

Utah State University

DigitalCommons@USU

---

Publications

Crop Physiology Lab

---

2012

## Effect of Silicon on Plant Growth and Drought Stress Tolerance

Kaerlek W. Janislampi

Follow this and additional works at: [https://digitalcommons.usu.edu/cpl\\_pubs](https://digitalcommons.usu.edu/cpl_pubs)



Part of the [Plant Sciences Commons](#)

---

### Recommended Citation

Janislampi, Kaerlek W., "Effect of Silicon on Plant Growth and Drought Stress Tolerance" (2012).

*Publications*. Paper 3.

[https://digitalcommons.usu.edu/cpl\\_pubs/3](https://digitalcommons.usu.edu/cpl_pubs/3)

This Other is brought to you for free and open access by the Crop Physiology Lab at DigitalCommons@USU. It has been accepted for inclusion in Publications by an authorized administrator of DigitalCommons@USU. For more information, please contact [digitalcommons@usu.edu](mailto:digitalcommons@usu.edu).



EFFECT OF SILICON ON PLANT GROWTH  
AND DROUGHT STRESS TOLERANCE

by

Kaerlek W. Janislampi

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science  
(Crop Physiology)

Approved:



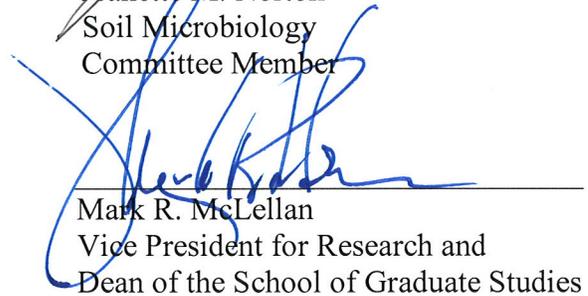
Bruce Bugbee  
Crop Physiology  
Major Professor



Jeanette M. Norton  
Soil Microbiology  
Committee Member



Paul R. Grossl  
Biogeochemistry  
Committee Member



Mark R. McLellan  
Vice President for Research and  
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY  
Logan, Utah

2012

Copyright © Kaerlek Janislampi 2012

All Rights Reserved

## ABSTRACT

## Effect of Silicon on Plant Growth and Drought Stress Tolerance

by

Kaerlek W. Janislampi, Master of Science

Utah State University, 2012

Major Professor: Dr. Bruce Bugbee  
Department: Plants, Soils, and Climate

Silicon is not considered an essential nutrient, but it is typically abundant in soils and can be taken up in large amounts by plants. Silicon is known to have beneficial effects when added to rice and several other plants. These effects include disease and insect resistance, structural fortification, and regulation of the uptake of other ions. In this study, the effect of silicic acid fertilization on the growth and drought tolerance of four crop plants (corn, wheat, soybean, and rice) was analyzed. Plants were studied using three cultivation techniques: 1) hydroponic solution and subjected to salt stress, 2) low-silicon soil-less medium (peat) and subjected to gradual drought stress, and 3) low-silicon soil-less medium (peat) and subjected to acute drought stress. Silicon was added both as reagent-grade  $\text{Na}_2\text{SiO}_3$  and as a siliceous liming agent (PlantTuff). Both forms of Si generally improved drought and salt stress tolerance, but the effects were inconsistent. Silicon increased corn dry mass by up to 18% and the effect was statistically significant ( $p < 0.05$ ) in two out of three techniques. Silicon increased water use efficiency in corn by up to 36% and the effect was statistically significant ( $p < 0.05$ ) in one out of two techniques. In the

acute drought stress technique, silicon increased wheat dry mass by 17% and the effect was statistically significant ( $p < 0.05$ ). Silicon increased soybean and rice dry mass by 20 to 30%, but the effect was not statistically significant. Silicon in oldest corn leaves increased from 0.4% to 3% as Si increased from less than 0.01 to 0.8 mM in the hydroponic solution. There was a statistically significant effect of silicon supply on the concentration of some other nutrients, but the effect was often not great enough to be considered biologically important. Rice accumulated the greatest concentration of foliar silicon, corn and wheat were intermediate, and soybean accumulated the least. Collectively, these results indicate an effect of silicon in drought and salinity stress tolerance, but additional studies on the rate and onset of drought are needed to determine interacting factors and better understand the inconsistent results.

(100 pages)

## PUBLIC ABSTRACT

## Effect of Silicon on Plant Growth and Drought Stress Tolerance

by

Kaerlek W. Janislampi, Master of Science

Utah State University, 2012

Silicone is a silicon-containing synthetic polymer. Silicon is a metalloid chemical element. Silicon is not considered an essential nutrient for plants, but it is typically abundant in soils and can be taken up in large amounts by plant roots. Silicon is known to have beneficial effects when added to the soil in which rice and several other plants are cultivated. These beneficial effects include disease and insect resistance, plant structural fortification, and regulation of the uptake of other plant nutrients. Silicic acid is the form of silicon in soils that is available to plants. In this study, the effect of silicic acid fertilization on the growth and drought tolerance of four crop plants (corn, wheat, soybean, and rice) was analyzed. Plants were cultivated in hydroponic solution and subjected to salt stress by the addition of salt to the hydroponic solution. Plants were also cultivated in a low-silicon soil-less medium (sphagnum peat moss) and subjected to drought stress by decreasing irrigation. Silicon fertilization generally improved drought and salt stress tolerance, but the effects were inconsistent. Silicon increased total corn plant mass by up to 20% and the effect was statistically significant ( $p < 0.05$ ) in two out of three techniques. Silicon increased water use efficiency (plant mass accumulated divided by mass of water used) in corn by up to 36% and the effect was statistically significant ( $p < 0.05$ ) in one out

of four trials. Collectively, these results indicate an effect of silicon in drought and salinity stress tolerance, but additional studies on the rate and onset of drought are needed to determine interacting factors and better understand the inconsistent results.

