's Selected Seeds, Fairfield, ME) and bok choy cultivar Black Summer (dark green bok choy, from Johnny's Selected Seeds, Fairfield, ME) were used. The seeding process and growth chamber operations were conducted as described in Chapter 1.

Seedlings were transplanted into the hydroponic boxes after 11 days of incubation in the growth chamber. For Experiment 1, sixty-four (8*8 rows in one hydroponic system per Si treatments) seedlings of equal sizes were selected for each Si treatment. Greenhouse temperature set point of 30 °C (day) and 25 °C (night) were

used during both experiments. During the daytime, the greenhouse air temperature often exceeded the set point because the greenhouse was equipped with only fan cooling and no evaporative cooling. This limitation prevented the use of a control treatment for plants that did not receive heat stress. The 36 non-guard plants in each hydroponic system were monitored and harvested over 6 consecutive harvests. During each harvest, six plants from each Si treatment were removed from the hydroponic box and evaluated for root length, shoot and root fresh and dry weight, and numbers of true leaves. Shoot height of plants used for the fifth (6 plants) and sixth (6 plants, final) harvest were measured daily. After measuring the dry weight, plant shoots and roots were separated and grounded using a grinder (Arthur H. Thomas scientific apparatus, Philadelphia, PA). All plant shoot or root samples from each treatment were combined for tissue analysis. The experiments were terminated at 25 days after transplant (DAT) for bok choy, and 20 DAT for lettuce due to plants collapsing from being tall and fragile. Tissue analysis for essential elements and Si were carried out using plants from the final harvest by ashing the samples (AOAC 900.02B) and mixing with aqua regia before performing inductively coupled plasma atomic emission spectroscopy (ICP-AES) at a commercial testing lab (PLT-1, MMI Labs, Athens, GA).

Experiment 2 was carried out following the exact same protocol as Experiment 1, except for three differences: 1) in addition to measuring the plant height, plant width (plant radius at its widest width) was also measured, 2) the plant height and width were measured every other day, and 3) the experiment was terminated at 25 DAS for

both lettuce and bok choy.

For both experiments, plant growth parameters were analyzed using MS Excel. T-tests were performed comparing each treatment with the control to reveal the contrast among treatments. Graphs of tissue analysis data were done using MS Excel for each nutrient.

Basil grown under lower temperature conditions

To evaluate the efficacy of Si amendments on hydroponically grown basil under cold stress, three similar greenhouse experiments were conducted. The first experiment started with seeding on Jan. 19, 2018. The second experiment started with seeding on Mar. 18, 2018, and the third experiment started with seeding on Feb. 11, 2019. Organic basil 'Genovese' (Johnny's Selected Seeds, Fairfield, ME) was used as the cultivar for all three experiments. The procedure of seeding, transplanting and maintaining during the growing period are mostly identical to the lettuce and bok choy experiments described previously. The set points for the three experiments are listed below. The environmental set points used for the three experiments were identical unless stated otherwise. Specific details of the experiments include:

1) Number of plants during seeding: two sheets (276 cells) per Si level.
2) Transplant: 128 plants per Si level, using 2 hydroponic boxes for each Si level. Seedlings were transplanted 15 days after sowing due to slower growth under the cold environment.
3) Greenhouse environment set points: Experiment 1: constant 18 °C across all

DAT. Experiment 2 and 3: constant 23 °C across all DAT.

4) Growth period: 30 DAT. Total of 45 days after sowing.
5) Growth parameters evaluated: The 36 non-guard plants from each hydroponic system were monitored and harvested over 6 consecutive harvests. During each harvest, 12 plants from each treatment (6 from each box) were removed from the hydroponic system and evaluated for root length, shoot and root fresh and dry weight, and numbers of true leaves. Shoot height of the 12 plants (from both hydroponic systems) used for the final harvest were measured daily.
6) Tissue analysis: Plants from Experiment 1 were not collected and analyzed due to plants being very small and the experiment did not yield meaningful results. For Experiment 2, all plant shoot or root samples from the same treatment were combined into one sample for tissue analysis. For Experiment 3, shoot and root tissue from each individual harvested plant was analyzed, but nitrogen and sulfur analysis for root samples failed due to the small sample mass for individual plants.
7) Statistical analysis: For Experiment 3, the tissue analysis was performed for each individual plant. The analysis was carried out in a stepwise fashion using SAS 9.4 (SAS Institute, Cary, NC). The first step was a single degree of freedom contrast to compare all Si treated groups against the no Si control to determine whether there were any treatment effects. The second step was a regression analysis to reveal the rate effect of the Si amendments. The third

step was a linear regression against application rates to reveal whether the Si treatment yielded linear effects in the content of each element in plant tissue. The contrast results were represented by *P*-values, with $P < 0.05$ indicated statistical significance. The linear regression was represented by both *P*-values and R-squared values, with the higher R-squared values indicating a better fitting linear relationship between the Si treatment levels and the content of each nutrient in the plant tissue.

2.3 Results

Lettuce and bok choy grown under higher temperature conditions

The greenhouse environmental data for the experiments with lettuce and bok choy grown under higher temperature conditions are presented in Figure 4 and Table 3 for Experiment 1, and Figure 5 and Table 4 for Experiment 2. During the day time, the greenhouse was often heated with abundant sunlight and was only relying on two ventilation fans for cooling. As a result, the greenhouse day temperature usually fluctuated substantially and often exceeded the 30 °C set point. During the night, the greenhouse temperature remained around 25 °C when the outside temperature was below 25 °C, which was the temperature set point for the greenhouse. The rootzone nutrient solution temperature fluctuated with the greenhouse air temperature but the changes occurred more slowly. The average day/night air temperature across the entire growth period were 30.6/25.1 °C (Expt. 1), and 29.1/22.5 °C (Expt. 2).

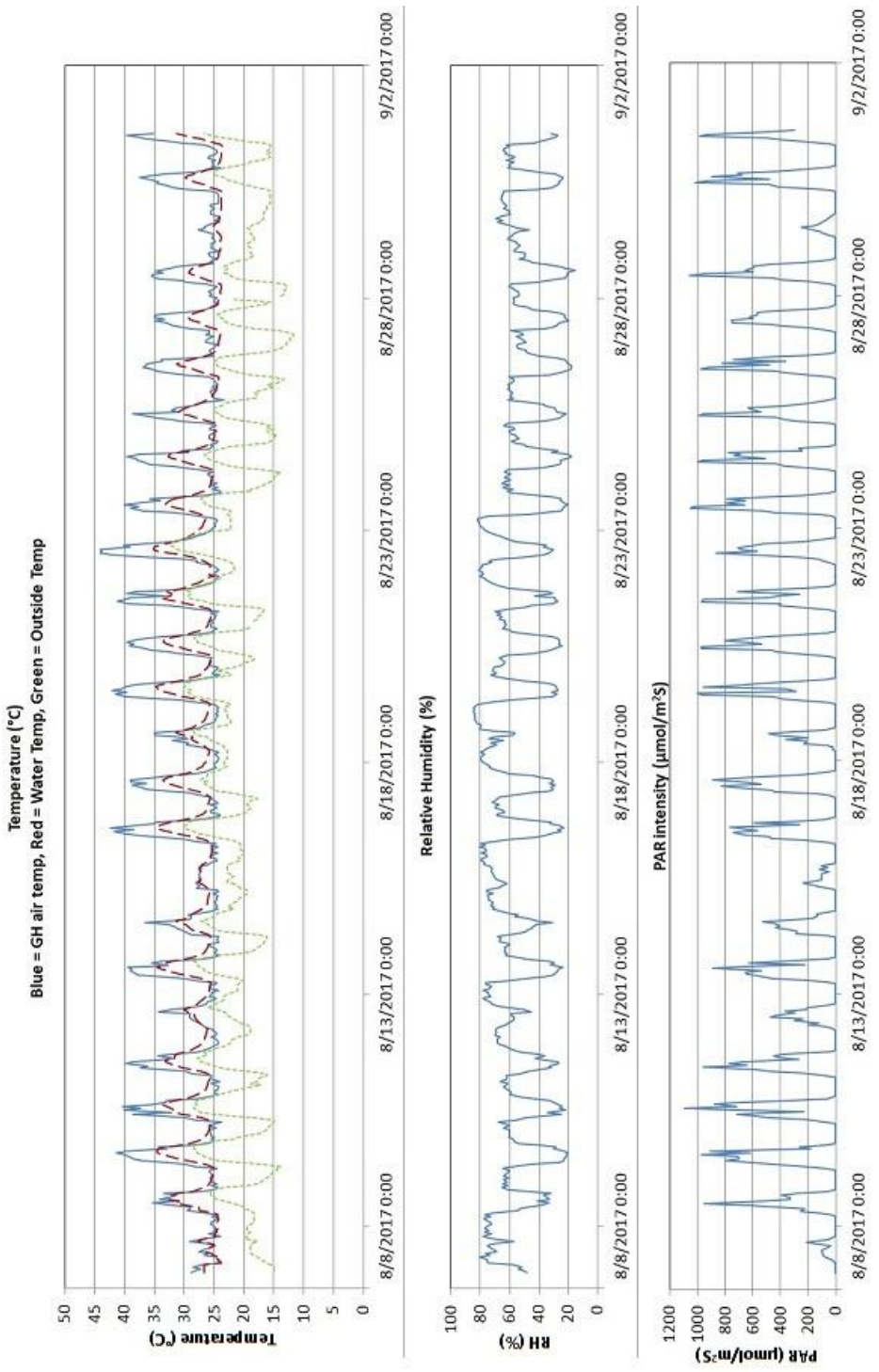


Fig. 4. Lettuce and bok choy heat Experiment 1: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). From Aug. 6, 2017, first day after transplant to Aug. 31, 2017 (25 DAT).

Table 3. Lettuce and bok choy heat Experiment 1: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Aug. 6, 2017, first day after transplant to Aug. 31, 2017 (25 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
7-Aug	1	25.9	24.9	25.4	24.4	18.6	18.8	74.1	4.2
8-Aug	2	29.9	28.3	29.0	27.7	22.2	20.2	50.5	18.4
9-Aug	3	32.7	25.1	30.1	25.9	24.0	17.2	49.6	24.2
10-Aug	4	31.7	25.0	29.9	26.1	24.4	18.8	51.3	19.9
11-Aug	5	31.3	24.6	29.9	26.5	24.0	20.9	57.2	20.1
12-Aug	6	28.1	24.8	27.9	25.9	22.5	21.9	69.6	11.1
13-Aug	7	32.4	25.0	30.1	26.0	24.9	17.3	52.6	21.7
14-Aug	8	28.0	24.7	27.7	26.0	21.8	20.8	64.9	14.4
15-Aug	9	26.9	24.7	26.9	25.4	21.9	20.4	74.8	5.9
16-Aug	10	33.2	24.9	30.2	26.0	25.8	19.0	56.8	21.6
17-Aug	11	32.1	24.7	29.8	26.2	24.5	23.0	62.3	21.4
18-Aug	12	29.0	24.6	28.2	26.0	25.0	22.8	78.4	9.4
19-Aug	13	34.0	25.0	30.6	26.0	27.0	21.2	57.1	22.0
20-Aug	14	32.2	24.9	29.6	25.7	24.1	17.9	53.8	22.0
21-Aug	15	32.9	24.6	29.9	25.8	25.7	22.8	63.5	19.1
22-Aug	16	34.5	25.1	30.7	27.2	28.0	23.4	65.8	17.9
23-Aug	17	28.5	25.2	26.2	25.4	19.3	15.6	54.2	22.1
24-Aug	18	31.3	25.2	28.6	25.0	22.4	15.3	47.1	21.0
25-Aug	19	29.9	25.3	27.6	24.6	21.6	16.1	49.2	18.7
26-Aug	20	30.5	25.5	27.3	24.2	20.7	12.6	43.2	20.7
27-Aug	21	29.7	25.4	26.6	24.0	20.5	14.8	45.5	19.7
28-Aug	22	29.8	25.1	26.5	24.0	20.2	19.0	45.2	21.7
29-Aug	23	25.4	25.1	24.3	23.8	17.8	15.8	61.2	4.1
30-Aug	24	30.5	25.3	26.5	23.8	21.0	16.4	50.4	22.7
31-Aug	25	34.0		27.3		20.8			28.0
Average		30.6	25.1	28.3	25.5	22.8	18.8	57.4	18.1
St. Dev.		2.5	0.7	1.8	1.1	2.6	3.0	10.0	6.3

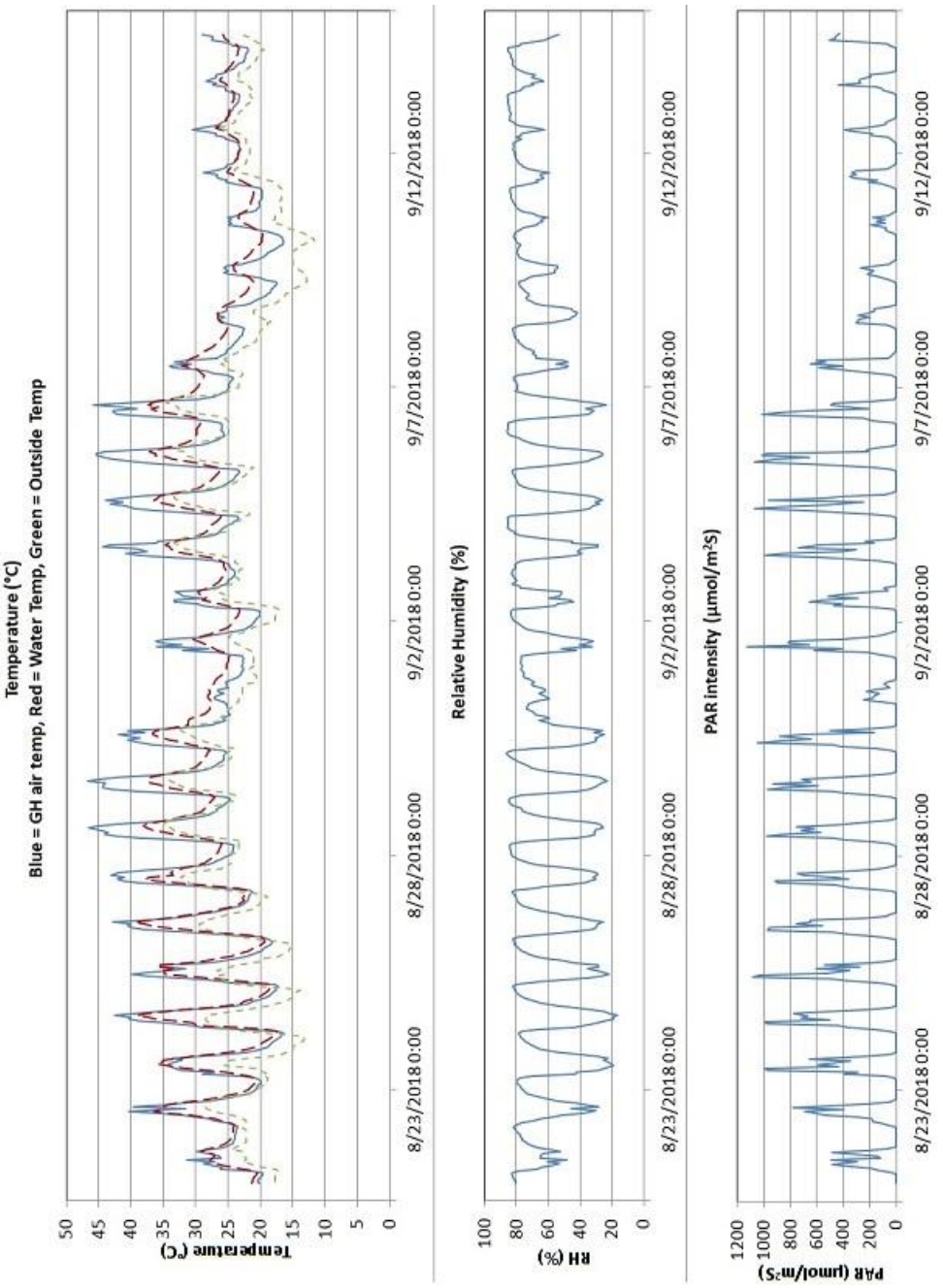


Fig. 5. Lettuce and bok choy heat Experiment 2: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). From Aug. 21, 2018, first day after transplant to Sep. 15, 2018 (25 DAT).

Table 4. Lettuce and bok choy heat Experiment 2: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Aug. 21, 2018, first day after transplant to Sep. 15, 2018 (25 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
21-Aug	1	26.6	23.8	26.3	24.3	21.8	22.3	71.6	11.2
22-Aug	2	30.7	27.2	30.0	27.1	25.5	23.7	57.2	16.3
23-Aug	3	28.3	17.4	28.3	18.7	20.1	14.8	57.7	19.4
24-Aug	4	31.2	18.2	29.3	19.3	24.3	15.6	59.4	21.4
25-Aug	5	30.4	19.0	29.6	20.4	23.3	16.3	62.1	21.8
26-Aug	6	32.5	22.0	30.8	22.7	26.1	20.1	63.9	20.8
27-Aug	7	34.0	24.7	31.2	26.7	28.8	23.8	66.7	18.7
28-Aug	8	33.3	25.7	31.0	28.2	28.7	25.5	69.1	22.5
29-Aug	9	37.1	25.8	33.2	28.6	30.8	25.0	63.9	22.2
30-Aug	10	34.5	25.1	32.8	28.5	29.0	24.9	56.1	22.6
31-Aug	11	25.3	22.7	27.3	25.2	22.1	21.3	72.5	4.9
1-Sep	12	27.9	20.6	27.2	23.8	22.6	17.7	68.6	17.8
2-Sep	13	28.7	24.4	27.3	25.6	24.2	23.4	73.0	14.7
3-Sep	14	34.7	24.6	30.9	27.2	29.2	23.8	69.4	20.3
4-Sep	15	34.7	24.0	32.1	27.2	29.2	22.6	64.3	21.5
5-Sep	16	35.1	25.9	32.5	29.7	29.1	25.1	67.8	22.0
6-Sep	17	24.7	24.8	25.1	29.4	20.7	23.3	75.6	19.8
7-Sep	18	28.1	23.2	29.7	25.9	24.1	20.2	72.4	11.9
8-Sep	19	24.2	18.3	25.6	22.1	19.1	14.7	67.4	8.0
9-Sep	20	21.7	16.9	22.7	20.2	14.3	13.0	74.3	5.0
10-Sep	21	22.0	19.8	21.9	21.2	16.7	16.7	78.1	4.3
11-Sep	22	25.1	23.3	23.7	23.4	20.9	21.8	76.9	8.5
12-Sep	23	26.4	23.7	25.2	24.3	23.8	22.0	81.7	6.9
13-Sep	24	25.7	22.3	25.2	23.7	22.3	20.4	79.2	7.8
14-Sep	25	26.0	20.5	25.1	22.8	21.5	20.6	74.8	12.8
15-Sep	26	28.3	20.0	26.0	23.0	22.7	17.3	69.2	19.9
Average		29.1	22.5	28.1	24.6	23.9	20.6	68.9	15.5
St. Dev.		4.3	2.9	3.2	3.2	4.2	3.7	7.0	6.5

Observing the growth patterns during Experiment 1, the lettuce plants (Black Seeded Simpson, leaf lettuce) grown under high temperature showed severe heat stress symptoms, such as fast growth, elongated stems and internodal distance, etiolation of pale-colored and thinner leaves, and early bolting. Around 20 DAT, many lettuce plants started to collapse due to the stress symptoms, and thus the experiment was terminated. The bok choy plants (Asian Delight, white stem type bok choy) had visually longer and thicker stems (data not recorded), faster growth and early bolting (Figure 6). The root morphology of both the lettuce and bok choy plants remained normal. This phenomenon was consistent and no visually significant differences among each Si treatment group was observed.

Fig. 6. Experiment 1: Visual comparison of lettuce ‘Black Seeded Simpson’ (left) and bok choy ‘Asian Delight’ (right) plants from the 75 ppm Si treatment during the fourth harvest (Aug. 26 2017, 20 DAT). It was visually apparent that the lettuce plants had pale leaves, elongated stems and internodal distance. The bok choy plants had longer and thicker stems and showed signs of early bolting.

During Experiment 1, the plant height of lettuce and bok choy was recorded every day and the growth curve is shown in Figure 7. For both lettuce and bok choy, there were no statistically significant differences in shoot height when comparing 0, 25 and 75 ppm Si treated plants at the end of the experiment.

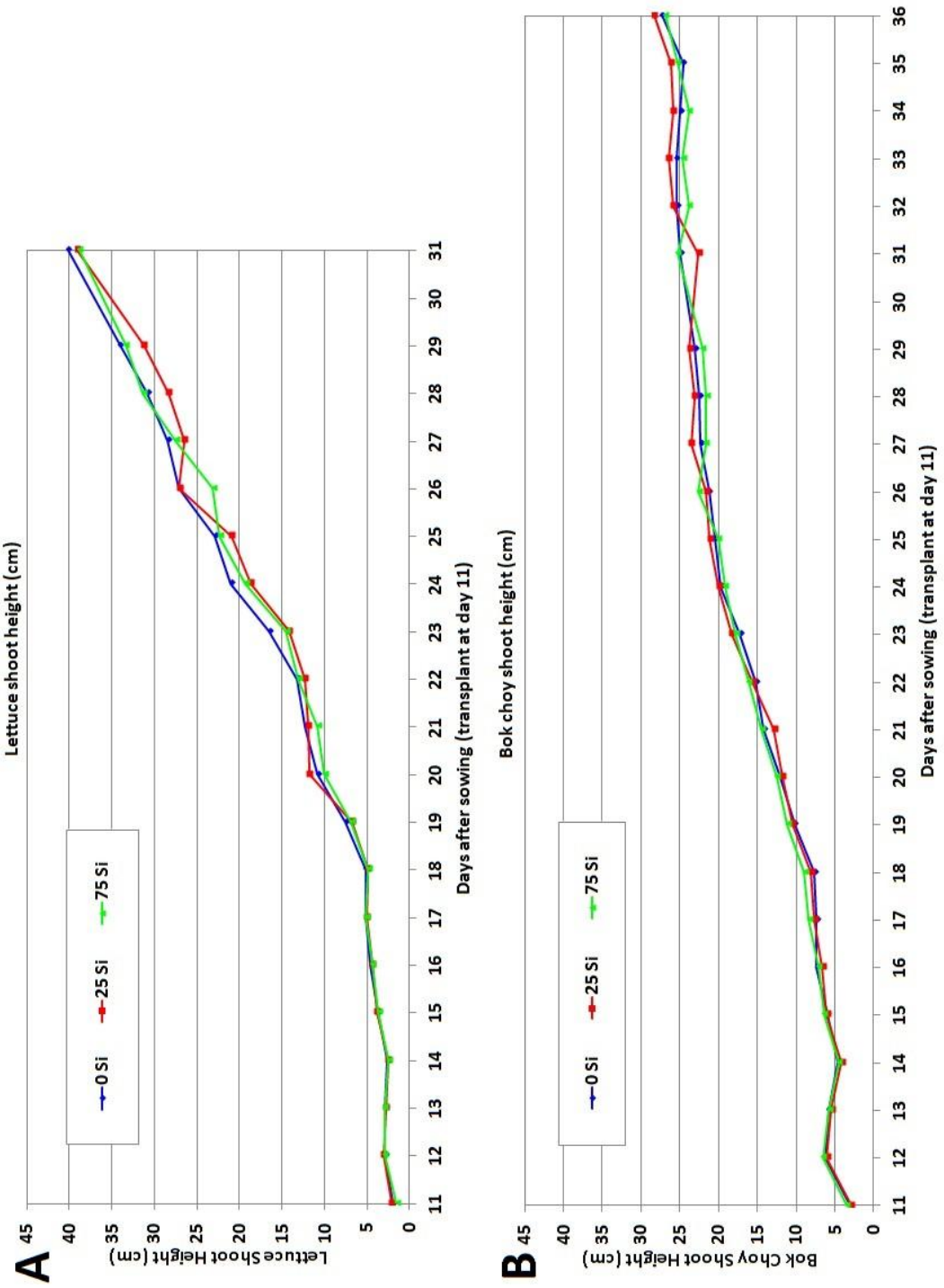


Fig. 7. Lettuce and bok choy heat Experiment 1: Average shoot height for the control, 25 and 75 ppm Si treatments applied to lettuce 'Black Seeded Simpson', A) and bok choy 'Asian Delight', B) plants across the entire growth period [20 days after transplant (DAT) for lettuce and 25 DAT for bok choy]. Shoot height was measured daily after transplant. n = 12.

For lettuce, shoot fresh weight of the control and the 75 ppm Si treatment were very similar and were both lower than the 25 ppm Si treatment but the differences were statistically non-significant. Similar trends were also observed for shoot dry weight and the number of true leaves. Root length, fresh and dry weight also showed similar trends, whereas the 25 ppm treatment group had marginally but statistically non-significant higher values compared with the control and 75 ppm Si treatments, which were very similar (Table 5). Overall, all growth parameters were not different (statistically non-significant) among the control, 25 and 75 ppm Si treatments. The Si treatments did not reduce the heat stress symptoms, nor did they increase the marketable yield of 'Black Seeded Simpson' leaf lettuce when grown under heat environment.

Table 5. Lettuce and bok choy heat Experiment 1: growth parameters for the control, 25 and 75 ppm Si treatments for lettuce plants 'Black Seeded Simpson' at the final harvest (20 DAT, n = 6). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	40.17	26.0	44.5	2.88	1.69	0.11	12.7
SD	4.12	6.00	6.49	1.67	0.37	0.06	1.63
25 Si	39.00	30.7	51.6	4.27	2.09	0.16	14.3
SD	3.16	7.66	8.88	1.73	0.49	0.07	2.50
75 Si	38.83	23.8	44.8	2.85	1.70	0.11	13.7
SD	2.99	8.04	17.83	1.57	0.75	0.06	2.34
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.595	0.269	0.143	0.187	0.083	0.187	0.207
Ctrl vs 75 Si	0.537	0.609	0.967	0.975	0.992	0.975	0.413
25 Si vs 75 Si	0.927	0.163	0.426	0.167	0.252	0.167	0.644

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

For bok choy, the differences in shoot fresh weight between each Si treatment were more obvious. The 25 ppm Si treatment resulted in the highest fresh weight, followed by the control and the 75 ppm Si treatment. The differences between the control and the 25 or 75 ppm Si treatments were non-significant, but the difference between the 25 and 75 ppm Si treatments were close to statistical significance for the shoot and root fresh weights. A similar trend was also observed for shoot dry weight. During the final harvest, the roots of adjacent plants were entangled and difficult to separate. The 75 ppm Si treatment resulted in the longest roots, closely followed by the 25 ppm Si treatment. The control treatment had the shortest roots and the differences between the control and 75 ppm Si treatment were statistically significant. However, different trends were observed for root fresh and dry weight data: the 75 ppm Si treatment had the highest root mass, followed by the control and the 25 ppm Si treatment. These differences among the treatments were all statistically non-significant. The number of true leaves were very similar for each treatment (Table 6). Overall, the Si treatments used for ‘Asian Delight’ white stem bok choy did not provide significant benefits in reducing heat stress symptoms, nor did they increase the marketable yield.

Table 6. Lettuce and bok choy heat Experiment 1: growth parameters for the control, 25 and 75 ppm Si treatments for bok choy plants 'Asian Delight' at the final harvest (25 DAT, n = 6). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	27.33	42.2	64.5	3.09	3.51	0.17	11.5
SD	2.07	14.18	16.82	1.64	1.00	0.09	0.55
25 Si	28.33	57.2	76.0	2.14	3.91	0.14	12.2
SD	2.42	26.59	14.01	1.75	0.58	0.09	0.75
75 Si	26.83	58.3	56.1	4.66	3.11	0.26	11.7
SD	1.94	8.41	13.47	4.68	0.69	0.24	0.82
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.460	0.259	0.226	0.358	0.426	0.497	0.113
Ctrl vs 75 Si	0.675	**0.043**	0.503	0.467	0.440	0.431	0.688
25 Si vs 75 Si	0.265	0.922	**0.050**	0.261	0.057	0.281	0.296

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

Both lettuce and bok choy absorbed Si in small quantities in their shoots and roots under heat. As Si non-accumulators, the amount of Si absorbed by lettuce and bok choy in shoots and roots was very minimal (0.01 – 0.07%). The changes in mineral composition in the lettuce and bok choy were very similar when treated with Si. The amount of nitrogen increased with the addition of Si, the result of using nitric acid to control the pH of the nutrient solution. Phosphorus levels in shoots and roots were not very different among the treatments. The level of potassium and calcium in the shoots and roots increased as the Si level increased, the result of the additional potassium that was added to the Si treatments (from K_2SiO_3). In both lettuce and bok choy, when the Si treatment level is increased, less magnesium, sulfur, iron, and copper was absorbed by the shoots and roots. The amount of manganese in plant tissue also increased as more Si was added. This phenomenon was more apparent in lettuce than in bok choy. The amount of molybdenum in both shoots and roots of lettuce decreased as more Si was added, but in bok choy the amount of the molybdenum increased (Figures 8, 9, 10 and 11). Overall, the Si amendments did not substantially affect the mineral composition of leaf lettuce and white stem bok choy plants when grown under heat environment and amended with Si.

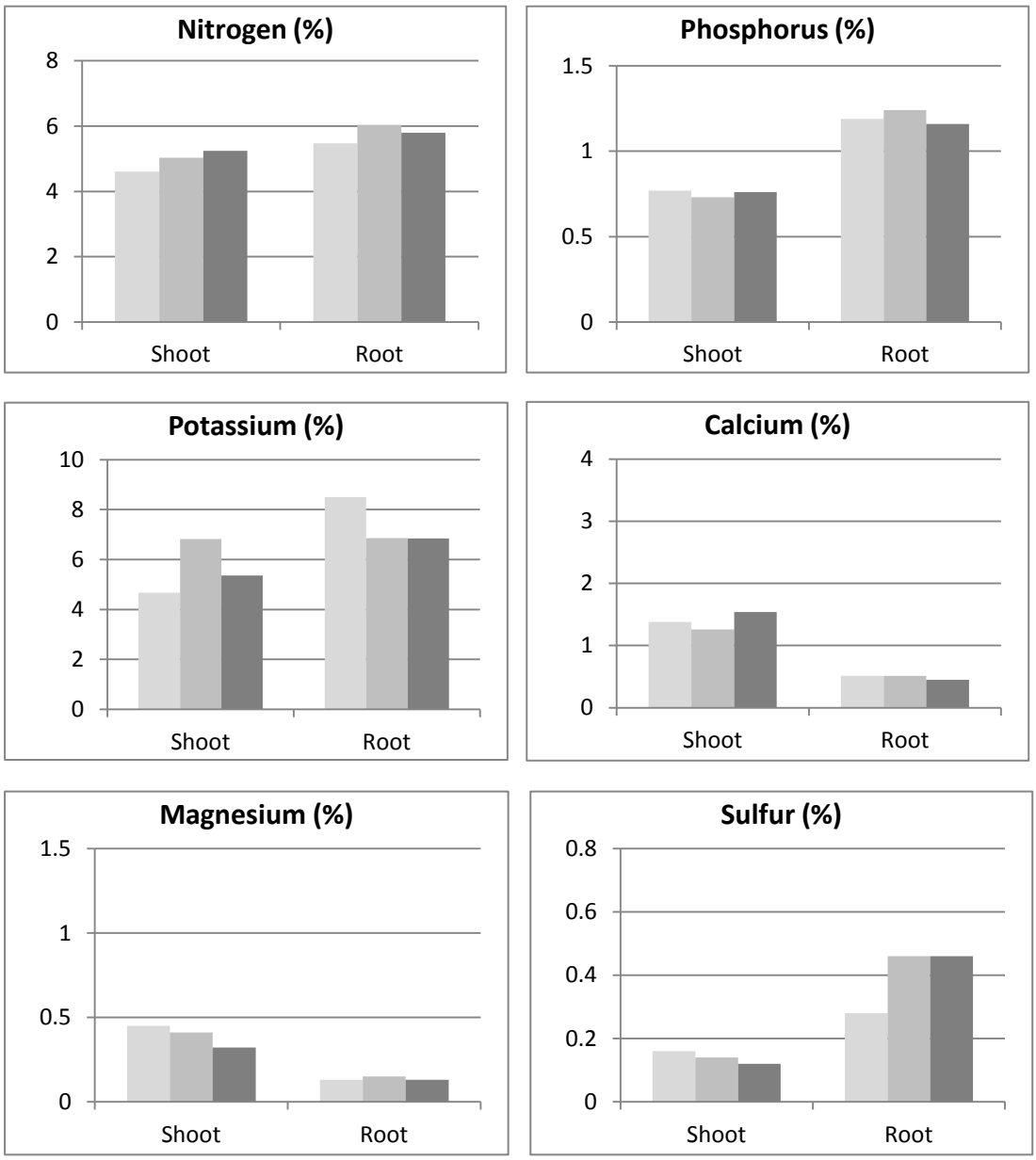


Fig. 8. Lettuce and bok choy heat Experiment 1: macronutrient tissue analysis of lettuce 'Black Seeded Simpson' shoots and roots harvested at 20 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

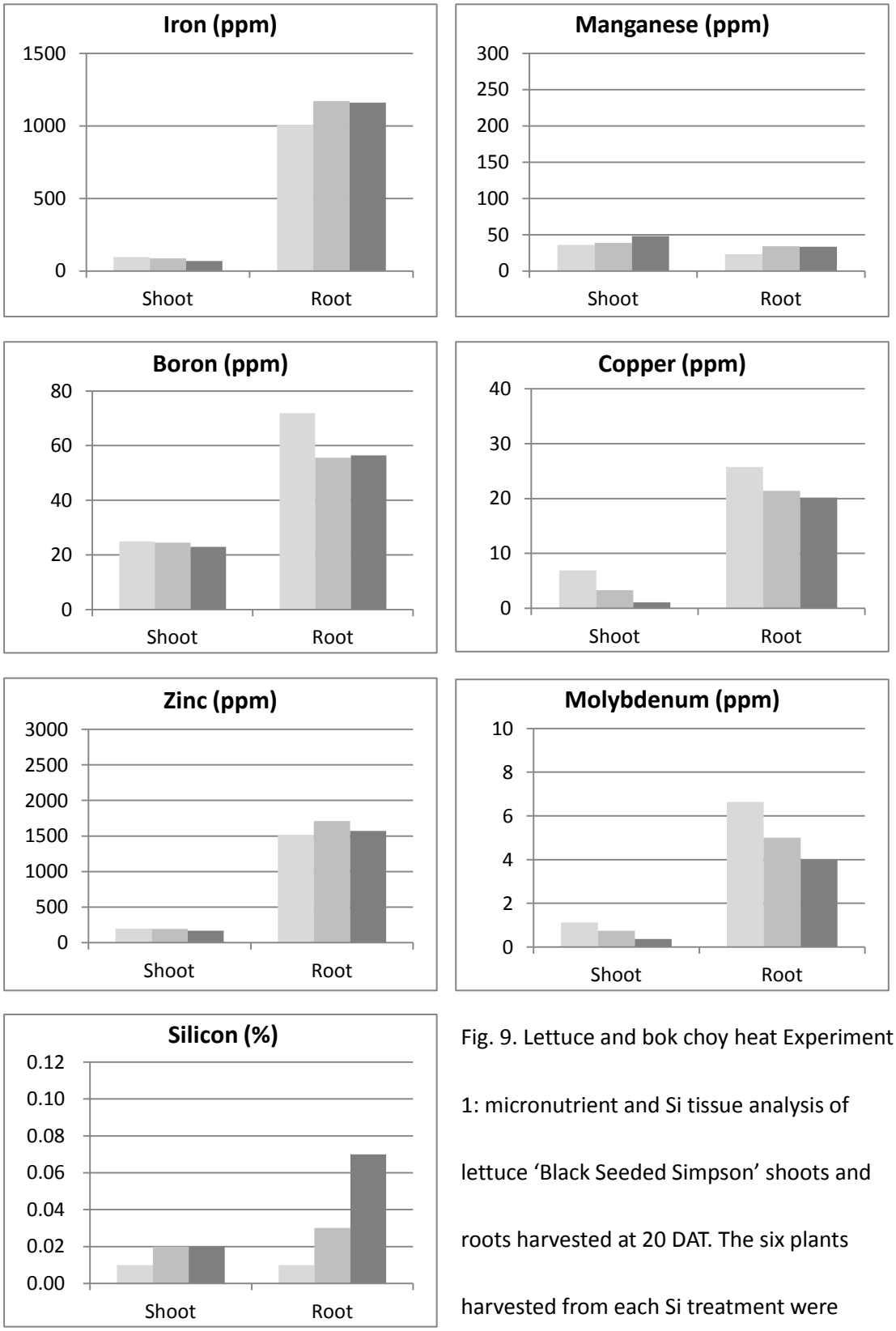


Fig. 9. Lettuce and bok choy heat Experiment 1: micronutrient and Si tissue analysis of lettuce 'Black Seeded Simpson' shoots and roots harvested at 20 DAT. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

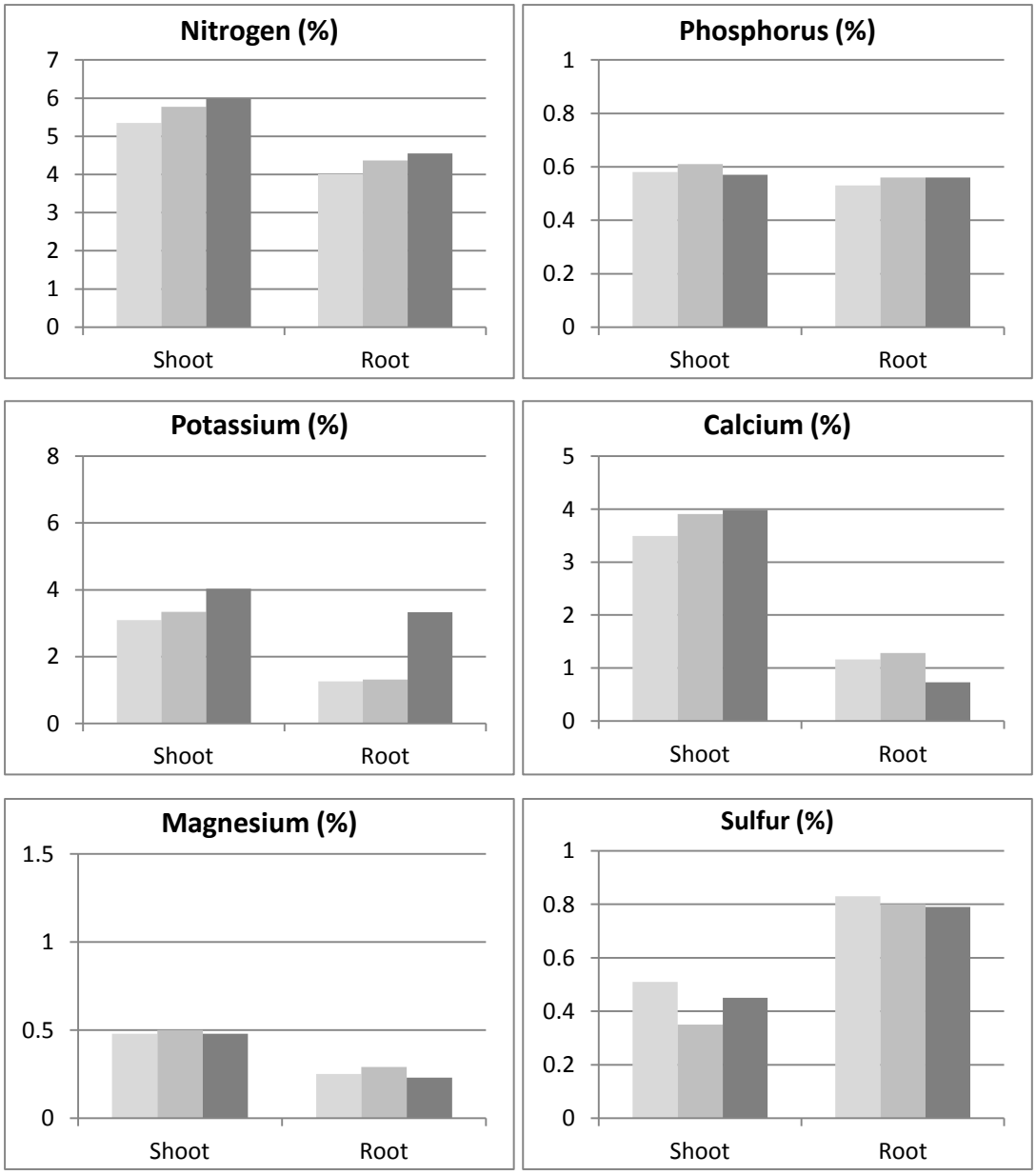


Fig. 10. Lettuce and bok choy heat Experiment 1: macronutrient mineral composition analysis of bok choy 'Asian Delight' shoots and roots harvested at 25 DAT. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

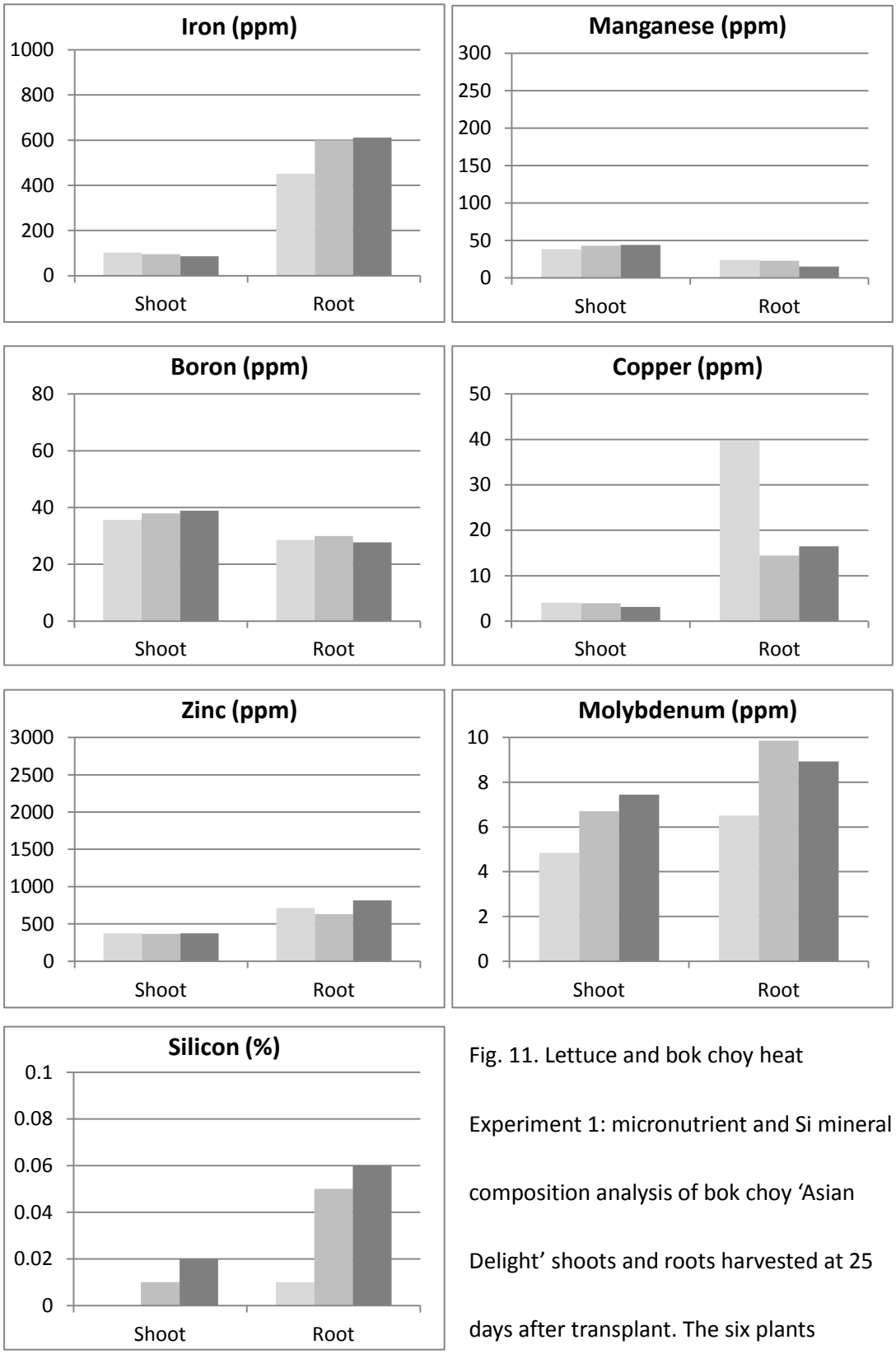


Fig. 11. Lettuce and bok choy heat Experiment 1: micronutrient and Si mineral composition analysis of bok choy 'Asian Delight' shoots and roots harvested at 25 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

During Experiment 2, the heat stress symptoms were less severe for the ‘Rex’ butterhead lettuce. The general morphology of the lettuce plant remained normal. The plant did not exhibit extended stem length and remained its normal shape (basal rosette). The size and thickness of the leaves was similar to lettuce grown under normal conditions. The ‘Black Summer’ dark green bok choy showed severe symptoms of heat stress, including longer and thicker stems, and a generally taller statue. The root morphology of both lettuce and bok choy remained normal. All these phenomena were consistent and no significant visual differences were observed among the Si treatments (Figure 12).

Fig. 12. Visual comparison of lettuce ‘Rex’ (left) and bok choy ‘Black Summer’ (right) plants from the 75 ppm Si treatment harvested at the fourth harvest (Sep. 16, 2018, 20 days after transplant) of Experiment 2. The morphology of the lettuce plants remained normal after the heat exposure. The bok choy plants showed typical heat stress symptoms such as longer and thicker stems and a generally taller statue.

At the end of Experiment 2 (25 DAT), there were no statistically significant differences in shoot sizes among all Si treatments. The shoot height and width were recorded every other day, and the growth curves are presented in Figures 13 and 14. The shoot height and width results were very similar among all Si treatments. For bok choy, the 25 ppm Si treatment had statistically significantly taller plants than the control and 75 ppm Si treatments, but the shoot widths were not significantly different among all treatments.

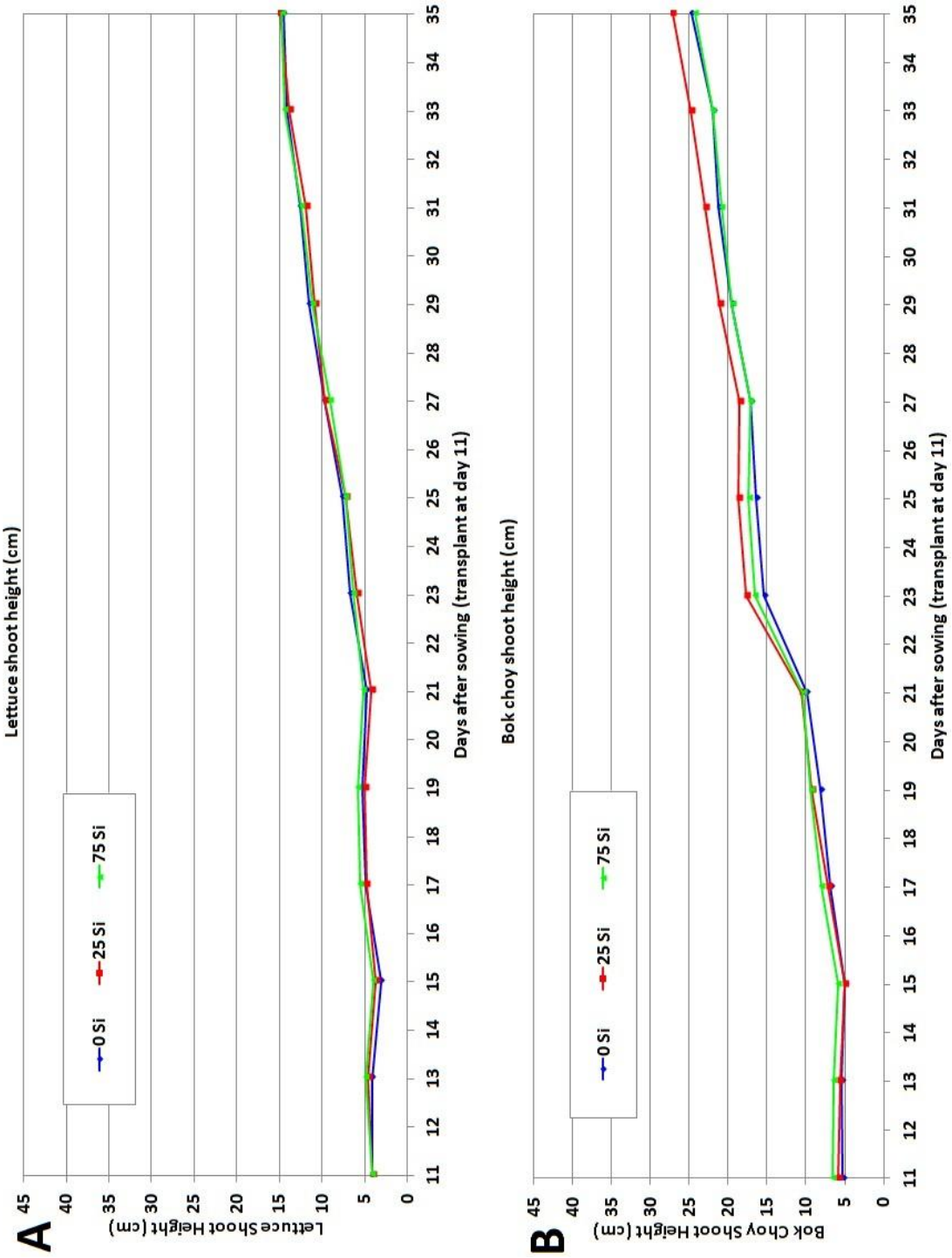


Fig. 13. Lettuce and bok choy heat Experiment 2: Average shoot height for the control, 25 and 75 ppm Si treatments applied to lettuce 'Rex' (A) and bok choy 'Black Summer' (B) plants across the entire growth period (25 days after transplant for both lettuce and bok choy). Shoot height was measured every other day after transplant. n = 12.

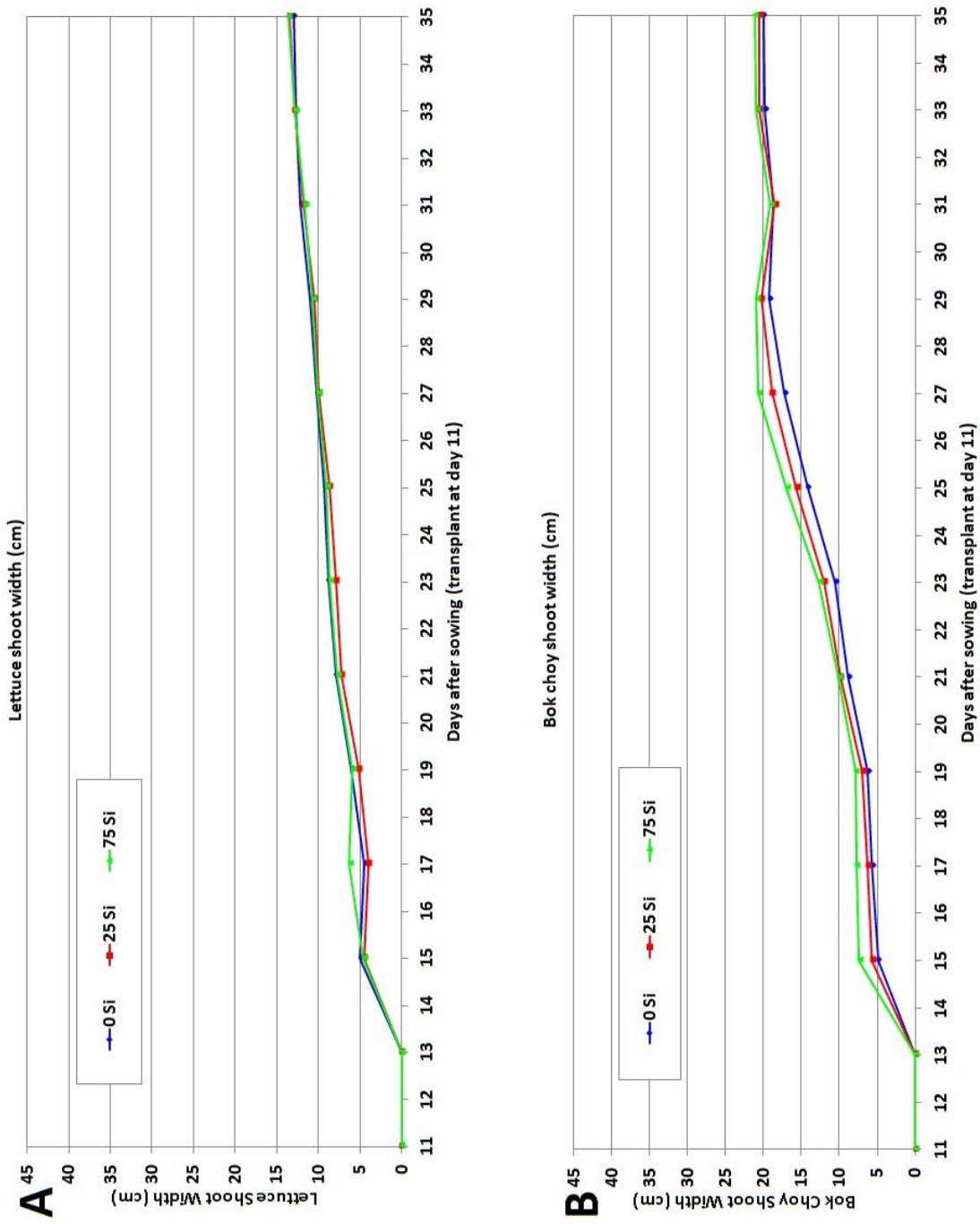


Fig. 14. Lettuce and bok choy heat Experiment 2: Average shoot width for the control, 25 and 75 ppm Si treatments applied to lettuce 'Rex', (A) and bok choy 'Black Summer' (B) plants across the entire growth period (25 days after transplant for both lettuce and bok choy). Shoot width was measured every other day after transplant. n = 12.

For lettuce, the differences in shoot fresh weight between each treatment were very minor. Shoot fresh weight of the control treatment was only marginally heavier than the 25 ppm Si treatment and the 75 ppm Si treatment. Similar trends were also observed for shoot dry weight and the numbers of true leaves. There were no statistically significant differences in shoot growth parameters among all Si treatments. The root length of the 25 ppm Si treatment was significantly higher than the control and the 75 ppm Si treatment, but the root fresh and dry weights were very similar among all treatments. This was due to the fact that the roots were tangled, and separation of the roots during harvest reduced the accuracy of the root length measurements. There were no significant differences in the root fresh and dry weights among all treatments (Table 7). Overall, the Si treatments did not show much effect on 'Rex' butterhead lettuce when grown under heat environment.

Table 7. Lettuce and bok choy heat Experiment 2: growth parameters for the control, 25 and 75 ppm Si treatments for lettuce plants 'Rex' at the final harvest (25 DAT, n = 12). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**SW**	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)			(g)				(-)
0 Ctrl	14.46	12.99	56.7	103.3	6.28	4.01	0.31	26.6
SD	1.07	1.33	7.21	12.42	1.03	0.50	0.06	3.03
25 Si	14.93	13.42	64.7	95.0	5.94	3.91	0.29	26.3
SD	1.14	1.45	6.53	12.83	0.95	0.51	0.05	1.97
75 Si	14.73	13.64	55.7	95.5	5.83	4.11	0.30	26.8
SD	1.26	1.55	7.85	13.97	1.49	0.63	0.07	2.17
Contrast (*P*-value a = 0.05)								
Ctrl vs 25 Si	0.303	0.464	**0.009**	0.124	0.403	0.628	0.343	0.813
Ctrl vs 75 Si	0.582	0.284	0.748	0.167	0.398	0.680	0.599	0.819
25 Si vs 75 Si	0.675	0.718	**0.006**	0.926	0.837	0.404	0.774	0.560

[x]SH = shoot height, SW = shoot width, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

For bok choy, shoot fresh and dry weights followed a similar trend as the shoot height: the 25 ppm Si treatment had the highest values. The differences between the control and the 75 ppm Si treatment were small. The 25 ppm Si treatment also had the highest root length, and root fresh and dry weights. The control treatment had the next highest values. The 75 ppm Si treatment had the lowest root length, and root fresh and dry weights (Table 8). Overall, the Si treatments applied to 'Black Summer' dark green bok choy did not provide significant benefits in reducing heat stress symptoms, nor did they increase the marketable yield.

Table 8. Lettuce and bok choy heat Experiment 2: growth parameters for the control, 25 and 75 ppm Si treatments for bok choy plants 'Black Summer' at the final harvest (25 DAT, n = 12). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**SW**	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)			(g)				(-)
0 Ctrl	24.75	20.00	66.8	164.9	6.85	8.09	0.36	14.1
SD	2.22	1.54	11.41	33.68	3.01	1.42	0.12	1.56
25 Si	27.17	20.50	70.8	212.0	7.36	8.64	0.38	14.6
SD	2.21	2.28	17.08	46.90	5.56	1.69	0.24	1.08
75 Si	24.25	21.17	66.1	185.2	5.79	7.55	0.23	14.1
SD	1.66	2.04	18.29	50.15	3.11	1.95	0.12	2.02
Contrast (*P*-value a = 0.05)								
Ctrl vs 25 Si	**0.014**	0.536	0.499	**0.010**	0.782	0.393	0.864	0.374
Ctrl vs 75 Si	0.539	0.129	0.916	0.260	0.404	0.455	**0.012**	1.000
25 Si vs 75 Si	**0.002**	0.458	0.518	0.189	0.403	0.159	0.077	0.460

[x]SH = shoot height, SW = shoot width, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

The absorption of Si and changes in mineral composition in ‘Rex’ butterhead lettuce and ‘Black Summer’ dark green bok choy were very similar compared to Experiment 1. A small amount of Si was absorbed by the plants. The level of nitrogen in the tissue of both lettuce and bok choy increased with the addition of Si, as a result of adding nitric acid to the nutrient solution for pH control. Phosphorus levels in the shoots and roots of lettuce and bok choy were not substantially different among all treatments. The amount of potassium and calcium in the shoots and roots of lettuce and bok choy increased when Si concentration increased, as a result of using K_2SiO_3 as the Si source. When the Si concentration increased, less magnesium, sulfur, iron, and copper were absorbed by the shoots and roots of lettuce, but this trend was not observed in bok choy. The concentration of manganese in both lettuce and bok choy was increased as more Si was added. The concentration of molybdenum in lettuce shoots and roots decreased as more Si was added, this trend was reversed in bok choy (Figures 15, 16, 17 and 18). Overall, the Si treatments did not substantially affect the mineral composition of both cultivars of lettuce and bok choy when grown under heat environment during both experiments.

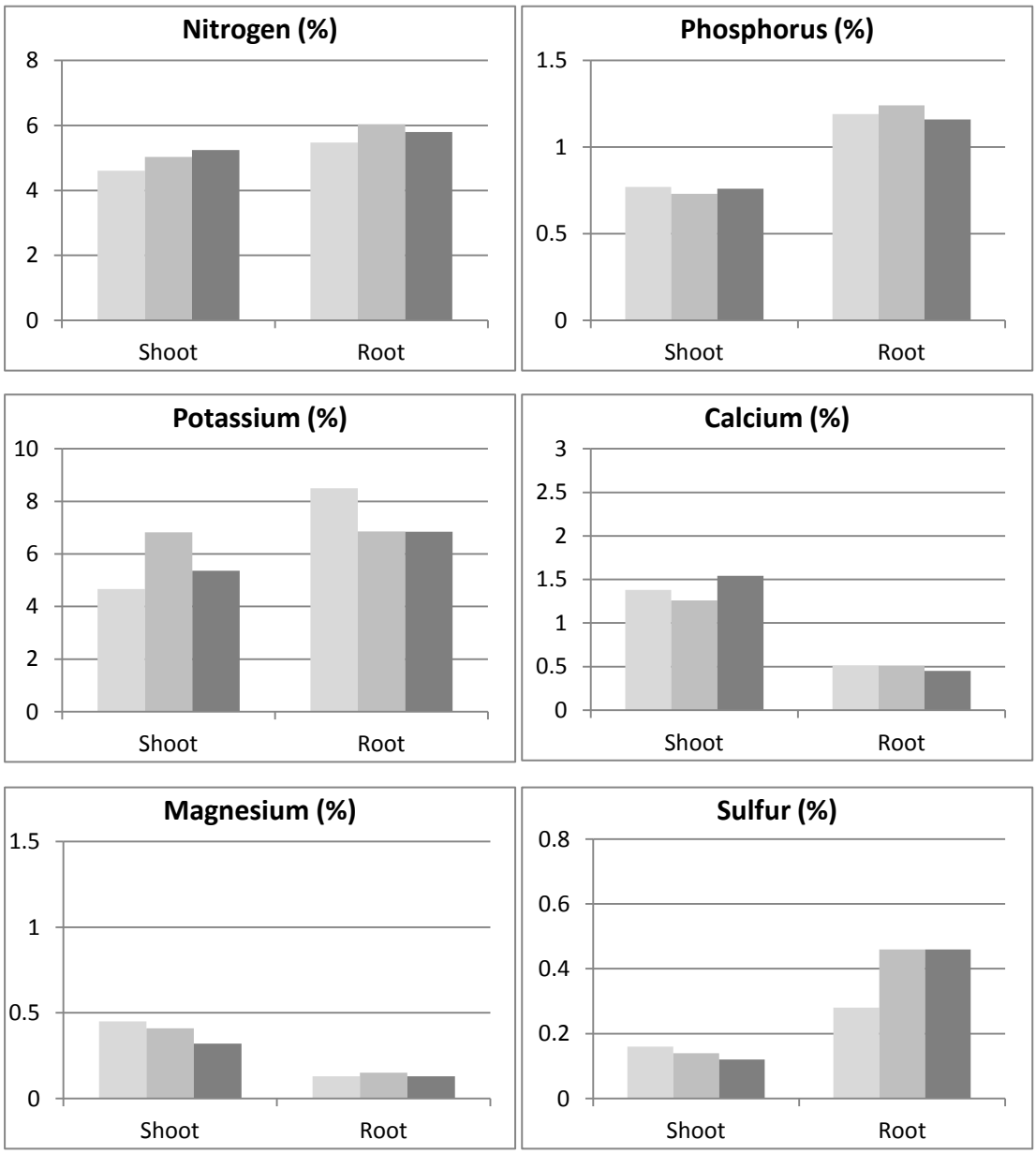


Fig. 15. Lettuce and bok choy heat Experiment 2: macronutrient tissue analysis of lettuce 'Rex' shoots and roots harvested at 25 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

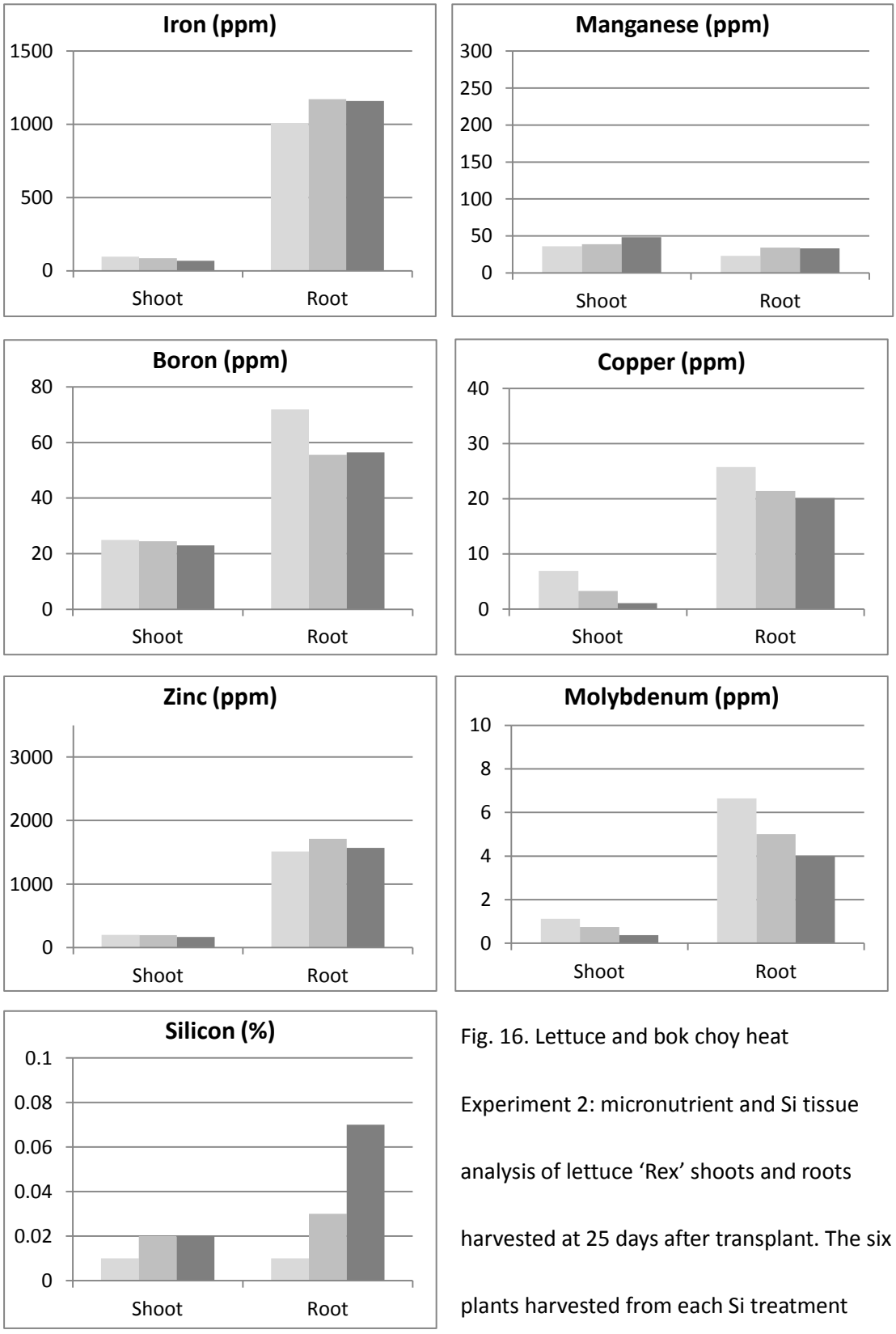


Fig. 16. Lettuce and bok choy heat Experiment 2: micronutrient and Si tissue analysis of lettuce 'Rex' shoots and roots harvested at 25 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

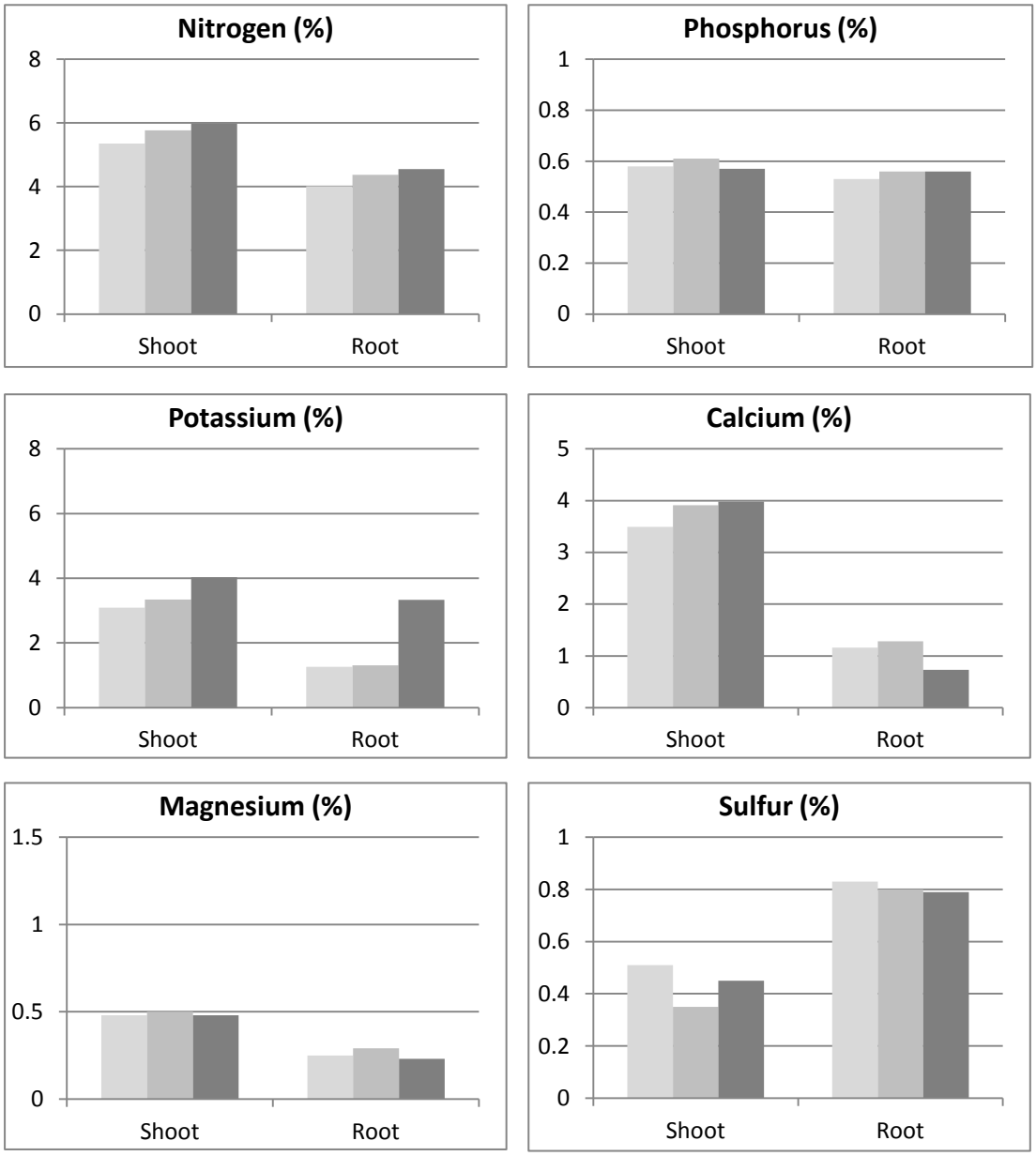


Fig. 17. Lettuce and bok choy heat Experiment 2: macronutrient mineral composition analysis of bok choy 'Black Summer' shoots and roots harvested at 25 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

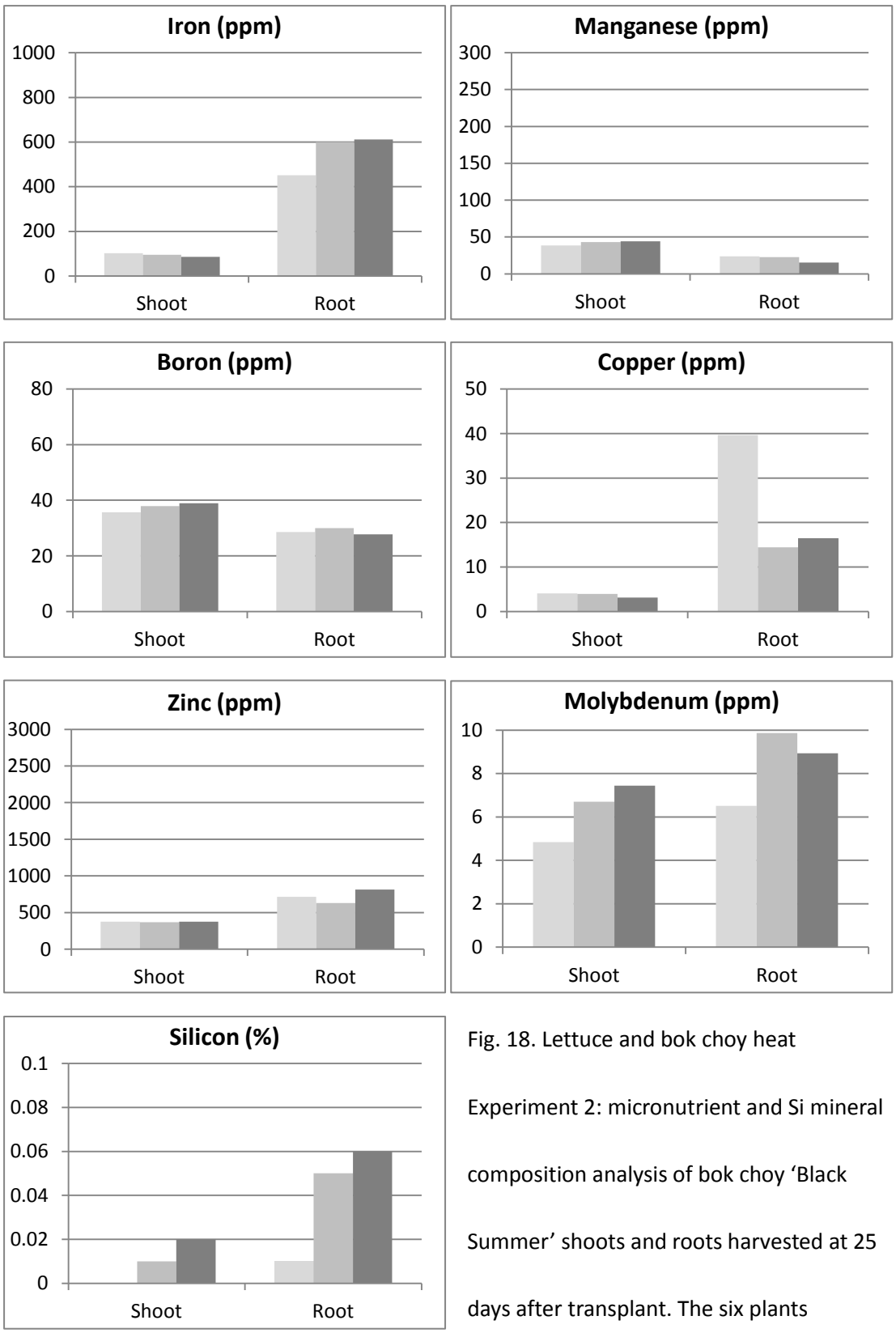


Fig. 18. Lettuce and bok choy heat Experiment 2: micronutrient and Si mineral composition analysis of bok choy 'Black Summer' shoots and roots harvested at 25 days after transplant. The six plants harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

Basil grown under lower temperature conditions

The basil cold experiments were conducted during the winter months (Experiment 1 seeding date: Jan. 19, 2018, end date: Feb. 24, 2018; Experiment 2 seeding date: Mar. 18, 2018, end date: May 3, 2018; Experiment 3 seeding date: Feb. 11, 2019, end date: Mar. 28, 2019). The greenhouse was equipped with two natural gas heaters and was able to maintain the temperature set point during both day and night after transplant. The greenhouse air and nutrient solution temperatures were maintained at 18 °C during both day and night for Experiment 1, and at 23 °C during both day and night for Experiments 2 and 3. The greenhouse environmental data is presented in Figure 19 and Table 9 (Experiment 1), Figure 20 and Table 10 (Experiment 2) and Figure 21 and Table 11 (Experiment 3).

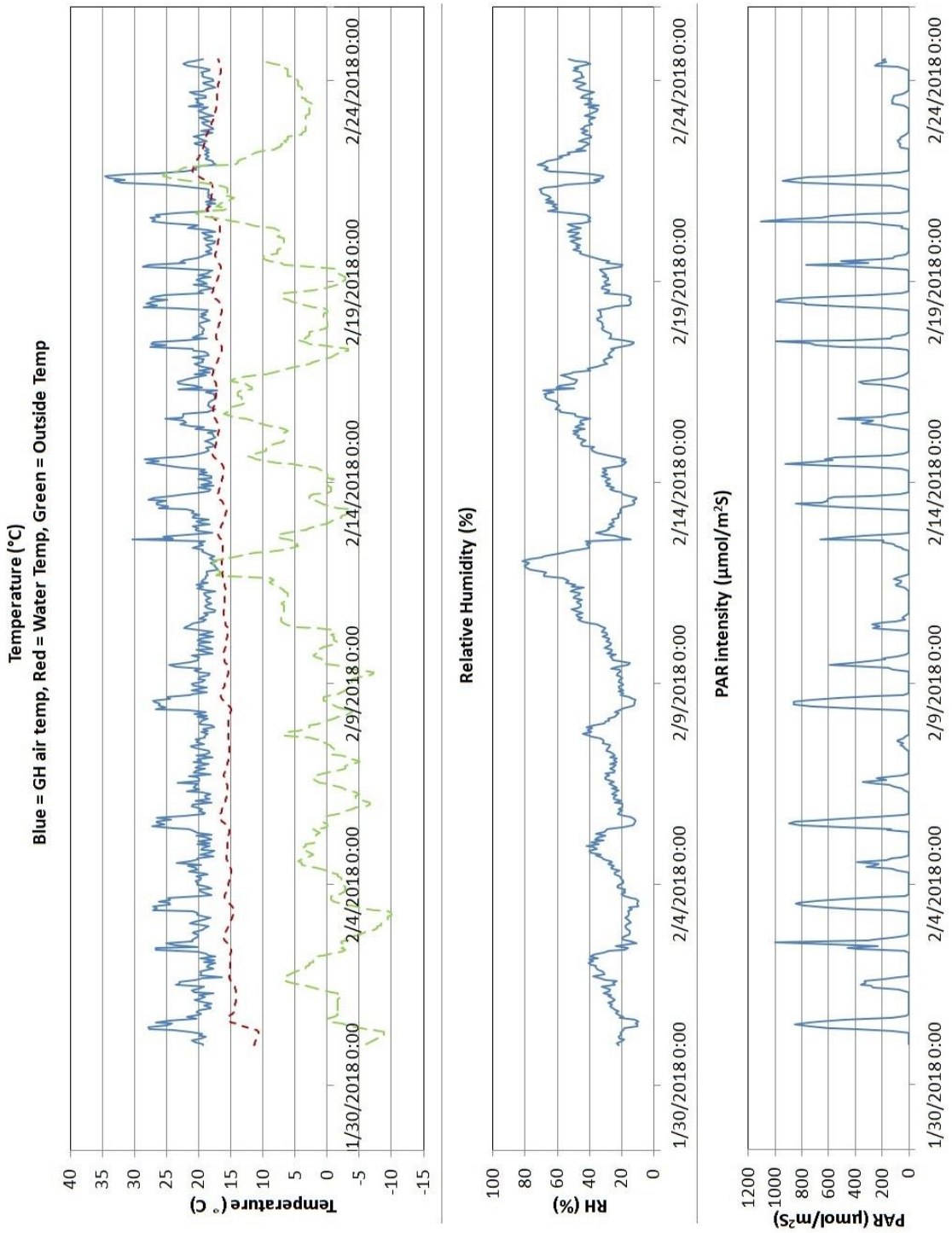


Fig. 19. Basil cold Experiment 1: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). From Jan. 31, 2018, first day after transplant to Feb. 24, 2018 (25 DAT).

Table 9. Basil cold Experiment 1: calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Jan. 31, 2018, first day after transplant to Feb. 24, 2018 (25 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
31-Jan	1	22.6	19.1	13.5	14.2	-3.6	-1.7	20.6	16.0
1-Feb	2	20.0	19.6	14.8	14.9	3.5	3.2	32.5	6.9
2-Feb	3	21.8	19.9	15.5	15.0	-3.2	-8.7	17.7	13.2
3-Feb	4	22.7	19.2	15.3	15.2	-4.0	-2.3	16.9	17.1
4-Feb	5	19.7	19.1	15.4	15.5	2.3	2.4	32.1	5.9
5-Feb	6	22.8	19.5	16.0	15.9	-0.1	-5.4	20.6	17.2
6-Feb	7	20.0	19.5	15.7	15.4	-0.8	-3.6	26.2	5.8
7-Feb	8	19.5	19.3	15.3	15.3	0.2	0.3	32.2	1.6
8-Feb	9	23.0	19.7	15.8	15.7	-1.6	-4.2	18.7	17.9
9-Feb	10	20.4	19.1	15.8	15.7	-0.9	-0.9	23.1	9.0
10-Feb	11	19.7	18.4	15.9	15.9	4.9	6.5	43.3	4.5
11-Feb	12	18.9	18.0	16.1	16.3	11.1	16.3	63.9	2.5
12-Feb	13	20.9	19.7	16.5	16.1	6.0	-0.8	31.5	7.8
13-Feb	14	23.0	19.0	16.4	16.4	-0.1	-0.1	22.0	17.0
14-Feb	15	22.1	18.4	17.0	17.1	7.3	7.2	34.3	15.6
15-Feb	16	20.6	18.1	17.3	17.4	11.7	13.4	54.4	7.8
16-Feb	17	20.4	19.6	121.8	16.9	7.3	0.9	43.7	5.7
17-Feb	18	21.6	19.0	16.9	16.6	1.1	0.3	28.0	13.4
18-Feb	19	23.4	19.3	17.2	17.0	3.6	-2.3	25.3	21.0
19-Feb	20	20.6	18.4	17.0	16.9	6.1	6.9	41.0	8.9
20-Feb	21	22.1	18.1	17.7	18.1	14.7	15.7	56.4	14.7
21-Feb	22	25.5	18.2	19.4	20.1	20.8	12.9	55.4	17.2
22-Feb	23	19.3	18.9	18.8	17.7	5.8	3.2	41.4	2.2
23-Feb	24	19.4	18.7	17.1	16.7	3.4	5.2	43.8	3.2
24-Feb	25	20.0		16.6		8.3	6.3	49.4	5.8
Average		21.2	19.0	20.6	16.3	4.2	2.8	35.0	10.3
St. Dev.		1.6	0.6	21.1	1.2	6.0	6.6	13.6	6.0

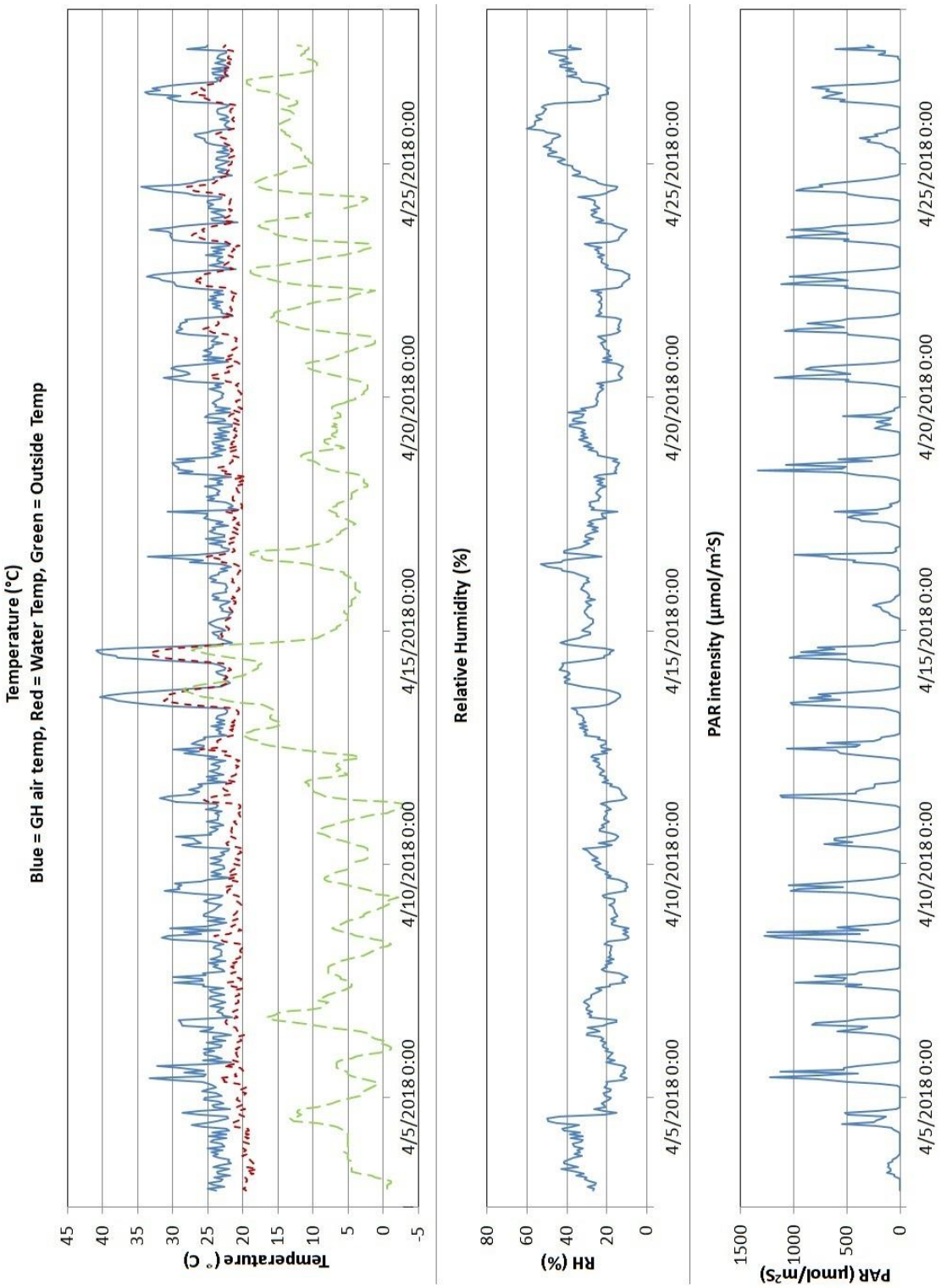


Fig. 20. Basil cold Experiment 2: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). From Apr. 3, 2018, first day after transplant to May 3, 2018 (30 DAT).

Table 10. Basil cold Experiment 2: calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Apr. 3, 2018, first day after transplant to May 3, 2018 (30 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
3-Apr	1	21.4	21.4	19.2	19.7	4.0	5.1	35.5	4.0
4-Apr	2	23.3	22.3	20.4	20.3	9.5	7.6	28.1	11.2
5-Apr	3	24.2	20.8	21.3	20.5	4.3	0.1	17.2	23.9
6-Apr	4	24.0	21.5	21.2	20.8	9.1	8.5	26.5	14.7
7-Apr	5	23.8	20.8	21.0	20.6	6.3	2.9	17.5	16.6
8-Apr	6	24.0	20.4	21.6	20.6	3.7	0.6	14.9	23.9
9-Apr	7	24.1	21.3	21.4	21.0	4.3	3.2	19.5	21.2
10-Apr	8	23.1	20.5	21.2	20.6	5.3	1.0	20.7	15.5
11-Apr	9	25.0	21.1	22.7	21.0	6.9	6.1	19.4	20.5
12-Apr	10	25.1	21.5	22.6	21.2	13.9	15.4	23.9	17.7
13-Apr	11	30.4	22.1	26.3	22.2	22.5	19.9	30.1	21.1
14-Apr	12	30.5	21.5	27.2	22.4	19.6	6.8	29.6	23.0
15-Apr	13	21.7	21.2	21.5	21.0	4.5	4.3	30.2	5.1
16-Apr	14	24.5	21.2	22.0	21.1	12.7	6.3	34.3	12.5
17-Apr	15	23.0	20.9	21.1	20.7	5.8	3.3	24.5	11.1
18-Apr	16	25.1	21.6	21.7	21.2	8.0	7.2	23.6	22.3
19-Apr	17	29.9	20.8	23.4	20.6	18.0	3.3	29.3	7.7
20-Apr	18	25.4	20.8	22.1	21.1	7.3	3.3	18.2	24.2
21-Apr	19	26.4	21.1	23.0	21.4	11.2	8.0	19.6	23.3
22-Apr	20	27.6	21.1	24.0	21.7	13.5	5.8	18.1	24.7
23-Apr	21	27.0	21.2	24.0	21.7	12.9	5.8	21.0	24.6
24-Apr	22	26.2	22.1	23.7	21.7	13.6	11.1	32.9	20.6
25-Apr	23	24.1	22.2	22.2	21.4	13.4	13.5	51.3	7.3
26-Apr	24	28.6	21.8	24.4	22.0	16.0	10.4	32.1	23.8
27-Apr	25	23.3	21.8	21.8	21.4	11.3	10.4	45.4	7.3
28-Apr	26	38.6	22.2	23.8	21.8	15.6	11.5	41.6	18.1
29-Apr	27	25.2	21.3	22.6	21.6	11.2	7.3	29.2	18.1
30-Apr	28	24.3	21.3	22.0	21.3	9.8	7.4	27.6	15.8

(Table 10 continued)

1-May	29	31.0	22.0	26.4	22.3	19.4	15.8	23.4	24.9
2-May	30	34.7	22.8	29.9	23.2	25.6	21.4	25.9	25.4
3-May	31	35.5	24.8	31.4	24.3	27.5	21.9	33.9	23.9
Average		26.5	21.5	23.1	21.4	11.8	8.2	27.3	17.9
St. Dev.		4.1	0.8	2.7	0.9	6.4	5.8	8.5	6.6

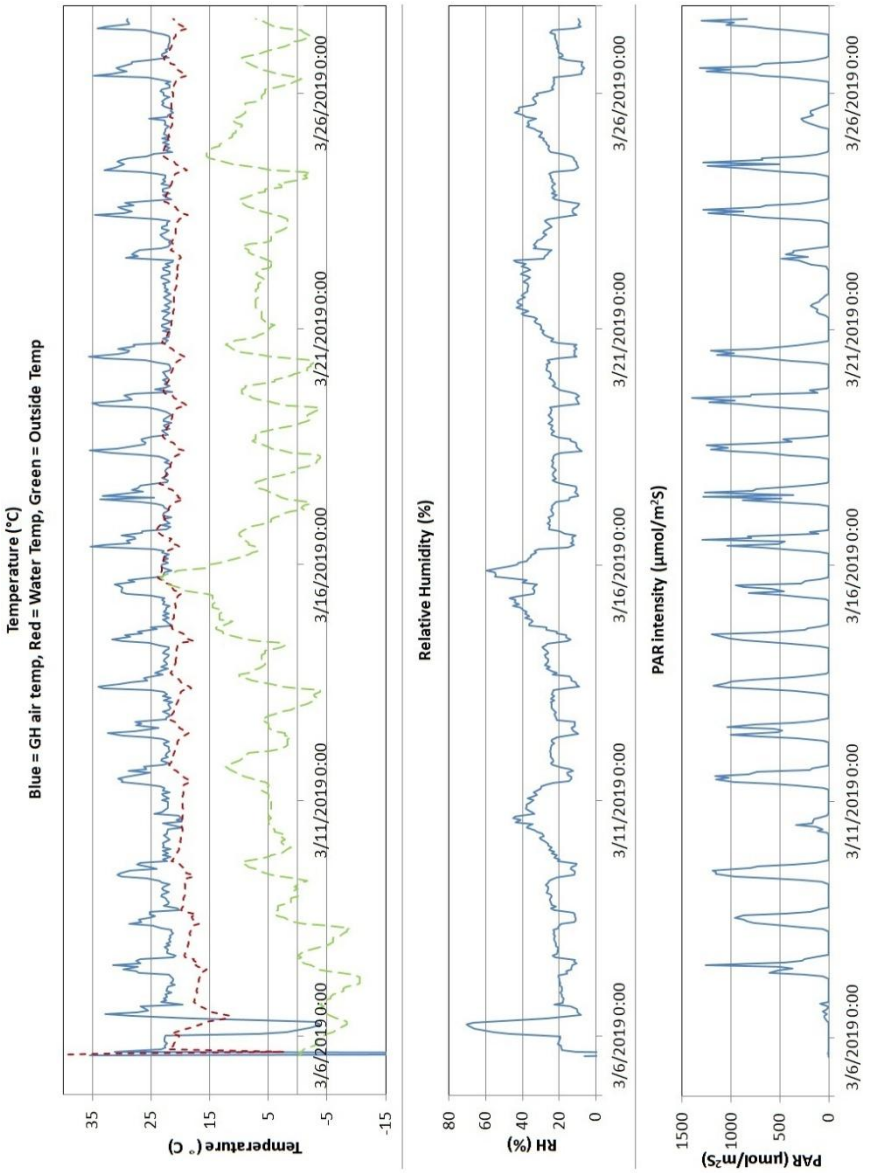


Fig. 21. Basil cold Experiment 3: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). The out of scale data at the beginning indicating the datalogger was installed on the Mar. 5, 2019 and the environmental data was missing for the previous days. Data ranges from Mar. 5, 2019 (6 DAT) to Mar. 27, 2018 (28 DAT). The dip of the greenhouse air temperature on the night of Mar 6th indicates the frost event that happened inside the greenhouse due to an unexpected power outage.