## CONTENTS

	Page
ABSTRACT.....	iii
PUBLIC ABSTRACT.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	x
CHAPTER	
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	12
3. MATERIALS AND METHODS.....	21
4. RESULTS.....	26
5. DISCUSSION AND CONCLUSIONS.....	55
LITERATURE CITED.....	63
APPENDICES.....	76
A. CONVERSION OF ELECTRICAL CONDUCTIVITY TO OSMOTIC POTENTIAL IN DILUTE NUTRIENT SOLUTION.....	77
B. DROUGHT STRESS LYSIMETER SYSTEM.....	78
C. MOISTURE DISTRIBUTION IN 20-L CONTAINERS OF GROWING MEDIUM.....	82
D. PRELIMINARY STUDIES USING LYSIMETER SYSTEM.....	83
E. INITIAL RESEARCH ON SILICON AS A BENEFICIAL PLANT NUTRIENT.....	84
F. NUTRIENT SOLUTIONS USED IN HYDROPONIC STUDIES.....	87

## LIST OF TABLES

Table		Page
3 – 1	Average greenhouse conditions during two hydroponic trials.....	22
3 – 2	Average greenhouse conditions during two 2-L container trials.....	25
4 – 1	3-factor ANOVA table from 2 blocks (replicates in time) of average container dry mass per plant compiled.....	27
4 – 2	2-factor ANOVA table from 2 blocks (replicates in time) of 4 replicate plants dry mass per plant compiled.....	27
4 – 3	3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers percent dry mass.....	28
4 – 4	3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers percent roots.....	29
4 – 5	3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers water use efficiency.....	30
4 – 6	ANOVA table from Trial 1 dry mass data at 4 <sup>th</sup> harvest.....	35
4 – 7	ANOVA table from Trial 1 dry mass data at 5 <sup>th</sup> (final) harvest.....	35
4 – 8	ANOVA table from Trial 1 water use efficiency data.....	38
4 – 9	ANOVA table from Trial 2 water use efficiency data.....	38
4 – 10	3-factor ANOVA table from corn dry mass data from both trials.....	42
4 – 11	2-factor ANOVA table from drought-stressed corn dry mass data from both trials.....	42
4 – 12	3-factor ANOVA table from wheat dry mass data from both trials.....	46
4 – 13	2-factor ANOVA table from drought-stressed wheat data from both trials.....	46
4 – 14	3-factor ANOVA table from soybean dry mass data from both trials.....	49

4 – 15	2-factor ANOVA Table from drought-stressed soybean dry mass data from both trials.....	49
4 – 16	3-factor ANOVA table from rice dry mass data from both trials.....	52
4 – 17	2-factor ANOVA table from drought-stressed rice dry mass data from both trials.....	52
5 – 1	Overall statistical significance of results.....	60
A – 1	Comparison of osmotic potential with electrical conductivity in a dilute nutrient solution.....	77
C – 1	Mean volumetric water content (VWC or $\theta_v$ ) of two replicate containers.....	82

## LIST OF FIGURES

Figure	Page
1 – 1	Photograph of silicon dioxide..... 1
1 – 2	Effect of silicon (Si) on rice growth and yield..... 2
1 – 3	Plot of the solubility relationships of the different forms of silica, in terms of activity, not concentration..... 3
1 – 4	Diagram of apoplastic and symplastic pathways in plant roots..... 5
1 – 5	Representation of the movement of silicon from the soil solution through plant vascular system to leaf tips..... 6
1 – 6	Diagram of silicon deposits between cuticle and epidermal cells in a plant leaf..... 7
1 – 7	Net returns for various crops in the nation..... 9
1 – 8	Comparison of transpiration and biomass in wheat..... 9
3 – 1	Corn cultivated in 6 containers of hydroponic solution..... 21
3 – 2	Corn in containers at 19 days after planting on weighing lysimeters..... 22
3 – 3	Concentration of silicon in leachate from containers of peat with varying amendments..... 23
3 – 4	Four groups of 24 2-L containers per species..... 25
4 – 1	Whole-plant dry mass from two blocks of 4 replicate hydroponic plants..... 26
4 – 2	Whole-plant percent dry mass from two blocks of 4 replicate hydroponic plants..... 28
4 – 3	Percent root mass from two blocks of 4 replicate hydroponic plants..... 29
4 – 4	Water use efficiency (g dry mass per kg of water transpired) from two blocks of 4 replicate hydroponic plants..... 30

4 – 5	Silicon concentration in the oldest leaves, newest leaves, and roots of hydroponic plants harvested from both trials and subjected to no stress or -0.40 MPa osmotic (salt) stress.....	31
4 – 6	Corn nutrient concentration in the oldest leaves of plants from both hydroponic trials.....	32
4 – 7	Silicon concentration in older leaves of corn grown in Trial 1 and Trial 2.....	33
4 – 8	Corn in Trial 1 at 43 days after planting.....	33
4 – 9	Corn dry mass per plant from 5 harvests in Trial 1.....	34
4 – 10	Corn dry mass per plant from 6 harvests in Trial 2.....	35
4 – 11	Percent dry mass from 6 harvests in Trial 1.....	36
4 – 12	Percent dry mass from 6 harvests in Trial 2.....	36
4 – 13	Cumulative transpiration of 4 replicate containers per treatment in Trial 1.....	36
4 – 14	Mass of 4 replicate containers per treatment in Trial 1.....	37
4 – 15	Cumulative transpiration of 4 replicate containers per treatment in Trial 2.....	37
4 – 16	Mass of 4 replicate containers per treatment in Trial 2.....	37
4 – 17	Corn water use efficiency (g aboveground dry mass per kg of water transpired) by treatment consisting of 4 replicate containers in Trial 1 (left) and Trial 2 (right).....	38
4 – 18	Silicon concentration and solution pH in leachate from post-harvest containers.....	38
4 – 19	Nutrient concentration in the oldest leaves of corn harvested at 46 days after planting in Trial 1.....	39
4 – 20	Nutrient concentration in the oldest leaves of corn harvested at 44 days after planting in Trial 2.....	40
4 – 21	Silicon concentration in plant tissues.....	41
4 – 22	Aboveground corn dry mass from two trials in peat.....	42
4 – 23	Drought-stressed corn plants supplied with 0.2 and 0.8 mM silicon, at 23 days after planting during Trial 1.....	43

4 – 24	Nutrient concentration in the oldest fully-expanded leaves of corn plants in Trial 1 and Trial 2.....	44
4 – 25	Aboveground wheat dry mass from two trials in peat.....	45
4 – 26	Drought-stressed wheat plants supplied with 0.2 and 0.8 mM silicon, at 48 days after planting during Trial 1.....	46
4 – 27	Nutrient concentration in the oldest fully-expanded leaves of wheat plants in Trial 1.....	47
4 – 28	Aboveground soybean dry mass from two trials in peat.....	48
4 – 29	Drought-stressed soybean plants supplied with 0.2 and 0.8 mM silicon, at 27 days after planting during Trial 1.....	49
4 – 30	Nutrient concentration in the oldest fully-expanded leaves of soybean plants in both trials.....	50
4 – 31	Aboveground rice dry mass from two trials in peat.....	51
4 – 32	Drought-stressed rice plants supplied with 0.2 and 0.8 mM silicon, at 27 days after planting during Trial 1.....	52
4 – 33	Nutrient concentration in the oldest fully-expanded leaves of soybean plants in both trials.....	53
B – 1	System for monitoring and control of load cell balance mass.....	78
B – 2	Side view diagram showing the attachment of rigid platforms to a beam sensor load cell to facilitate mass measurements.....	79
B – 3	Transpiration equation.....	81
D – 1	Foliar silicon content in older leaves of plants grown in a preliminary trial in peat/vermiculite.....	83

## CHAPTER 1

### INTRODUCTION

#### Silicon as a nutrient

The word silicon is derived from the Latin word *silex*, meaning flint. The word was originally used to denote any hard rock. In modern English, silicon refers to the element and silica refers to a compound in which each molecule of silicon is chemically bound to two oxygen molecules ( $\text{SiO}_2$ ; silicon dioxide).



Figure 1 – 1. Photograph of silicon dioxide

The earth's crust contains a large proportion of silicon (Ingri 1978; Iler 1978). This silicon is mostly in the form of silicates and aluminosilicates. In soil solutions, silicon is found mostly in the form of silicic acid ( $\text{Si}(\text{OH})_4$ ) (Lindsay 1979; Epstein 1994). In plants, silicon is found mostly as silicon dioxide. Although silicon can be found in great abundance on this planet, its biological functions are not as well studied as those of other elements such as oxygen, hydrogen, and carbon (Wainwright 1997). As a note of interest, the element germanium is an analog for silicon in biological terms, but germanium can cause low-level toxicity in plants (Ma *et al.* 2002).

Silicon has been responsible for the human disease pneumonoultramicroscopic silicovolcanoconiosis (more commonly known as silicosis).