Table 11. Basil cold Experiment 3: calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Mar. 5, 2019 (6 DAT) to Mar 27, 2019 (28 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
5-Mar	6	-48.3	6.9	-33.0	19.6	-1.7	-5.6	2.4	3.8
6-Mar	7	20.6	20.4	15.6	16.1	-5.6	-6.7	21.4	13.5
7-Mar	8	24.6	22.1	17.7	18.4	-3.2	-6.5	19.0	21.6
8-Mar	9	23.9	22.5	18.6	19.2	0.4	0.3	21.0	24.9
9-Mar	10	25.2	22.4	19.7	20.1	5.0	2.2	21.1	10.6
10-Mar	11	21.6	22.6	19.6	19.6	4.3	5.1	36.2	20.6
11-Mar	12	25.6	22.5	20.3	20.9	8.9	5.1	21.7	23.3
12-Mar	13	24.3	22.2	20.6	20.5	3.8	-1.5	19.5	26.2
13-Mar	14	25.0	22.6	20.3	20.7	4.6	5.6	22.1	22.5
14-Mar	15	25.2	22.5	20.4	20.9	10.1	13.8	28.7	18.5
15-Mar	16	26.6	22.6	22.0	22.9	19.7	12.4	42.2	24.3
16-Mar	17	26.4	22.4	22.5	22.3	8.2	2.2	21.5	25.3
17-Mar	18	26.2	22.3	21.8	21.8	3.7	-1.2	19.0	28.7
18-Mar	19	26.4	22.3	21.4	21.6	3.8	-0.9	19.8	26.8
19-Mar	20	26.3	22.3	21.3	21.5	5.0	0.3	20.0	27.0
20-Mar	21	26.7	22.6	21.4	21.7	6.6	5.2	22.7	9.8
21-Mar	22	24.3	22.5	21.0	20.6	11.6	6.5	39.1	7.1
22-Mar	23	23.8	22.4	20.8	20.8	6.5	3.1	31.3	24.4
23-Mar	24	26.6	22.4	21.2	21.5	6.3	2.4	19.8	28.8
24-Mar	25	26.9	22.6	21.6	22.0	9.9	11.6	21.5	11.1
25-Mar	26	22.5	22.4	21.5	21.2	9.2	4.5	33.7	24.2
26-Mar	27	26.8	22.3	21.1	21.3	5.5	1.0	16.5	28.4
27-Mar	28	28.4				8.0	13.5	13.6	
Average		25.0	22.3	20.5	20.7	5.9	3.1	24.7	21.3
St. Dev.		1.8	0.5	1.6	1.5	5.2	5.3	7.4	6.8

Basil cold Experiment 1 (conducted using a constant temperature of 18 °C) resulted in very stunted plant growth across all treatments. At the end of the experiment (30 DAT), the average height of the basil plants was 2.31 cm (control treatment, SD = 0.38 cm), 1.79 cm (25 ppm Si treatment, SD = 0.35 cm) and 3.12 cm (75 ppm Si treatment, SD = 0.71 cm), well below the acceptable size for commercial growers. Although the Si treated plants showed increased plant size, the plants failed to achieve a marketable size by a substantial margin after 30 days of growth at 18 °C. No plant tissue analysis was conducted.

The results from basil cold Experiments 2 and 3 (both conducted at a constant temperature of 23°C) resulted in comparable trends in plant growth (Figure 22). At the end of the experiments (30 DAT), the 75 ppm Si treated plants were 43.6% (significant, Experiment 2) and 28.4% (significant, Experiment 3) taller compared to the control plants (Figure 23). Shoot dry weights of the 75 ppm Si treated plants were 75.5% (significant, Experiment 2) and 25.1% (non -significant, Experiment 3) higher than the control plants. Similar trends were observed for shoot fresh and dry weight and the number of true leaves. The differences in root length, fresh and dry weights were less pronounced than the shoot data. During the final harvest, the fully grown roots of individual plants were tangled with roots from other plants, resulting in difficult root separation. For all measured root parameters at the end of the experiment, the differences among the control, 25 ppm Si treatment and 75 ppm Si treatment plants were non-significant for both experiments (Tables 12 and 13).

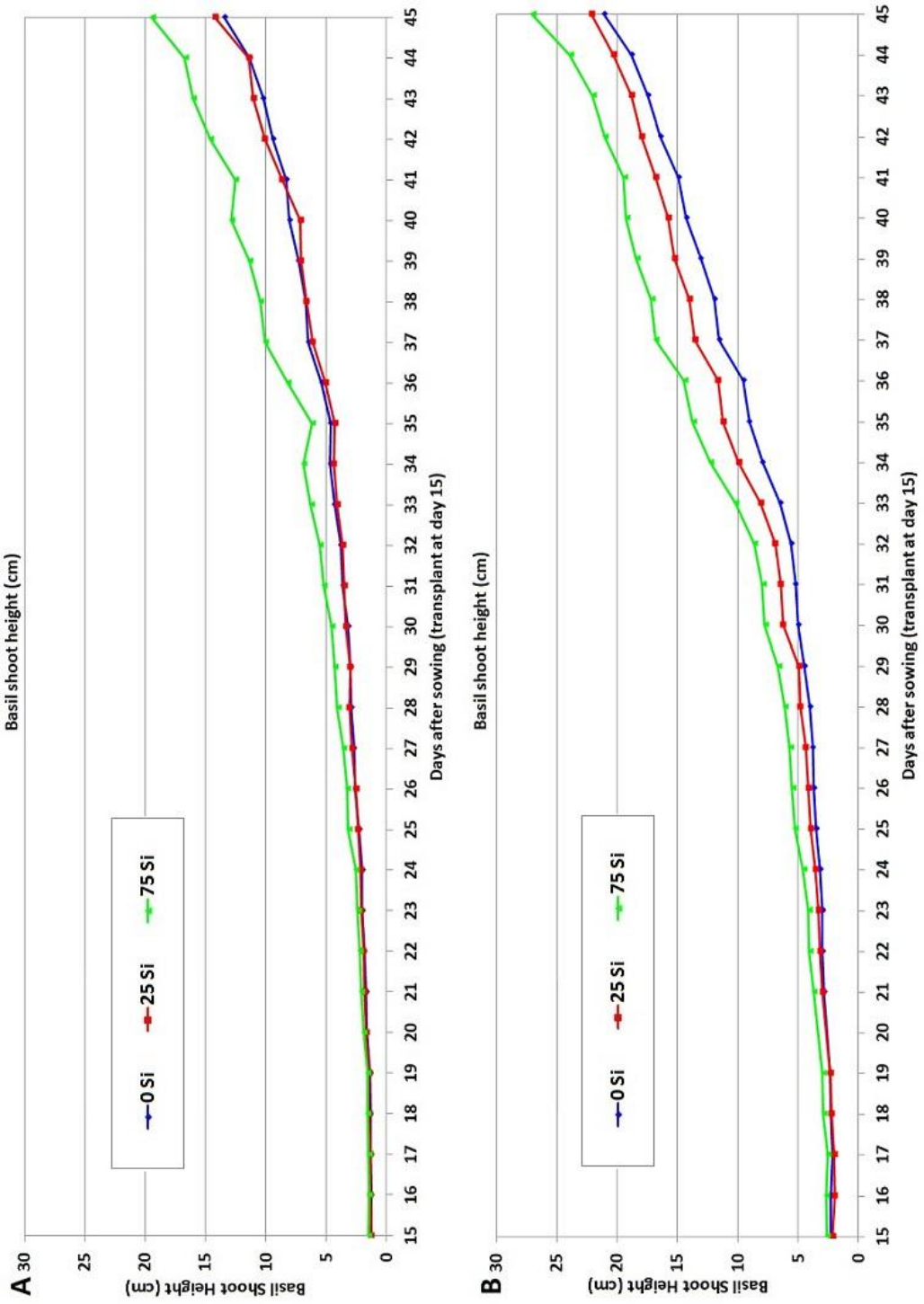


Figure 22: Average shoot height for the control, 25 and 75 ppm Si treatments applied to basil 'Genovese' across the entire growth period (30 DAT for both experiments). A = Experiment 2. B = Experiment 3. Plant height was measured daily after transplant. n = 12.

Table 12. Basil cold Experiment 2: growth parameters for the control, 25 and 75 ppm Si treatments for basil plants 'Genovese' at the final harvest (30 DAT, n = 12, six plants each from two identical ponds). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	13.5	49.9	16.7	8.2	1.53	0.35	51.6
SD	2.95	7.56	5.55	3.20	0.58	0.13	11.89
25 Si	14.2	52.0	19.2	8.9	1.70	0.36	60.7
SD	2.47	5.51	5.63	2.24	0.45	0.08	16.03
75 Si	19.5	54.4	29.9	14.0	2.69	0.56	74.6
SD	2.71	6.90	7.56	6.75	0.71	0.26	12.20
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.605	0.557	0.414	0.643	0.545	0.847	0.251
Ctrl vs 75 Si	**0.002**	0.260	**0.003**	0.070	**0.006**	0.091	**0.004**
25 Si vs 75 Si	**0.003**	0.481	**0.012**	0.096	**0.011**	0.098	0.096

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

Table 13. Experiment 3: growth parameters for the control, 25 and 75 ppm Si treatments for basil plants 'Genovese' at the final harvest (30 DAT, n = 9 for the control treatment, and n = 12 for the 25 and 75 ppm Si treatments, six plants each from two identical ponds). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	21.1	38.0	15.7	7.7	1.31	0.28	25.8
SD	6.02	4.64	6.77	3.31	0.59	0.12	7.31
25 Si	22.2	37.3	16.2	7.1	1.28	0.21	30.2
SD	2.98	3.09	3.80	1.59	0.32	0.06	5.94
75 Si	27.1	34.2	19.9	8.5	1.64	0.34	32.5
SD	6.95	6.04	6.06	3.37	0.59	0.14	6.22
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.644	0.715	0.855	**0.003**	0.881	0.164	0.161
Ctrl vs 75 Si	**0.050**	0.117	0.164	0.066	0.220	0.298	**0.041**
25 Si vs 75 Si	**0.040**	0.125	0.090	0.181	0.078	**0.011**	0.357

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 $mg \cdot L^{-1}$. SD = Standard deviation. Bolded values indicate $P \leq 0.05$.

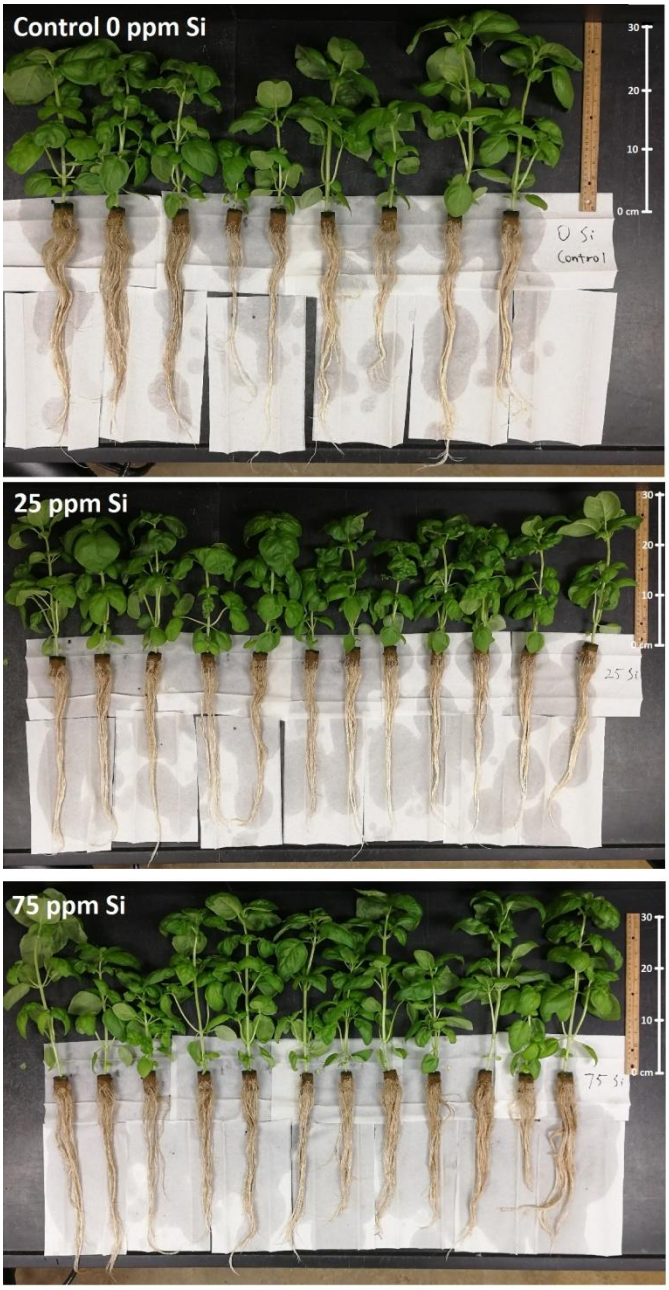


Fig. 23. Visual comparison of basil plants harvested at the end of the growth period (30 DAT) of basil cold Experiment 3. The average shoot and root length for the control treatment: 21.1 and 38.0 cm; the 25 ppm Si treatment: 22.2 and 37.3 cm; and the 75 Si treatment: 27.1 and 34.2 cm. Twelve plants were harvested from the 25 and 75 ppm Si treatments. Only nine plants were harvested from the control treatment due to plant death from an unexpected frost event at 6 DAT. Statistical analysis of the differences are illustrated in Table 13.

During Experiment 3, the greenhouse experienced an unexpected power outage for 7 hours during the night of the 6 DAT, and the air temperature inside the greenhouse dropped from 23°C to -1°C (21°C to 11°C for the nutrient solution temperature). Plants from all treatments had between 4 to 6 true leaves. Interesting cold hardiness results were observed afterward. Nineteen out of 116 (total of 128 plants, minus the 12 plants from the first harvest on the 5 DAT) plants from the control treatment showed severe frost damage symptoms. The entire shoot became water soaked and turned dark green, started to rot and never recovered. Most interestingly, only one plant from the 25 ppm Si treatment eventually died from the frost damage, and every plant from the 75 ppm Si treatment survived the frost event (Figures 24A and B). Some of the surviving plants showed less severe frost damage: the tip of the plant and newly emerging leaves had parts turned brown (Figure 24C). This was observed on 42 (out of 97 for control), 28 (out of 115 for 25 ppm Si) and 57 (out of 116 for 75 ppm Si) surviving plants. During the successive growing stages, all surviving plants exhibited normal morphology of the newly developed leaves and the rest of the plant, with only the one pair of leaves damaged during the frost event exhibiting a deformed shape (Figure 24D).

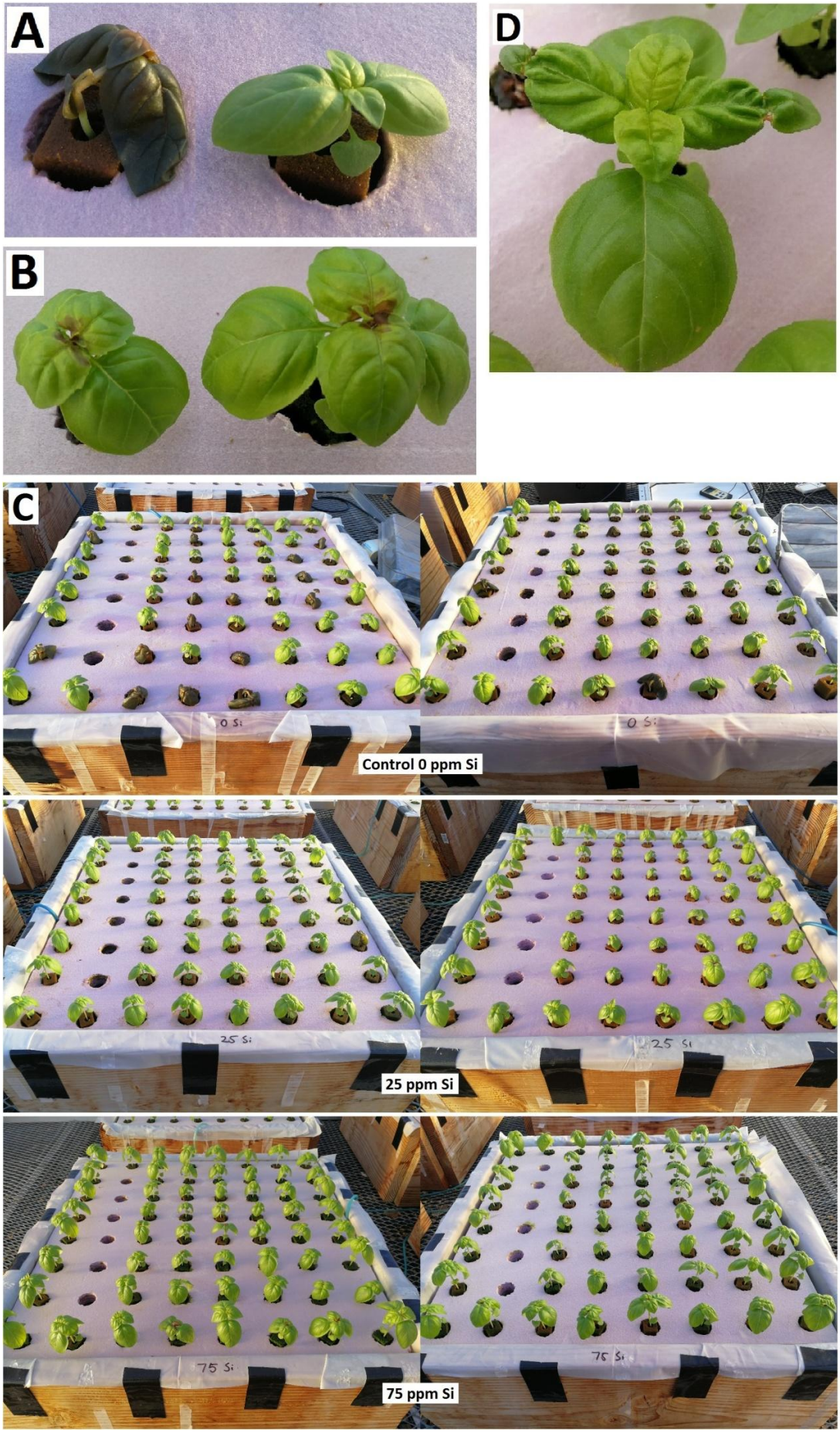

A
B
C
D
0 Si
0 Si
Control 0 ppm Si
25 Si
25 Si
25 ppm Si
75 Si
75 Si
75 ppm Si

Fig. 24. Symptoms of frost damage (from 23 °C down to -1 °C over a 7-hour period, 6 DAT) on basil plants. A: Severe frost damage symptoms: The entire plant became water soaked and turned dark green/brown. The photo was taken from the 0 Si treatment. B: Moderate frost damage symptoms: growing tip and newly emerging leaves turned brown. The photo was taken from the 25 Si treatment. C: Pictures of the growing boards showing the 0, 25 and 75 ppm Si treatments. Si treated plants mostly survived the frost event, whereas the control group experienced substantial losses. Photos shown in A, B, and C were made 6 hours after the frost event. D: A moderately damaged plant 10 days after the frost event (16 DAT). The damaged leaves had a twisted shape, but the morphology of the overall plant and newer leaves remained normal. The photo was taken from the 0 Si treatment.

Despite being a Si non-accumulator, basil plants absorbed Si in small quantities in both shoots and roots under cold environment. With the increase of Si amendment level, less magnesium, sulfur, manganese, copper, and zinc were absorbed by the plant shoots and roots. The level of nitrogen, potassium, and calcium in shoots and roots were not significantly different among the control, 25 and 75 ppm Si treatment plants, even though more potassium and nitrogen were added to the Si amended plants as a result of the use of K_2SiO_3 and nitric acid. Phosphorus level in shoots and roots were also not significantly different among each treatment, which was not consistent with previous studies on Si accumulator species grown in soil (Lepolu et al., 2016; Provance-Bowley et al, 2010). The level of molybdenum in the shoots was not significantly different among all treatments, but Si treated plants had a significantly higher molybdenum content in their roots. The level of iron and boron in the shoots and roots was not affected by the Si treatment (Figures 25, 26 and Table 14). Overall, the Si treatments did not substantially affect the mineral composition of basil plants grown in a cold environment.

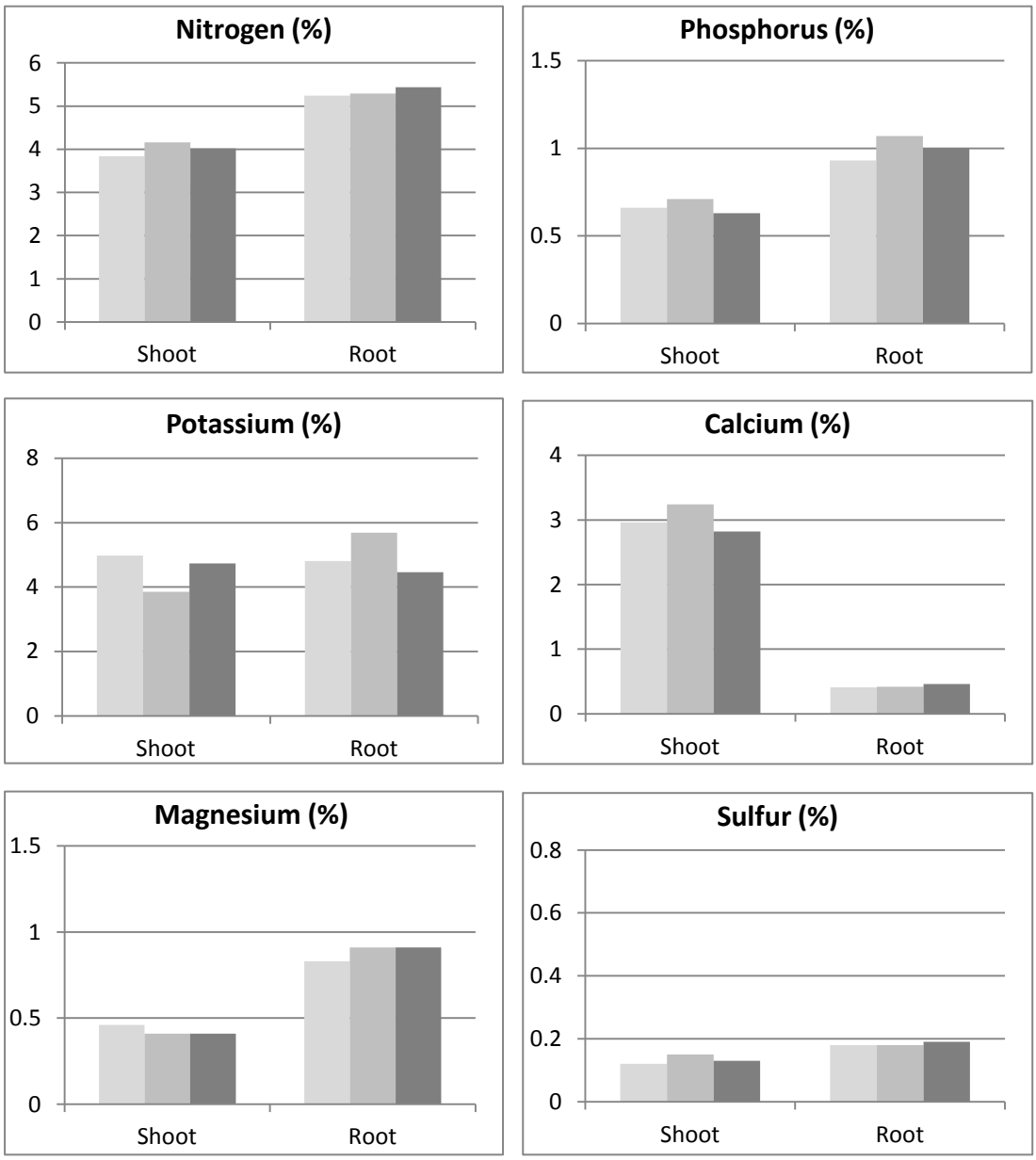
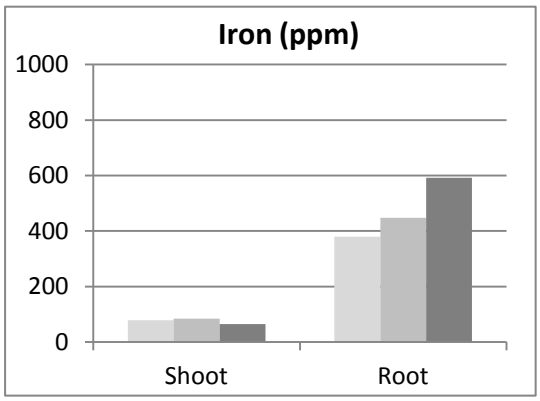
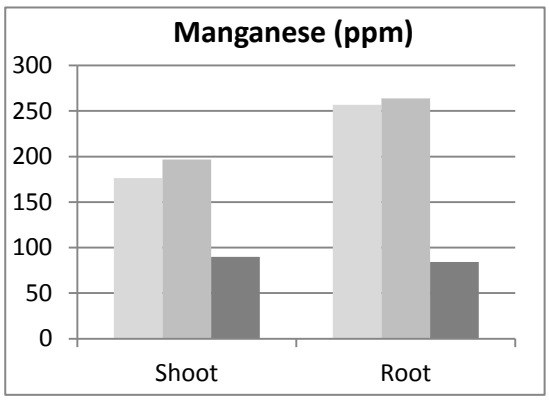
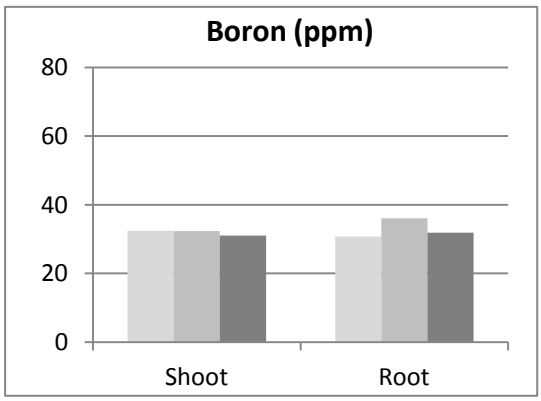
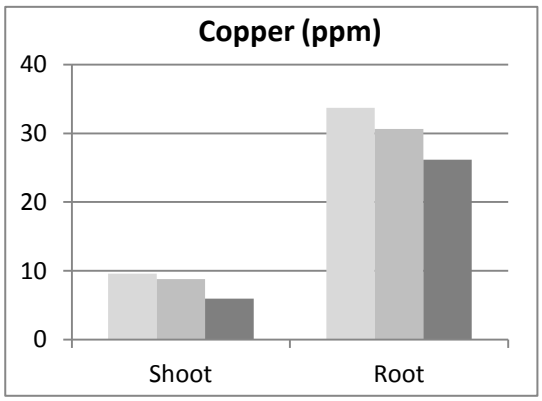


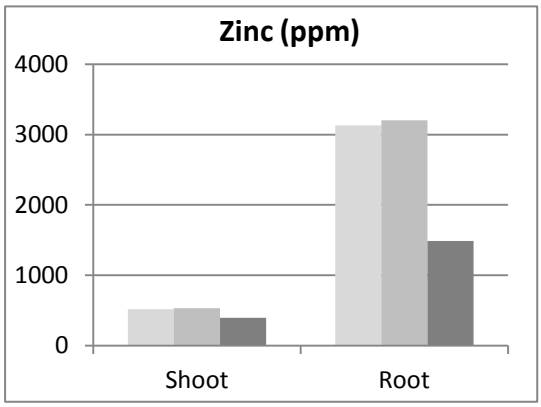
Fig. 25. Basil cold Experiment 2: macronutrient mineral composition analysis of basil 'Genovese' shoots and roots harvested at 30 days after transplant. The 12 plants harvested from each Si treatments were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

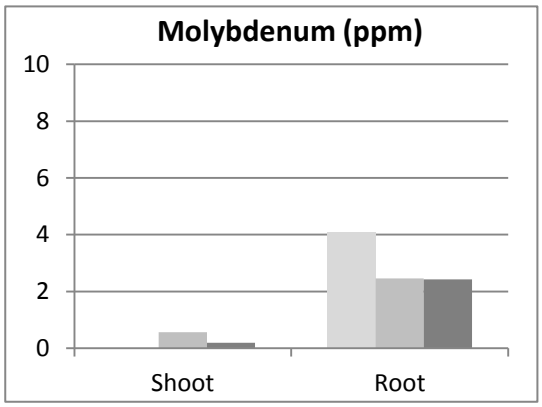












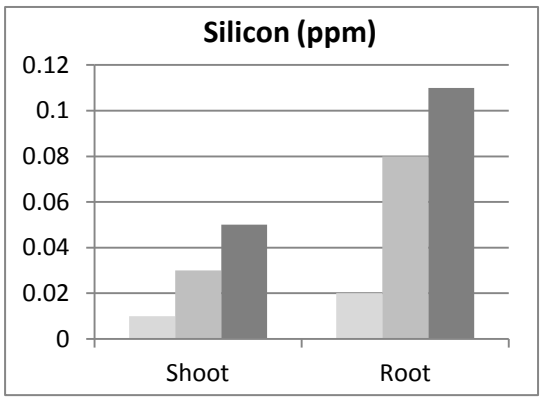


Fig. 26. Basil cold Experiment 2: micronutrient and Si mineral composition analysis of basil 'Genovese' shoots and roots harvested at 30 days after transplant. The 12 plants harvested from each Si treatments were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = Control, grey = 25 ppm Si and dark grey = 75 ppm Si.

Table 14. Basil cold Experiment 3: A stepwise analysis of the mineral composition of basil shoots (top) and roots (bottom) harvested at 30 days after transplant. n = 12. The treatment effect indicates whether Si treatments overall resulted in statistically significant differences compared to the non-Si-treated control. The rate effect indicates whether the application rates (0, 25 or 75 ppm) of Si treatment resulted for any significant effects. The linear regression analysis indicates whether the Si treatments yielded linear effects in the content of each element in the plant tissue. Nitrogen and sulfur analysis for root samples were not conducted due to the small sample sizes from individual plants, indicated by N/A. Bolded values indicate $P \leq 0.05$.

	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Mo	Si
	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm	%
0 Si shoot	4.92	0.77	5.50	2.92	0.43	0.19	85.16	85.23	28.21	6.42	422.05	1.71	0.01
25 Si shoot	5.15	0.80	6.52	3.00	0.41	0.18	109.52	71.05	30.04	6.51	373.30	1.86	0.04
75 Si shoot	4.76	0.68	6.06	2.63	0.36	0.16	81.87	61.74	29.18	4.41	287.23	1.57	0.06
							P -value (a = 0.05)						
Treatment Effect	**0.0431**	**0.0072**	**0.0330**	0.0601	0.5055	0.4655	**0.0020**	0.7980	**0.2485**	0.0532	0.3471	0.2869	**0.0278**
Rate Effect	**0.0078**	**0.0101**	0.1272	0.0816	**0.0088**	**0.0161**	**0.0179**	**0.0382**	0.6722	**0.0061**	**0.0006**	0.6923	**<0.0001**
Linear Regression	**0.0385**	**0.0281**	0.1841	0.1989	**0.0081**	**0.0044**	0.4389	0.1163	0.3200	**0.0016**	**0.0014**	0.6709	**<0.0001**
							R-Square						
Linear Regression	0.1492	0.1662	0.0644	0.0604	0.2325	0.2639	0.0223	0.0888	0.0366	0.3129	0.3205	0.0068	0.6894
0 SI root	N/A	0.94	7.07	0.50	1.36	N/A	441.25	117.00	24.97	29.97	1359.14	6.65	0.04
25 Si root	N/A	0.80	7.74	0.48	1.11	N/A	375.61	88.00	24.90	26.52	899.17	10.94	0.09
75 Si root	N/A	0.90	6.29	0.53	1.00	N/A	441.77	58.44	24.48	19.97	725.91	12.57	0.11
							P -value (a = 0.05)						
Treatment Effect	N/A	**0.0029**	**0.0068**	**0.0417**	0.1613	N/A	0.1171	0.9674	0.8955	0.6755	**0.0026**	**0.2124**	**<0.0001**
Rate Effect	N/A	**0.0027**	**0.0034**	**0.0473**	**<0.0001**	N/A	0.2411	**0.0001**	0.8844	0.2364	**<0.0001**	**0.0021**	**<0.0001**
Linear Regression	N/A	0.9876	**0.0198**	0.0744	**<0.0001**	N/A	0.6368	**<0.0001**	0.6249	0.0868	**<0.0001**	**0.002**	**<0.0001**
							R-Square						
Linear Regression	N/A	<0.0001	0.1631	0.0991	0.4640	N/A	0.0073	0.4370	0.0078	0.0917	0.6611	0.2683	0.6599

2.4 Discussion and conclusions

In this study, the effects of Si nutrition in lettuce, bok choy, and basil against challenging temperature environments were examined. When two different cultivars of lettuce and bok choy were grown under the heat environment (above 30 °C), the leaf lettuce 'Black Seeded Simpson' exhibited typical heat stress symptoms, but the butterhead lettuce 'Rex' was less affected. Both white stem bok choy 'Asian Delight' and dark green bok choy 'Black Summer' exhibited typical heat stress symptoms. In both cultivars of lettuce and bok choy, Si treatment did not significantly affect the size, mass, and morphology of the plant shoots and roots when grown under heat environment.

For 'Genovese' basils grown under the cold environment, the results showed that basil plants treated with Si resulted in increased cold hardiness. One surprising finding was that Si amendments increased the frost tolerance of the basil plants during the early stages of growth. After the unintentional frost event, Si treated basil plants had higher survival rates. This could mean that basil plants can withstand a short event of rapid temperature drop, such as a cold night during the earlier growth stages. This could potentially indicate that when Si is included in the nutrient program, basil plants grown in the field could be sown earlier, resulting in an earlier harvest date.

The experiments were conducted in floating hydroponic systems to allow for accurate control of the concentration of Si and other mineral nutrients, and uniform

results across replications were obtained because the hydroponic systems were placed closely together at the same location in the greenhouse. But plants grown in the field face a much different environment than in a hydroponic system in the greenhouse, including temperature differences between air and root zone, Si and other nutrient availability to the plants, soil type, water availability, and much more. Field experiments in the future are necessary to confirm the beneficial effects of Si nutrition to basil plants.

During this study, Si treatments did not significantly enhance the basil plant growth when the temperature was held at 18 °C. The effect of Si was more significant at a constant temperature of 23 °C, but this study did not look into the effect of Si due to a different day/night temperature scenario. Another attempt this study did not conduct was to apply a lower temperature during the seedling stage (before transplant), and then gradually increase the average day and night temperatures during the later growing periods to simulate an outdoor growing season. For greenhouse growers, amending the nutrient solution with Si can potentially reduce the heating cost without sacrificing substantial plant vigor during the winter season. To be able to provide sound information to growers, such as the optimal greenhouse temperature settings when utilizing Si in a nutrient program, or the best Si application rates, a deeper understanding of Si nutrition in basil grown under lower temperature conditions is needed. Both researchers and growers must gain more experience before implementing such practices.

In general, the Si treatment did not significantly affect the mineral composition

in the shoots and roots of lettuce, bok choy, and basil. The addition of potassium from K_2SiO_3 and nitrogen from using nitric acid to reduce pH from K_2SiO_3 did not influence the level of potassium and nitrogen in the shoots and roots of lettuce, bok choy, and basil. No major changes for macro and micro-nutrients in the shoots and roots of lettuce, bok choy and basil were observed.

When treated with Si, lettuce and bok choy absorbed very small quantities of Si (0.01 – 0.07%) in their shoots and roots under heat environment. Basil grown under lower temperature conditions absorbed more Si (maximum of 0.11%) than lettuce and bok choy. In all three species, the rates of Si application and Si absorption in plant tissue had a positive linear relationship and it was not cultivar dependent. Overall, the Si absorption of lettuce and bok choy grew under heat environment, and basil when grown under cold environment was very minimum, when compared against Si accumulators in general, which can accumulate more than 5% of dry weight as Si (Datnoff et al., 2001).

When under certain biotic or abiotic stresses, some Si non-accumulator species can start Si influx and accumulation in their tissue, and thus obtain beneficial effects against stresses. This scenario was only observed in very few cases and is thought to be plant-stress specific. The mechanism of stress selectivity is unclear (Zellner et al., 2011). Tobacco is a typical Si non-accumulator and usually has a Si content of less than 300 mg per kg of dry tissue (<0.03%). When infected with tobacco ringspot virus (TRV), Si transport from the roots can be induced and Si content in tissue can increase (0.045% in the shoots, and 0.11% in the roots), resulting in a delay of

disease onset (Zellner et al., 2011). In potato, a typical Si non-accumulator, drought stress induced up-regulation of Si transporter genes and resulted in Si accumulation in the tuber peel (Vijaya et al., 2016). In the case of lettuce and bok choy, heat environment did not induce a substantial influx and accumulation of Si, nor did it provide any benefits to the plants against heat stress.

For basil grown under lower temperature conditions, influx and accumulation of Si was observed in both shoots and roots, and an increase in plant size was observed. The Si treated plants also showed significant hardiness and survival rates against an unexpected frost event. But this result was generated from a non-designed, unexpected event and was not repeated. The study was also limited due to available funding and did not use individual plants as samples for tissue analysis during Experiment 2. Solid conclusions can only be drawn if this study is repeated in the future.

Lettuce, bok choy, and basil represented the plant family Asteraceae, Brassicaceae, and Lamiaceae in this study. In this study, Si nutrition was shown to be beneficial to basil grown under lower temperature conditions. For the other mint family plants, including mint, thyme, sage, rosemary, and many other economically important herb species, Si nutrition could be a potential candidate for growers who need better plant growth under unfavorable environmental conditions. Since there are multiple OMRI (Organic Materials Review Institute) certified Si sources, such as wollastonite, Si nutrition can also be very beneficial to organic growers.

During the final harvest of lettuce and bok choy heat Experiment 2, lettuce and

bok choy from each Si treatments were tasted by the author. The taste and texture of the lettuce ‘Rex’ was normal. The texture of the bok choy ‘Black Summer’ was firm and hard to chew. It also tasted more bitter than normal. The taste of the lettuce and bok choy was not noticeably different among all Si treatments.

When tasting the control and Si amended basil plants at the end of basil cold Experiments 2 and 3, no obvious differences were observed. But a formal taste panel evaluation was not conducted. Since basil is mostly consumed for its unique taste, olfactory properties and essential oil composition, analysis of key volatile and nutritional compounds and a taste panel evaluation should be conducted as a part of future research.

Chapter 3

Effect of silicon in lettuce, bok choy, and basil under stress from mechanical damage (cut and grow back)

3.1 Introduction

Cut and grow back, sometimes also known as cut and come again, is a common harvesting method for crops such as leafy green vegetables and herbs. Plants can be harvested and regrown multiple times over a longer growing period without re-sowing, and such practice can produce a higher yield for growers or gardeners with a limited planting space or time for sowing (Royal Horticultural Society, 2019). For plants that grow in a rosette form, such as kale, collards, chard, and leaf lettuce, new leaves emerge from the center of the plant and the older leaves are pushed to the outside edges. When the outside leaves in the rosette are harvested, the center growing point will keep producing more leaves. The plant can be harvested multiple times during growing seasons before losing its vigor (Voyle, 2014). Many non-rosette plants are also suitable for cut and grow back, such as basil, broccoli, and chives.

There are two common practices for cut and grow back. One involves selectively harvesting the oldest, outside leaves while they are still young and flavorful for every single plant, while keeping the center of the plant for producing new leaves. Non-rosette plants such as basil can also utilize this method. This practice requires a

high labor input. Another practice is to mow the field/bed at a certain height and leave the base of the plant to regrow (Voyle, 2014). One example for garlic chives (*Allium tuberosum*), is to mow the field to the base of the plant. Garlic chives can be harvested three to four times a year over several years after establishment (MacKenzie and Kooyman, 2018).

Si can bring benefits to plants under stress from mechanical damage such as lodging and shearing. Plants can absorb and accumulate Si under the epidermal tissues, and the Si deposition ultimately forms a polymerized layer that can increase cellular rigidity, resulting in a stronger physical structure for the plant tissue (Datnoff et al., 2001; Hansen et al., 1976; Ma and Yamaji, 2006; Raven, 1983; Zellner and Leisner, 2013).

Si uptake can be induced by biotic or abiotic stresses. In rice, a typical Si accumulator, Si accumulation was observed after inoculation with the blast fungus *Magnaporthe oryzae* (Brunings et al., 2009). Stress-induced Si accumulation can even be found in some non-accumulator species, such as Arabidopsis (Fauteux et al., 2005), potato (Vijaya et al., 2016) and tobacco (Zellner et al., 2011). But whether the stress from mechanical damage can induce Si uptake into the plant tissue remains unknown. The objectives of this study were: 1) To evaluate the effect of Si nutrition on the growth and morphology of lettuce, bok choy and basil to a mature, marketable size under optimal growing conditions, 2) To evaluate whether Si absorption in lettuce, bok choy and basil can be induced by stress from mechanical damage such as from a cut and grow back treatment, and 3) To evaluate if Si nutrition can alter the mineral

nutrition composition in the shoots and roots of lettuce, bok choy and basil under optimal growing conditions and after the cut and grow back treatment.

3.2 Materials and methods

Lettuce and bok choy

To evaluate the efficacy of Si amendments on hydroponically grown lettuce and bok choy under stress from mechanical damage, one single experiment was conducted starting with seeding on Oct. 26, 2016. Lettuce cultivar Black Seeded Simpson (leaf lettuce, W. Atlee Burpee & Co, Warminster, PA) and bok choy cultivar Asian Delight (white stem bok choy, Johnny's Selected Seeds, Fairfield, ME) were used for this experiment. During the experiment, the lettuce and bok choy experiments were conducted simultaneously. Three sheets of Oasis cubes of lettuce and bok choy were sown and incubated for 11 days in the growth chamber as described in Chapter 1. Sixty-four (8*8 rows in one hydroponic system per Si treatments) seedlings of equal sizes for each Si treatment were selected for transplanting into the hydroponic systems in the greenhouse. Greenhouse temperature set points of 20°C (day) and 18°C (night) were used during the experiment.

The 64 lettuce and bok choy plants from each Si treatment were divided into "cut" (one half of the growing board) and "uncut" (the other half of the growing board) groups. The first cut was performed on 35 DAT. Before the cut treatment, the

shoot height of 12 plants was recorded for each Si treatment, and six non-guard plants from both cut and uncut groups were harvested and combined into one sample of shoot and root for tissue analysis.

The first cut treatment was performed after the first harvest. All plants from the "cut" group were cut with a pair of scissors at 5 cm above the growing board as shown in Figure 27A-C. On 60 DAT (25 days after the first cut), six non-guard plants from both cut and uncut groups were harvested for tissue analysis. Each harvested plant was separated into the new shoot (newly grown out parts), old shoot (old leaf and stem tissues below the 5 cm cut line left from the previous cut) and roots (Figure 27D). The above plant parts of all six harvested plants from each Si treatment were combined into one sample of new shoot, old shoot and root for tissue analysis. After the harvest, all plants from the "cut" group were cut 5 cm above the growing board again. The last of the six non-guard plants from both cut and uncut groups were harvested on 85 DAT (25 days after the second cut) for tissue analysis. The plant parts were prepared using the same methods as during the second harvest. At this stage, the lettuce and bok choy plants started bolting (Figure 27E).