However, among chromalveolates and plants silicon has been considered beneficial in some cases (Datnoff *et al.* 2001). Silicon is considered an essential nutrient for a group of algae known as diatoms (Ketchum 1954; Round *et al.* 1990; Kinrade *et al.* 2001; Kinrade *et al.* 2002; Volcani 1978). As for vascular plants, silicon is only considered essential for the Equisetaceae family (Chen and Lewin 1969). However, silicon is

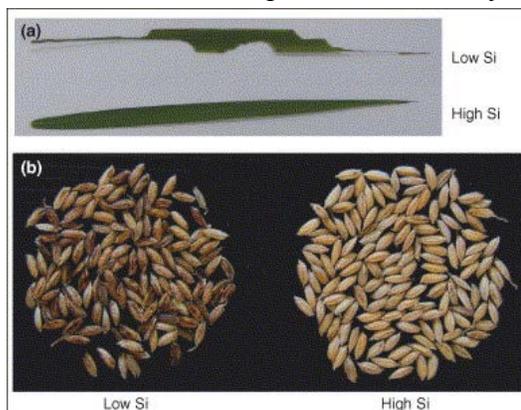


Figure 1 – 2. Effect of silicon (Si) on rice growth and yield. (a) Rice with low levels of Si is susceptible to insect attack; (b) a low level of Si accumulation results in grain discoloration owing to infection by multiple fungal pathogens. From Ma and Yamaji 2006.

absorbed by most plants, in greater or lesser quantities (Weiss and Herzog 1978; Epstein 1999; Epstein 2002; Epstein and Bloom 2005).

In particular, graminaceous crops are known to absorb silicon (Carpita 1996; Rafi and Epstein

1999; Tamai and Ma 2003; Appendix E). The

interactions of silicon with rice (Fig. 1 – 2)

have been of particular interest (Savant *et al.*

1997; Deren 2001; Mengel and Kirkby 2001;

Ma and Takahashi 2002; Tamai and Ma 2003; Rodrigues *et al.* 2004). See Figure 1 – 2

for an example of the importance of silicon to rice growth and yield. Sugarcane, a known

silicon accumulator, has also been of interest (Clements 1964, 1965; Savant *et al.* 1999).

### Silicon in soil

Most soils contain a substantial percentage of silicon, generally about 31% (Sposito 1989). In soil solutions, silicon is found mostly as uncharged monomeric silicic acid at concentrations from about 0.1 mM to 0.6 mM (Epstein 1994), or up to about 0.8 mM at equilibrium (Lindsay 1979) when the solution pH is below 9 (Ma and Takahashi

2002). A few soil factors can affect the availability of silicon for plant uptake. According to Jones and Handreck (1967) “there is a marked effect of pH on the solubility of silica in soils,” but this effect is not in the range of pH in which most plants are cultivated. Weathering is the main factor in the availability of silicon in soils. As weathering increases, available silicon is generally depleted. This phenomenon occurs mostly in tropical regions of the earth. Figure 1 – 3 provides a visual description of the average speciation of silicon

according to pH. Details of the speciation of silica in soils are provided by Lindsay (1979), but a brief review follows:

At a lower pH, silicic acid ( $\text{H}_4\text{SiO}_4$ ) is more soluble and less likely to dissociate. Silicic acid ( $\text{H}_4\text{SiO}_4$ ) is in equilibrium with soil  $\text{SiO}_2$  at pH 3.10 and at a concentration of 0.794 mM. Silicic acid is in equilibrium with silicate ions that polymerize at pH 9.71, and at a concentration of 0.794 mM. A 1.0000 mM solution of  $\text{H}_4\text{SiO}_4$  would dissociate into 0.9999 mM  $\text{H}_4\text{SiO}_4$  and 0.0001 mM  $\text{H}_3\text{SiO}_4^-$  at pH 6.00. The same solution would dissociate into 0.404 mM  $\text{H}_4\text{SiO}_4$  and 0.596 mM  $\text{H}_3\text{SiO}_4^-$  at pH 10.00. Uptake of silicon from soil increases with increasing soil water content (Hemmi 1933; Williams and Shapter 1955). The presence of aluminum and iron oxides in the soil has been shown to decrease the amount of soluble Si in the soil solution (Jones and Handreck 1967). High

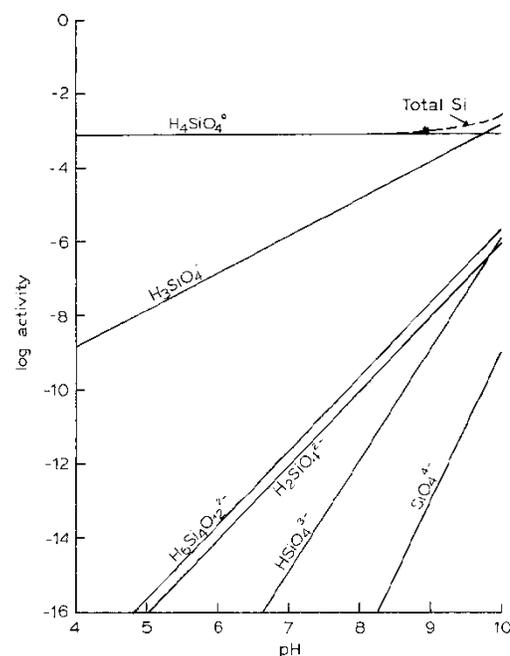


Figure 1 – 3. Plot of the solubility relationships of the different forms of silica, in terms of activity, not concentration. From Lindsay (1979).

concentration of silicic acid (e.g. >120–140 ppm or 1.2–1.5 mM) in the soil solution can lead to polymerization to colloidal silica (Jones and Handreck 1967).

Some weathered acidic soils contain less available silicon than others. Lindsay (1979) states that “in highly weathered soils, free SiO<sub>2</sub> may become depleted from soils leaving sesquioxides of iron and aluminum as the major residual minerals.” In such soils, silicon amendments can be important for optimal crop yields (Alvarez *et al.* 1988, Korndörfer and Lepsch 2001).

### Silicon uptake in plants

The essentiality of silicon for plant growth has long been a question of interest to plant nutrition researchers. Uptake of silicon varies by species and by plant group (Jones and Handreck 1967; Ma *et al.* 2001; Richmond and Sussman 2003). In some plants, such as many species of the Gramineae family, uptake of silicon appears to be passive (Jones and Handreck 1967). In other plants, such as dicotyledons, uptake of silicon appears to be excluded by the roots (Jones and Handreck 1967). Originally, rice was considered a special case. Rice plants appear to perform active uptake of silicon (Ma and Yamaji 2006; Van Soest 2006), at least in hydroponic solutions. However, using modern analytical techniques, it is possible that many plants may soon be seen to have some form of silicon uptake or rejection mechanism in cortical cell membranes (Richmond and Sussman 2003). Mitani and Ma (2005) note a silicon transporter in cortical cell membranes of several species of crops, noting also a greater density of the transporter in rice as compared to cucumber and tomato. Ma and Yamaji (2006) suggest that there is a gene that encodes “a Si uptake transporter in rice.” Cornelis *et al.* (2010) suggest that silicon uptake is passive in forest trees.

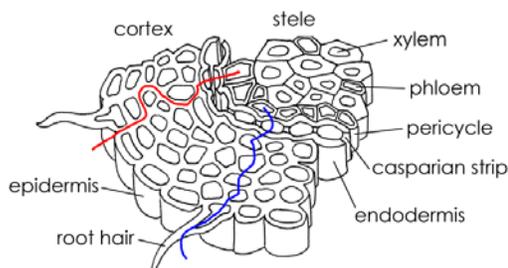


Figure 1 – 4. Diagram of apoplastic and symplastic pathways in plant roots. The apoplastic pathway (red) includes intercellular spaces and cell wall spaces. The symplastic pathway (blue) includes the cytoplasm and plasmodesmata.

or by passive diffusion. Wiese *et al.* (2007) state that xylem loading is of greater importance than uptake in the root symplast when it comes to silicon uptake. Figure 1 – 4 displays the two pathways by which solutes may enter the xylem of a plant root. For most plants, the concentration and amount of silicon in the plant tends to increase with plant age (Jones and Handreck 1967).

#### Deposition of silicon in tissues

Once accumulated inside a plant, silicon can “lend rigidity and roughness to the walls” of plant cells (Epstein and Bloom 2005) as well as provide other beneficial effects (Van Soest 2006). Studies indicate that silicon is transported passively in the transpiration stream, and is deposited at sites of high transpiration (Wiese *et al.* 2007). There is no evidence of silicon being mobile within plants. It is hypothesized that as water is transpires from the plant, silicic acid accumulates and forms colloidal silicic acid, then amorphous silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ) (also referred to as silica gel, phytoliths, or

Following root uptake from the rhizosphere,<sup>1</sup> silicic acid is loaded into the xylem. Ma and Yamaji (2006) state that xylem loading is “mediated by a kind of transporter” in rice, but

not in cucumber and tomato, in which dicotyledon species xylem loading appears to be passive. Other species are most likely to mediate xylem loading either by a transporter

<sup>1</sup> The rhizosphere is the volume of soil solution that is in contact with and accessible by uptake mechanisms of plant roots

opal), which polymerizes at high concentrations ( $> 2\text{mM}$ ), thus creating a rigid polymer within the plant (Jones and Handreck 1967; Gao *et al.* 2006; Ma and Yamaji 2006).

Figure 1 – 5 provides a graphic example of the movement of silicon from soil to leaf tips.

Silicon has been shown to enhance growth and yield, promote upright stature, prevent lodging, promote favorable exposure of leaves

to light, provide resistance to bacterial and fungal diseases (Fawe *et al.* 2001; Voogt and

Sonneveld 2001), provide resistance to herbivores (Coors 1987), low temperatures

(Epstein 1999, 2001), salinity (Hamayun *et al.*

2010; Lee *et al.* 2010), heavy metal toxicity

(Valamis and Williams 1967; Neumann and

zur Nieden 2001; Voogt and Sonneveld 2001; Liang *et al.* 2005), and aluminum toxicity

(Barcelo *et al.* 1993; Cocker *et al.* 1998; Kidd *et al.* 2001), and influence nitrogen,

phosphorus, and the composition of other elements in plant tissue (Bollard and Butler

1966; Epstein and Bloom 2005). Not only has rhizosphere supply of silicon been

effective, but “silicon supplementation in the form of external foliar treatments has

proven to increase the pathogen resistance of plant species that do not take up silicon

efficiently” (Richmond and Sussman 2003). Industrial by-products containing silicon

have been used to benefit plants. “Siliceous blast-furnace slags” have been added to soils

to increase rice disease resistance (Jones and Handreck 1967; Savant *et al.* 1996; Pereira

*et al.* 2004).