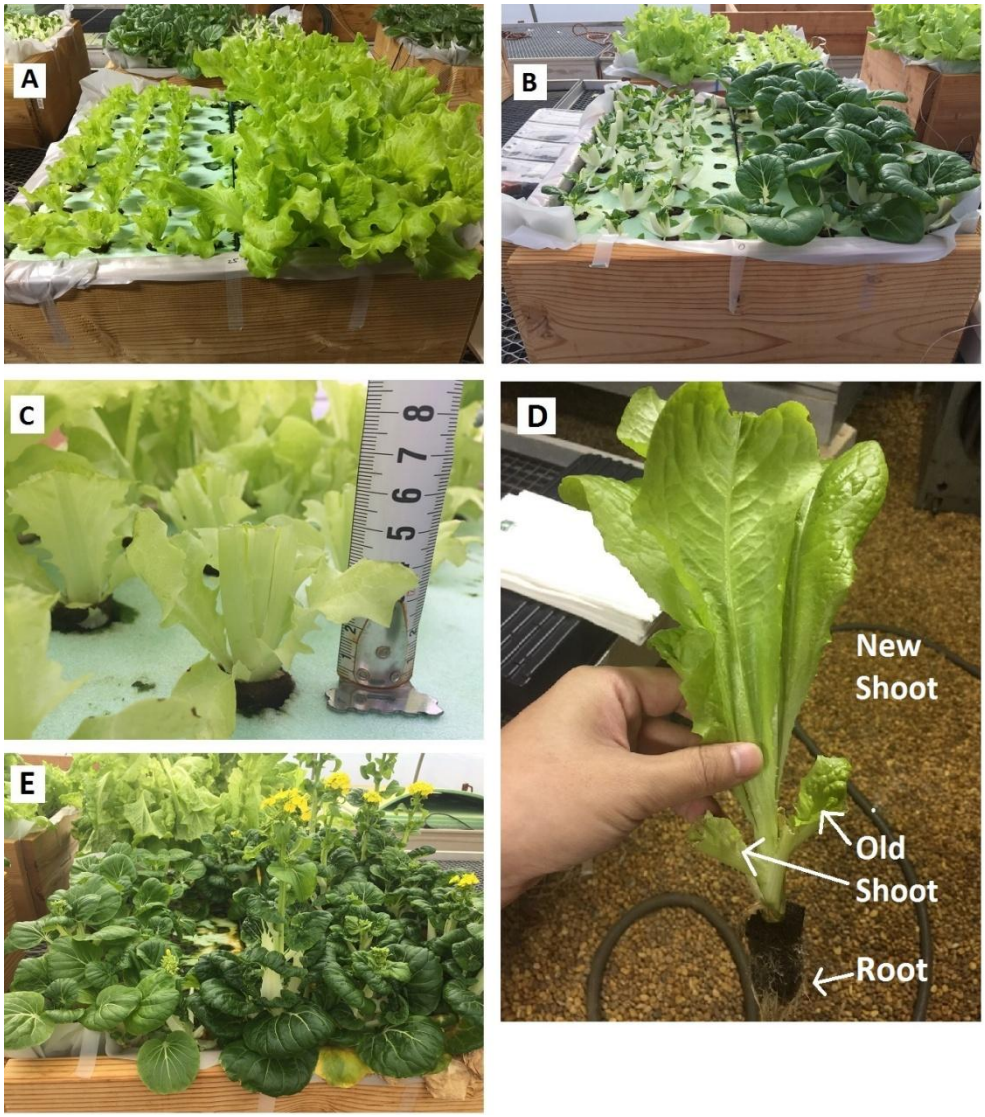


Fig. 27. A and B: Cut and grow back procedures for lettuce and bok choy plants. The growing board of lettuce 'Black Seeded Simpson' and bok choy 'Asian Delight' were separated into the cut (left) and uncut (right) groups. C: During the cutting process, plants from the cut section were cut with scissors at 5 cm above the growing board. D: The harvested plants were disassembled into the new shoots, old shoots and roots for separate tissue analysis after the second harvest. E: Bolting bok choy plants at the time of the third harvest. Photos of A, B and C were taken after the first cut [35 days after transplant (DAT)]. Photo D was taken during the second harvest (60 DAT). Photo E was taken during the third harvest (85 DAT).

The plant parts from all harvests were dried in an oven for 4 days followed by grinding (Arthur H. Thomas scientific apparatus, Philadelphia, PA). Tissue analysis for essential nutrients and Si was carried out by ashing the samples (AOAC 900.02B) and mixing with aqua regia before performing ICP-AES at a commercial testing lab (PLT-1, MMI Labs, Athens, GA). Tissue analysis of old shoots and roots for all samples was not possible due to the small sample mass. Nutrient content in the shoots were graphed using MS Excel for each nutrient.

Basil

To evaluate the efficacy of Si amendments in hydroponically grown basil under stress from mechanical damage, one experiment was conducted starting with seeding on Oct. 12, 2017. Organic basil 'Genovese' (Johnny's Selected Seeds, Fairfield, ME) was used for this experiment. Three sheets of Oasis cubes of basil were sown and incubated for 11 days in the growth chamber as described in Chapter 1. Sixty-four (8*8 rows) seedlings of equal sizes for each Si treatment were selected for transplanting into the hydroponic systems in the greenhouse. Greenhouse temperature set points of 30°C (day) and 28°C (night) were used during the experiment.

The 64 basil plants from each Si treatment were divided into "cut" (one half of the growing board) and "uncut" (the other half of the growing board) groups. The first cut was performed on 30 DAT. Before the cut treatment, six non-guard plants from both cut and uncut groups of each Si treatment were harvested. The shoot

height, root length, shoot and root fresh and dry weight, and number of true leaves were recorded to reveal any differences in plant growth among the Si treatments. The plant parts were combined into one sample of shoots and roots each for tissue analysis.

The first cut treatment was performed after the first harvest. All plants from the "cut" group were cut with a pair of scissors 5 cm above the growing board, leaving only one branch below the cut point. On 55 DAT (25 days after the first cut), six non-guard plants from both cut and uncut groups were harvested and combined into one sample of shoots and roots for tissue analysis. All plants from the "cut" group were cut at 3 cm above the first "Y split" node, leaving only one branch on each remaining branch as described in Figure 28. After the second cut treatment, the uncut plants started bolting. The third harvest that was originally scheduled for 80 DAT was not performed.

The tissue analysis was carried out using the same methods as described earlier for the lettuce and bok choy experiment. The nitrogen and sulfur analysis for the roots were not conducted due to the small sample mass. The tissue analysis data were graphed using MS Excel for each nutrient.

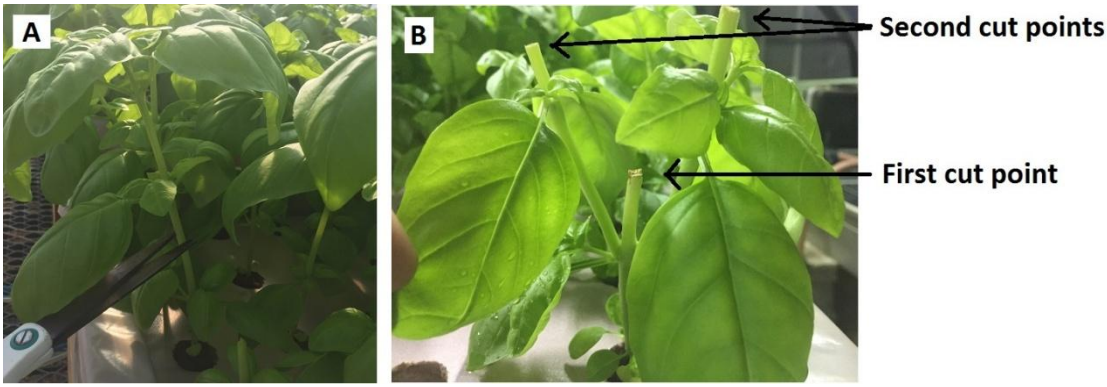


Fig. 28. Cut and grow back procedure for basil plants. The first cut (A) was performed on 30 DAT by cutting off the shoots 5 cm above the growing board, leaving only one branch below the cut point. The basil plant kept growing from the first branch onward, forming a “Y split” structure. The second cut (B) was performed on 55 DAT by cutting off the shoot tissues 3 cm above the first “Y split”. Unlike the lettuce and bok choy experiment, the basil did not have any “old shoot” tissue parts that failed to grow after the cuts.

3.3 Results

Lettuce and bok choy

The environmental data acquisition during the experiment was interrupted several times due to a bad datalogger program. A portion of the data covering 68 to 77 DAT (Dec. 19, 2016 – Dec. 28, 2016) is presented in Appendix C.

On 35 DAT, the lettuce plants with different Si amendment levels showed statistically significant differences in plant height. With 12 plants from each treatment evaluated, the average height of the control plants was 13.25 cm (SD = 1.90 cm), 16.38 cm (SD = 3.54 cm, $P = 0.015$ vs. Ctrl) for the 25 ppm Si treated plants, and 20.54 cm (SD = 2.92 cm, $P < 0.001$ vs. Ctrl) for the 75 ppm Si treated plants. The bok choy plants showed statistically non-significant differences in height but still followed the general trend that the Si treated plants were taller. The average height of the control plants was 14.63 cm (SD = 1.72 cm), 15.46 cm (SD = 1.01 cm, $P = 0.165$ vs. Ctrl) for the 25 ppm Si treated plants, and 15.63 cm (SD = 1.11 cm, $P = 0.107$ vs. Ctrl) for the 75 ppm Si treated plants. This plant growth evaluation at the first harvest predicted the effect of Si treatments on plant growth to a mature, marketable stage under optimal growing conditions. The Si treatments significantly and linearly increased the height of lettuce plants. This phenomenon was less obvious for bok choy.

During the first harvest, there were no differences in the nutrient composition between the cut and uncut groups of lettuce (Figures 29 and 30) and bok choy

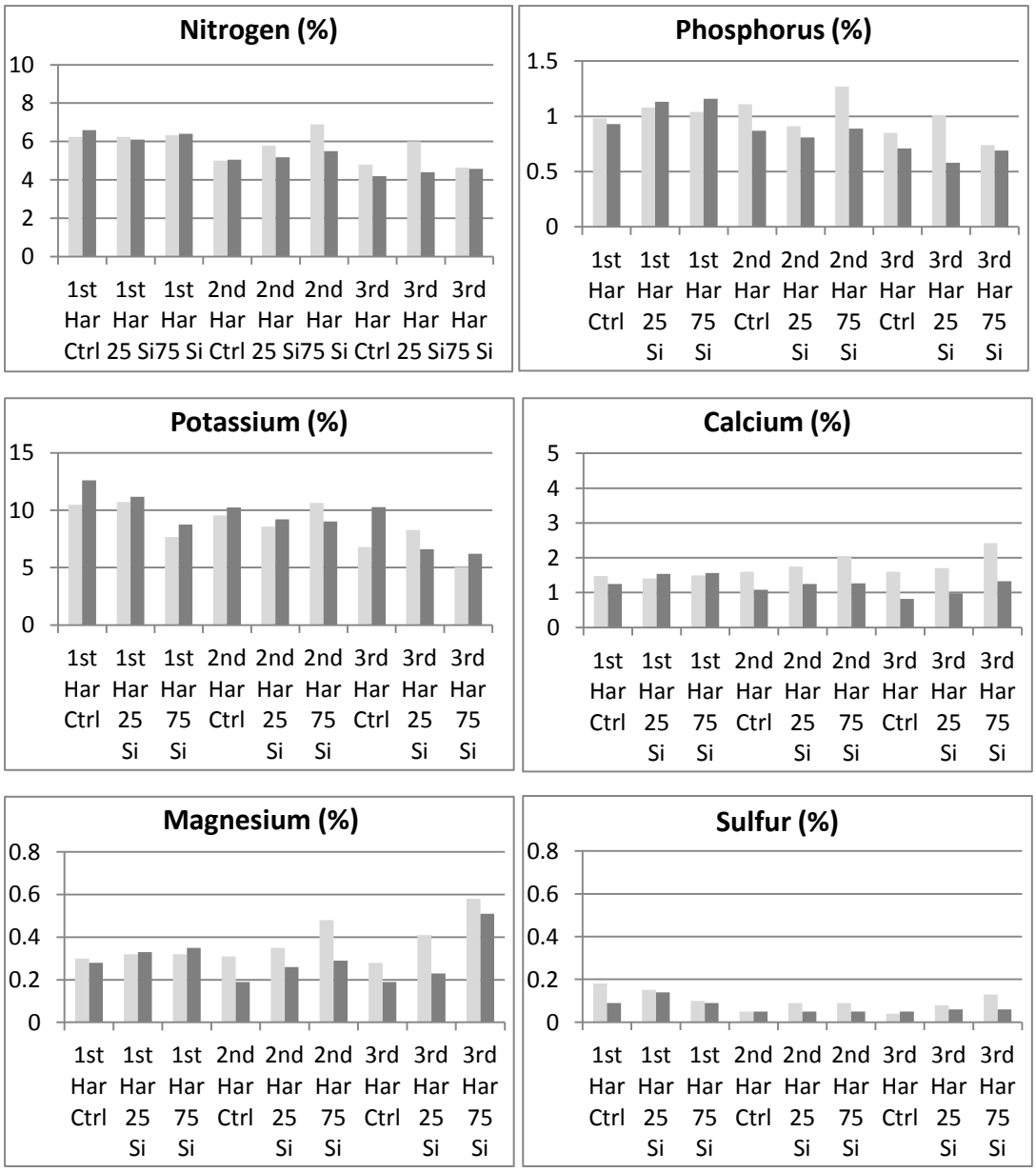


Fig. 29. Macronutrient analysis of lettuce 'Black Seeded Simpson' shoot tissue harvested at 35 DAT (first harvest), 60 DAT (second harvest) and 85 DAT (third harvest) treated with 0, 25 and 75 ppm of Si. Six plants were harvested from each Si treatment and the new shoots were sampled using ICP-AES. Light grey = plants that were cut and allowed to grow back. Dark grey = uncut plants.

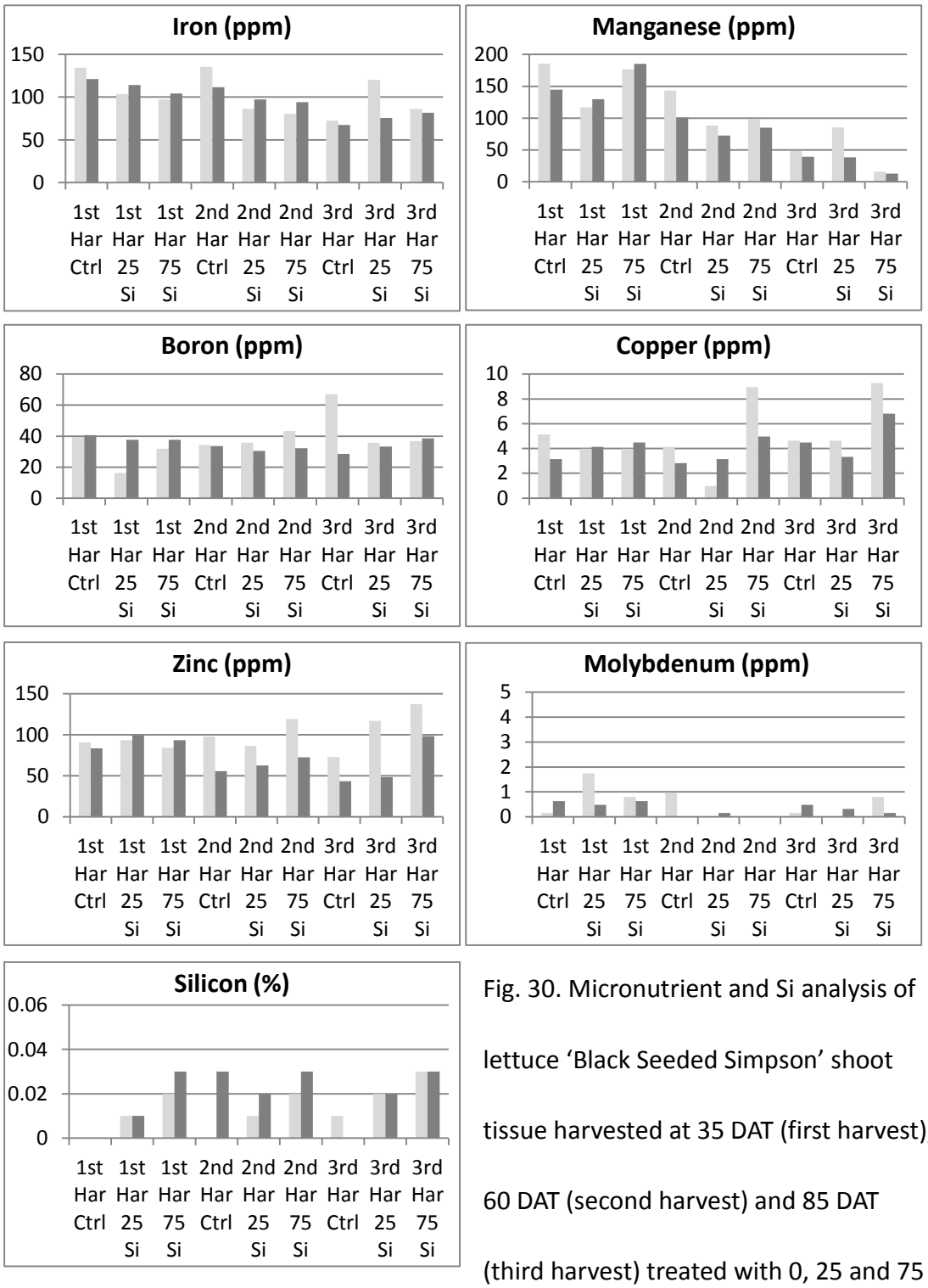


Fig. 30. Micronutrient and Si analysis of lettuce 'Black Seeded Simpson' shoot tissue harvested at 35 DAT (first harvest), 60 DAT (second harvest) and 85 DAT (third harvest) treated with 0, 25 and 75 ppm of Si. Six plants were harvested from each Si treatment and the new shoots were sampled using ICP-AES. Light grey = plants that were cut and allowed to grow back. Dark grey = uncut plants.

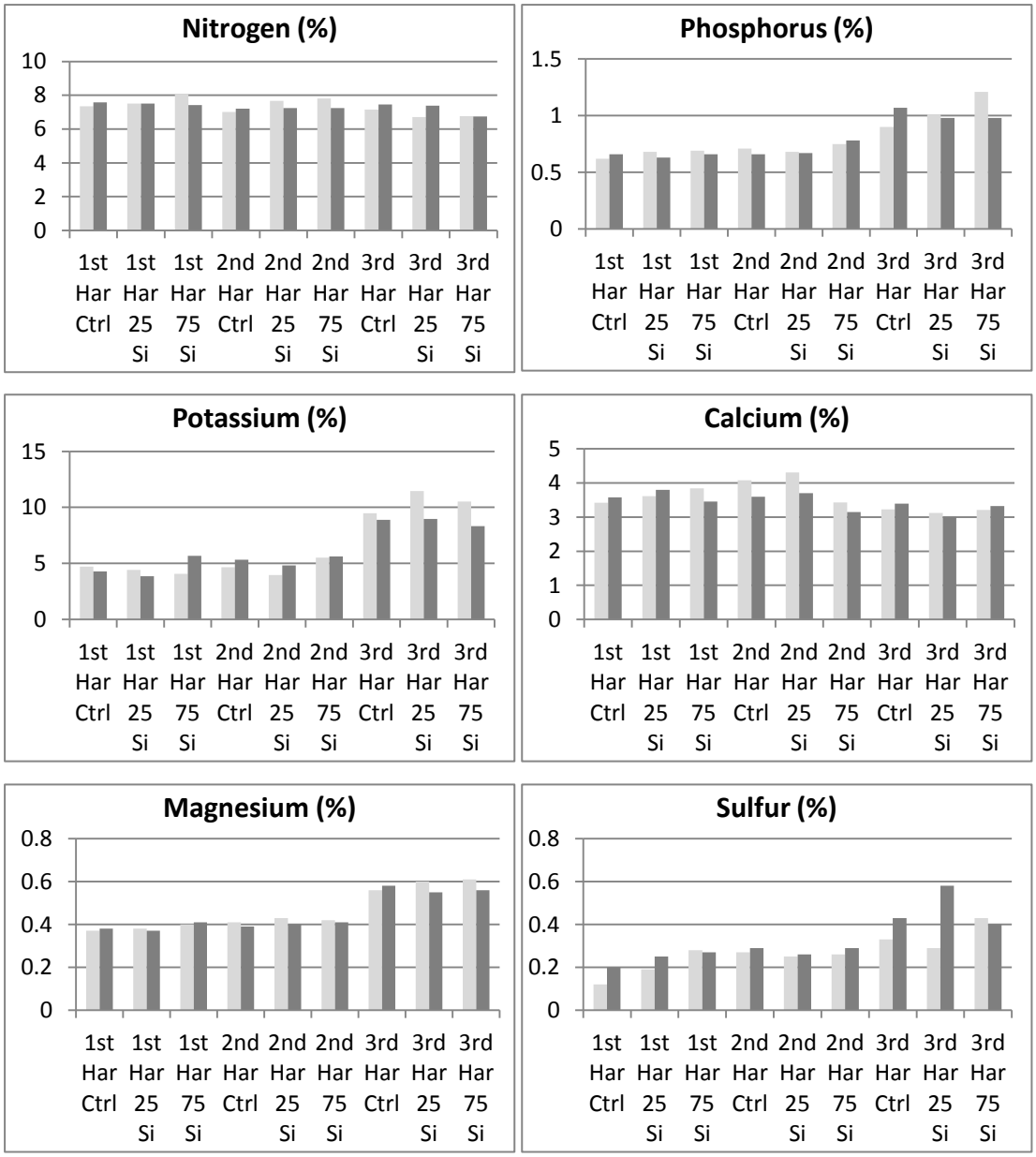


Fig. 31. Macronutrient analysis of bok choy 'Asian Delight' shoot tissue harvested at 35 DAT (first harvest), 60 DAT (second harvest) and 85 DAT (third harvest) treated with 0, 25 and 75 ppm of Si. Six plants were harvested from each Si treatment and the new shoots were sampled using ICP-AES. Light grey = plants that were cut and allowed to grow back. Dark grey = uncut plants.

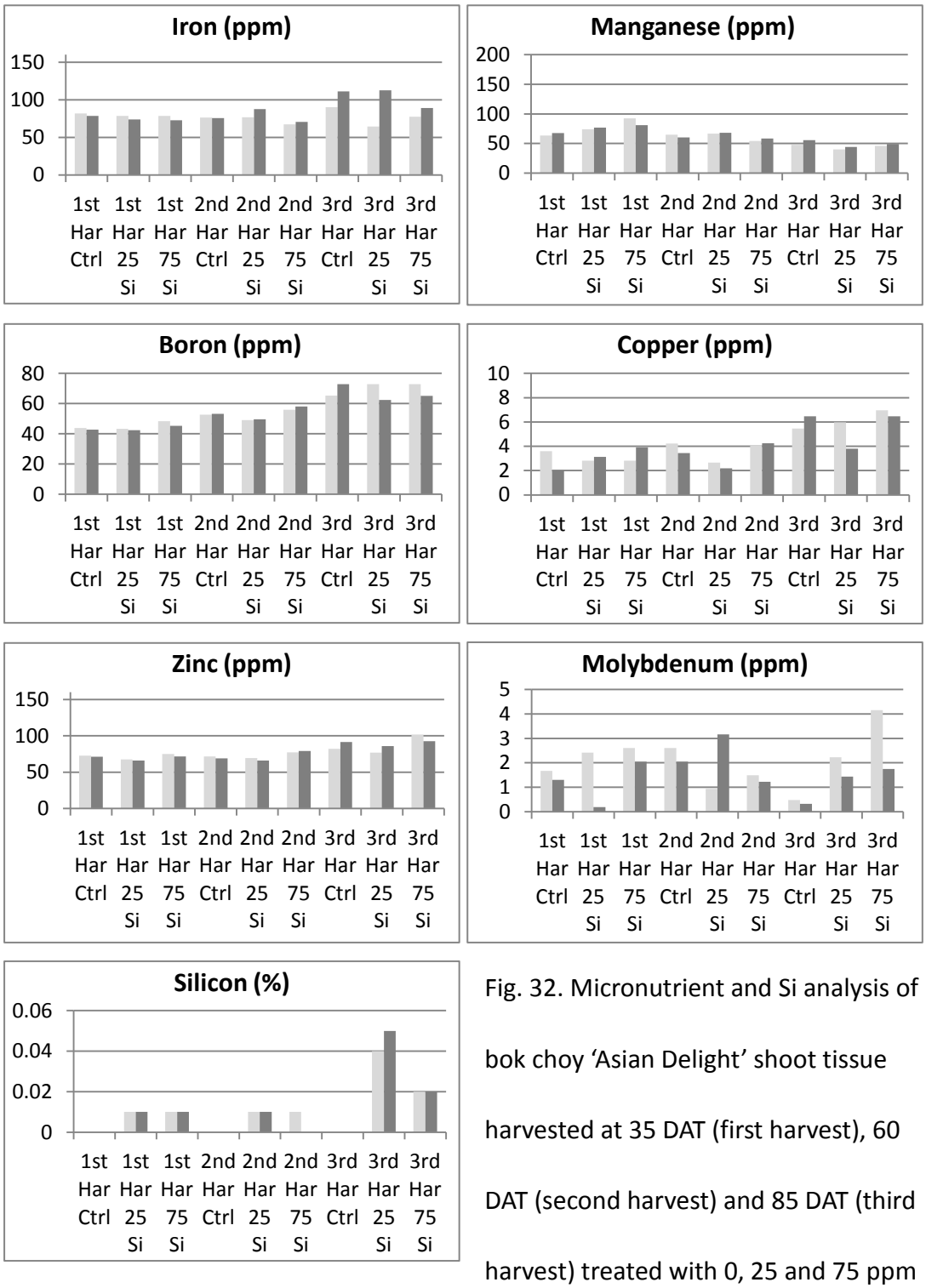


Fig. 32. Micronutrient and Si analysis of bok choy 'Asian Delight' shoot tissue harvested at 35 DAT (first harvest), 60 DAT (second harvest) and 85 DAT (third harvest) treated with 0, 25 and 75 ppm of Si. Six plants were harvested from each Si treatment and the new shoots were sampled using ICP-AES. Light grey = plants that were cut and allowed to grow back. Dark grey = uncut plants.

(Figures 31 and 32) since they were grown in the same hydroponic system and under the same conditions. In lettuce shoot tissue collected at the first harvest, the level of nitrogen and phosphorus were consistent among all Si treatments. Surprisingly, with the addition of K_2SiO_3 from the Si treatment, the level of potassium decreased as the Si level increased. The same trend was also observed for the iron content. The level of magnesium showed the opposite trend, namely its level increased as the Si level increased. There were no obvious differences in the contents of calcium, sulfur, manganese, boron, copper, zinc, and molybdenum among all Si treatments. In bok choy, most of the nutrient contents were consistent among all Si treatments except for sulfur and manganese, which showed an increased amount with increased Si. The differential content of potassium and iron as observed in the lettuce plants were not detected in bok choy. Both lettuce and bok choy plants absorbed a very small amount of Si (maximum of 0.03%) in the shoots when treated with Si.

As the plants grew older, the content of some nutrients started to alter as can be seen by comparing the first, second and third harvest. In general, the level of nitrogen, phosphorus, potassium, iron, and manganese decreased in lettuce shoot tissue. Different trends were observed in bok choy, where the level of phosphorus, potassium, magnesium, sulfur, iron, boron, and copper increased as the plant grew older. The level of nitrogen, calcium, manganese, and zinc remained relatively similar across the different plant ages. Whether lettuce and bok choy plants absorbed more Si when growing older cannot be accurately assessed because the level of Si accumulation in the tissue was very small, resulting in the possibility that the data

could have been affected by instrumental errors.

During the second and third harvest, differences in nutrient content between the cut and uncut plants were observed. In lettuce shoot tissue, the levels of calcium, magnesium, and zinc were much higher in the cut plants than the uncut plants. In the bok choy shoot tissue, increased iron and decreased molybdenum levels were observed in the uncut plants during the third harvest. All the other nutrients remained similar in nutrient content comparing the cut and uncut groups. As the Si level increased, an overall increase in calcium, magnesium, and zinc, and a decrease in potassium, iron, and manganese were observed in lettuce. In bok choy, no substantial increases or decreases in nutrient content were observed (Figures 29, 30, 31 and 32).

Basil

During the basil experiment, the greenhouse control system was able to maintain the temperature set point during both day and night after transplant. The greenhouse air temperature and the nutrient solution temperature were maintained at 30 °C during the day and 28 °C during the night. The greenhouse environmental data are shown in Figure 33 and Table 15 (before the first harvest), and Figure 34 and Table 16 (between the first and second harvest).

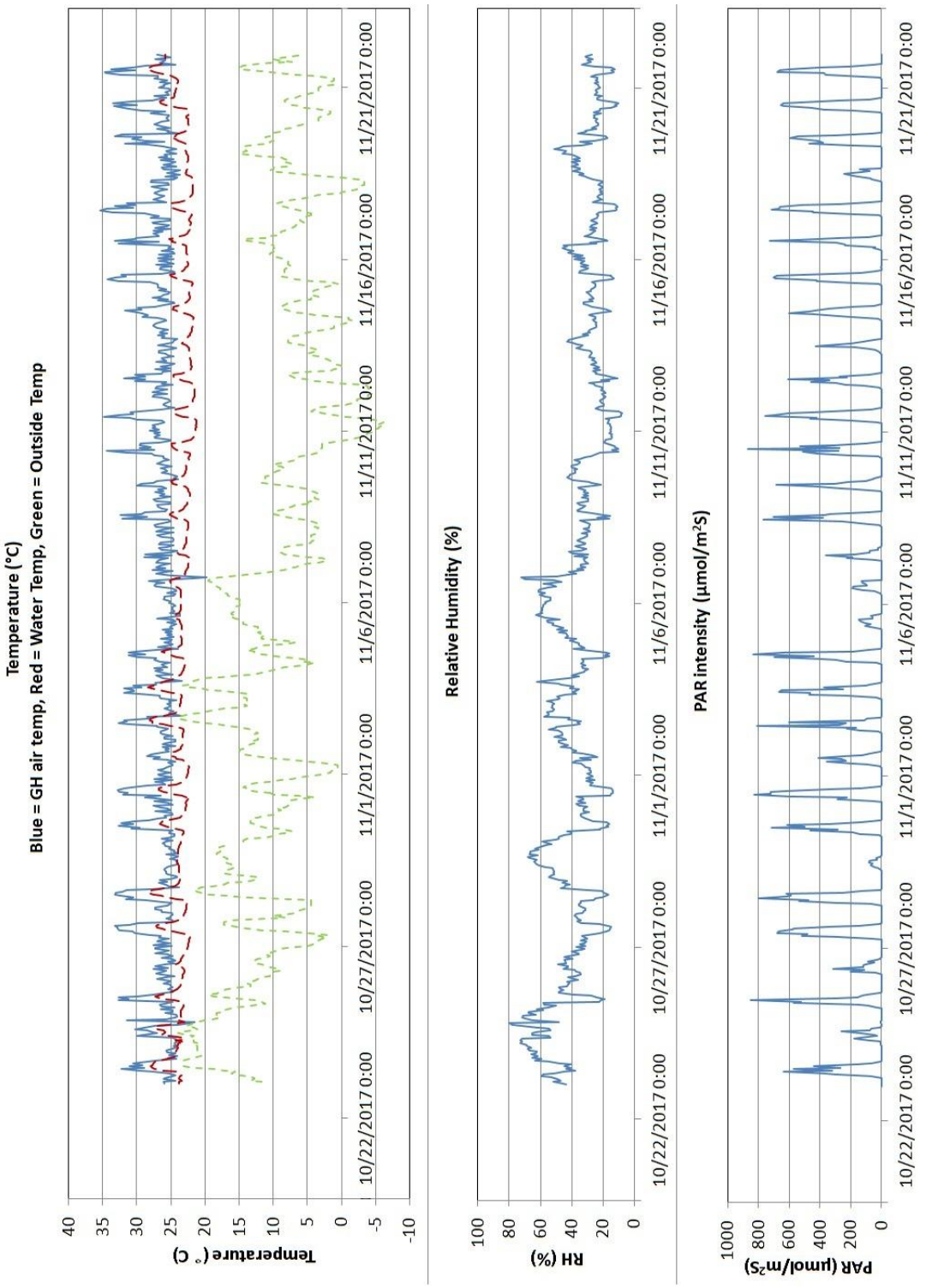


Fig. 33. Basil cut and grow back experiment: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). Data covered the period from Oct. 23, 2017 (first DAT) to the first harvest on Nov. 21, 2017 (30 DAT).

Table 15. Basil cut and grow back experiment: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), from Oct. 23, 2017, first DAT to the first harvest on Nov. 21, 2017 (30 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
23-Oct	1	27.5	24.8	25.8	24.0	20.4	21.2	57.3	10.7
24-Oct	2	26.6	26.1	25.0	24.5	21.8	20.3	64.5	4.4
25-Oct	3	27.1	25.8	24.6	23.3	15.2	11.9	40.1	11.6
26-Oct	4	25.8	26.3	23.3	22.4	10.9	5.0	38.5	4.2
27-Oct	5	29.0	26.1	24.6	22.8	10.9	5.9	29.1	16.6
28-Oct	6	28.7	25.2	25.3	23.8	15.2	14.8	38.2	16.2
29-Oct	7	24.9	25.5	24.0	23.6	17.2	14.0	59.0	1.7
30-Oct	8	27.3	26.2	24.2	22.8	11.3	8.4	29.9	14.4
31-Oct	9	28.6	26.3	24.3	22.5	10.3	2.3	25.7	16.3
1-Nov	10	26.1	25.4	23.8	23.3	10.4	12.6	31.6	7.0
2-Nov	11	28.0	25.6	25.5	23.6	19.2	14.5	47.5	13.5
3-Nov	12	28.2	26.3	25.6	23.2	18.8	9.1	40.3	12.3
4-Nov	13	27.5	25.5	24.2	23.5	9.4	11.5	33.5	12.9
5-Nov	14	25.2	25.2	23.8	23.5	14.9	15.5	54.7	3.7
6-Nov	15	25.6	26.4	23.9	22.5	17.3	6.1	47.3	4.0
7-Nov	16	26.3	26.0	23.0	22.6	6.1	4.0	32.7	4.6
8-Nov	17	27.2	26.2	23.1	22.4	5.2	3.9	30.3	11.4
9-Nov	18	26.4	25.9	23.4	22.6	9.2	8.2	35.3	8.7
10-Nov	19	28.6	27.0	23.0	21.4	1.8	-5.1	16.4	13.8
11-Nov	20	28.6	26.6	22.8	21.6	-0.2	-3.9	17.5	14.8
12-Nov	21	27.5	26.1	22.8	22.2	2.3	2.3	23.2	10.7
13-Nov	22	25.7	26.5	22.6	21.8	5.9	0.6	31.3	4.6
14-Nov	23	27.4	26.2	23.1	22.2	5.4	3.6	26.5	10.9
15-Nov	24	28.0	25.7	23.2	22.7	6.5	9.2	29.6	12.3
16-Nov	25	27.1	26.3	23.3	22.2	10.4	6.1	29.8	9.4
17-Nov	26	29.2	26.6	23.3	21.8	22.7	17.3	19.8	14.4

Table 15 continued

18-Nov	27	25.6	25.5	22.4	22.7	5.3	12.6	36.4	4.0
19-Nov	28	27.4	26.4	23.3	22.5	10.3	3.7	27.5	9.5
20-Nov	29	28.8	26.5	24.5	24.2	5.4	2.2	20.9	13.4
21-Nov	30	28.6	26.1	26.4	25.8	9.4	5.9	24.2	12.5
Average		27.3	26.0	23.9	22.9	11.0	8.1	34.6	10.2
St. Dev.		1.2	0.5	1.0	0.8	6.3	6.6	12.4	4.5

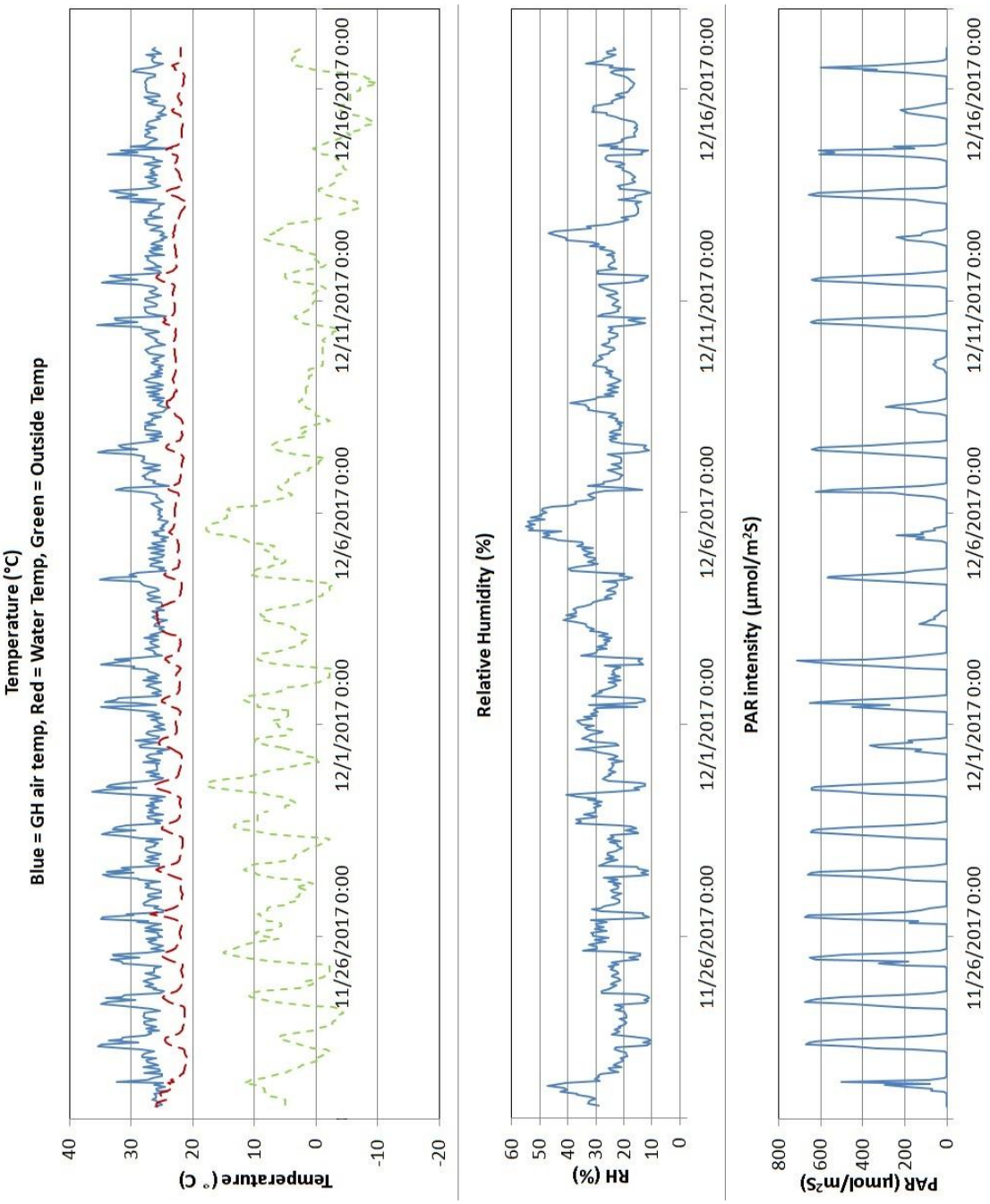


Fig. 34. Basil cut and grow back experiment: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). Data covers the period between Nov. 22, 2017 (first DAT) and the second harvest on Dec. 16, 2017 (55 DAT).

Table 16. Basil cut and grow back experiment: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), between Nov. 22, 2017 (30 DAT) and the second harvest on Dec. 16, 2017 (55 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
22-Nov	31	26.2	26.7	23.0	21.1	8.0	0.6	29.5	5.1
23-Nov	32	29.2	28.3	22.7	22.2	1.9	0.1	19.7	13.7
24-Nov	33	29.1	26.5	22.8	21.7	4.4	-0.8	20.8	13.4
25-Nov	34	28.4	26.2	23.1	22.0	8.8	7.9	25.7	12.8
26-Nov	35	27.8	26.4	23.2	21.8	6.8	2.6	23.4	11.1
27-Nov	36	29.0	26.5	23.2	21.7	7.0	1.2	21.4	12.6
28-Nov	37	28.8	26.3	23.0	22.0	7.8	5.3	27.2	12.8
29-Nov	38	28.1	26.5	23.3	21.9	10.1	2.5	23.2	12.3
30-Nov	39	26.8	26.0	23.5	22.5	5.6	5.8	30.5	7.6
1-Dec	40	28.7	26.5	23.1	22.3	7.9	-0.8	32.3	11.3
2-Dec	41	27.8	26.1	22.8	23.0	4.9	2.1	25.9	10.3
3-Dec	42	25.7	26.5	25.2	22.0	6.2	-0.8	32.3	2.3
4-Dec	43	27.7	25.9	22.8	22.3	5.2	7.2	29.6	9.9
5-Dec	44	25.4	25.6	22.9	22.8	14.6	12.8	46.8	3.5
6-Dec	45	26.8	26.6	22.6	21.8	5.2	0.9	25.3	7.2
7-Dec	46	28.9	26.5	22.5	21.8	3.8	0.3	20.9	11.9
8-Dec	47	26.8	26.3	23.3	23.0	-0.9	1.3	26.8	4.8
9-Dec	48	26.1	26.4	22.8	22.9	-0.5	-1.6	26.1	1.2
10-Dec	49	28.1	26.3	23.4	23.1	0.7	0.3	22.8	10.5
11-Dec	50	28.3	26.2	23.8	22.7	1.6	0.9	23.4	11.4
12-Dec	51	25.6	26.3	22.9	21.8	5.7	-4.2	28.4	3.9
13-Dec	52	28.3	26.4	22.3	22.6	-3.2	-4.2	17.8	11.6
14-Dec	53	27.8	27.0	22.7	21.7	-2.2	-7.3	19.6	10.5
15-Dec	54	26.0	27.0	22.4	21.6	-5.5	-8.0	22.6	3.6
16-Dec	55	26.9	26.3	22.1	22.0	0.4	2.0	24.1	8.2
Average		27.5	26.5	23.0	22.2	4.2	1.0	25.8	8.9
St. Dev.		1.2	0.5	0.6	0.5	4.6	4.5	5.9	3.9

On 30 DAT, the Si treated basil plants exhibited statistically significant differences for multiple growth parameters (Table 17). The 75 ppm Si treated plants were the tallest among all treatments, and the difference was statistically significant compared to the control treatment. The 75 ppm Si treated plants also had the highest shoot fresh and dry weight, but the differences were not statistically significant compared against the control. The number of true leaves for the 75 ppm Si treatment was the highest among all treatments, and almost reached a statistical significance against the control ($P = 0.052$). The 75 ppm Si treated plants had the longest roots but the differences among all treatments were not statistically significant. The large standard deviation in the root length data was the result of tangled up roots with adjacent plants and reduced the accuracy of root measurement. The differences in the root fresh and dry weight among all Si treatments were statistically non-significant. Overall, the Si treatment substantially increased the shoot growth of basil plants under optimal growing conditions, and the root growth was less affected.

Table 17. Growth parameters for the control, 25 and 75 ppm Si treatments for basil plants 'Genovese' at the first harvest (30 DAT, n = 6). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	21.5	43.0	11.5	2.06	0.69	0.07	12.2
SD	4.94	11.69	4.33	0.89	0.28	0.03	4.60
25 Si	23.5	43.5	12.8	2.56	0.78	0.09	14.2
SD	3.08	8.81	3.24	0.76	0.22	0.03	4.17
75 Si	24.3	46.3	13.2	2.18	0.75	0.07	14.7
SD	3.76	12.24	2.93	0.73	0.22	0.02	4.07
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.101	0.868	0.240	**0.043**	0.239	**0.020**	0.121
Ctrl vs 75 Si	**0.031**	0.346	0.130	0.609	0.464	0.587	0.052
25 Si vs 75 Si	0.405	0.370	0.709	0.086	0.599	**0.042**	0.676

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

Similar to lettuce and bok choy, during the first harvest, there were no obvious differences in the nutrient composition between the cut and uncut basil plants as they were grown in the same hydroponic system. This was reflected in the tissue analysis data (Figures 35 and 36). During the first harvest, the levels of nitrogen, phosphorus, potassium, magnesium, sulfur, boron, copper, and molybdenum were consistent among different Si treatments. The phenomenon of differential potassium uptake depending on Si treatment as found for lettuce was not observed in basil. Si treatments reduced calcium accumulation in the shoots but not in the roots. The level of manganese and zinc were decreased as the Si level increased in the roots but not in the shoots.

Similar to lettuce and bok choy, as the basil plants grew older, the content of some nutrients started to alter as shown by comparing the first and second harvest. In general, the level of nitrogen, phosphorus, potassium, sulfur, iron, and manganese started to decrease in the shoot tissue. Boron content was increased in the shoots but not in the roots at the second harvest. The level of calcium, magnesium, and zinc remained relatively similar across the different plant ages. Despite increased Si accumulation in the plant tissue when amended with more Si, the level of Si in both shoots and roots were similar for the two harvests, indicating that the plants did not take up and accumulate more Si in the shoots and roots as they grew older when the nutrient solution was amended with Si.

During the second harvest, differences in nutrient contents between the cut and uncut groups were observed. The levels of nitrogen, phosphorus, potassium, calcium,

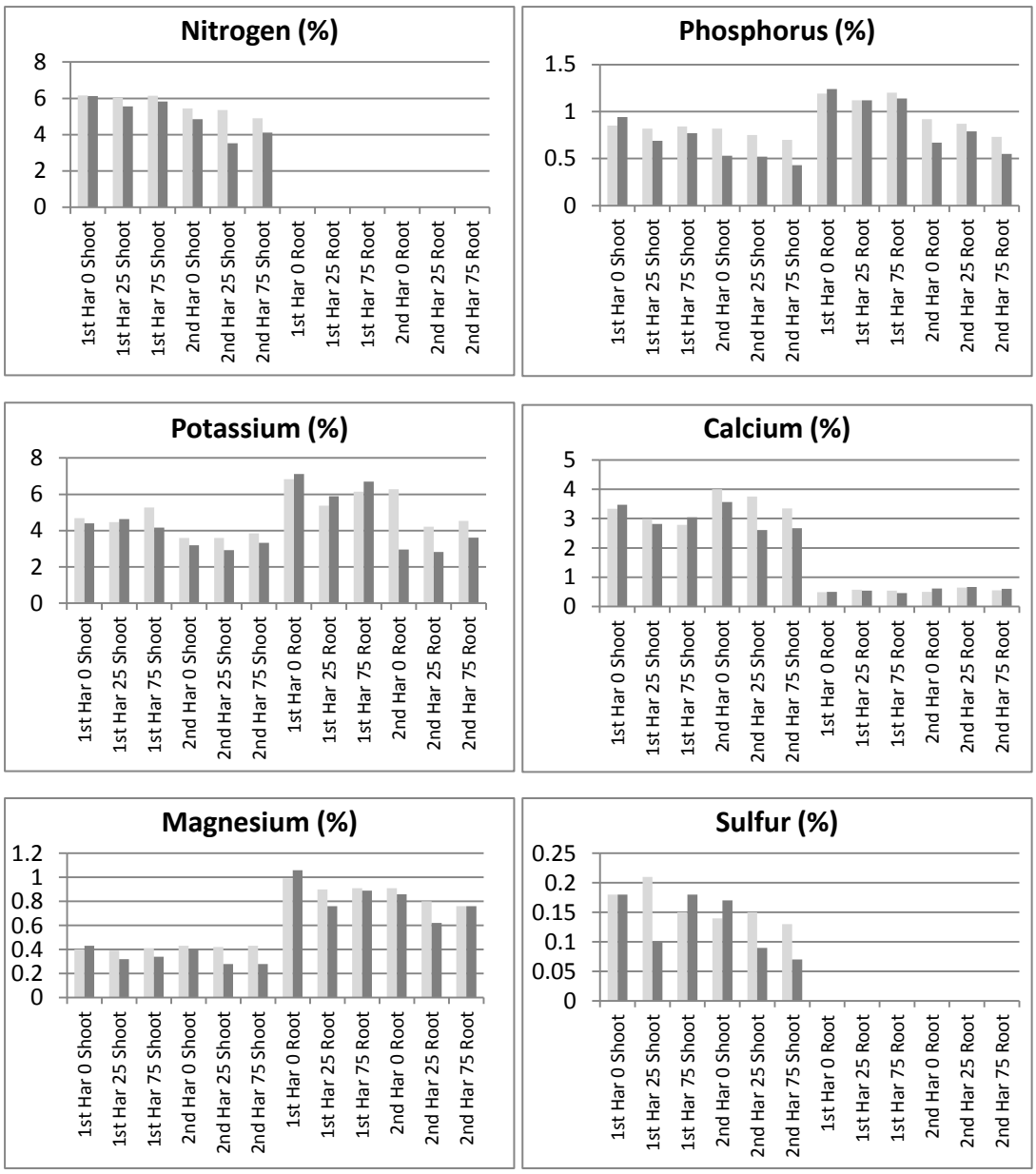


Fig. 35. Macronutrient analysis of basil 'Genovese' shoot and root tissue harvested at 30 DAT (first harvest) and 55 DAT (second harvest) treated with 0, 25 and 75 ppm of Si. The six plants harvested from each Si treatment were combined into one sample of shoot and root for ICP-AES. Light grey = plants cut and allowed to grow back. Dark grey = uncut plants.

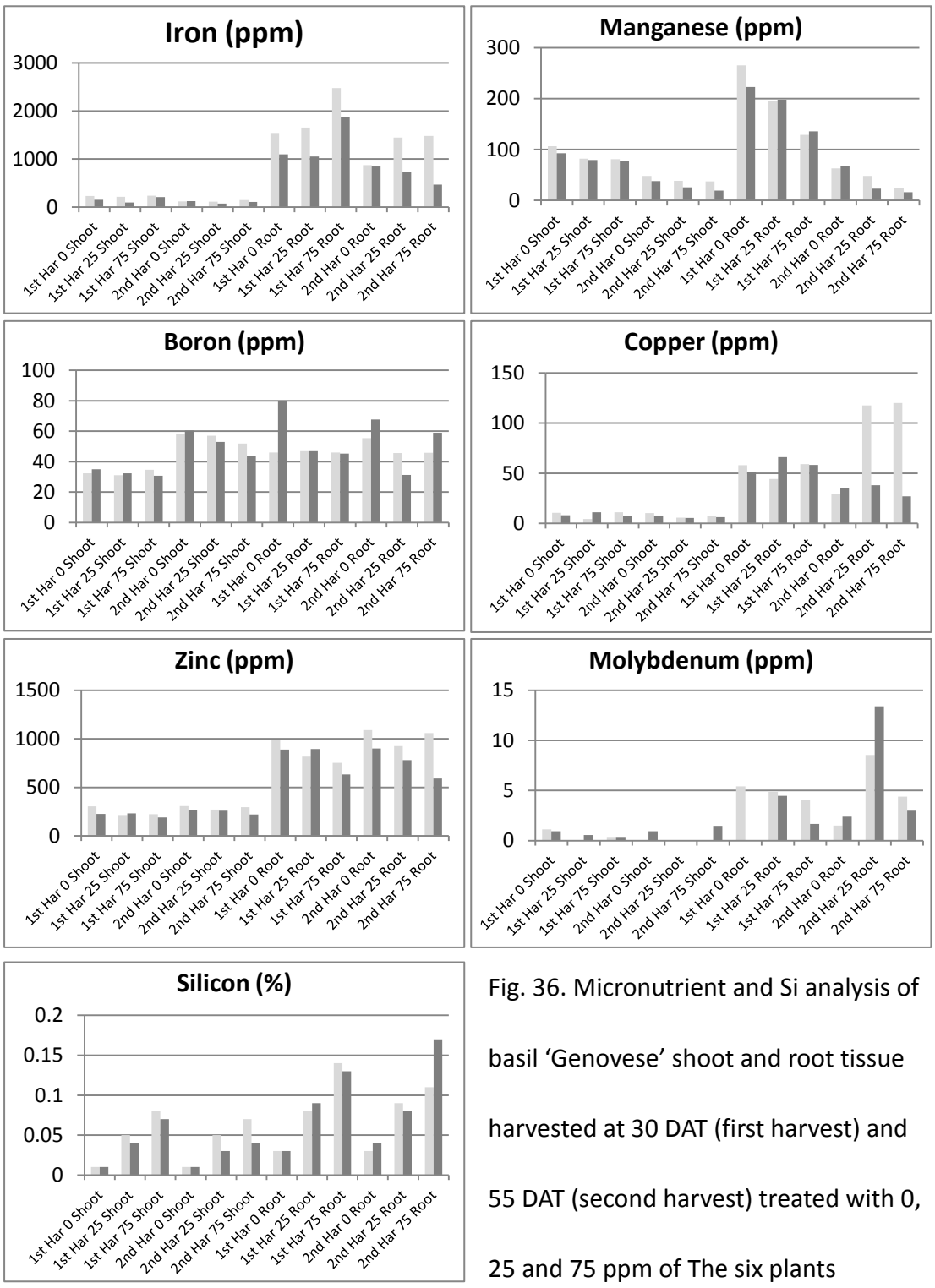


Fig. 36. Micronutrient and Si analysis of basil 'Genovese' shoot and root tissue harvested at 30 DAT (first harvest) and 55 DAT (second harvest) treated with 0, 25 and 75 ppm of The six plants harvested from each Si treatment were combined into one sample of shoot and root for ICP-AES. Light grey = plants cut and allowed to grow back. Dark grey = uncut plants

magnesium, manganese, and zinc were higher in both the shoots and roots of the cut group compared to the uncut group. This phenomenon was not Si dependent. For the cut group only, iron was accumulated in both shoots and roots of the Si treated plants but not in the non-treated control plants, indicating that Si treatments induced iron uptake when basil plants were stressed from mechanical damage. The level of Si in the cut and uncut groups was not different for both shoots and roots at the second harvest, indicating that the cut and grow back practice did not induce Si uptake and accumulation in the shoots or roots of basil.

3.4 Discussion and conclusions

In conclusion, when grown under optimal conditions, despite little Si accumulation, lettuce and bok choy benefited from Si treatment as was reflected in the increased plant shoot size and weight. Basil also benefited from the Si treatment, as was reflected in the increased shoot size but not in the roots. But the increases in shoot size and weight could also have been the result of additional potassium (18.87% more at 25 ppm Si, and 40.10% more at 75 ppm Si) and nitrogen (2.92% more at 25 ppm Si, and 8.76% more at 75 ppm Si) from adding additional K_2SiO_3 and nitric acid to the nutrient solutions amended with Si.

At a mature stage (first harvest), the Si amendment did not strongly influence the nutrient composition of lettuce plants, with the exception of calcium, magnesium, and zinc. Bok choy and basil plants were less sensitive to the Si treatments than lettuce, since most of the nutrient contents were not altered by the Si amendments. As the plant ages, differential absorption of several nutrients in lettuce and basil was observed, and this phenomenon was less strong in bok choy. These changes in the nutrient composition when plant ages were not the result of Si treatments. The cut and grow back treatment also generated some changes in the nutrient content of lettuce and basil, which again was less strong in bok choy. These nutrient content differences between the cut and uncut plants were also not the result of Si treatments, with the only exception that in basil, the Si treatments increased the iron content in both shoots and roots. The cut and grow back practice did not affect the Si

contents in both lettuce and bok choy shoots, and basil shoots and roots as the plants grew older.

As Si non-accumulators, the amount of Si absorbed by lettuce and bok choy in the shoots were very minimal (maximum of 0.03% Si in shoots) compared with Si accumulators in general, which can have more than 5% of dry weight as Si (Datnoff et al., 2001). In basil, Si was accumulated in both shoots and roots at a level higher than most Si non-accumulators. Compared with the lettuce and bok choy grown for this experiment, basil absorbed more Si when amended with Si (maximum of 0.08% in shoots and 0.14 % in roots) when grown under optimal growing conditions. This is a relatively high content for a Si non-accumulator compared with lettuce, bok choy and other non-accumulators such as tobacco (<300 mg Si per kg of dry tissue, Zellner et al., 2011).

Although not especially beneficial to cut and grow back practice, growers can still benefit from adding Si to their nutrient programs when in need for faster plant growth, and other benefits such as increasing the pH of the nutrient solution, or increasing the resistance to different biotic and abiotic stresses. Potential other benefits of Si, such as increasing the resistance against temperature or disease stresses, can be found in other chapters in this dissertation.

Chapter 4

Effects of silicon nutrition on lettuce, bok choy, and basil under various disease stresses

4.1 Introduction

Lettuce powdery mildew (LPM), caused by the obligate biotrophic parasitic ascomycete fungus *Golovinomyces cichoracearum* (previously known as *Erysiphe cichoracearum*), is often considered a minor and secondary disease of lettuce production in coastal areas, but can be a major concern for lettuce production in arid areas, impacting the quality of lettuce and causing economical losses (Blancard et al., 2006; Koike and Saenz, 1996). The first report of LPM was in 1941 at the Salinas Valley, CA (Pryor, 1941). Afterwards, LPM epidemics have been reported in drier, warmer areas of the US such as the southern part of the Salinas Valley and Arizona (Ryder, 1999). More recently, LPM was also identified and reported on Long Island, NY in 2013 (McGrath, 2013). The fungus grows ectophytically and can infect both mature leaves and seedlings. LPM spots gradually enlarge and eventually coalesce into patches that cover most foliar areas. Severely infected leaves may become necrotic, dry out and die (Blancard et al., 2006; Koike et al., 2007). LPM can retard plant growth, and eventually cause plant death (Lebeda and Mieslerova, 2003).

The life cycle of *G. cichoracearum* involves both sexual and asexual stages (Figure

37). The asexual conidia of *G. cichoracearum* can survive overwinter. The germination and germ tube formation requires 8 – 10 hrs, followed by a penetration stage of 10 to 17 hrs. The whole infection process can take 120 hrs (Schnathorst, 1959a). It has been reported that the LPM can initiate infection by releasing ascospores from the chasmothecia that were formed during the previous infection season, and the chasmothecia can burst open upon absorption of water on the plant surface (Schnathorst, 1959b).

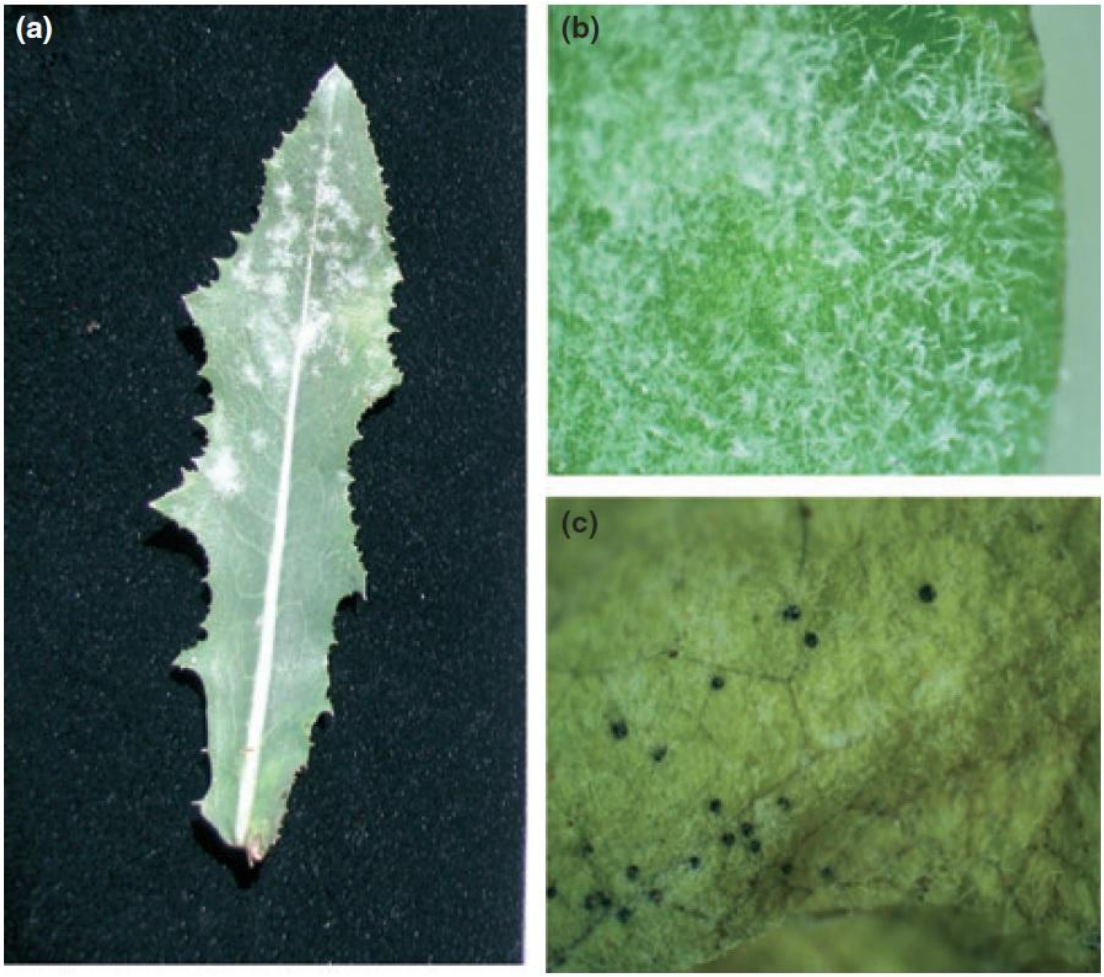


Fig. 37. Symptom and sign of lettuce powdery mildew (*Golovinomyces cichoracearum*) on lettuce. (a): Infected leaves covered with spots; (b): Conidiophores on infected lettuce leaf surface; (c): Chasmothecia. Figures from Lebeda and Mieslerova (2011).

The greenhouse environment provides the ideal conditions for LPM development, especially during the fall and winter months. The conidia of *G. cichoracearum* are spread by air and can germinate at temperatures between 5 and 33 °C. The optimal temperature for disease development is in the range of 18 – 25 °C. The disease development is slowed at 10 – 15 °C and at 30 °C (Sogelova, 2007). Humidity also plays an important role in LPM development. The conidia of *G. cichoracearum* can germinate at lower humidity (50 – 75% RH) with an optimal RH of 95 – 98%. But prolonged exposure to free water on the plant surface can inhibit the growth of *G. Cichoracearum* and reduce LPM development (Schnathorst, 1965). The light intensity can also affect LPM development. Reduced light intensity can cause the LPM resistant lettuce cultivar Great Lakes to become susceptible (Schnathorst, 1960).

Currently, the most widely used methods for controlling LPM are to use resistant cultivars and chemical control. Using the QTL mapping methods, Simko et al. (2014) found that leaf and butterhead lettuce were the most resistant, while crisphead lettuce was the most sensitive to LPM. Sulfur, QoI and myclobutanil were very effective against LPM when applied early and often (Matheron and Porchas, 2000). However, fungicide resistance in some powdery mildews to some groups of fungicides have been reported, for example, barley/corn powdery mildew (*Blumeria graminis* f. Sp. *tritici*) resistance to strobilurin fungicides (Erichsen, 1999).

Among the popular Brassicaceae vegetables, bok choy attracts few fungal diseases but many insect pests, such as diamondback/cabbage moth (*Plutella*

xylostella), cabbage looper (*Trichoplusia ni*), flea beetle (*Phyllotreta spp.*), cutworms (*Agrotis spp.*), leaf miners (*Phytomyza horticola*) and aphids (species from Aphididae). The insect pests feeding on bok choy can be broadly classified as chewing (Coleoptera, Lepidoptera, Hymenoptera), piercing or sucking (Heteroptera, Homoptera, Thysanoptera) and putrefying (Diptera) (Hegedus and Erlandson, 2012). The natural defense mechanisms of Brassicaceae plants include different physical and biochemical defense strategies, such as surface waxes, trichomes, toxic secondary plant metabolites and volatile compounds (Kumar and Singh, 2015).

Pests such as diamondback moth have been a major problem for not only bok choy but all Brassicaceae crops. As of today, the most common methods used by farmers in the developing countries are synthetic insecticides due to their efficacy and availability, but this approach has become more difficult due to the rapid development of insecticide resistance, and to their effects on natural enemies of the pests. This problem is especially acute for poorer farmers in Asia and Africa, who lack access to the up-to-date knowledge and the newest insecticides, and so tend to rely heavily and overuse older, more toxic, broad-spectrum insecticides (Grzywacz et al., 2009).

Plants can react to mechanical damage by altering the expression of genes that contribute to tissue defense and repair (Reymond and Farmer, 1998). Wounding caused by insect chewing can induce similar responses in the plant. A study in Arabidopsis (Reymond et al., 2000) showed that damage from physical wounding by puncturing and feeding by the cabbage butterfly (*Pieris rapae*) both caused induction

in many of the same stress-response genes. Meanwhile, there were several genes that were induced specifically by either puncture wounds (such as pathogenesis-related genes and aromatic metabolite synthesis) or insect feeding (such as hevein-like protein), indicating that insect elicitors were altering the plant's responses in addition to its mechanical wounding responses. Another study in lima bean (Mithofer et al., 2005) showed that continuous mechanical wounding induced the emission of a volatile organic compound blend qualitatively similar to that as from feeding by caterpillars (*Spodoptera littoralis*). These findings suggest that a controlled and reproducible mechanical tissue damage can resemble the insect's feeding process, and can be used as a tool for analyzing the role of physiological responses by plants against insect herbivory (Mithofer et al., 2005).

Basil downy mildew (BDM), caused by obligate parasitic oomycetes *Peronospora belbahrii*, was first observed and reported in Florida in 2007 (Roberts et al., 2009). BDM quickly spread to the northeast, southeastern, central and pacific states of the US, and became a major threat to all of the sweet basil acreage in the US (Wyenandt et al., 2015). Economical losses from BDM were estimated to be in the tens of millions of dollars. As a result, the acreage of field-grown basil was reduced in the US (Wyenandt et al., 2015).

The optimal temperature for BDM development is 20 °C (Garibaldi et al., 2007). The germination of *P. belbahrii* requires high humidity and leaf wetness. The sporangia enter the host via direct penetration of the leaf surface and via the

stomata. After infection, the sporangiophores exit through the stomata and produce sporangia, the secondary inoculum (Figure 38 A-B) (Koroch et al., 2013). The sporangia develop on the underside of the leaf, forming a dark brown fungal mass. The leaf becomes yellow, eventually necrotic and the leaf may fall off (Figure 38 C-D). In the field or greenhouse environment, BDM can develop and spread rapidly under favorable conditions such as high humidity, mild temperatures, poor air circulation and extended durations of leaf wetness (Garibaldi et al., 2007; Wyenandt et al., 2015). BDM may also be spread via contaminated seeds (Farahani-Kofoet et al., 2012).

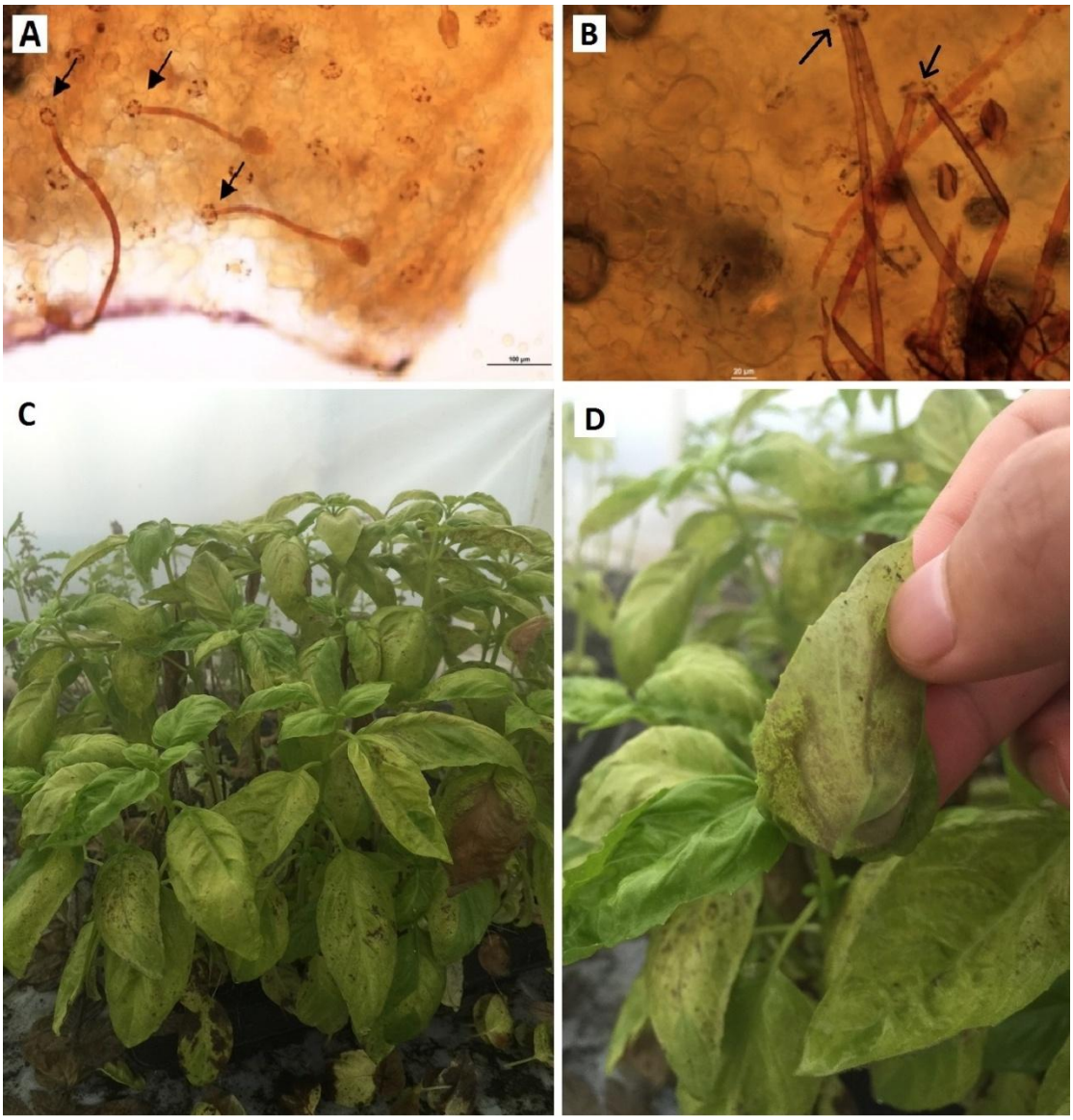


Fig. 38. Basil downy mildew. A: Sporangia enter the host through stomata (arrows). B: Sporangiophores emerging from stomata (arrows). C: Basil plants heavily infected with basil downy mildew. The upperside of leaves appears yellow and necrotic with substantial defoliation. D: Underside of the basil leaf covered with sporangia. The grey-brown appearance is a typical sign of basil downy mildew. Figures A and B: from Koroch et al. (2013). Figures C and D: from Yuan Li.

The current effective methods of controlling BDM include using monitoring and forecasting programs, resistant cultivars, cultural practices of growing basil when and where BDM is less likely to occur, and controlling humidity in the greenhouse environment (Wyenandt et al., 2015). Dr. Margaret McGrath from Cornell University has developed a spreadsheet-based online monitoring program since 2009. This system records and tracks the disease incidences across the US, providing a forecast for disease movement, and thus growers stay informed about the BDM threat (McGrath et al., 2010).

Among all basil species, sweet basil (*O. basilicum*) was determined to be the most susceptible to BDM. The economically less important basil species, such as the citrus (*O. x citriodorum*), spice (*O. americanum*), and holy type (*O. tenuiflorum*) basil were the least susceptible to BDM (Homa et al., 2016). The genes responsible for BDM resistance in *O. americanum* were identified (dominant, *Pb1A* and *Pb1A'*) and transferred into *O. basilicum* at 2017 (Ben-Naim et al., 2017). Led by Drs. Jim Simon and Andy Wyenandt at Rutgers University and aided by their graduate student Robert Pyne, extensive efforts have been undertaken to develop BDM resistant basil cultivars using traditional breeding techniques. At the end of the year 2018, four BDM resistant (two were also Fusarium Wilt resistant) basil varieties were released (US patent 10,159,212).

There are fungicides that are currently registered for BDM control. However, options for greenhouse and organic growers remain limited. Resistance to mefenoxam, a widely used fungicide has been reported in Isreal and Italy (Cohen et

al., 2013). Several phosphorous acid fungicides that were once effective against BDM have lost their efficacy (Cohen et al., 2017). It is expected that *P. belbahrii* will develop resistance to more fungicides in the future.

More environmental and consumer friendly control for disease (e.g., LPM and BDM) and insect pests of multiple crops needs to be developed. An alternative solution to chemical controls is to boost plant nutrition and strengthen the plant's self-defense against fungal invasion. When absorbed from the roots, soluble Si has been reported to accumulate under the leaf cuticle and form a protective layer of polymerized silica gel. This Si layer can increase the mechanical strength of the plant surface and serve as a barrier against fungal penetration (Datnoff et al., 2001; Etesami and Jeong, 2018; Ma and Yamaji, 2006; Zellner and Leisner, 2013). As a result, Si nutrition has been reported to boost the plant's defense against fungal diseases, such as in rice blast (Sun et al., 2010; Winslow et al., 1997), wheat powdery mildew (Belanger et al., 2002; Provance-Bowley et al., 2010) and tomato powdery mildew (Gilardi et al., 2011).

The objectives of this study were 1) To evaluate the efficacy of Si treatment when lettuce, bok choy, and basil are grown under LPM, insect damage (mimicked by mechanical wounding), and BDM, respectively; and 2) To evaluate if these typical disease stresses in lettuce, bok choy, and basil can induce Si uptake and accumulation in the plant shoots and roots. The information generated from this study would reveal the feasibility of controlling fungal diseases in lettuce, bok choy,

and basil by adding Si as a plant nutrient, as well as help improving hydroponic nutrient solution recipes.

4.2 Materials and methods

Lettuce Powdery Mildew

To evaluate the effect of Si treatments on lettuce infected with LPM, two experiments were conducted starting on Mar. 18, 2018 (Experiment 1) and May 11, 2018 (Experiment 2).

For LPM Experiment 1, lettuce cultivar Rex (Boston butterhead type, LPM sensitive, from Johnny's Selected Seeds, Fairfield, ME) were used. For LPM Experiment 2, lettuce cultivars Rex (described above) and Salanova (green oakleaf type, LPM sensitive, from Johnny's Selected Seeds, Fairfield, ME) were used. The seeding process and growth chamber operations were conducted as described in Chapter 1. Thirty-two (4*8 rows in one hydroponic system per Si treatments, for Experiment 1) and 24 (4*6 rows in one hydroponic system per Si treatments, for Experiment 2) seedlings of equal sizes and growth stages from each Si treatments were transplanted to the floating hydroponic systems after 11 days of incubation in the growth chamber. Greenhouse temperature was set to 23°C day and 20°C night (16 Hr photoperiod) for the entire growth period. The greenhouse environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR and DLI)

were monitored and recorded as described in Chapter 1.

Lettuce seedlings were inoculated right after transplant. For LPM Experiment 1, two heavily LPM infected lettuce plants grown in pots were obtained from Dr. Margery McGrath (Long Island Horticultural Experiment Station, Cornell University, Riverhead, NY) three days before the inoculation. During the inoculation, an inoculum of LPM was collected from the infected plants by brushing off the dusty fungal material (sporulation) into a bowl. The fungal material was spread evenly to all shoots of the newly transplanted lettuce seedlings using a brush. This procedure was repeated five days after the first inoculation. For LPM Experiment 2, nine heavily LPM infected lettuce plants were obtained from Dr. Margery McGrath. The transplanted seedlings received the same inoculation procedures with an additional inoculation at 10 days after the first inoculation, for a total of three inoculations. The inoculum plants were also placed next to the hydroponic systems to increase the disease spread. During both experiments, a control group of “no disease” was not included due to the fact that powdery mildew is an airborne disease. Disease progression was monitored and recorded periodically. Disease levels were assessed by estimating the leaf area covered by mildew spots and patches on a percent coverage basis.

Mechanical damage to simulate insect feeding on bok choy

To evaluate the effect of Si on bok choy under insect stress, one experiment was conducted starting on Jan. 19, 2018. Bok choy cultivar Black summer (dark green bok choy, from Johnny’s Selected Seeds, Fairfield, ME) was used for this experiment. The

greenhouse environmental data were recorded the same way as described for the LPM experiments. The seeding and transplant procedures, and the environmental set points were identical to the LPM Experiment 1 as described above.

The bok choy plants received puncture treatments on 30 DAT. Sixteen plants on the left side of the growing board were punctured, whereas the 16 plants on the right side served as control groups and remained untouched. For the punctured plants, about 10% of the leaf area was removed by punching multiple holes through the leaf using a hole puncher. After the treatment, the plants were grown for another 15 days. During the final harvest, shoots and roots of both punctured and control plants (16 plants each) were separated, and the shoots and roots of all 16 plants from each Si treatments (0, 25 and 75 ppm Si) were combined into 1 sample for tissue analysis. The plant samples were dried in an oven for four days, then processed using a grinder (Arthur H. Thomas Scientific Apparatus, Philadelphia, PA). Tissue analysis for essential elements and Si were carried out by ashing the samples (AOAC 900.02B) and mixing with aqua regia before performing inductively coupled plasma atomic emission spectroscopy (ICP-AES) at a commercial testing lab (PLT-1, MMI Labs, Athens, GA). Tissue analysis data were graphed using MS Excel for each element.

Basil Downy Mildew

To evaluate the effect of Si on basil plants grown under BDM, two experiments were conducted starting on Oct. 26, 2015 (Experiment 1) and Nov. 11, 2018

(Experiment 2). Organic basil var. ‘Genovese’ (Johnny’s Selected Seeds, Fairfield, ME) was used for both experiments. The greenhouse environmental data were recorded the same way as described for the LPM experiments.

Before BDM Experiment 1, basil plants infected with BDM were detected and collected in the field from New Brunswick, New Jersey on Sep. 2015. A separate disease chamber in a different greenhouse was built (located on Cook Campus, Rutgers University) using PVC pipes and single-layer polyethylene sheets. Two hydroponic systems and a humidifier were placed in the disease chamber for growing 64 ‘Nufar’ (Johnny’s Selected Seeds, Fairfield, ME) basil plants that served as the disease inoculum (Figure 39). The BDM was kept in the disease chamber for Experiment 1.

Fig. 39. ‘Nufar’ basil plants grown in a disease chamber built for preserving BDM collected in the field for BDM Experiment 1.

For BDM Experiment 1, six sheets of Oasis cubes were sown and grown in the growth chamber for 11 days. Four hydroponic systems (64 plants each) for each Si treatment were transplanted, representing four replications of the same Si treatment. One set of the replications was placed in a separate chamber covered with transparent 2-mil polyethylene sheets in the same greenhouse to prevent aerial BDM disease spread, serving as a healthy control group. The experimental group consisted of the other three replications that were placed together on the growing bench following a completely randomized design (Figure 40). The greenhouse temperature was set to 25 °C day and 22 °C night (16 Hr photoperiod). The basil plants were grown for four weeks to reach a mature size before BDM inoculation. BDM is a strong disease that can destroy crops quickly. Disease inoculation at the mature stage ensured the basil plants survived the disease long enough and the disease progression data could be collected before the plants succumbed to the disease.

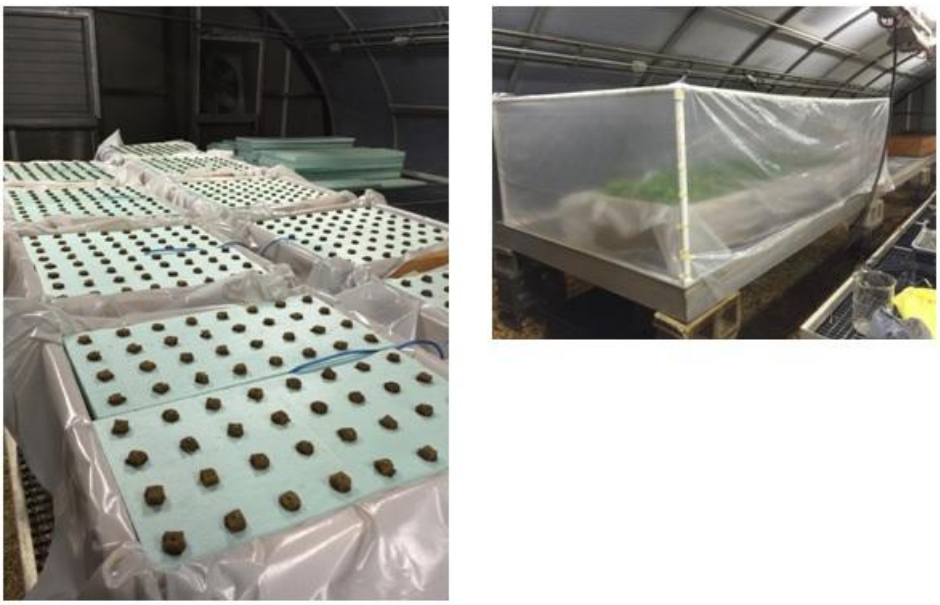


Fig. 40. Complete randomized design for BDM Experiment 1. Left: the disease group consists of three hydroponic systems for each of the 0, 25 and 75 ppm Si treatments. Right: the healthy control group for one of the Si treatments placed in a chamber covered with a polyethylene sheet near the infected plants. Each hydroponic system holds 64 plants (28 guard plants).

Diseased leaves were collected from the infected plants kept in the disease chamber. The inoculation was conducted one time to the basil plants on 28 DAT. The infected leaves were agitated in a bottle with DI water for 5-10 min to create a suspension of fresh *Peronospora belbahrii* spores. The suspension was filtered with a 40 µm nylon mesh filter (Fisher Scientific, Pittsburgh, PA). The above steps were repeated once. The suspension was centrifuged at 6000 rpm for 10 minutes, the pellet was collected and re-suspended in DI water. Spore concentration was checked using a hemocytometer slide under a microscope and diluted to 25,000 spores/mL for inoculation. This suspension was applied to plant shoot by saturating the leaf surface with a mist bottle. The greenhouse humidity was kept as close to 100% as possible by misting the entire greenhouse using a mist faucet connected to a water hose. Disease progression was monitored every other day post inoculation (DPI).

BDM was first observed at 15 DPI. Disease progression was monitored and recorded twice (15 and 20 DPI). The method of evaluating the disease was adopted and modified from the approach described in Pyne et al. (2014) and Wyenandt et al. (2015). Ten leaves (six leaves from the bottom, mature section, and four leaves from the newly emerged top section) from each plant were assessed, and every single leaf was rated from 0 to 5 based on the percentage of the underside leaf area covered by fungal sporulation (Figure 41). On 25 DPI, most plants had barely any leaves left, and disease evaluation was impossible to conduct. The experiment was therefore terminated on 25 DPI.

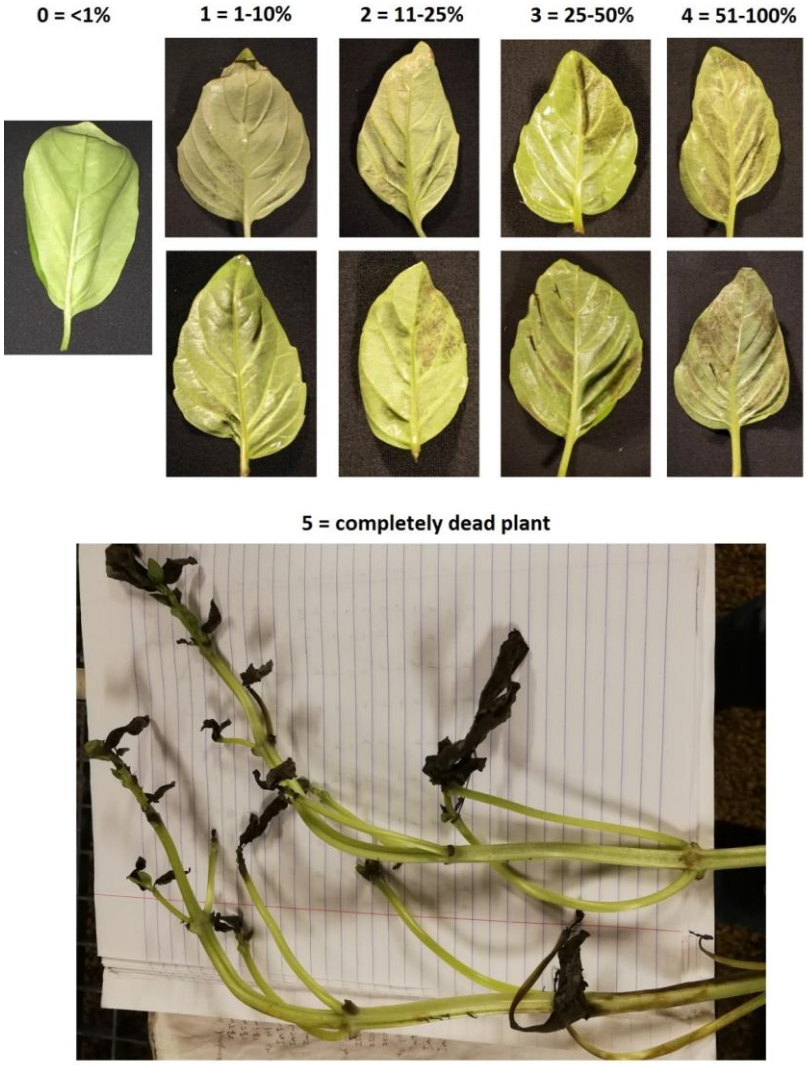


Fig. 41. BDM disease evaluation method of single leaves adopted from Wyenandt et al. (2015) by estimating the underside leaf area covered by BDM sporulation. The disease was assessed using an ordered categorical of 0 to 5 scale. 0 = Healthy leaf with no visible infections. 1 = less than 10% of leaf area was covered with sporulation, 2 = 11 to 25% of leaf area, 3 = 26 to 50% of leaf area, and 4 = more than 50%. A score of 5 indicates a completely dead plant with no leaves attached. Ten leaves (six older and four newer) were evaluated from every single plant. The disease index of a single plant was calculated by averaging the score of all ten leaves evaluated. The disease index of each Si treatment was calculated by averaging all 192 plants per treatment.

A disease index for each Si treatment was calculated based on the disease rating of every single leaf. The disease index for a single plant was the average of the ten leaves evaluated. The disease index for a single hydroponic system was the average of the 64 plants in it (includes the guard plants). The disease index of each Si treatment was the average of the three hydroponic systems, totaling 192 plants.

At the end of the experiment (25 DPI, 53 DAT), 20 plants from each Si treatment in the healthy control group were selected and their growth parameters (shoot height, root length, shoot, and root fresh and dry weight, and number of true leaves) were recorded. All plants from both healthy and infected groups were harvested. The harvested plants were separated into leaves, stems and roots. Plant parts from each Si treatments were combined into a single sample. All harvested plant parts were dried in an oven for four days, then processed using a grinder (Arthur H. Thomas Scientific Apparatus, Philadelphia, PA). Tissue analysis for essential elements and Si were carried out by ashing the samples (AOAC 900.02B) and mixing with aqua regia before performing inductively coupled plasma atomic emission spectroscopy (ICP-AES) at a commercial testing lab (PLT-1, MMI Labs, Athens, GA). The growth parameters and Si contents were analyzed and graphed using MS Excel.

Before the start of Experiment 2, BDM infected plants were preserved in a separate chamber similar to Experiment 1 in a different greenhouse (located inside the NJAES Research Greenhouses, Cook Campus, Rutgers University). The chamber was equipped with a humidifier for maintaining a high disease pressure.

For Experiment 2, three sheets of Oasis cubes were sown and grown in the

growth chamber for 11 days. For each Si treatment, two hydroponic systems [each with a capacity of 32 (4 by 8) plants] were transplanted with seedlings of equal sizes and growth stages. The healthy control group divided over three Si treatments was placed on the growing bench. The diseased group divided over three Si treatments was placed in a chamber covered with transparent 2-mil plastic sheets and equipped with a humidifier and box fan. The plants were grown for four weeks before inoculation. Before inoculation, eight plants from each Si treatment were harvested to evaluate their growth parameters at a mature, marketable stage (shoot height, root length, shoot and root fresh weight, and number of true leaves). The disease inoculation process was identical to Experiment 1.

BDM was first observed on the 8 DPI. The 18 non-guard plants from each Si treatment were evaluated every other day. The experiment was terminated on the 20 DPI. The disease progression curves were graphed using MS Excel. To compare the disease progression among each Si treatment, a permutation test was conducted using R (method designed by Russel Thompson and Gordon Smyth) to calculate all pairwise comparisons between two groups of disease progression curves. Accurate P-values were obtained by using a large permutation value (10,000) (Elso et al., 2004; Baldwin et al., 2007).

At the end of the experiment, eighteen plants from each Si treatments of both healthy and infected groups were harvested and separated into leaves, stems, and roots. Plant parts from each Si treatments were combined into 1 sample. Sample preparation, tissue analysis, and statistical analysis were carried out using the same

methods as described for Experiment 1.

4.3 Results

Lettuce powdery mildew

During LPM Experiment 1, the lettuce showed no sign of powdery mildew infection until 30 days after the final inoculation (35 DAT). The experiment was therefore terminated. During LPM Experiment 2, lettuce plants started to show very minor infection 30 days after the final inoculation (40 DAT), estimated at less than 10 mildew spots per hydroponic system (Figure 42). The lettuce plants were too crowded to observe every single leaf. The plants were kept and observed for another 15 days. The disease level did not increase further. The experiment was terminated on 55 DAT, no disease data were collected. The greenhouse environmental data for both of the experiments are shown in Appendix C.

Fig. 42. LPM Experiment 2: The lettuce plants showed very minor powdery mildew disease infection after three (0, 5 and 10 DAT) inoculation attempts. Left: Salanova green oakleaf lettuce grown in the floating hydroponic system. Right: close observation showing that very few powdery mildew lesions were detected across all Si treatments. Both photos were taken from the control treatment at 40 DPI.

Mechanical damage to simulate insect feeding on bok choy

The bok choy experiment was conducted during the winter months and the greenhouse was able to maintain the temperature set points during both day and night after transplant. The greenhouse environmental data are shown in Figures 43 (before puncture), 44 (after puncture), and Table 18.

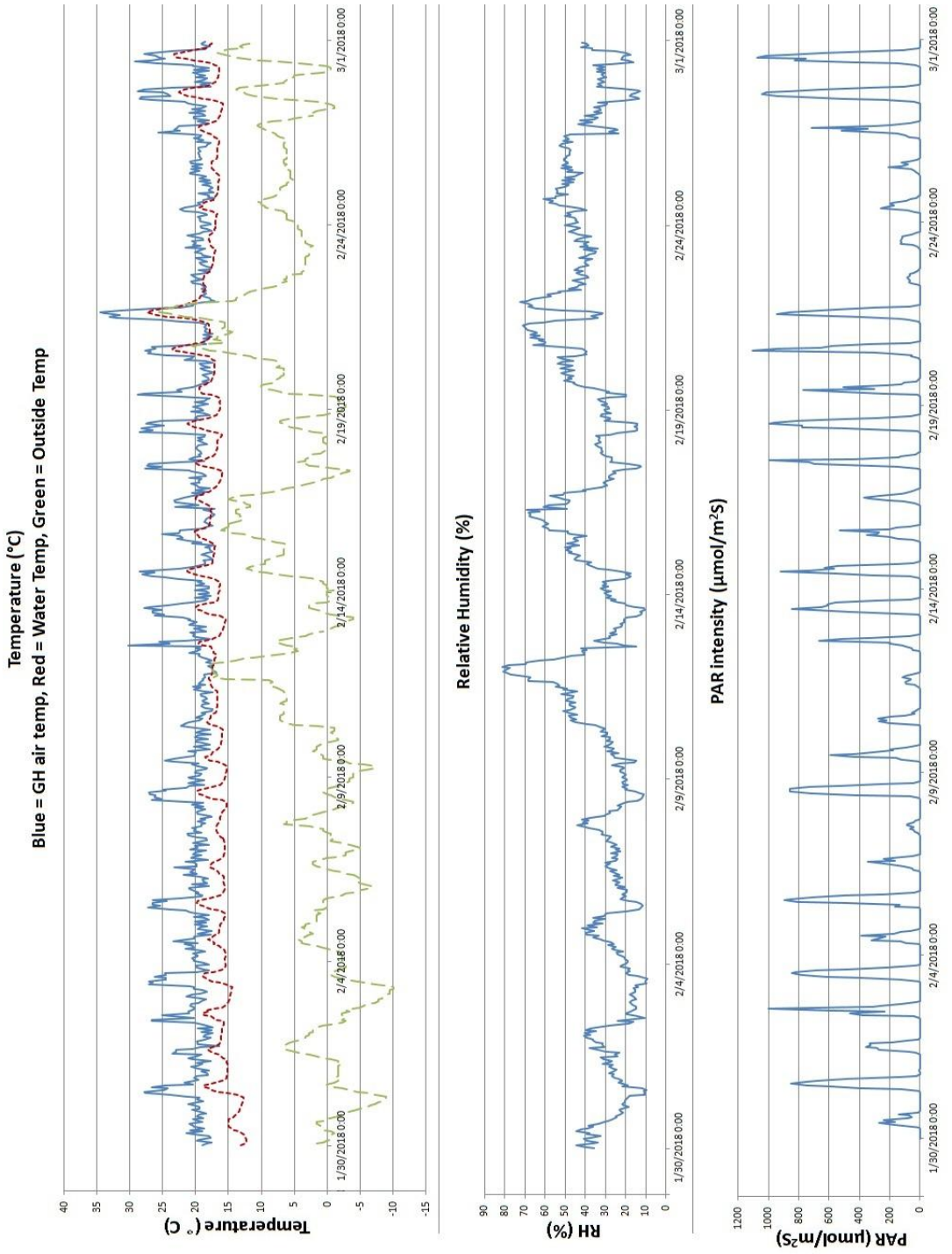


Fig. 43. Bok choy hole punch Experiment: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). Data ranges between Jan. 30, 2018, the first DAT and Feb. 28, 2018 (30 DAT, when the puncture was conducted).