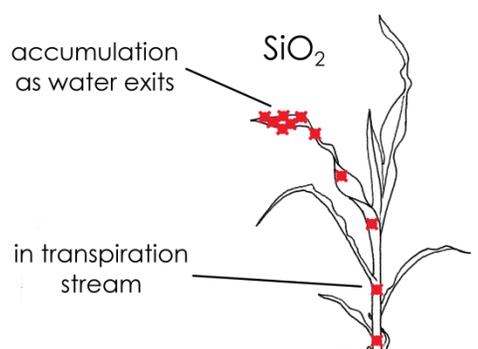


Figure 1 – 5. Representation of the movement of silicon from the soil solution through plant vascular system to leaf tips. Red symbols represent concentrations of silicon.

## Drought stress physiology and silicon

Drought stress can damage plant cell membranes, and cell wall architecture, as well as inhibit photosynthesis and cell division (Hsiao 1973; Taiz and Zeiger 2006). Hsiao (1973) states that “many of the changes observed under nutrient or water deficiencies seem to represent general patterns of modulation in plants under adversity.” Here, four specific changes are briefly discussed.

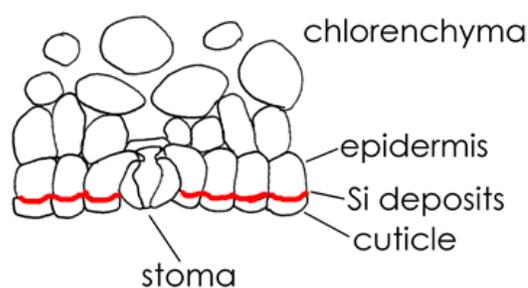


Figure 1 – 6. Diagram of silicon deposits (red) between cuticle and epidermal cells in a plant leaf.

1) Plant cuticle “contributes to minimize uncontrolled water loss at stomatal closure” (Riederer and Schreiber 2001). The water loss that occurs through cuticular transpiration “accounts for only 5 to 10% of the total leaf transpiration” (Taiz and Zeiger 2006). “As Si is deposited beneath the cuticle of the leaves

forming a Si-cuticle double layer (Figure 1 – 6), the transpiration through the cuticle may decrease by Si deposition” (Ma 2004). Silicon deposits 2.5  $\mu\text{m}$  thick between the cuticle (generally 0.1  $\mu\text{m}$  thick in rice) and endodermal cells have been found in rice (Ma and Takahashi 2002). Silicon deposits have also been found in guard cells around stomata in blueberry (Morikawa and Saigusa 2004). Silicon can reduce the transpiration rate by 30% in rice, which has a thin cuticle (Ma 2004).

2) There is also some evidence that silicon may be involved in the osmotic adjustment of plants. Kaya *et al.* (2006) found that 2 mM  $\text{Na}_2\text{SiO}_3$  increased leaf relative water content by 26.5% in water-stressed corn (50% of FC) grown in peat/perlite/sand. Gunes *et al.*

(2008a) and Crusciol *et al.* (2009) found that silicon increased proline<sup>2</sup> (a key solute in osmotic adjustment) content in stressed plant tissue. However, Kaya *et al.* (2006), Lee *et al.* (2010), and Shen *et al.* (2010) found the opposite. Sonobe *et al.* (2010) also suggest a silicon-induced effect of osmotic adjustment in sorghum roots.

3) Silicification of trichomes has been observed in plants (Sangster *et al.* 1983; Hodson *et al.* 1985). It is possible that silicon-fortified trichomes act as antennae that absorb short-wave radiation and emit long-wave radiation to aid in the cooling of leaves. However, it is also possible that silicon-fortified trichomes increase the leaf-atmosphere boundary layer, thus creating a larger energy transfer gradient.

4) Silicon accumulation has been observed mostly in areas of high transpiration in plants. However, there is some evidence that silicon accumulation in and/or on plant roots may serve as part of drought tolerance mechanisms. Yeo *et al.* (1999) hypothesized a silicon-mediated decrease of root apoplastic leakage to the stele in rice roots grown in saline nutrient solution. Lux *et al.* (2002) found high root endodermal silicification in a drought-tolerant sorghum cultivar, and proposed that this silicification may be related to the drought tolerance.

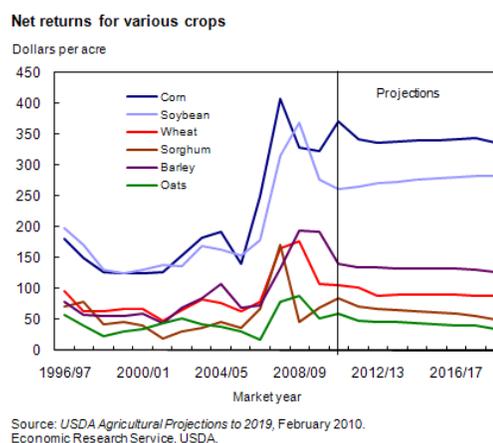


Figure 1 – 7. Net returns for various crops in the nation. R reproduced from USDA Feed Grain Baseline ... [updated 2010]

<sup>2</sup> Proline is an amino acid formed in leaf tissues subjected to drought stress, and is thought to function as an osmolyte (Kameli and Losel 1993; Fumis and Pedras 2002; Verbruggen and Hermans 2008).