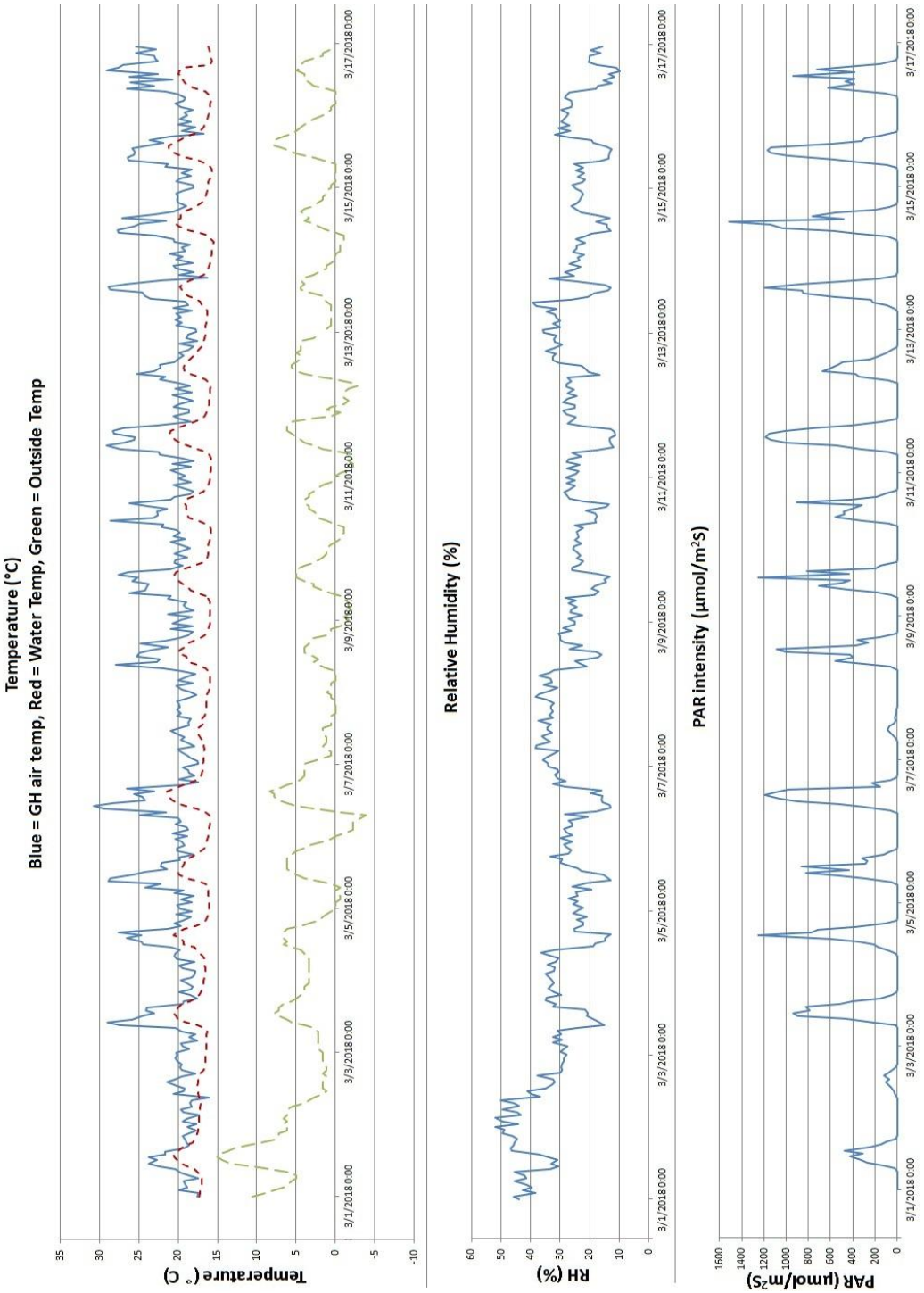


Fig. 44. Bok choy hole punch Experiment: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). Data ranges between Mar. 1, 2018, (30 DAT, when the puncture was conducted) and Mar. 16, 2018 (45 DAT, final harvest).

Table 18. Bok choy hole punch Experiment: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), between Jan. 30, 2018, the first DAT and the final harvest on Mar. 16, 2018 (45 DAT).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI
30-Jan	1	19.8	19.9	14.2	13.1	-0.1	-6.1	29.7	4.6
31-Jan	2	22.6	21.4	16.0	15.6	-3.6	-3.0	20.6	16.0
1-Feb	3	20.0	18.7	16.6	16.0	3.5	2.5	32.5	6.9
2-Feb	4	21.8	19.9	16.8	14.7	-3.2	-8.7	17.7	13.2
3-Feb	5	22.7	19.2	16.7	15.5	-4.0	-2.3	16.9	17.1
4-Feb	6	19.7	19.1	16.7	15.9	2.3	2.4	32.1	5.9
5-Feb	7	22.8	19.5	17.5	15.7	-0.1	-5.2	20.6	17.2
6-Feb	8	19.8	19.5	16.3	15.6	-1.9	-3.6	26.2	5.8
7-Feb	9	19.5	19.3	16.4	15.7	1.7	0.3	32.2	1.6
8-Feb	10	23.0	19.7	17.3	15.4	-1.6	-4.2	21.7	17.9
9-Feb	11	20.4	19.1	16.8	16.0	-0.9	-0.9	25.8	9.0
10-Feb	12	19.7	18.4	17.2	16.7	4.9	6.5	43.3	4.5
11-Feb	13	18.9	18.0	17.5	17.3	11.1	16.3	63.9	2.5
12-Feb	14	20.9	19.7	17.6	15.8	6.0	-1.0	31.5	7.8
13-Feb	15	23.0	19.0	17.7	16.4	-0.1	-0.1	22.0	17.0
14-Feb	16	22.1	18.4	18.6	17.2	7.3	7.2	34.3	15.6
15-Feb	17	20.5	18.1	18.6	17.7	7.7	13.4	54.4	7.8
16-Feb	18	20.1	19.6	18.4	16.2	11.3	0.9	43.7	5.7
17-Feb	19	21.6	19.0	17.8	16.6	1.1	0.3	28.0	13.4
18-Feb	20	23.4	19.3	18.5	16.3	3.6	-2.3	25.3	21.0
19-Feb	21	20.6	18.4	18.1	17.1	6.1	7.0	41.0	8.9
20-Feb	22	22.1	18.1	19.9	17.9	14.7	15.7	56.4	14.7
21-Feb	23	25.5	18.2	22.5	19.1	20.8	12.9	55.4	17.2
22-Feb	24	19.3	18.9	18.3	17.3	5.8	3.2	41.4	2.2
23-Feb	25	19.4	18.7	17.6	16.9	3.4	5.3	43.8	3.2

Table 18 continued

24-Feb	26	20.0	18.6	17.8	16.5	22.7	17.3	49.2	5.8
25-Feb	27	19.3	18.7	17.0	16.6	6.1	6.4	49.9	2.8
26-Feb	28	20.7	19.5	17.8	16.1	8.1	1.0	37.2	9.4
27-Feb	29	23.4	19.3	19.1	16.5	8.4	3.3	26.0	22.9
28-Feb	30	22.9	18.6	19.7	17.4	10.5	11.4	29.6	22.1
1-Mar	31	20.6	18.6	18.7	17.3	10.1	6.3	43.2	8.9
2-Mar	32	19.6	19.4	17.1	16.9	2.1	2.1	34.2	2.2
3-Mar	33	21.9	19.0	18.4	16.7	5.1	3.4	28.8	17.9
4-Mar	34	21.6	19.4	18.0	16.1	4.7	0.1	24.6	14.0
5-Mar	35	21.9	19.5	18.1	16.1	4.1	-1.0	25.4	15.3
6-Mar	36	22.7	18.9	18.7	16.8	4.2	2.3	26.3	24.2
7-Mar	37	19.4	19.4	16.8	16.1	0.8	0.5	34.4	1.6
8-Mar	38	21.2	19.4	17.3	16.0	1.7	-1.5	25.9	18.2
9-Mar	39	22.8	19.5	18.2	16.0	2.4	0.2	22.2	19.5
10-Mar	40	21.8	19.5	17.7	15.9	2.1	-1.0	23.9	15.3
11-Mar	41	23.8	19.5	18.5	16.0	2.8	-0.8	21.9	27.6
12-Mar	42	20.9	19.0	17.7	16.5	3.4	1.2	29.3	13.8
13-Mar	43	21.5	19.6	17.7	15.8	2.3	0.0	25.6	15.9
14-Mar	44	22.2	19.3	18.1	16.0	1.9	0.4	21.3	21.9
15-Mar	45	22.7	19.5	18.5	16.1	4.4	1.3	23.1	24.3
16-Mar	46	24.3	24.5	17.5	17.4	2.7	0.2	17.3	19.2
Average		21.4	19.3	17.8	16.4	4.5	2.4	32.2	12.6
St. Dev.		1.6	1.0	1.2	0.9	5.5	5.8	11.5	7.2

During the bok choy hole punch experiment, the punctured plants did not exhibit any visual differences in height, color and general morphology on both shoots and roots 15 days after the puncture treatments (observations only, no data recorded).

When comparing the mineral content, there was little difference between the punctured and non-punctured control plants. None of the essential nutrients exhibited obvious differences other than that the iron and copper level in the roots were marginally higher for the punctured plants compared to the control plants. The nutrient content in the shoot tissue among all the Si treatments were similar. Among the root samples, the 25 ppm Si treated plants exhibited substantially lower phosphorus, potassium, magnesium, and iron, and high levels of sulfur and molybdenum (Figures 45 and 46).

When treated with Si, the bok choy plants showed a very minor increase in Si accumulation in the shoots for both punctured and non-punctured plants. The increase of Si content in the roots was more obvious than shoots, but the overall Si content was very minimal (maximum of 0.13%). The non-punctured control plants exhibited marginally higher Si content than the puncture treated plants across all Si treatments, and this trend was consistent in both shoots and roots.

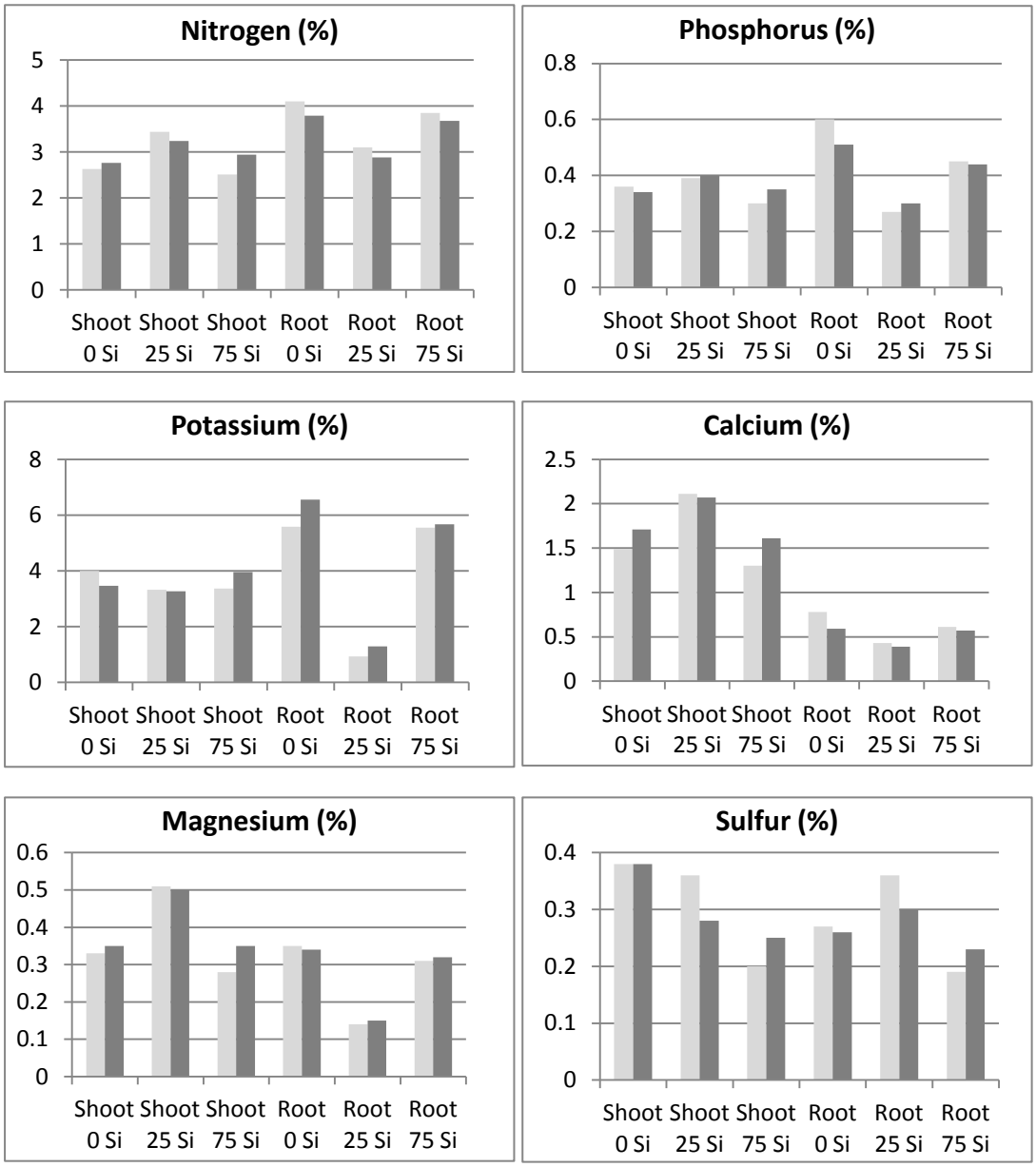


Fig. 45. Bok choy hole punch Experiment: Macronutrient analysis of bok choy 'Black Summer' shoot and root tissue treated with 0, 25 and 75 ppm of Si harvested at 15 days after the puncture treatment (35 DAT). The 18 plants harvested from both punctured and control groups of each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = non-punctured plants. Dark grey = punctured plants.

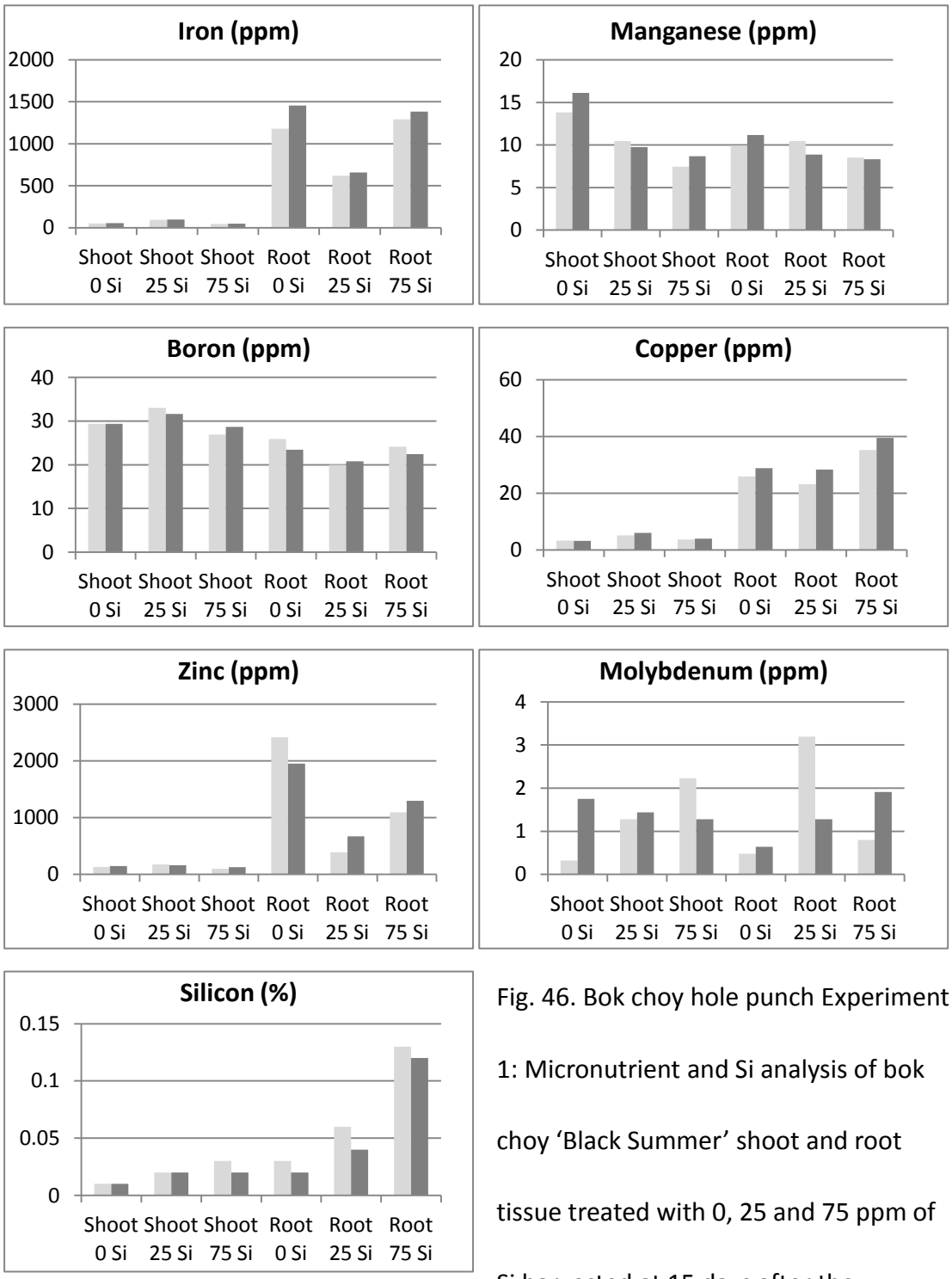


Fig. 46. Bok choy hole punch Experiment 1: Micronutrient and Si analysis of bok choy 'Black Summer' shoot and root tissue treated with 0, 25 and 75 ppm of Si harvested at 15 days after the puncture treatment (35 DAT). The 18 plants harvested from both punctured and control groups of each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = non-punctured plants. Dark grey = punctured plants.

Basil Downy Mildew

The BDM experiments were conducted during the winter seasons of 2015 and 2018. During BDM Experiment 1, the environmental data acquisition system was not well established yet. The environmental data from Nov. 6, 2015 (first DAT) to Dec. 28, 2015 (53 DAT) are not available. During BDM Experiment 2, the datalogger was damaged before the experiment started and the data table had time values missing randomly. The datalogger broke down entirely on the day of inoculation (Dec. 19, 2018) and no data were collected by the datalogger after that. The experiment continued without a backup datalogger. The environmental data from the first DAT (Nov. 22, 2018) to the day before inoculation (Dec. 18, 2018) are presented in Figure 47 and Table 19. The environmental data during the post-inoculation days (Dec. 19, 2018 to Jan. 8, 2019) are not available.

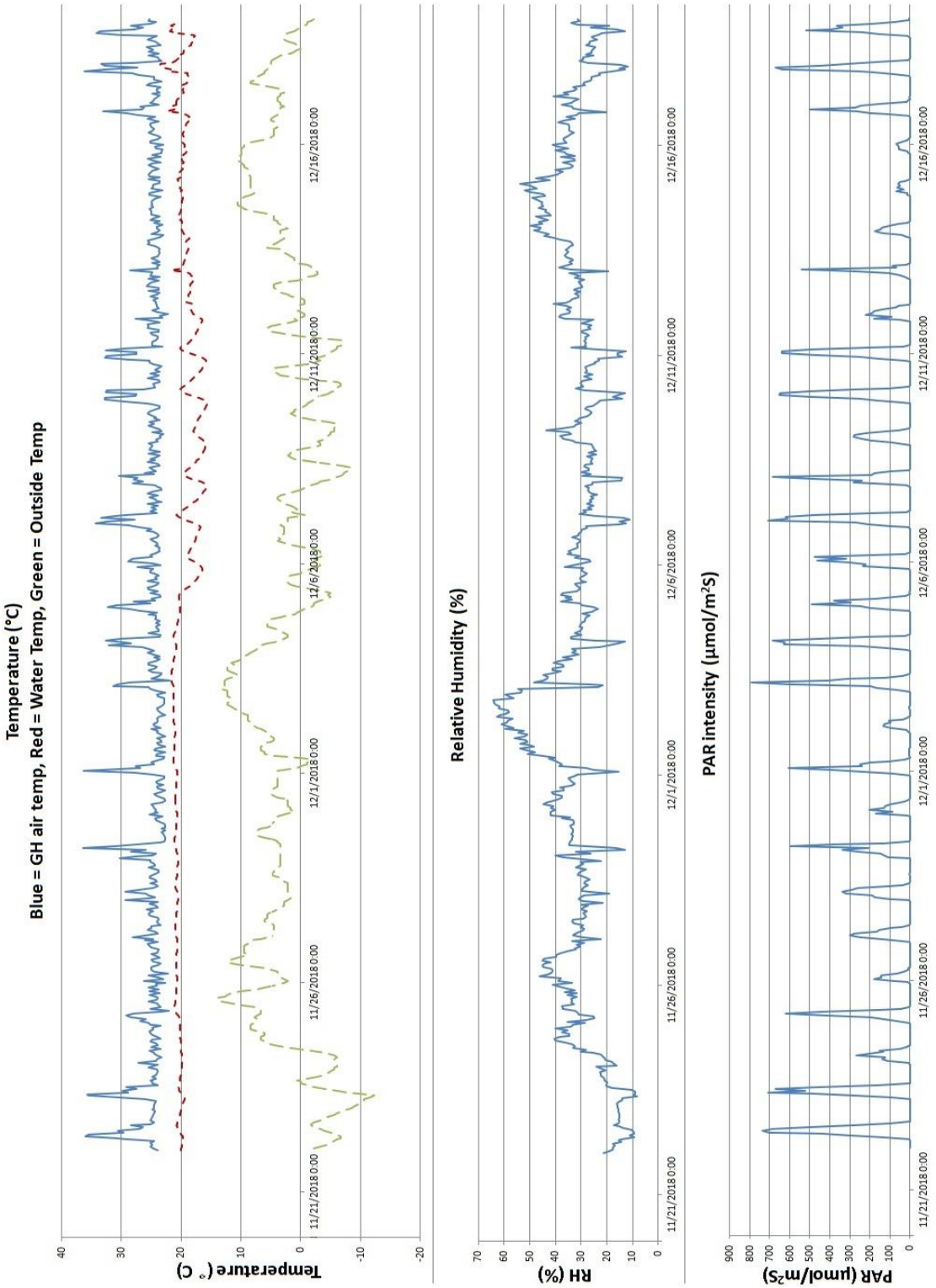


Fig. 47. BDM Experiment 2: Greenhouse (GH) environmental data, including greenhouse air temperature, outside air temperature, hydroponic nutrient solution temperature, relative humidity and light intensity (PAR). Data ranges between Nov. 22, 2018 (first DAT) to Dec. 18, 2018 (the day before BDM inoculation).

Table 19. BDM Experiment 2: Calculated averages of daily greenhouse air temperature (°C), hydroponic solution water temperature (°C), outside temperature (°C), greenhouse relative humidity (%) and daily light integral [DLI, mol/(m^2d)] of the growing period (day = 6 am – 22 pm, night = 22 pm – 6 am), between Nov. 22, 2018 (first DAT) and Dec. 18, 2018 (the day before BDM inoculation).

Dates	DAT	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI
22-Nov	1	27.5	25.1	22.3	19.8	-4.3	-9.1	14.2	14.3
23-Nov	2	26.9	26.2	22.1	21.5	-4.5	-5.0	17.8	12.3
24-Nov	3	24.4	26.9	21.3	22.0	3.2	7.8	32.1	4.1
25-Nov	4	24.7	24.5	21.8	21.9	9.8	3.3	34.6	9.4
26-Nov	5	24.4	26.6	21.4	22.2	9.0	6.5	36.5	2.9
27-Nov	6	24.6	25.8	21.0	22.0	4.6	2.2	29.4	6.5
28-Nov	7	24.6	26.7	21.2	22.4	3.4	3.3	29.8	6.6
29-Nov	8	24.6	24.2	21.9	22.1	4.8	1.9	35.1	7.2
30-Nov	9	23.5	28.0	21.5	22.8	4.0	0.9	35.1	4.0
1-Dec	10	23.5	24.0	22.0	22.5	5.0	7.7	37.6	7.8
2-Dec	11	23.5	27.3	22.3	23.3	11.2	12.6	53.2	2.9
3-Dec	12	24.6	28.6	21.6	22.8	10.3	4.2	33.6	8.7
4-Dec	13	24.8	26.0	20.4	21.5	2.9	-3.7	30.1	11.5
5-Dec	14	24.7	25.7	18.9	20.6	-0.1	-3.0	31.6	6.6
6-Dec	15	24.9	29.6	19.2	22.1	2.3	1.2	27.0	8.5
7-Dec	16	24.6	27.3	18.4	21.3	1.1	-7.0	24.8	11.9
8-Dec	17	25.2	24.1	18.4	19.8	2.2	-5.2	29.9	8.3
9-Dec	18	25.1	29.0	18.3	21.9	-1.0	-5.6	26.0	7.3
10-Dec	19	25.1	28.5	18.4	21.6	0.1	-6.0	24.9	10.8
11-Dec	20	24.8	24.4	20.1	20.5	1.5	0.0	31.1	9.6
12-Dec	21	24.5	24.9	19.9	21.9	1.4	0.0	31.9	4.3
13-Dec	22	24.5	24.0	20.7	21.7	3.4	3.8	39.7	3.8
14-Dec	23	24.3	24.2	21.5	21.8	8.9	8.4	47.1	1.6
15-Dec	24	24.4	24.2	21.1	21.2	9.8	6.2	36.7	1.7
16-Dec	25	25.7	24.5	21.4	21.7	4.1	3.4	34.1	6.1

Table 19 continued

17-Dec	26	27.0	25.4	21.5	21.2	6.0	2.5	25.1	10.2
18-Dec	27	26.7	24.0	21.1		0.9	-4.1	25.6	9.0
Average		24.9	25.9	20.7	21.7	3.7	1.0	31.6	7.3
St. Dev.		1.0	1.7	1.3	0.8	4.2	5.5	7.9	3.4

The growth evaluation for BDM Experiment 1 was conducted after the disease evaluation at 53 DAT. For Experiment 2, the growth evaluation was conducted before the disease inoculation at 28 DAT. For both experiments, the basil plants had reached a mature, marketable size. The 75 ppm Si treated basil plants exhibited superior growth in both experiments compared against the 25 ppm Si and control treatments. The 25 ppm Si treated and the control plants exhibited very similar growth. The height and fresh weight and number of true leaves of the shoots of the 75 ppm Si treated plants were 9.6%, 14.4% and 37.4% (Experiment 1), and 12.2%, 3.3% and 20.7% (Experiment 2) higher than the control plants, respectively. The root length and fresh weight were 29.1%, 79.2% (Experiment 1) and 1.0% and 42.0% (Experiment 2) higher than the control plants, respectively.

Table 20. BDM Experiment 1: growth parameters for the control, 25 and 75 ppm Si treatments for basil plants 'Genovese' before BDM inoculation (53 days after transplant, n = 20). Shoot and root dry weight were not recorded during the experiment. The shoot and root dry weight in this table were calculated values from the fresh weight at mature stage, based on the data from all basil experiments described in this dissertation. SDW = SFW * 0.08348. RDW = RFW * 0.03814. T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**[z]	**RDW**[z]	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	34.3	41.9	37.5	5.38	3.13	0.21	19.0
SD	8.74	23.17	22.75	3.92	1.90	0.15	11.0
25 Si	33.1	49.8	32.5	6.94	2.71	0.26	18.6
SD	7.45	19.35	20.87	5.89	1.74	0.22	12.25
75 Si	37.6	54.1	42.9	9.64	3.58	0.37	26.1
SD	6.03	17.37	22.29	5.69	1.86	0.22	13.65
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.655	0.225	0.524	0.352	0.478	0.331	0.916
Ctrl vs 75 Si	0.223	0.077	0.471	**0.010**	0.448	**0.009**	0.106
25 Si vs 75 Si	**0.016**	0.447	**0.009**	**0.025**	0.136	0.149	**0.002**

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 $mg \cdot L^{-1}$. SD = Standard deviation. Bolded values indicate $P \leq 0.05$.
[z]Calculated from fresh weight data

Table 21. BDM Experiment 2: growth parameters for the control, 25 and 75 ppm Si treatments for basil plants 'Genovese' before BDM inoculation (28 days after transplant, n = 8). T-tests were performed to evaluate the growth responses to the Si treatments.

Treatment	**SH**[x]	**RL**	**SFW**	**RFW**	**SDW**	**RDW**	**NL**
(ppm Si)[y]	(cm)		(g)				(-)
0 Ctrl	24.5	50.5	15.0	2.19	1.25	0.08	14.5
SD	2.99	7.89	2.96	0.55	0.25	0.02	2.52
25 Si	23.3	37.8	11.6	2.35	0.97	0.09	12.0
SD	2.06	6.45	2.30	0.53	0.19	0.02	4.90
75 Si	27.5	51.0	15.5	3.11	1.29	0.12	17.5
SD	1.73	4.40	1.01	0.21	0.08	0.01	1.00
Contrast (*P*-value a = 0.05)							
Ctrl vs 25 Si	0.604	**0.048**	0.127	0.685	0.127	0.685	0.410
Ctrl vs 75 Si	0.121	0.916	0.751	**0.036**	0.751	**0.036**	0.092
25 Si vs 75 Si	**0.020**	**0.018**	**0.035**	0.058	**0.035**	0.058	0.108

[x]SH = shoot height, RL = root length, SFW and RFW = shoot and root fresh weight, SDW and RDW = shoot and root dry weight, NL = number of true leaves.
[y]1 ppm = 1 mg·L^{-1}. SD = Standard deviation. Bolded values indicate P ≤ 0.05.

During BDM Experiment 1, at 15 DPI, the disease progression was considered at an early stage, and the disease progression differences among the control, 25 and 75 ppm Si treatments were non-significant. At 20 DPI, plants across all treatments were heavily infected. The disease evaluation showed that the 25 and 75 ppm Si treated plants were significantly less infected by 16.1% and 23.7%, respectively, compared to the control treatment. Visually, the disease level across all treatments was intense, and the Si treated plants did not exhibit visually superior BDM tolerance (Figure 48). At 25 DPI, all plants across all treatments were almost completely defoliated and were considered dead. The experiment was therefore terminated at that point.

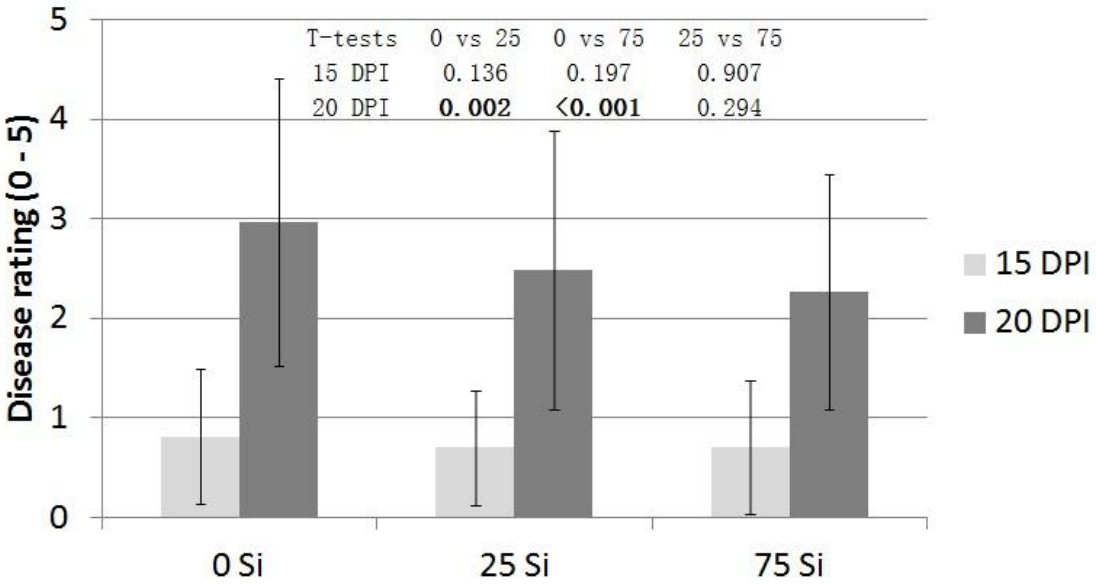


Fig. 48. BDM Experiment 1: Disease progression (n = 192 plants) at 15 and 20 DPI. Error bars indicate ± standard deviation. Ten leaves from each plant were evaluated based on a 0 – 5 scale, with 0 indicating a healthy leaf, and 5 indicating a completely dead plant. T-tests were conducted on the data for the 0, 25 and 75 Si treated plants at 15 and 20 DPI to reveal statistical significances (*P*-value a = 0.05).

During BDM Experiment 2, the disease was first spotted at 8 DPI. The disease quickly developed afterward and disease evaluation was performed every other day (Figure 49). At 20 DPI, most of the basil plants in all treatments were completely defoliated, and the experiment was therefore terminated.

Comparing the overall disease progression, the 25 and 75 ppm Si treated plants had significantly less disease during the earlier stages (8 to 10 DPI). The 25 and 75 ppm Si treated plants had 39.6% and 68.5% less disease than the control plants at 8 DPI, respectively. During the later disease progression stage (12 to 16 DPI), the differences of disease presence among the Si treated plants and the control plants were still statistically significant but to a lesser extent. The 25 and 75 ppm Si treated plants had 6.5% and 6.2% less disease than the control plants at 16 DPI, respectively. Near the end of the experiment (18 to 20 DPI), the disease presence across all treatments was very similar and was statistically non-significant (Figure 50 and Table 22). When comparing the overall disease progression curves, the 25 and 75 ppm Si treated plants were very similar ($P = 0.987$), and were both statistically significant compared to the control treatment ($P = 0.0045$ for 25 ppm Si, and 0.0002 for 75 ppm Si).

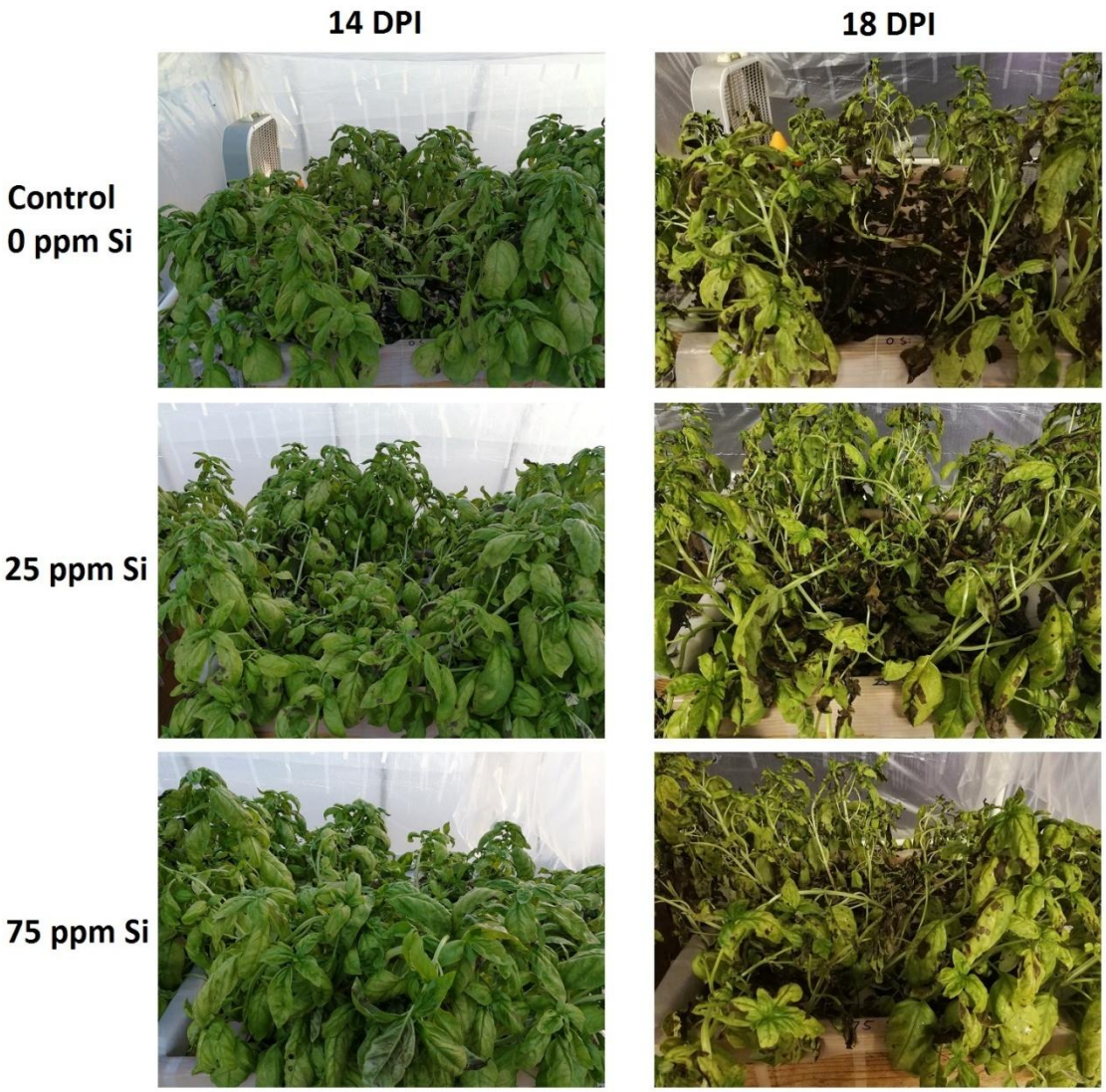


Fig. 49. BDM disease progression during Experiment 2. BDM was first spotted at 8 DPI and quickly developed inside the enclosed chamber. Extensive defoliation was observed during each disease evaluation after 10 DPI. At 20 DPI, the plants across all Si treatments were mostly defoliated and the experiment was therefore terminated.

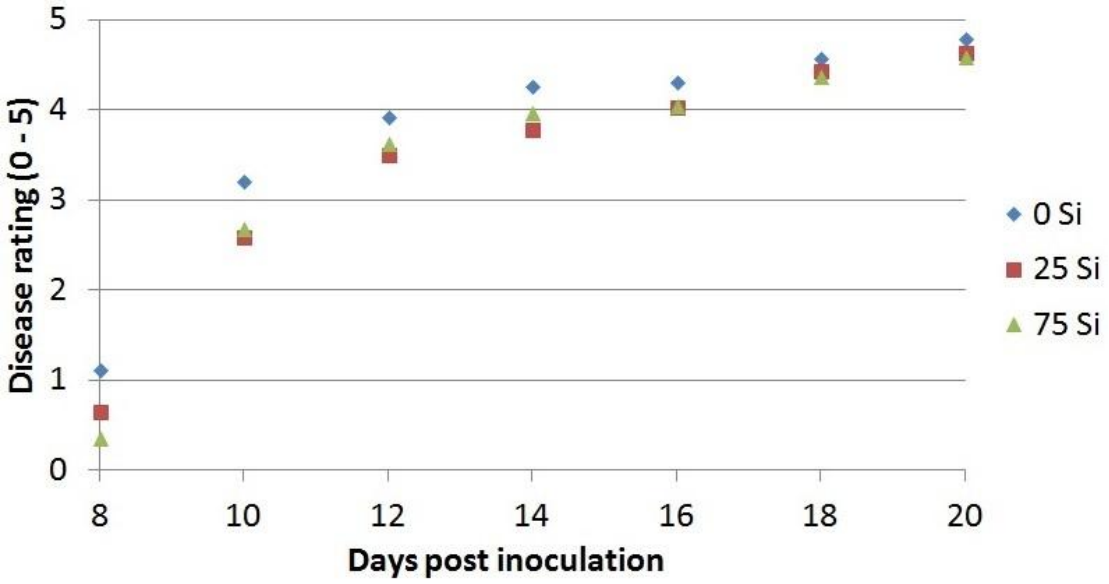


Fig. 50. BDM Experiment 2: Disease progression (n = 18 plants) for the control, 25 and 75 ppm Si treatments from 8 to 20 DPI. Ten leaves from each plant were evaluated based on a 0 – 5 scale, with 0 indicating a healthy leaf, and 5 indicating a completely dead plant.

Table 22. BDM Experiment 2: Disease progression (n = 18 plants) from 8 to 20 DPI. Ten leaves from each plant were evaluated based on a 0 – 5 scale. T-tests were conducted among the 0, 25 and 75 Si treatments to reveal statistical significance (*P*-value, a = 0.05). A permutation test was conducted using software package R to reveal the statistical significances (*P*-value, a = 0.05) between the disease progression curves for each Si treatment (n = 18 curves). Bolded values indicate P ≤ 0.05.

DPI	**8**	**10**	**12**	**14**	**16**	**18**	**20**
0 ppm Si Ctrl	1.11	3.21	3.92	4.27	4.32	4.58	4.79
SD	0.54	0.46	0.40	0.34	0.30	0.38	0.27
25 ppm Si	0.67	2.60	3.52	3.80	4.04	4.45	4.64
SD	0.53	0.45	0.56	0.47	0.45	0.47	0.48
75 ppm Si	0.35	2.68	3.63	3.98	4.05	4.38	4.59
SD	0.43	0.48	0.37	0.26	0.15	0.27	0.33
T-tests (*P*-value a = 0.05)							
Ctrl vs 25 Si	**0.016**	**<0.001**	**0.017**	**0.002**	**0.038**	0.350	0.256
Ctrl vs 75 Si	**<0.001**	**0.002**	**0.028**	**0.007**	**0.002**	0.073	0.051
25 Si vs 75 Si	0.059	0.613	0.483	0.166	0.902	0.590	0.706
Disease progress curve comparison (*P*-value a = 0.05)							
Ctrl vs 25 Si	**0.005**						
Ctrl vs 75 Si	**<0.001**						
25 Si vs 75 Si	0.987						

The tissue analysis was conducted twice for BDM Experiment 1. The first tissue analysis was conducted by digesting with heated hydrofluoric acid, then followed by heropoly blue colorimetric method at a commercial testing lab (IB220, Brookside Labs, New Bremen, OH). The results indicated a substantial amount of Si in the control samples. The same samples were sent to MMI labs for a re-test using ICP-AES, and showed zero to a very small amount of Si in the control sample, proving that the high Si content in the control samples in the first analysis were instrument errors by the testing lab.

During both experiments, the healthy and diseased basil plants did not show substantial differences in both macro- and micro-nutrient content, with the only exception that the BDM induced the absorption and accumulation of iron in leaves, stems and roots across all treatments. There were no obvious differences in the Si content in leaves, stems, and roots when comparing the healthy and diseased plants across all treatments, indicating the BDM did not induce the absorption and accumulation of Si in basil (Figures 51, 52, 53 and 54).

The Si accumulation in the stems was substantially less than in the leaves and roots across all treatments and both healthy and infected plants (Figures 52 and 54). This agrees with the theory that when Si is absorbed by the roots, the deposition of Si happens at the site of evapotranspiration, where the soluble monosilicic anions are deposited into polymerized Silica gel and accumulate under the leaf cuticles.

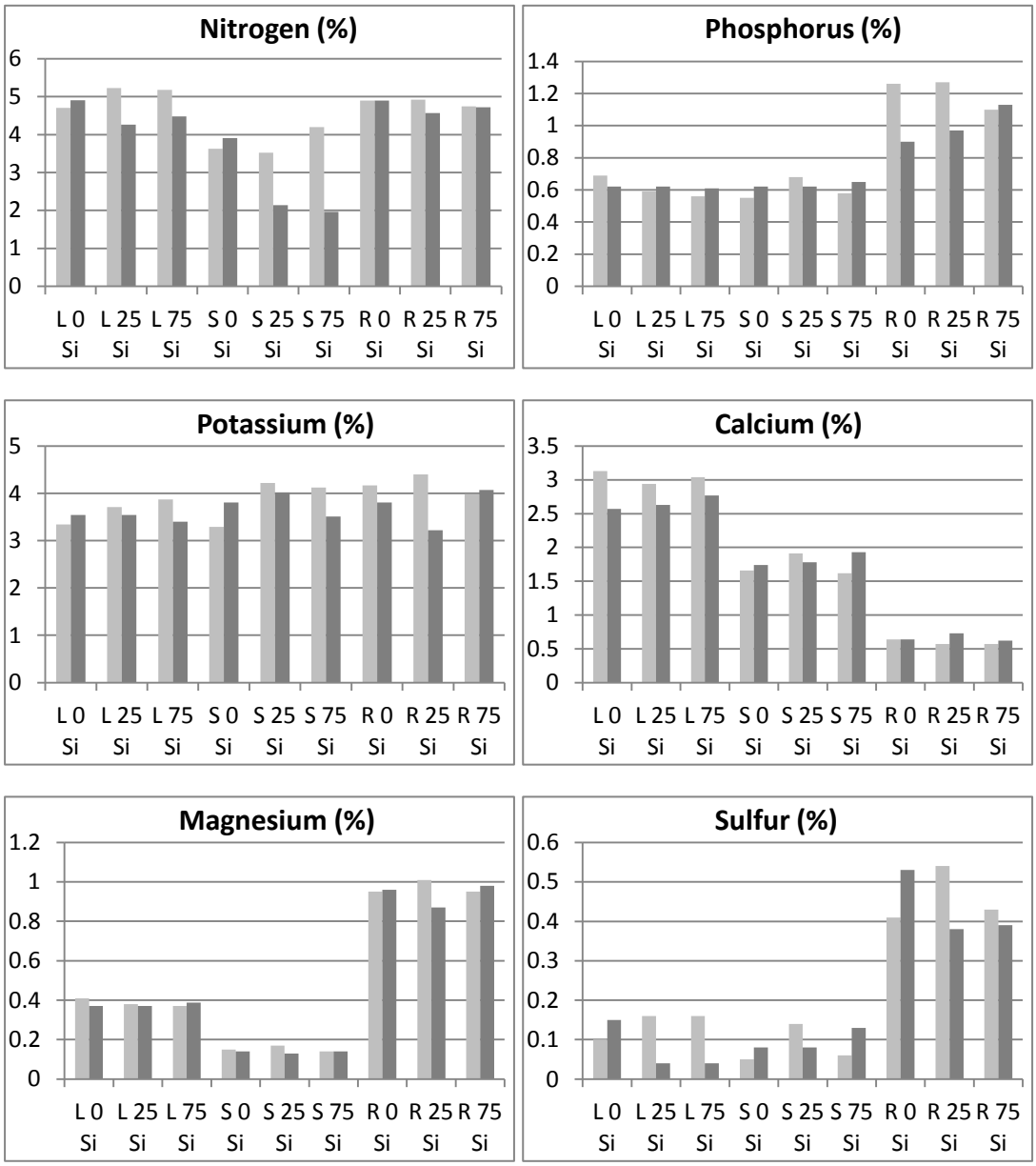


Fig. 51. BDM Experiment 1: Macronutrient analysis of basil 'Genovese' leaf, stem and root tissue treated with 0, 25 and 75 ppm of Si harvested at 25 DPI. The 60 plants (20 plants from each hydroponic system) harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = healthy plants. Dark grey = diseased plants. L = leaf, S = stem and R = root.

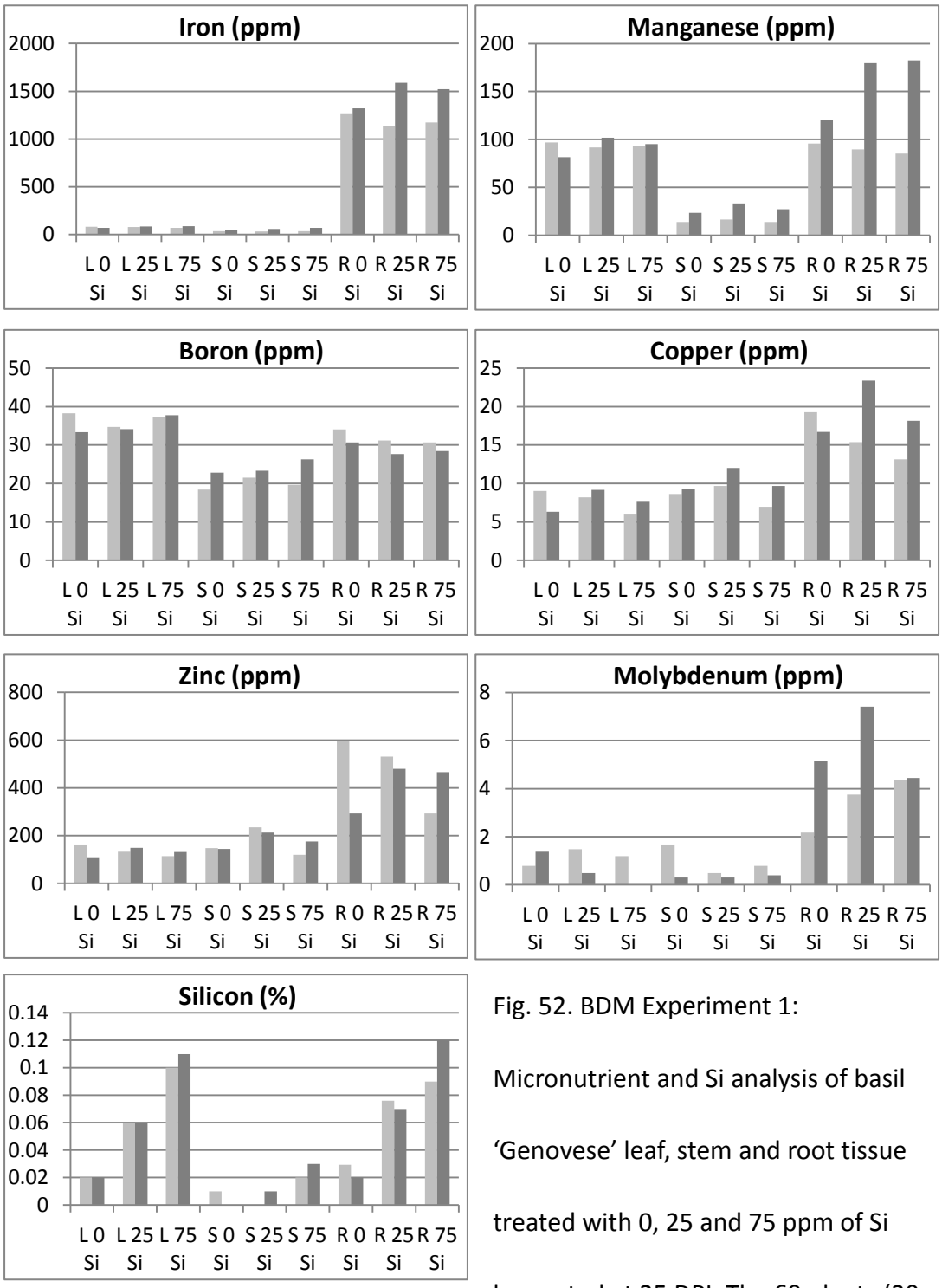


Fig. 52. BDM Experiment 1: Micronutrient and Si analysis of basil 'Genovese' leaf, stem and root tissue treated with 0, 25 and 75 ppm of Si harvested at 25 DPI. The 60 plants (20 plants from each hydroponic system) harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = healthy plants. Dark grey = diseased plants. Dark grey = diseased plants. L = leaf, S = stem and R = root.

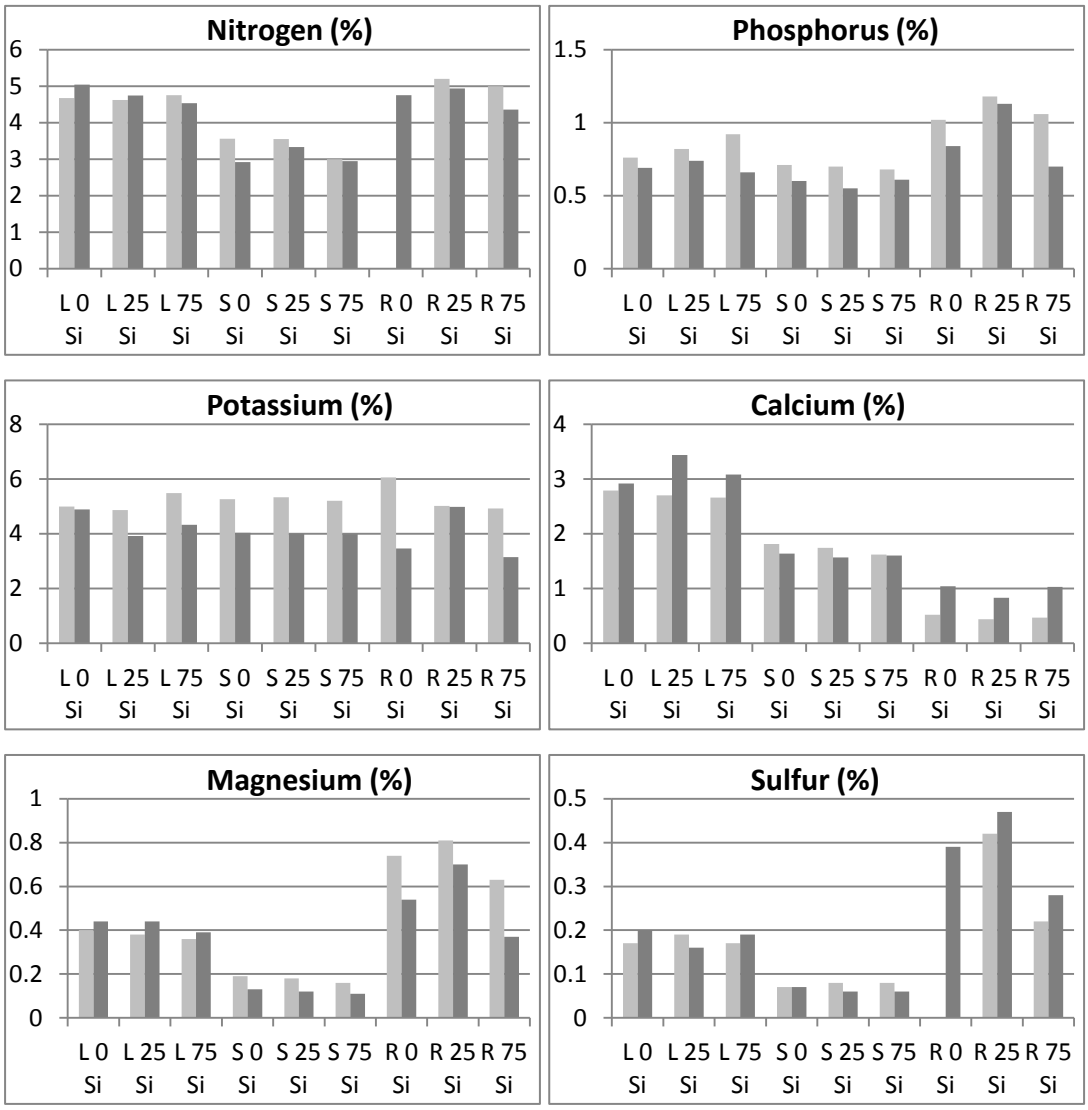


Fig. 53. BDM Experiment 2: Macronutrient analysis of basil 'Genovese' leaf, stem and root tissue treated with 0, 25 and 75 ppm of Si harvested at 25 DPI. The 60 plants (20 plants from each hydroponic system) harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. The nitrogen and sulfur analysis for the healthy 0 Si plant root were not successful. Light grey = healthy plants. Dark grey = diseased plants. Dark grey = diseased plants. L = leaf, S = stem and R = root.

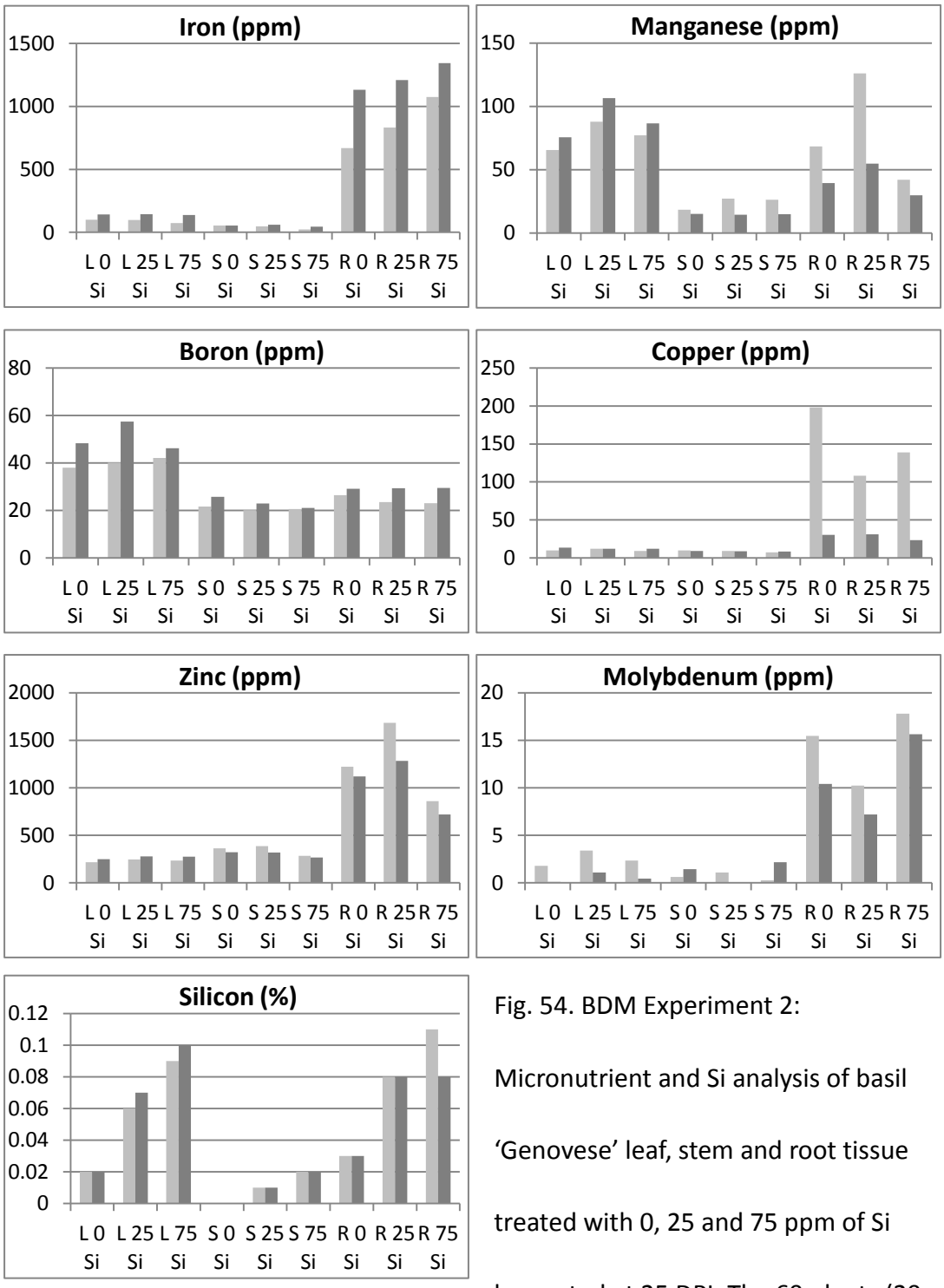


Fig. 54. BDM Experiment 2: Micronutrient and Si analysis of basil 'Genovese' leaf, stem and root tissue treated with 0, 25 and 75 ppm of Si harvested at 25 DPI. The 60 plants (20 plants from each hydroponic system) harvested from each Si treatment were combined into one sample (n = 1; shoots and roots separated) for ICP-AES. Light grey = healthy plants. Dark grey = diseased plants. Dark grey = diseased plants. L = leaf, S = stem and R = root.

4.4 Discussion and conclusions

The lettuce (‘Rex’ and ‘Salanova oakleaf’) used in this experiment were the most LPM sensitive cultivars known today, as suggested by Dr. Margaret McGrath (Cornell University) who is currently conducting a trial study investigating the sensitivity of different lettuce cultivars to LPM. The optimum temperature and humidity for LPM development were 18-25 °C and 95-98%, and the environmental conditions in the greenhouse during the experiment were suitable for LPM development.

The cultivars used in this study have a rosette morphology and grew wide rather than tall. During LPM Experiment 2, at 40 DPI the plants were in contact with each other, and evaluating every single leaf became impossible. LPM disease progression appeared to stop develop further from 40 to 55 DPI. Since LPM is spread by airborne spores, the experiment could be improved by having plants more widely spaced. Better air circulation inside the plant canopy can potentially increase the disease spread as well. Although LPM can infect the lettuce plant at any growing stage, LPM favors infecting older leaves compared to younger leaves. The inoculation method could potentially be applied to the plants at the mature rather than the seedling stage to increase the level of infections. Increasing the plant spacing also allows for additional biomass accumulation.

When conducting the tissue tests for bok choy, the entire above ground plant parts were harvested (combining the stem and leaves). As the BDM tissue analysis indicated, basil stems and leaves can contain substantially different amounts of Si

because the Si deposition mostly occurs at the site of evapotranspiration. The puncture treatment removed approximately 10% of the leaf area while the stems were left intact. To improve this experiment, the leaf tissue should be cut away from the stems during the harvest, and the tissue analysis should be conducted for leaves, stems, and roots separately.

During both BDM experiments, the Si treatments significantly delayed disease onset, but the effects were small when the plants were under heavy disease pressure. Although the results were statistically significant, it is hard to conclude whether the Si treatments can be an effective anti-fungal agent as desired by commercial growers. During both BDM experiments, the environmental conditions were optimal for BDM development and spread. BDM outbreaks in the field or in a commercial greenhouse often occur under less ideal environments and thus have slower development rates. The effects of Si nutrition during low disease pressure was not evaluated in this study.

In order to provide growers with adequate knowledge about applying Si as a beneficial micronutrient, different disease scenarios need to be studied, such as different disease levels, basil types and cultivars, and different environmental conditions. The cultivar used for this study was the original Italian sweet basil 'Genovese'. Other popular sweet basil cultivars, such as the hybrid Nufar, and other types of economically relevant basils such as the citrus (*O. x citriodorum*), holy (*O. tenuiflorum*) and the Thai (*O. basilicum thyrsiflora*) should also be studied.

This study was conducted in floating hydroponic systems to allow for accurate

control of the concentration of Si and other mineral nutrients, but field or greenhouse pot production provide a much different environment for both the plants and the pathogens. A field or greenhouse pot study is necessary in order to understand the impacts of soil- or growth media applied Si treatments on BDM, soil or media chemistry, plant Si absorption, and the residual effects of Si treatments on successive or rotation crops.

Both of the BDM experiments suffered from not having a well recorded environmental data across the entire experiment period due to instrument issues. The experiments continued with a crippled datalogger due to the limited availability of BDM inoculum at the time. As a result, conclusions of plant growth and disease progression regarding the environmental conditions can only be drawn arbitrarily. Despite water being constantly dripping from the greenhouse roof, it was not certain if the environmental humidity have reached 100%, which was crucial for BDM development. A secondary or back-up data logging system is strongly suggested in future experiments.

During both BDM experiments, the 75 ppm Si treatment did not exhibit significantly superior effects of disease suppression compared with the 25 ppm Si treatment. Based on the result that the BDM did not induce Si absorption and accumulation across the Si treatments, it is possible that the disease suppression was the result of changes at the physiological level from the Si treatment, rather than Si deposition and the formation of a protective layer. This could result from one or several mechanisms such as: 1) Regulation of stress-related phytohormones such as

abscisic acid (ABA), jasmonic acid (JA) and ethylene, or 2) Regulation of stress-related gene expressions. A closer look at the hormonal profile and the selected transcriptome by comparing the [-BDM, -Si], [-BDM, +Si], [+BDM, -Si] and [+BDM, +Si] treatments could lead to a deeper understanding of the beneficial effects of Si nutrition in Si non-accumulator plant species.

The effects of Si nutrition in lettuce, bok choy, and basil grow while exposed to LPM, insect damage (mimicked by mechanical hole puncture) and BDM, respectively, were investigated in this study. The effects of Si treatment on lettuce infected with LPM could not be assessed because the disease did not develop in both the control and the Si treatments during both experiments. The Si treatments did not result in any obvious effects on bok choy plants suffering mechanical wounding. No differences in plant size or morphology were observed. The mechanical wounding treatment did not induce any Si absorption and accumulation in the shoot and root tissue of bok choy. During both BDM experiments, the Si treatments significantly reduced the BDM disease progression. During both BDM experiments, the diseased basil plants did not exhibit any increase in Si accumulation in their leaves, stems or roots compared with the control (no Si) treatment.

Chapter 5

Summary and conclusions

The goal of this dissertation research was to reveal the potential beneficial effects of Si nutrition in three Si non-accumulator leafy green vegetable species (lettuce, bok choy, and basil, representing the common leafy green vegetable families of Asteraceae, Brassicaceae, and Lamiaceae). A summary of all the experiments conducted in this dissertation is represented in Figure 55.

In this dissertation, several different types of stresses were introduced to the plants, including temperature stresses (heat stress to lettuce and bok choy, and cold stress to basil), cut-and-grow-back stresses (lettuce, bok choy and basil), and biotic stresses (lettuce powdery mildew, insect chewing on bok choy, and basil downy mildew). The plants were grown hydroponically for careful control of nutrient and Si levels available to the plants, and eliminating the natural variability of nutrient availability in soils. The Sonneveld nutrient solution (Table 2) was incorporated with 0, 25 and 75 ppm of Si (using K_2SiO_3) for the Si treatments. For the Si amended nutrient solutions, extra nitric acid was added to maintain the optimum pH, thus the solutions contained additional nitrogen and potassium. The seedlings were incubated in a growth chamber equipped with incandescent and fluorescent lights. After transplanting into deep flow hydroponic systems in the greenhouse, no supplemental lighting was used.

	Temperature stress			Physical damage			Disease		
	 Heat (>30°C)	 Heat (>30°C)	 Cold (23°C)	   Cut and grow back			 Powdery Mildew	 Wounding, mimicking insect damage	 Downy Mildew
Repetitions	2	2	3	1	1	1	2	1	2
Did Si increase plant growth under stress?	✗	✗	✓	N/A	N/A	N/A	Unable to infect lettuce with LPM, experiments failed	✗	✗
Did Si increase the plant's stress resistance?	✗	✗	✓	N/A	N/A	N/A		N/A	✓
Did the stress increase Si uptake?	✓	✓	✓	✗	✗	✗		✗	✗

Fig. 55. A summary of all the experimental results from this dissertation, including the temperature stress experiments, cut and grow back experiments, and disease experiments.

When grown under optimal conditions, the Si treated lettuce, bok choy, and basil plants had increased plant shoot size and weight at the mature stage. Tissue analysis indicated that Si treatments only marginally influenced the macro- and micronutrient composition in both shoots and roots of lettuce, bok choy, and basil. The effects were the strongest in lettuce compared to bok choy and basil. As Si non-accumulators, lettuce and bok choy absorbed a very small amount of Si in their shoots (as high as 0.03%). Basil absorbed more Si in the shoots (as high as 0.08%), which is a high Si content for a Si non-accumulator.

During the temperature stress experiments, two cultivars of lettuce (leaf lettuce Black Seeded Simpson and butterhead lettuce Rex) and bok choy (white stem bok choy Asian Delight and dark green bok choy Black Summer) were exposed to heat stress (above 30 °C). The Si treatments did not result in significant differences in size, biomass, and morphology in both cultivars of lettuce and bok choy. The Si treatments also did not affect the macro- and micronutrient composition in the shoots and roots of both cultivars of lettuce and bok choy. The additional nitrogen and potassium in the Si treated nutrient solutions did not influence the level of potassium and nitrogen in the shoots and roots of lettuce and bok choy. As a result of the Si treatments, lettuce and bok choy absorbed very small quantities of Si (0.01 – 0.07%) in their shoots and roots under heat stress. Overall, the lettuce and bok choy plants grown under heat stress did not benefit from Si nutrition.

For ‘Genovese’ basils grown under the cold stress, when grown at 18°C, despite the 75 ppm Si treated basil plants were the tallest, all Si treatments resulted in

undesirable small plant sizes. When grown at 23 °C, the Si treatments significantly increased cold hardiness of basil. The 75 ppm Si treated basil plants had significantly better growth in both shoots and roots. After an unanticipated frost event during the early growth stage (-1 °C for 7 hours at 6 DAT), both 25 and 75 ppm Si treated basil plants showed substantially higher survival rates. The Si treatments did not influence the macro- and micronutrient composition in shoots and roots of basil grown under cold stress. The cold stress increased Si absorption and accumulation in small quantities in basil shoots and roots.

During the cut and grow back experiments, Si treated lettuce, bok choy and basil plants did not show any enhanced Si absorption and accumulation after a cut and grow back harvest. Among all Si treatments, comparing the cut and uncut plants, the macro- and micronutrient composition were mostly unchanged, with the exception that in basil, the Si treatments increased the iron content in both shoots and roots after the cut and grow back. Overall, the Si treatments did not result in any obvious differences in nutrient composition, including Si, after the cut and grow back treatments for lettuce, bok choy and basil.

The lettuce powdery mildew (LPM) experiments were conducted twice but both times the inoculations failed to develop enough disease pressure on the lettuce plants. As a result, the effects of Si treatment on lettuce infected with LPM could not be assessed.

In order to mimic the mechanical wounding from insects on bok choy, 10% of the leaf area was removed by puncturing holes using a paper hole punch. Two weeks

after the treatment, no visual differences in height, color and general morphology on both shoots and roots of bok choy was observed. The puncture treatment also did not result in any differences in macro- and micronutrient content, nor in additional absorption and accumulation of Si in both shoots and roots. The Si treatments did not result in any beneficial effects for the mechanically wounded bok choy plants.

For basil infected with downy mildew (BDM), both experiments showed that the Si treatments, to a small degree but significantly, delayed the BDM disease onset and progression. The 75 ppm Si treatment did not result in significantly superior disease suppression compared to the 25 ppm Si treatment. BDM did not increase Si absorption and accumulation in basil across all Si treatments. These results indicate that the disease suppression effect from Si treatments could be the result of Si induced changes at the physiological level, rather than of Si deposition and the formation of a protective layer.

During both BDM experiments, the basil plants were placed in a disease-optimized, enclosed environment and the plants were under substantial disease pressure. To evaluate whether Si treatments can be an effective BDM control agent as desired by commercial growers, additional experiments need to be conducted on different scenarios of disease pressure, environmental conditions, basil cultivars, and field production.

In general, Si treatments did not provide any benefits in biomass accumulation and stress resistance to lettuce and bok choy plants grown under heat, cut and grow back, powdery mildew and insect stresses. On the other hand, basil benefited from Si

nutrition. The Si treatments provided basil plants with cold hardiness when grown under mild cold stress (23 °C) and increased the survival rate of young basil plants during a short frost event. Si treatments also provided basil with some BDM resistance.

This dissertation research provided valuable information for further understanding the physiology and mechanisms of Si absorption, accumulation, its efficacy in stress resistance, and its effects on plant growth for three typical Si non-accumulator leafy green vegetable species exposed to biotic and abiotic stresses. The information generated from this research can provide the plant nutrient industry and growers, especially organic growers, with insights into the beneficial effects of silicon nutrition for Si non-accumulators crops.

APPENDICES

1. Datalogger program

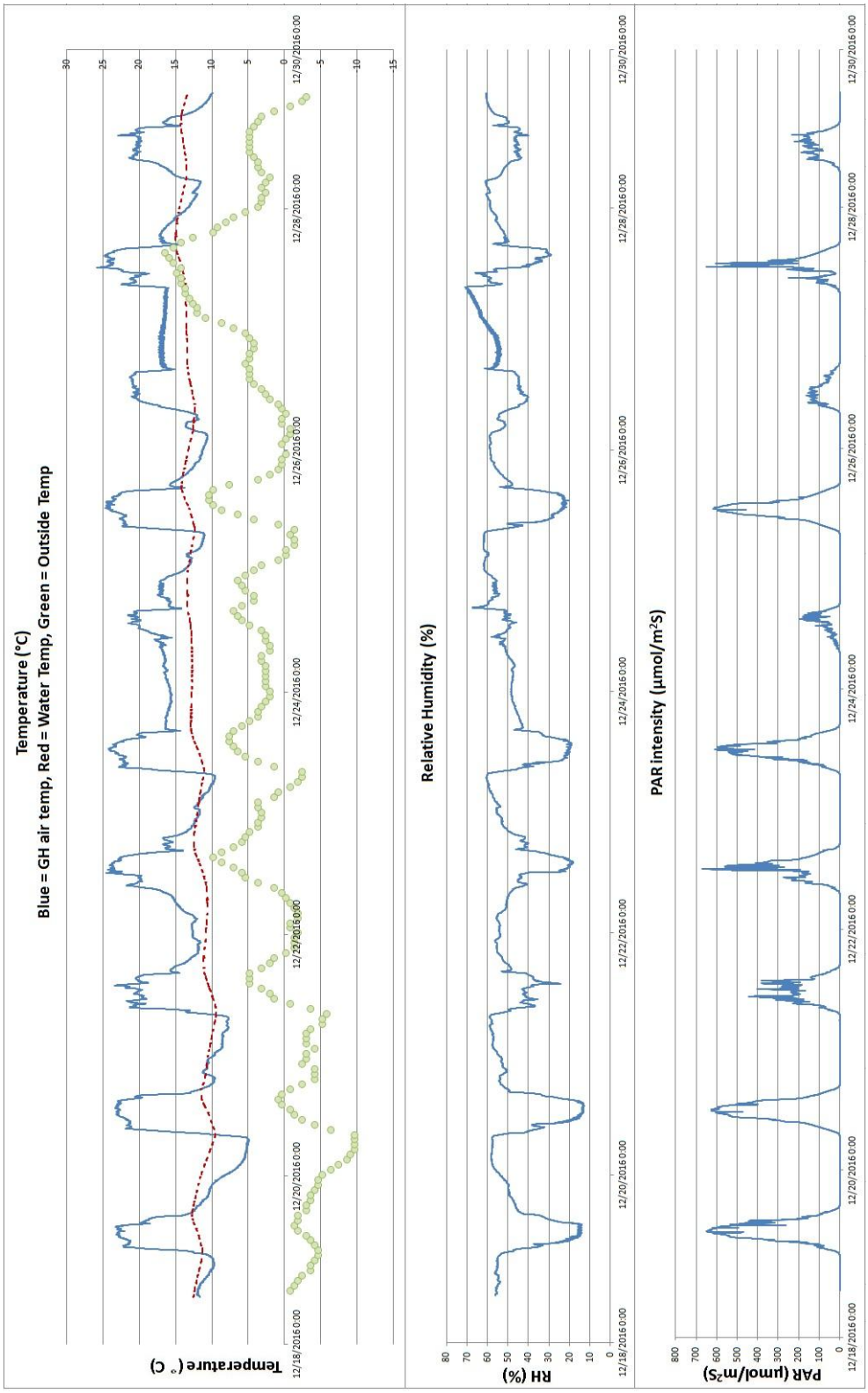
```
'CR1000

'Created by Short Cut (3.1)


'Declare Variables and Units

Public BattV

Public PTemp_C

Public AirTC

Public RH

Public Temp_C

Public Temp_C_2

Public Temp_C_3

Public Temp_C_4

Public PAR_Den

Public PAR_Tot

Public PAR_Den_2

Public PAR_Tot_2


Units BattV=Volts
```

```
Units PTemp_C=Deg C
Units AirTC=Deg C
Units RH=%
Units Temp_C=Deg C
Units Temp_C_2=Deg C
Units Temp_C_3=Deg C
Units Temp_C_4=Deg C
Units PAR_Den=umol/s/m^2
Units PAR_Tot=mmol/m^2
Units PAR_Den_2=umol/s/m^2
Units PAR_Tot_2=mmol/m^2

'Define Data Tables
DataTable(DEC2016,True,-1)
    DataInterval(0,1,Min,10)
    CardOut(0,-1)
    Sample(1,AirTC,FP2)
    Sample(1,RH,FP2)
    Sample(1,Temp_C,FP2)
    Sample(1,Temp_C_2,FP2)
    Sample(1,Temp_C_3,FP2)
    Sample(1,Temp_C_4,FP2)
```

```
    Sample(1,PAR_Den,FP2)
    Totalize(1,PAR_Tot,IEEE4,False)
    Sample(1,PAR_Den_2,FP2)
    Totalize(1,PAR_Tot_2,IEEE4,False)
EndTable


'Main Program
BeginProg
    'Main Scan
    Scan(1,Sec,1,0)
        'Default Datalogger Battery Voltage measurement 'BattV'
        Battery(BattV)
        'Default Wiring Panel Temperature measurement 'PTemp_C'
        PanelTemp(PTemp_C,_60Hz)
        'HMP50/HMP60 Temperature & Relative Humidity Sensor measurements
'AirTC' and 'RH'
        VoltSE(AirTC,1,mV2500,1,0,0,_60Hz,0.1,-40)
        VoltSE(RH,1,mV2500,2,0,0,_60Hz,0.1,0)
        If (RH>100) And (RH<108) Then RH=100
        'Type T (copper-constantan) Thermocouple measurements 'Temp_C'
        TCDiff(Temp_C,1,mV2_5C,2,TypeT,PTemp_C,True,0,_60Hz,1,0)
        'Type T (copper-constantan) Thermocouple measurements 'Temp_C_2'
```

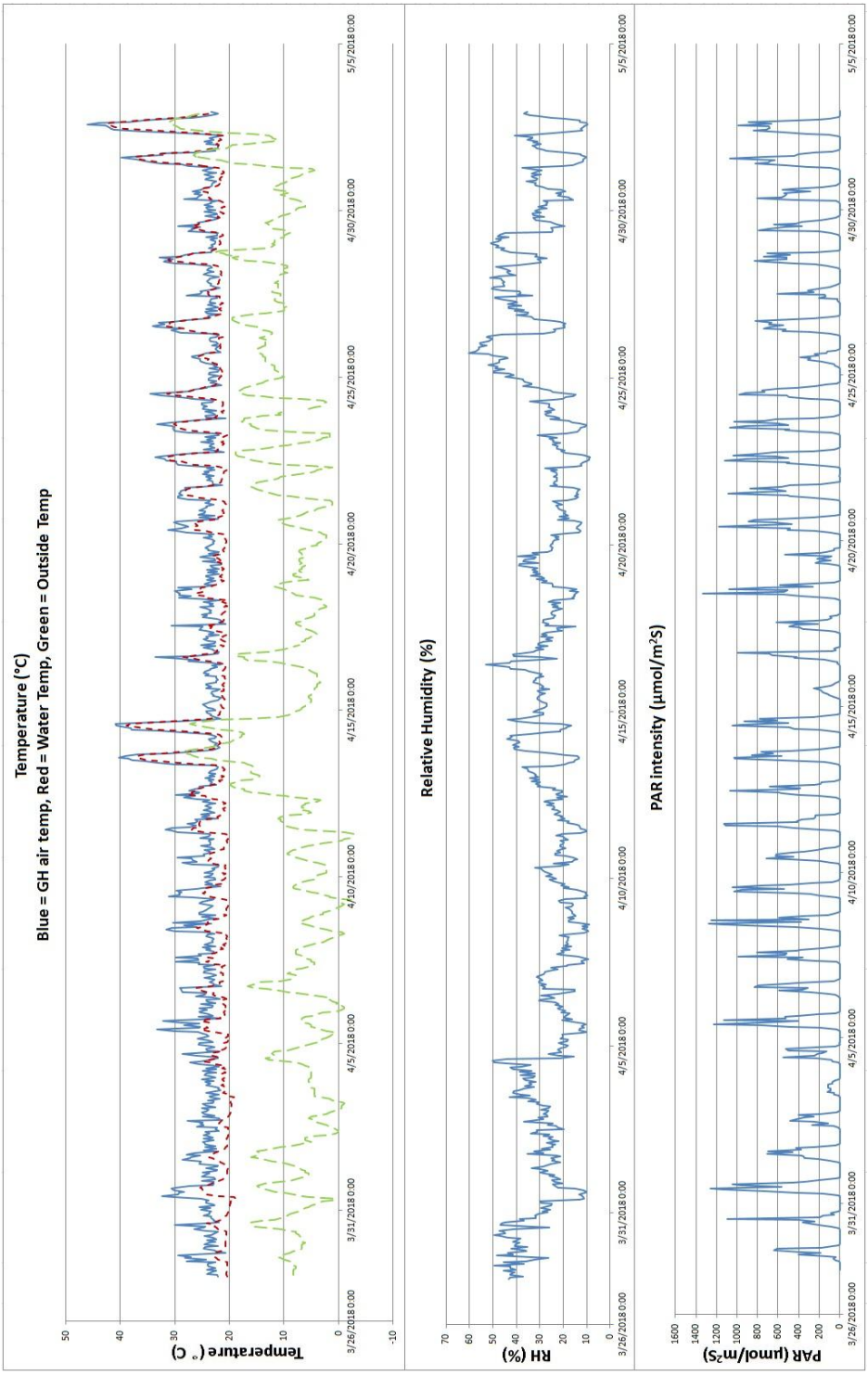
```
        TCDiff(Temp_C_2,1,mV2_5C,3,TypeT,PTemp_C,True,0,_60Hz,1,0)
        'Type T (copper-constantan) Thermocouple measurements 'Temp_C_3'
        TCDiff(Temp_C_3,1,mV2_5C,4,TypeT,PTemp_C,True,0,_60Hz,1,0)
        'Type T (copper-constantan) Thermocouple measurements 'Temp_C_4'
        TCDiff(Temp_C_4,1,mV2_5C,5,TypeT,PTemp_C,True,0,_60Hz,1,0)
        'LI190SB Quantum Sensor measurements 'PAR_Tot' and 'PAR_Den
        VoltDiff(PAR_Den,1,mv2_5,6,True,0,_60Hz,1,0)
        If PAR_Den<0 Then PAR_Den=0
        PAR_Tot=PAR_Den*1.655629
        PAR_Den=PAR_Den*1655.629
        'LI190SB Quantum Sensor measurements 'PAR_Tot_2' and 'PAR_Den_2
        VoltDiff(PAR_Den_2,1,mv2_5,7,True,0,_60Hz,1,0)
        If PAR_Den_2<0 Then PAR_Den_2=0
        PAR_Tot_2=PAR_Den_2*1.655629
        PAR_Den_2=PAR_Den_2*1655.629
        'Call Data Tables and Store Data
        CallTable DEC2016
    NextScan
EndProg
```

2. Environmental data collected between 68 and 77 DAT (Dec. 19, 2016 – Dec. 28, 2016) during the lettuce and bok choy cut-and-grow-back experiment.

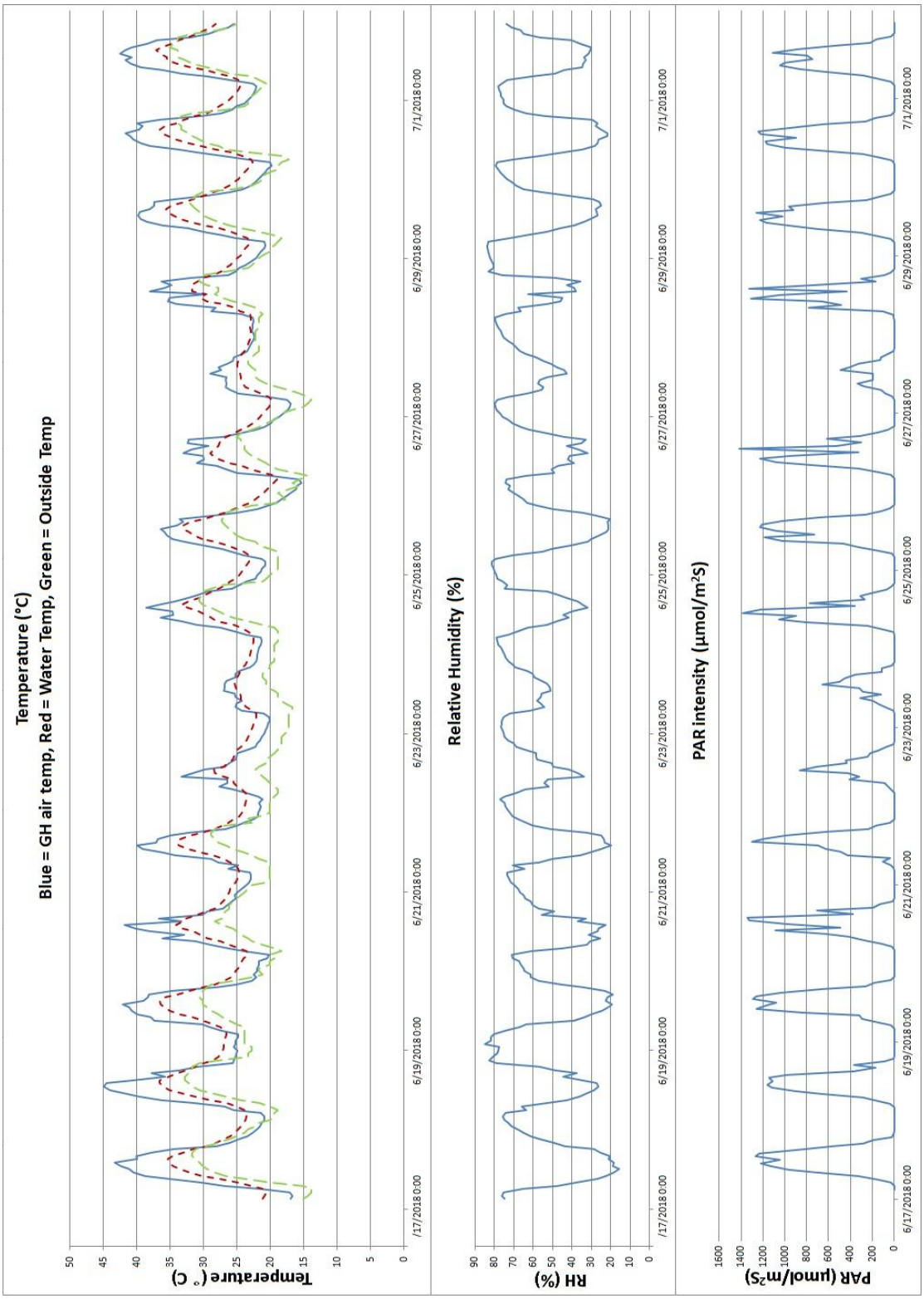


Dates	Days after sowing	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
19-Dec	68	15	7	12	11	-3	-7	44	9
20-Dec	69	15	13	11	10	-3	-3	44	10
21-Dec	70	16	13	10	11	1	-1	49	5
22-Dec	71	18	11	12	12	5	3	46	6
23-Dec	72	18	16	12	13	4	3	42	8
24-Dec	73	18	13	13	13	4	3	56	2
25-Dec	74	18	12	13	13	5	0	47	8
26-Dec	75	16	17	13	14	3	9	54	3
27-Dec	76	19	13	14	14	14	4	54	4
28-Dec	77	17	10	14	13	3	-4	56	3

3. Environmental data collected during the lettuce powdery mildew experiment. Experiment 1: 1 to 35 DAT (Mar. 29, 2018 – May 2, 2018). Experiment 2: A portion of the data from 26 to 40 DAT (June 17, 2018 – July 1, 2018).



Dates	Days after sowing	Avg Day Air temp	Avg Night air temp	Avg Day water temp	Avg Night water temp	Avg Day outside temp	Avg Night outside temp	Avg RH of the day	DLI (mol/day)
29-Mar	11	24.4	23.5	21.5	20.6	8.8	6.7	40.2	9.4
30-Mar	12	24.2	24.2	21.6	21.1	11.5	9.6	36.0	11.3
31-Mar	13	27.4	23.7	22.9	20.5	9.4	6.3	19.9	25.4
1-Apr	14	25.2	23.8	21.8	20.3	12.2	6.3	26.3	16.4
2-Apr	15	24.0	24.0	20.6	19.7	2.5	-0.1	27.9	11.1
3-Apr	16	23.1	23.6	20.6	21.1	4.0	5.1	35.5	4.0
4-Apr	17	24.5	24.3	22.3	20.6	9.5	3.8	28.1	11.2
5-Apr	18	26.1	24.0	22.3	20.8	4.0	0.1	17.2	23.9
6-Apr	19	24.9	23.6	22.8	21.2	9.1	8.5	26.5	14.7
7-Apr	20	25.0	24.2	22.5	20.8	6.3	2.9	24.0	16.6
8-Apr	21	26.5	24.3	22.9	20.5	3.7	0.6	14.9	23.9
9-Apr	22	25.7	23.6	22.8	21.1	4.3	3.2	19.5	21.2
10-Apr	23	25.0	24.3	22.6	20.6	6.1	1.0	20.7	15.5
11-Apr	24	26.5	23.8	24.2	21.1	6.9	6.1	19.4	20.5
12-Apr	25	25.4	23.4	24.1	21.3	13.9	15.4	27.5	17.7
13-Apr	26	30.5	23.1	29.1	21.7	22.5	19.9	30.1	21.1
14-Apr	27	25.5	23.4	29.2	21.3	10.1	6.8	29.6	23.0
15-Apr	28	23.0	23.5	21.2	21.1	4.5	4.3	30.2	5.1
16-Apr	29	24.8	23.8	23.4	21.2	12.7	6.3	34.3	12.5
17-Apr	30	23.8	24.0	22.1	20.9	5.8	3.3	24.5	11.1
18-Apr	31	26.2	23.4	23.5	21.4	8.0	7.2	23.6	22.3
19-Apr	32	23.4	24.1	21.7	20.8	6.7	3.3	29.3	7.7
20-Apr	33	26.7	24.1	23.9	20.9	7.3	3.3	18.2	24.2
21-Apr	34	26.5	24.0	24.9	21.1	11.2	8.0	19.6	23.3
22-Apr	35	27.5	23.9	26.0	21.1	13.5	5.3	18.1	24.7
23-Apr	36	27.2	23.9	25.6	21.2	12.9	5.3	21.0	24.6
24-Apr	37	26.1	22.9	25.1	21.6	13.6	11.1	32.9	20.6
25-Apr	38	23.9	23.0	23.1	21.7	13.4	13.5	51.3	7.3
26-Apr	39	28.2	23.3	26.7	21.6	16.0	10.4	32.1	23.8
27-Apr	40	23.4	23.3	22.4	21.7	11.3	10.5	45.4	7.3
28-Apr	41	26.6	23.0	25.7	21.8	15.6	11.8	41.6	18.1
29-Apr	42	25.0	24.5	23.9	23.0	11.3	10.1	29.2	18.1
30-Apr	43	24.5	23.7	23.2	21.3	9.6	7.9	27.6	15.9
1-May	44	30.2	23.3	29.5	22.0	18.3	17.1	23.4	24.9
2-May	45	34.3	22.7	33.5	23.1	24.7	26.1	21.5	25.5


Temperature (°C)
Blue = GH air temp, Red = Water Temp, Green = Outside Temp
Temperature (° C)
Relative Humidity (%)
RH (%)
PAR intensity (µmol/m²S)
PAR (µmol/m²S)
6/17/2018 0:00
6/19/2018 0:00
6/21/2018 0:00
6/23/2018 0:00
6/25/2018 0:00
6/27/2018 0:00
6/29/2018 0:00
7/1/2018 0:00

4. Applying Wollastonite to Soil to Adjust pH and Suppress Powdery Mildew on Pumpkin

*** This section was previously published in the peer-reviewed scientific journal HortTechnology.

Li, Y., Both, A. J., Wyenandt, C. A., Durner, E. F., and Heckman, J. R. 2019. Applying Wollastonite to Soil to Adjust pH and Suppress Powdery Mildew on Pumpkin. HortTechnology. DOI: 10.21273/HORTTECH04391-19. ***

Introduction

Pumpkin is a globally important cash crop grown for the processing and fresh-market industries (Ingerson-Mahar et al., 2007). Nearly 2 billion pounds of pumpkin were harvested in the United States in 2017 (Gregory, 2018). One of the major problems associated with pumpkin is the risk of premature defoliation caused by foliar diseases such as powdery mildew. *Podosphaera xanthii* (formerly *Sphaerotheca fuliginea*) and *Erysiphe cichoracearum* are the two reported fungal species that can cause powdery mildew in cucurbit crops in the United States (Zitter et al., 1996). These pathogens can move long distances within the growing season, from southern to northern U.S. production areas (Zitter et al., 1996). Cucurbit powdery mildew infects leaves and vines at any growth stage, typically starting with the older leaves. Symptoms of powdery mildew include white colonies to large, coalesced white blotches on leaves causing chlorosis, and is eventually followed by loss of foliage. Powdery mildew can significantly reduce the yield of pumpkins both

in terms of fruit size and number (Mossler and Nesheim, 2014; Zitter et al., 1996). Conventional and organic cucurbit growers take substantial efforts to control or mitigate losses to powdery mildew. Weekly applications of a fungicide can result in significant increases in cost, equipment, time, and labor. Most conventional fungicides currently used for cucurbit powdery mildew control have a high risk for resistance development (Wyenandt et al., 2018). The risk of losing fungicide efficacy for controlling diseases such as cucurbit powdery mildew requires continued efforts to help mitigate disease development through alternative means. Organic growers have fewer effective control options and face greater challenges when dealing with this pervasive disease. Organic growers can grow resistant or tolerant pumpkin cultivars, but additional disease control options are needed.