## Economic value of corn

According to the United States department of Agriculture, “Corn is the most widely produced feed grain in the United States, accounting for more than 90 percent of total value and production of feed grains,” and “The United States is a major player in the world corn trade market, with approximately 20 percent of the corn crop exported to other countries (Corn overview ... [updated 2010]).” Figure 1 – 7 displays a comparison of corn value with the value of other feed crops in the United States.

## Relationship between transpiration and growth

Measurements of daily water use have long been used to determine daily plant growth. Figure 1 – 8 displays the linear increase in aboveground biomass produced by wheat as transpiration per unit vapor pressure deficit increases (based on Wilson and Jamieson 1985). Reduction in transpiration is a reliable indicator of water stress in plants

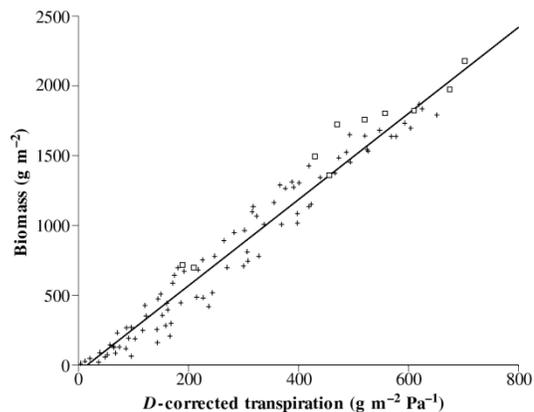


Figure 1 – 8. Comparison of transpiration and biomass in wheat. Adapted from Wilson and Jamieson 1985.

(Hsiao 1973). The sum of all water losses to the atmosphere from soil and plants is referred to as evapotranspiration. When evaporation from soil is eliminated, or reduced to an undetectable amount, the remaining water losses can be accounted for by transpiration from plants. Measurement of dry matter accumulation is equivalent to plant growth over the life of a plant. For this study, the effects of silicon fertilization on the growth drought tolerance of four species were under investigation.

## Objectives and hypotheses

The overall objective of this study was to determine the effect of silicon on growth, water use efficiency, and percent dry mass, during and following a period of drought or salt stress. As part of this objective, the following hypotheses were tested:

1.  $H_0$ : Dry mass of stressed plants fertilized with silicon is not statistically different than dry mass of stressed plants without supplemental silicon.

$H_1$ : There is a statistically significant difference in dry mass of stressed plants between plants fertilized with silicon and plants without supplemental silicon.

2.  $H_0$ : Dry mass of unstressed plants fertilized with silicon is not statistically different than dry mass of unstressed plants without supplemental silicon.

$H_1$ : There is a statistically significant difference in dry mass of unstressed plants between plants fertilized with silicon and plants without supplemental silicon.

3.  $H_0$ : Water use efficiency of stressed plants fertilized with silicon is not statistically different than water use efficiency of stressed plants without supplemental silicon

$H_1$ : There is a statistically significant difference in water use efficiency of stressed plants between plants fertilized with silicon and plants without supplemental silicon.

4.  $H_0$ : Water use efficiency of unstressed plants fertilized with silicon is not statistically different than water use efficiency of unstressed plants without supplemental silicon

H<sub>1</sub>: There is a statistically significant difference in water use efficiency of unstressed plants between plants fertilized with silicon and plants without supplemental silicon.

5. H<sub>0</sub>: Percent dry mass of stressed plants fertilized with silicon is not statistically different than percent dry mass of stressed plants without supplemental silicon.

H<sub>1</sub>: There is a statistically significant difference in percent dry mass of stressed plants between plants fertilized with silicon and plants without supplemental silicon.

6. H<sub>0</sub>: Percent dry mass of unstressed plants fertilized with silicon is not statistically different than percent dry mass of unstressed plants without supplemental silicon.

H<sub>1</sub>: There is a statistically significant difference in percent dry mass of unstressed plants between plants fertilized with silicon and plants without supplemental silicon.

## CHAPTER 2

### LITERATURE REVIEW

#### Silicon and drought stress

Water is vital to many biological systems and organisms. It supports many biological reactions by serving as a transport medium, a solvent, and a fuel for the hydrolysis of photosystem II. The limitation of water supply to plants is commonly referred to as drought, and plants respond in measureable ways to the limited supply.

Fertilization with mineral elements other than silicon has been shown to influence the drought tolerance of many crops. Purcell and King (1996) found that nitrogen improved soybean drought tolerance. Al-karaki *et al.* (1996) found that phosphorus facilitated osmotic adjustment in sorghum and bean, and Singh and Sale (2000) found similar results in white clover. A shraf *et al.* (2001) found that potassium fostered osmotic adjustment in pearl millet and similar results were found by Egilla *et al.* (2001) with hibiscus. Li *et al.* (2003) found that calcium increased antioxidant response in liquorice under drought stress.

Silicon appears to be involved in the fortification of plants against oxidation of cell membranes, leading to the protection of various plant structures and functions subjected to drought conditions. Silicon also appears to be part of the regulation of osmolytes within cells subjected to drought stress. In most cases, silicon does not appear to be beneficial to plants until some stress is imposed (Epstein and Bloom 2005). Since salinity in the rhizosphere is often associated with water deficit (Taiz and Zeiger 2006), studies of salt stress and drought stress are related. Studies of heat stress are also related,

since a decrease in evaporative cooling often results from a decrease in water that is available for evaporation.