An approach that has gained attention recently includes improved soil fertility management and optimized plant nutrition (Datnoff et al., 2007). In particular, the application of Si as part of a fertilization strategy has been studied for typical Si accumulator species such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and cucurbits (Belanger et al., 2003; Elawad and Green, 1979; Heckman et al., 2003; Lepolu et al., 2016; Provance-Bowley et al., 2010). A review by Datnoff (2014) summarized the current understanding of the physiological significance of Si in plants. Si increases plant resistance to fungal diseases by either increasing the Si content in epidermal tissue, thus forming a thickened Si–cellulose layer that is more resistant to fungal penetration, or by pathogenesis-mediated host defense responses (Zellner, 2017). In addition, a variety of crops, especially Si accumulators, showed

increases in biomass, Si accumulation, and disease or pest resistance when treated with plant-available Si (Zellner et al., 2011, 2019). Although not officially regarded as an essential plant nutrient, Si is now widely considered a beneficial element for many plants (Datnoff, 2014; Datnoff et al., 2001). Several plant growth media companies have started to incorporate Si in their soil-less products.

Both conventional and organic growers are interested in the types and application rates of approved Si materials that can adequately address disease problems. Acquiring naturally derived and approved organic sources of Si for organic production has become a priority. In previous studies, members of our group identified and investigated the properties of several Si mineral sources, including earth-mined minerals such as wollastonite, MontanaGrow (MontanaGrow, Bonner, MT); glacial rock flour; and human-processed minerals such as wood ash and steel mill slag (Heckman et al., 2003; Lepolu et al., 2016). We used pumpkin as a model crop and investigated the beneficial effects of different amounts of Si amendments, including each amendment's ability to neutralize soil acidity, enhance Si uptake, improve powdery mildew control, and increase plant biomass. Wollastonite, a naturally occurring mineral form of calcium silicate (Ca_2SiO_4), can provide all these tested beneficial effects to pumpkin plants. This product is naturally mined, and is listed by the Organic Materials Review Institute (OMRI; Eugene, OR) for use in organic production systems. We conducted experiments to understand further the effects of wollastonite on soil and plants under disease conditions, and to provide useful information to growers and the plant growth media industry. The objectives

of our study were 1) to find the optimal soil amendment rate for wollastonite to achieve the best suppression of powdery mildew, 2) to determine wollastonite's ability to neutralize soil acidity and change soil chemistry compared with regular limestone, and 3) to investigate the biomass accumulation in pumpkin plants resulting from wollastonite soil applications.

Rates for liming material application are often determined based on initial soil pH, target soil pH for the crop, and the liming requirement to reach that target. However, agronomists specializing in soil fertility not only need to provide sound advice on making optimum application rates of soil amendments, but also need to predict potential impacts on plant growth and crop mineral nutrition when target application rates are exceeded. Therefore, our greenhouse study was designed to include a wide range of wollastonite application rates, ranging from an unamended soil in need of liming, to a level that matched the lime requirement of the soil for growing pumpkin and most vegetable crops, as well as levels several orders of magnitude greater. Another reason for exploring greater application rates is that pumpkins are typically grown in widely spaced rows, permitting localized heavier application rates in the areas of seeding or transplanting that then are later dispersed by tillage. Application rates of wollastonite that might at first appear extremely high are more reasonable when one considers that future tillage can disperse the amendment across the field and extend the benefit to successive crops.

Materials and methods

Two similar experiments were conducted to evaluate the effectiveness of Si amendments. Expt. 1 started with seeding on 15 Apr. 2016. Expt. 2 started with seeding on 5 Dec. 2018. Expt. 1 was ended 35 d after seeding (DAS), whereas Expt. 2 was extended and ended 45 DAS.

A Readington loam (fine-loamy mixed, active, mesic Oxyaquic Fragiudalfs) soil was collected from the top 15 cm of soil at a local farm located in Hunterdon, NJ. This field had no recent history of limestone amendment or any chemical fertilizer or pesticide input, and had been managed based on organic farming techniques for at least the past 3 years. The collected soil was sieved through a homemade screen with square holes of 1 cm to remove pebbles and plant litter. The initial soil pH was 5.92 using the 1:1 soil volume-to-water ratio method. Soil tests for Si were performed using the method of Korndorfer (Datnoff et al., 2001). All extractions were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Individual 6.2-L plant containers (Poly-Tainer- Can #2; Nurseries Supplies, Orange, CA) were filled with 10 kg of the soil.

We used a limestone with a calcium carbonate equivalent of 93 [containing 22% calcium (Ca) and 1.2% magnesium (Mg); Limestone Products Corp., Sparta, NJ] for the limestone-amended treatments. To achieve a soil pH of 6.5, which is considered the optimum pH for growing pumpkins, the application rate for the limestone was calculated as 6.25 tons/acre based on initial pH and soil texture class.

We grew ‘Connecticut Field’ pumpkin plants (Stokes Seeds, Thorold, Ontario, Canada) in pots outdoors for 5 weeks to become naturally infected with cucurbit powdery mildew for use as a source of inoculum for Expt. 1. Expt. 1 was conducted in a double-layer, polyethylene-covered greenhouse located at the Rutgers University Vegetable Research Farm III in New Brunswick, NJ (lat. 40°27’45"N, long. 74°25’45"W; elevation, 21 m), with a constant temperature set point of 70 °F. Ten ‘Connecticut Field’ pumpkin seeds were sown in pots amended with different rates (6.25, 12.5, 25, or 50 tons/acre) of limestone or wollastonite [R.T. Vanderbilt Co., Norwalk, CT (OMRI listed)]. The control treatment consisted of pots filled with unamended soil. Before seeding, 10 g of blood meal (The Espoma Co., Millville, NJ) was mixed into the top 1 inch of the soil in all pots. The experiment was designed as a randomized complete block with four replications. The full set of treatments was distributed randomly within a block, with one naturally infected plant per block.

Pots were thinned to one pumpkin plant per pot 1 week after germination. Powdery mildew lesions started to become visible on the cotyledons at 15 DAS. The total number of lesions on each plant was counted every other day. Powdery mildew was present on most leaves by 25 DAS and the percentage of total leaf area affected was estimated visually every other day thereafter. The experiment was ended 35 DAS and all aboveground biomass from each pot was harvested. The biomass was dried at 68 °C for 5 d and weighed, and further analyzed for mineral composition using ICP-AES. To determine the Si content, the biomass samples were digested using 50% sodium hydroxide, followed by colorimetric analysis at Brookside

Laboratories (New Bremen, OH). Soil samples from all pots were collected by taking a soil core (2-cm diameter by 15-cm depth) from each pot immediately after biomass harvest, and the samples from each pot were tested individually using the Mehlich-3 soil test. To determine the soil Si level, all soil samples were digested with acetic acid followed by colorimetric analysis at Brookside Laboratories.

For Expt. 2, we collected squash leaves that were heavily infected with powdery mildew from an outdoor location in Bridgeton, NJ (lat. 39°52’05” N, long. 75°20’50” W; elevation, 36 m). The powdery mildew- infected leaves were placed among the pumpkin seedlings. The location, experimental design, and methods were identical to Expt. 1, except we used three additional lower amendment rates (0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 tons/ acre) for both limestone and wollastonite. Lesions of powdery mildew were first observed 15 DAS and the total number of lesions on each plant was counted every other day. We start to evaluate the percentage of total leaf area affected by 25 DAS. Expt. 2was ended45DAS (10 d later than Expt. 1) to compensate for the slower plant growth in December. Biomass was collected, processed, and analyzed as described for Expt. 1.

The area under the disease progress curve (AUDPC) was calculated for each treatment in each experiment to measure disease development over time. The AUDPC values for each treatment were calculated using the trapezoidal rule (Sparks et al., 2008). All experimental data, including disease progress, soil chemical levels, and plant elemental analysis were analyzed in a stepwise fashion using SAS (version 9.4; SAS Institute, Cary, NC). A single df contrast comparing all treatments to the

control was performed as a first step to determine whether there was a treatment effect. If a treatment effect was detected during this first step, a classic factorial analysis of amendments and rates was performed. The final step included linear and quadratic regression analyses of amendment rate.

Results, discussions and conclusions

The different start times for the two experiments resulted in different natural light conditions. The average daily light integral (DLI) inside the greenhouse during Expt. 1 was (± SD) 23.78 ± 11.30 $mol \cdot m^{-2} \cdot d^{-1}$, while the average DLI for Expt. 2 was (± SD) 8.16 ± 4.15 $mol \cdot m^{-2} \cdot d^{-1}$. These differences had an impact on plant growth and development (Fig. 4), as can be observed from the differences in average final plant dry weight (average for control group: 23.22 g/plant for Expt. 1, and 2.82 $g \cdot plant^{-1}$ for Expt. 2).

During both experiments, increasing the application rate of wollastonite increased the soil Si level significantly, while adding more limestone did not (Tables 1-2). Soil pH increased as the limestone and wollastonite application rates increased at similar rates, indicating that the acid neutralizing abilities of limestone and wollastonite are similar. The extracted soil Ca level increased with both liming materials, but the wollastonite amendments decreased the extracted soil Mg level as compared to limestone or the unamended soil.

Throughout the experiments, wollastonite amended pumpkin plants exhibited

lower disease levels, as shown by both powdery mildew colony counts and the percentages of leaf surface area coverage (Figs. 1-2). Based on AUDPC values, the disease level for all limestone treatments was not significantly different from the control group, but the wollastonite plants had fewer colonies and less surface area covered by powdery mildew (Fig. 1A-D). The time needed for colonies to coalesce and form large infected areas was delayed for the wollastonite treatments. To reach 50 colonies on the 6.25-ton/acre plants, wollastonite treated plants took 6.8 (Expt. 1) and 4.1 (Expt. 2) d longer than limestone plants. This indicates wollastonite delayed disease development, as shown in Fig. 3. However, higher levels of wollastonite application did not result in increased suppression of powdery mildew even as soil Si levels increased, as shown in Fig. 1. This result was consistent for both experiments.

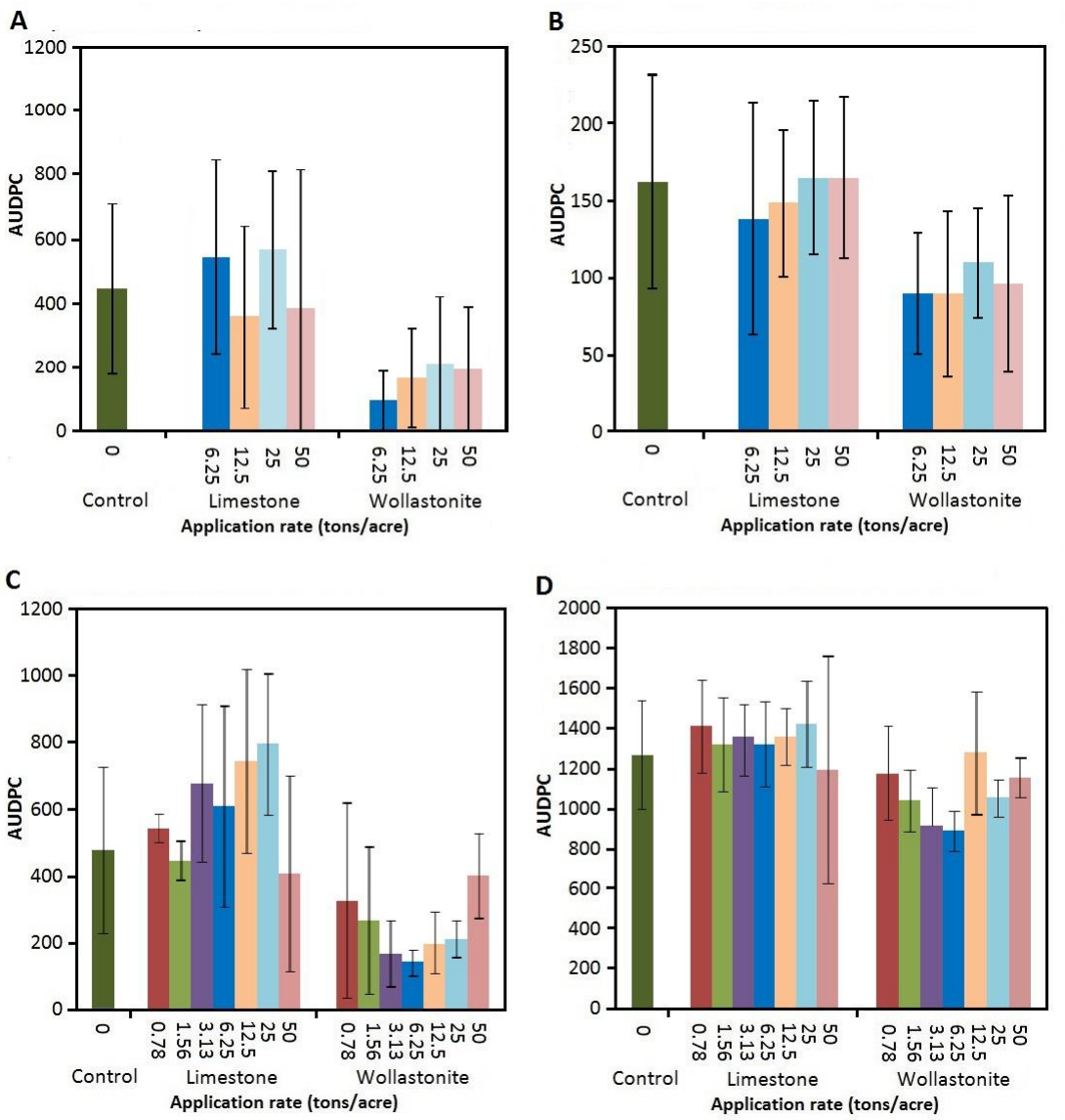


Fig. 1. Disease progression (n = 4) indicated by the areas under the disease progress curve (AUDPC) of powdery mildew on pumpkin. Error bars indicate ± SD. 1A (Expt. 1) and 1C (Expt. 2): Disease progression (spot count) during earlier growth stage [between 15 and 23 d after seeding (DAS)]. 1B (Expt. 1) and 1D (Expt. 2): Disease progression (area covered by coalescing colonies of powdery mildew) during the later growth stage (25 to 35 DAS for Expt. 1, 25 to 45 DAS for Expt. 2). 1 ton/acre = 2.2417 $Mg \cdot ha^{-1}$.

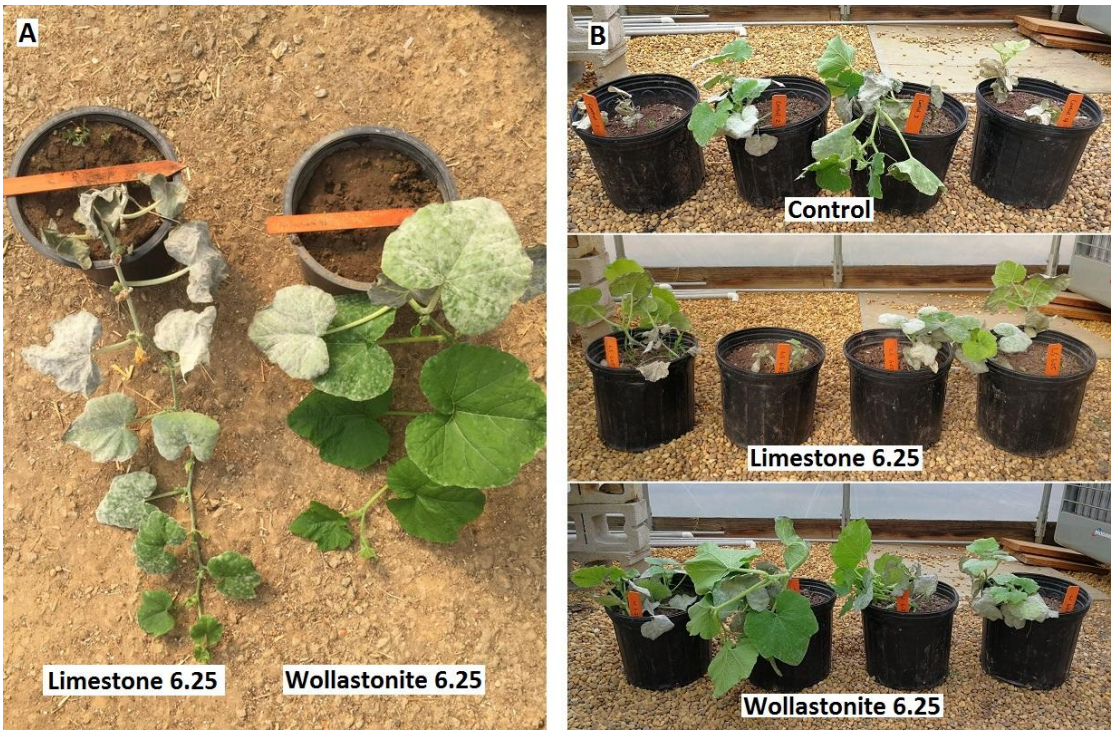


Fig. 2. Symptoms of powdery mildew development on pumpkin plants amended with A: limestone 6.25-ton/acre and wollastonite 6.25-ton/acre treatments [end of Expt. 1, 35 d after seeding (DAS)], and B: on plants from the control (no limestone or wollastonite applications), limestone 6.25-ton/acre and wollastonite 6.25-ton/acre treatments (end of Expt. 2, 45 DAS). 1 ton/acre = 2.2417 $Mg \cdot ha^{-1}$.

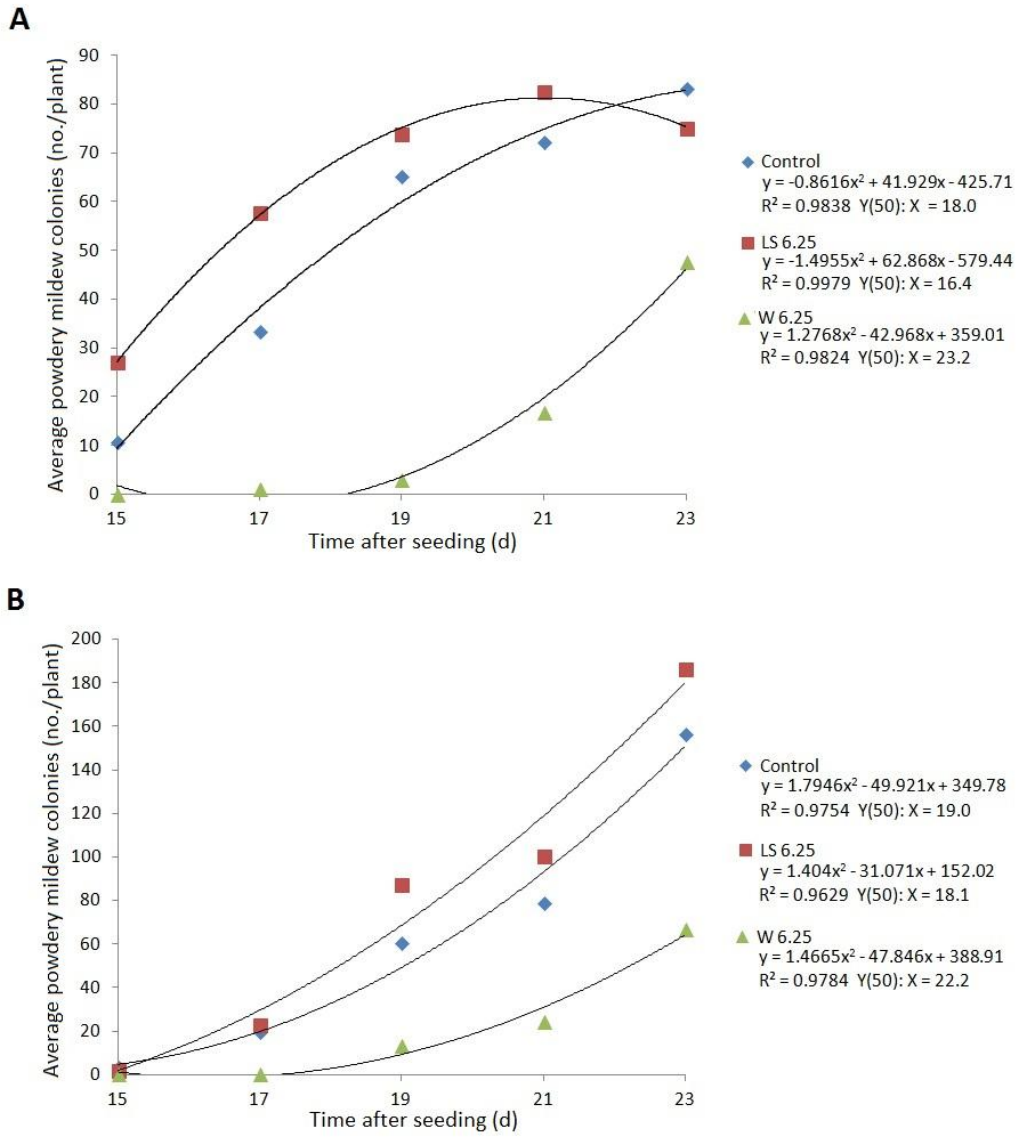


Fig. 3. Polynomial regression of disease progression during earlier (colony count) stage of powdery mildew on pumpkin [3A: Expt. 1, 3B: Expt. 2. Control (no application of limestone or wollastonite), 6.25 ton/acre limestone (LS 6.25) and 6.25 ton/acre wollastonite (W 6.25), DAS (d after seeding)]. A calculation for the time needed to reach an average of 50 powdery mildew colonies on the plants were made based on the equations shown. 1 ton/acre = 2.2417 $Mg \cdot ha^{-1}$.

Table 1. Effects of limestone (LS) or wollastonite (W) soil amendments on soil chemistry for a New Jersey Readington loam soil in pots grown with pumpkin, Expt. 1. Contrast and regression analyses were performed to evaluate the extracted soil mineral level in response to the different limestone and wollastonite application rates.

Application (tons/acre)[z]	CEC (meq/100 g)[y]	pH[x]	OM (%)	Si	S	P	K	Ca	Mg	Na	B	Fe	Mn	Cu	Zn
				(ppm)[w]						(mg·kg^{-1})[v]					
Control	20.2	5.3	7.9	53.7	21	184	79	1,794	264	58	0.68	158	45	6.2	4.8
LS 6.25	18.3	6.7	7.7	68.2	22	171	78	2,711	312	51	0.74	135	35	6.6	4.1
LS 12.5	20.7	7.0	7.5	72	24	174	86	3,352	302	51	0.73	128	35	7.3	4.3
LS 25	25.6	7.1	7.4	64.9	22	154	78	4,326	294	49	0.66	116	33	7.1	4.3
LS 50	32.5	7.2	7.4	51.4	23	144	89	5,620	310	49	0.76	110	32	7.2	4.8
W 6.25	19.5	6.7	7.8	218	22	164	81	3,057	240	48	0.84	129	37	7.2	4.2
W 12.5	17.9	7.3	7.5	348	25	160	83	2,996	215	50	0.66	121	35	7.3	4.4
W 25	20.0	7.5	7.4	434	24	155	84	3,402	212	49	0.77	119	35	7.1	4.5
W 50	23.0	7.6	7.1	467	26	146	83	4,008	196	44	0.73	106	31	6.4	4.1
Contrast significance (P value)															
Treatment effect	0.017	<0.001	0.000	<0.001	0.050	<0.001	<0.001	0.483	0.259	<0.001	0.264	<0.001	<0.001	<0.001	<0.001
Amendment effect	<0.001	<0.001	0.596	<0.001	0.150	0.015	<0.001	<0.001	0.951	0.270	0.377	0.002	0.030	0.613	0.252
Rate effect	<0.001	<0.001	<0.001	<0.001	0.069	<0.001	<0.001	<0.001	0.124	0.308	0.244	<0.000	<0.001	0.012	0.026
Amendment × rate interaction	<0.001	<0.001	0.285	<0.001	0.692	0.006	<0.001	0.003	0.167	0.645	0.139	0.011	0.016	0.004	<0.001
Regression significance (P value)															
Limestone linear	0.002	0.001	0.033	0.682	0.854	0.042	<0.001	0.056	0.712	0.597	0.148	<0.001	0.186	0.197	0.500
Limestone quadratic	0.233	0.005	0.085	0.056	0.874	0.343	0.046	0.050	0.503	0.698	0.122	0.007	0.634	0.262	0.457
Wollastonite linear	0.560	<0.001	0.039	<0.001	0.289	0.221	0.297	0.018	0.478	0.365	0.650	0.026	0.065	0.500	0.075
Wollastonite quadratic	0.104	<0.001	0.214	<0.001	0.446	0.725	0.405	0.104	0.507	0.185	0.696	0.585	0.577	0.102	0.039

[z]Control = no applications; 1 ton/acre = 2.2417 Mg·ha^{-1}.
[y]CEC = cation exchange capacity; 1 meq/100 g = 1 cmol·kg^{-1}.
[x]Mehlich-3 soil tests were performed on soil pH.
[w]Si = acetic acid extractable soil silicon; S = sulfur; 1 ppm = 1 mg·kg^{-1}.
[v]P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Na = sodium; B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; 1 mg·kg^{-1} = 1 ppm.
OM = organic matter.

Table 2. Effects of limestone (LS) or wollastonite (W) soil amendments on soil chemistry for a New Jersey Readington loam soil in pots grown with pumpkin, Expt. 2. Three additional lower rates were added in this experiment. Contrast and regression analyses were performed to evaluate the extracted soil mineral level in response to the different limestone and wollastonite application rates.

Application (tons/acre)[z]	CEC (meq/100 g)[y]	pH[x]	OM (%)	Si	S	P	K	Ca	Mg	Na	B	Fe	Mn	Cu	Zn
				(ppm)[w]							(mg·kg^{-1})[v]				
Control	14.5	4.9	6.1	37	18	183	148	905	161.3	69	0.36	134	51	12.8	4.3
LS 0.78	17.0	5.1	6.1	40	21	185	182	1,178	212.5	79	0.62	132	49	14.0	4.2
LS 1.56	15.0	5.4	5.7	43	19	186	176	1,299	220.0	74	0.47	128	43	14.6	3.9
LS 3.13	14.0	6.0	5.8	50	20	181	165	1,585	253.3	71	0.54	120	41	13.7	3.5
LS 6.25	15.8	6.6	5.8	57	23	189	181	2,205	272.8	75	0.68	118	39	14.8	3.5
LS 12.5	16.6	7.2	5.8	59	21	180	168	2,678	213.3	70	0.55	108	40	13.4	3.2
LS 25	28.4	7.1	5.5	55	25	174	183	4,898	222.8	74	0.65	95	33	14.0	3.5
LS 50	39.4	7.3	4.9	46	21	157	161	7,033	230.8	61	0.45	84	29	18.2	3.4
W 0.78	15.2	5.3	5.7	50	20	173	141	1,294	165.5	71	0.41	131	49	14.3	3.9
W 1.56	16.2	5.3	5.7	69	20	176	165	1,340	178.0	78	0.45	132	50	12.9	4.0
W 3.13	13.7	5.9	5.3	121	16	177	160	1,639	160.3	68	0.37	121	42	16.0	3.3
W 6.25	16.6	6.6	5.5	214	21	178	192	2,477	175.5	76	0.57	115	49	17.0	3.7
W 12.5	17.8	7.2	5.5	296	31	176	179	2,972	168.0	80	0.61	108	44	15.1	3.6
W 25	17.7	7.5	5.4	346	21	172	161	3,010	147.8	72	0.52	109	46	14.7	3.9
W 50	21.2	7.5	5.2	374	30	160	161	3,680	147.8	77	0.66	102	40	13.7	3.4
Contrast significance (P value)															
Treatment effect	<0.001	<0.001	<0.001	<0.001	0.071	0.078	0.015	<0.001	<0.001	0.185	0.003	<0.001	<0.001	<0.001	<0.001
Amendment effect	<0.001	0.100	0.002	<0.001	0.271	0.015	0.053	<0.001	<0.001	0.146	0.073	<0.001	<0.001	0.576	0.194
Rate effect	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.025	<0.001	<0.001	0.134	0.021	<0.000	<0.001	<0.001	<0.001
Amendment × rate interaction	<0.001	0.004	0.008	<0.001	0.004	0.544	0.019	<0.001	0.001	0.012	0.007	<0.001	0.002	<0.001	0.063
Regression significance (P value)															
Limestone linear	0.008	<0.001	0.595	<0.001	0.026	0.120	0.616	<0.001	0.706	0.969	0.124	<0.001	<0.001	0.019	0.005
Limestone quadratic	0.466	<0.001	0.112	<0.001	0.044	0.117	0.413	0.015	0.783	0.309	0.061	<0.001	0.025	<0.001	0.019
Wollastonite linear	0.033	<0.001	0.329	<0.001	0.162	0.786	0.121	<0.001	0.132	0.723	0.119	<0.001	0.678	0.236	0.816
Wollastonite quadratic	0.719	<0.001	0.893	<0.001	0.476	0.322	0.103	<0.001	0.470	0.829	0.390	0.001	0.775	0.125	0.542

[z]Control = no applications; 1 ton/acre = 2.2417 Mg·ha^{-1}.
[y]CEC = cation exchange capacity; 1 meq/100 g = 1 cmol·kg^{-1}.
[x]Mehlich-3 soil tests were performed on soil pH.
[w]Si = acetic acid extractable soil silicon; S = sulfur; 1 ppm = 1 mg·kg^{-1}.
[v]P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Na = sodium; B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; 1 mg·kg^{-1} = 1 ppm.
OM = organic matter.

When exposed to powdery mildew, wollastonite amended pumpkin plants accumulated significantly more biomass by the end of both experiments (Fig. 4). During Expt. 1, the highest accumulated plant biomass was observed for the 12.5-ton/acre of wollastonite amendment, but the value was only marginally higher than the 25 tons/acre treatment without being statistically significant (P = 0.930). During Expt. 2, the pathogen established itself much more quickly (data not shown), resulting in overall smaller plants and less uniform growth. The highest biomass was observed at 3.13 tons/acre of wollastonite, closely followed by the 6.25-and 25-ton/acre rates (P = 0.745 and 0.824, respectively). During both experiments, wollastonite treated plants had larger leaves, longer vines and were overall bigger, as shown in Figs. 2 and 4.

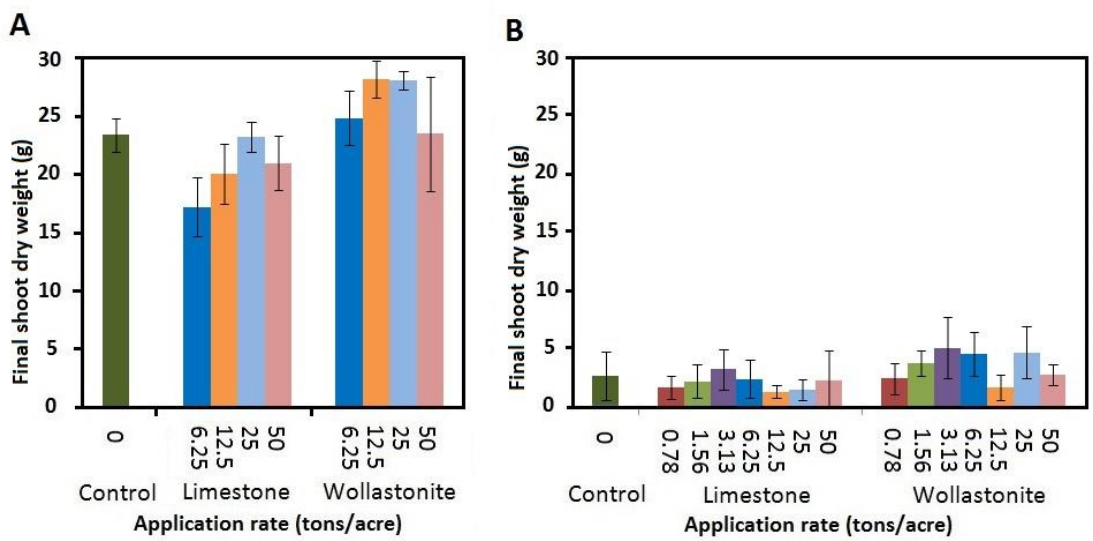


Fig. 4. Final shoot dry weight of pumpkin plants after infected with powdery mildew (4A: Expt. 1, 4B: Expt. 2, both n = 4). Error bars indicate ± SD. Expt. 1 was terminated at 35 d after seeding (DAS), while Expt. 2 was terminated at 45 DAS. 1 ton/acre = 2.2417 $Mg \cdot ha^{-1}$, 1 g = 0.0353 oz.

The Ca concentration in the plant tissue increased similarly with increasing amendment rates of limestone or wollastonite (Tables 3 and 4). In plants, the uptake of one cation often results in less uptake of other cations. We observed this too because less Mg was taken up as a result of the limestone or wollastonite treatments compared to the unamended control treatment. The wollastonite amendments also decreased potassium (K) uptake. Phosphorus (P) uptake increased in the plants subject to wollastonite amendments, but not in plants subjected to limestone amendments, which agrees with previous research (Tubaña and Heckman, 2015) that found that amending soil with Si can enhance P availability. The uptake of micronutrients is sensitive to changes in soil pH (Bryson et al., 2014). As expected, boron (B), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) concentrations in plant tissue decreased with limestone or wollastonite amendments (Tables 3 and 4).

Table 3. Complete elemental analysis of plant shoots cultivated from soil amended with limestone (LS), wollastonite (W) or no amendments (Control). Expt. 1. Contrast and regression were performed to evaluate foliar tissue nutrient content in response to different limestone and wollastonite application rates.

Application (tons/acre)[x]	Si	N	P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn
				(%)[y]						(ppm)[x]		
Control	0.24	4.5	0.40	2.3	4.9	1.6	0.28	62	110	197	7.1	65
LS 6.25	0.11	5.0	0.41	2.5	5.8	1.3	0.28	41	94	34	7.4	42
LS 12.5	0.08	4.5	0.34	2.0	6.5	1.3	0.26	31	81	28	6.4	40
LS 25	0.26	4.6	0.34	2.2	6.2	1.4	0.26	26	96	33	7.2	42
LS 50	0.09	4.4	0.33	2.0	6.7	1.3	0.25	28	85	34	6.8	39
W 6.25	0.63	4.6	0.44	1.9	5.6	1.2	0.25	26	86	41	7.1	46
W 12.5	0.48	4.4	0.47	1.6	6.5	1.3	0.24	16	78	35	6.6	33
W 25	0.42	4.5	0.45	1.5	6.7	1.3	0.24	13	80	34	6.2	26
W 50	0.41	4.3	0.42	1.6	6.9	1.3	0.23	11	69	31	6.0	21
Contrast significance (P value)												
Treatment effect	<0.001	0.949	0.004	0.836	0.453	0.520	0.682	0.231	0.424	0.005	0.279	0.739
Amendment effect	<0.001	0.118	<0.001	0.001	0.704	0.090	0.009	<0.001	0.079	0.052	0.050	<0.001
Rate effect	0.002	0.033	0.056	0.120	0.084	0.259	0.109	<0.001	0.294	0.044	0.056	<0.001
Amendment × rate interaction	<0.001	0.776	0.080	0.871	0.830	0.542	0.876	0.885	0.796	0.130	0.192	<0.001
Regression significance (P value)												
Limestone linear	0.002	0.149	0.123	0.564	0.746	0.091	0.232	0.004	0.782	0.724	0.909	0.856
Limestone quadratic	0.001	0.311	0.197	0.674	0.912	0.079	0.387	0.011	0.725	0.635	0.986	0.772
Wollastonite linear	0.005	0.762	0.677	0.014	0.116	0.381	0.808	<0.001	0.745	0.117	0.147	<0.001
Wollastonite quadratic	0.019	0.946	0.463	0.027	0.219	0.487	0.933	<0.001	0.997	0.306	0.287	<0.001

[x]Control = no applications; 1 ton/acre = 2.2417 Mg·ha^{-1}.
[y]An inductively coupled plasma atomic emission spectroscopy test was performed on plant shoot mineral composition. Si = silicon; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur.
[x]B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; 1 ppm = 1 mg·kg^{-1}.

Table 4. Complete elemental analysis of plant shoots cultivated from soil amended with limestone (LS), wollastonite (W) or no amendments (Control). Three additional lower application rates were added in Expt. 2. Contrast and regression were performed to evaluate foliar tissue nutrient content in response to different LS and W application rates.

Application (tons/acre)[x]	Si	N	P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn
				(%)[y]						(ppm)[y]		
Control	0.20	5.9	0.54	6.4	3.7	1.1	0.54	74	220	852	15.9	125
LS 0.78	0.17	6.3	0.60	5.1	4.4	1.2	0.56	84	177	725	16.1	107
LS 1.56	0.17	6.3	0.57	4.9	4.9	1.1	0.49	82	168	274	16.2	96
LS 3.13	0.11	6.0	0.53	4.6	5.8	1.1	0.48	49	137	80	13.9	70
LS 6.25	0.12	6.0	0.52	4.3	6.1	0.9	0.50	42	244	77	14.6	53
LS 12.5	0.09	6.1	0.53	4.7	6.4	0.7	0.51	34	167	72	14.3	47
LS 25	0.10	7.4	0.51	4.6	6.7	0.7	0.48	30	268	77	15.2	42
LS 50	0.08	5.0	0.46	4.6	6.9	0.7	0.48	28	209	77	15.8	39
W 0.78	0.24	6.2	0.48	5.2	4.5	1.1	0.46	87	157	1,035	14.0	104
W 1.56	0.28	5.9	0.49	5.3	5.0	0.9	0.41	66	152	475	14.3	93
W 3.13	0.32	5.3	0.47	4.5	5.5	0.7	0.41	44	140	82	12.5	55
W 6.25	0.23	5.9	0.51	4.6	6.2	0.7	0.39	29	185	63	12.3	36
W 12.5	0.26	5.8	0.59	3.4	7.3	0.6	0.44	28	151	64	11.8	38
W 25	0.26	5.8	0.65	3.9	7.4	0.5	0.39	17	161	49	10.5	21
W 50	0.26	5.6	0.63	4.0	8.2	0.6	0.44	16	169	57	12.2	22
Contrast significance (*P* value)												
Treatment effect	0.187	0.488	0.175	0.138	<0.001	<0.001	0.002	<0.001	0.917	<0.001	0.137	<0.001
Amendment effect	<0.001	0.134	0.525	0.105	0.006	<0.001	<0.001	0.011	0.023	0.287	0.001	0.003
Rate effect	0.036	0.150	0.574	0.002	<0.001	<0.001	0.187	0.000	0.078	<0.001	0.443	<0.001
Amendment × rate interaction	0.013	0.381	0.013	0.057	0.104	0.026	0.927	0.767	0.565	0.061	0.912	0.781
Regression significance (*P* value)												
Limestone linear	<0.001	0.080	0.260	0.333	<0.001	<0.001	0.485	<0.001	0.119	0.026	0.521	<0.001
Limestone quadratic	0.008	0.038	0.700	0.421	0.001	<0.001	0.649	<0.001	0.188	0.062	0.461	<0.001
Wollastonite linear	0.632	0.912	0.011	0.001	<0.001	<0.001	0.284	<0.001	0.722	0.006	0.019	<0.001
Wollastonite quadratic	0.692	0.928	0.052	0.004	<0.001	<0.001	0.248	0.001	0.898	0.020	0.036	<0.001

[x]Control = no applications; 1 ton/acre = 2.2417 Mg·ha^{-1}.
[y]An inductively coupled plasma atomic emission spectroscopy test was performed on plant shoot mineral composition. Si = silicon; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur; B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; 1 ppm = 1 mg·kg^{-1}.

Limestone amendments had no significant impact on plant uptake of Si (Tables 3 and 4). However, the plant Si content increased due to the wollastonite amendments compared to the unamended control treatment. Most interestingly, the highest concentration of Si in the plants was observed at lower application rates of wollastonite. During Expt. 1, as the application rate of wollastonite increased from 6.25 to 50 tons/acre, a significant decrease of the Si concentration in the plants was observed. During Expt. 2, the plant Si level was the highest at the 3.13-ton/acre treatment (Tables 3 and 4). Based on our results, there is no evidence that exceeding typical agronomic application rates of wollastonite (e.g., for the purpose of neutralizing soil acidity) will further increase Si uptake.

Similar to our previous work (Lepolu et al., 2016), the current study also found that wollastonite is both an effective liming material and an effective source of plant available Si. Therefore, soil and crops may benefit from wollastonite amendments. This study demonstrated that wollastonite applications increased soil pH, increased Si concentration in pumpkin plants, helped suppress powdery mildew, and enhanced plant P uptake. We observed an increase in biomass accumulation of pumpkin plants grown in Si-treated soil while under high powdery mildew pressure. While this study focused on pumpkin, many other crops, especially Si accumulator plants, such as grain crops, may be able to utilize wollastonite to help increase plant growth and yields and to better tolerate foliar diseases such as powdery mildew (Tubaña and Heckman, 2015). Although not tested in our study, other researchers have shown that Si amended crops had an increase in tissue firmness, and were less susceptible

to insect attacks (Datnoff, 2014; Datnoff et al., 2001).

Our results showed that wollastonite applications have similar liming effects as regular limestone, and marginally change soil chemistry compared to applying regular limestone. While it is more expensive, wollastonite can be used as a liming agent with the same effectiveness as common agricultural limestone. As with limestone applications, there was no additional benefit to powdery mildew suppression when the wollastonite application rates were increased beyond what is needed to reach the target soil pH. Higher application rates of wollastonite did not increase, but rather decreased the Si concentration in plants, as well as reduced the plant's ability to suppress powdery mildew. We do not know the reason why our plants exhibited lower Si uptake rates when more wollastonite was added to the soil beyond the application rate needed to reach the target soil pH. We do not think soil pH contributes to this phenomenon because both wollastonite and limestone amendments increased the soil pH equally well. Si needs to be root absorbed in order to change plant response to pathogen infection at both the physiological and molecular level. Our results showed that disease suppression was positively correlated with the Si concentration in plants, but not with the Si concentration in the soil. As a result, the observed increase in biomass did not have a linear relationship with an increased wollastonite level in the soil. While the impact of powdery mildew was the lowest at 3.13-and 6.25-ton/acre of wollastonite applications, shoot biomass was increased for not only the lower, but also the higher wollastonite applications as well. Our findings suggest that going beyond the normal

agronomic rate needed to reach the target soil pH for pumpkin is not necessarily harmful to biomass yield. Therefore, pumpkin growers with fields that already have an optimal soil pH could still apply a moderate rate of wollastonite to obtain the benefits of enhancing plant Si uptake for powdery mildew suppression.

To reveal the reason for why the lower wollastonite amendment rates resulted in higher Si concentrations in pumpkin, future research is needed to study plant growth and development under non-disease conditions, and possibly using different soil types. For a pot experiment conducted in a greenhouse, soil and air temperature are more similar than field cultivation, which the soil temperature is usually considerably lower than the air temperature. Soil moisture distribution can also be less controlled. Therefore, the solubility, availability and uptake of Si by plants grown in the field could be different from plants grown in pots in a greenhouse. Although wollastonite appears effective at reducing powdery mildew development in pumpkin, there could be other benefits from using wollastonite as liming material instead of limestone even in the absence of disease pressure. Moreover, our study was terminated before the fruiting stage, and careful evaluation of plant and fruit characteristics, as well as yields and tissue elemental analysis through the entire growth period of healthy pumpkin crops will be of great interest to growers. Any residual effects of wollastonite amendments to soils compared with limestone amendments over multiple cropping cycles with Si accumulator or non-accumulators will also be of interest to growers.

Naturally mined Si sources such as wollastonite can be used in organic farming.

We found that wollastonite can suppress powdery mildew development and neutralize soil acidity at the same time, but the optimal application rate must be carefully considered since we found that lower application rates yielded the best results. Organic growers are encouraged to inquire with certifiers and researchers before deciding on amendment rates for certified farmland. While some OMRI listed products are currently being used by organic growers, our previous research (Lepolu et al., 2016) has shown that several of these materials are effective as a source of Si, but are less effective as liming agents. In the case of wollastonite, if applied to acidic soils, the combined benefits of a liming agent with potential fungal disease suppression may be of great interest to organic growers.

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