#### Effect of silicon on growth

Ahmad *et al.* (1992) declared that the “addition of silicon caused significant recovery from salt stress” in wheat at different growth stages, including germination. Gong *et al.* (2003) found that 7.14 mmol Na<sub>2</sub>SiO<sub>3</sub> per 8 kg of soil resulted in an increase in wheat leaf area of 8.3 cm<sup>2</sup> per plant, an increase in dry mass of 45.3 mg per plant, and an increase in leaf thickness. According to Gong *et al.* (2005), the addition of 2.11 mmol Na<sub>2</sub>SiO<sub>3</sub> increased net assimilation rate by ~37 mmol C m<sup>-2</sup> s<sup>-1</sup> under drought conditions in potted wheat. Hattori *et al.* (2005) observed that 1.66 mM K<sub>2</sub>SiO<sub>3</sub> “ameliorated the decrease in dry weight under drought stress conditions,” by about 25% in sorghum grown in containers of sand. In other words, dry weight decreased by only about 54% rather than by about 79%. Kaya *et al.* (2006) found that 2 mM Na<sub>2</sub>SiO<sub>3</sub> increased root dry mass by 0.02 g per plant, and whole-plant dry mass by 0.74 g per plant in drought-stressed corn grown in a mixture of peat, perlite, and sand for 45 days. Romero-Aranda *et al.* (2006) demonstrated that the application of 2.5 mM Si resulted in an increase in leaf area in tomato plants treated with 80 mM (approximately -0.4 MPa Ψ<sub>s</sub>) NaCl. Eneji *et al.* (2008) found that 1000 mg kg<sup>-1</sup> potassium silicate (K<sub>2</sub>SiO<sub>3</sub>) application to the soil of four grass species under deficit irrigation (half of field capacity) “produced the greatest biomass yield responses across species,” as compared to calcium silicate (CaSiO<sub>3</sub>) or silica gel. According to Gunes *et al.* (2008a), sodium silicate applied to the soil mitigated the adverse dry mass reduction effects of drought in 6 of 12 sunflower cultivars. Pulz *et*

*al.* (2008) found that using calcium and magnesium silicates in the place of dolomitic limestone (in areas with acidic soil) increased potato plant height, decreased stem lodging (weak lower stems), and increased the yield of marketable tubers in drought conditions (soil  $\Psi = -0.05$  MPa). In addition to observations of reduced occurrence of stalk lodging and an increase of mean tuber mass in potatoes, Crusciol *et al.* (2009) found that the application of  $284.4 \text{ m g dm}^{-3}$  Ca and Mg silicate to the soil increased proline concentrations under drought conditions. Sonobe *et al.* (2009) found no effect of silicon on unstressed hydroponic sorghum, but found that 50 ppm (approximately 0.8 mM) silicon ameliorated dry mass reduction in hydroponic sorghum exposed to polyethylene glycol water stress. Chen *et al.* (2010) found that applying 1.5 mM silicon to drought-stressed rice significantly ( $P < 0.05$ ) increased total root length, surface area, volume, and root activity, even to the extent that these parameters were equivalent to those observed in non-stressed plants in many cases. Chen *et al.* (2010) also found that silicon increased photosynthetic rate on a per-leaf basis. Hamayun *et al.* (2010) concluded that silicon “was more effective in alleviating salinity than drought stress” in soybean. They found that the “adverse effects of NaCl and PEG on plant growth were alleviated by adding  $100 \text{ mg L}^{-1}$  and  $200 \text{ mg L}^{-1}$  Si to salt and drought stressed treatments” in terms of shoot length, shoot mass, root mass, and chlorophyll content. According to Lee *et al.* (2010), the addition of 2.5 mM Si to soybean plants “is beneficial in hydroponically grown plants as it significantly improves growth attributes and effectively mitigate the adverse effects of NaCl induced salt stress.” According to Shen *et al.* (2010), the addition of 1.7 mM Si significantly increased soybean dry mass by 26% when subjected to -0.5 MPa of PEG stress. Sonobe *et al.* (2010) report that 1.78 mM Si ( $\text{SiO}_2$ ) in a 15% PEG 6000 (v/v)

solution (to create -0.6 MPa) at 23 days increased shoot dry weight and second-nodal root diameter of Sorghum plants in hydroponic culture, even with decreased osmotic potential of roots. Ahmed *et al.* (2011) state that “silicon application may be useful to improve the drought tolerance of sorghum through the enhancement of water uptake ability.” Also of interest is that Bakhat *et al.* (2009) found that corn supplied with 0.8 mM Na<sub>2</sub>SiO<sub>3</sub> in solution culture under no stress conditions accumulated more leaf area and biomass than corn supplied with no silicon under the same conditions.

#### Effect of silicon on tissue strength

Agarie *et al.* (1998) report that “silicon prevents the structural and functional deterioration of cell membranes when rice plants are exposed to environmental stress,” and that silicon may also be “involved in the thermal stability in cell membranes.” Kaya *et al.* (2006) showed that 2 mM Na<sub>2</sub>SiO<sub>3</sub> decreased electrolyte leakage by 18.3% in water-stressed corn (50% of FC). According to Gunes *et al.* (2008a), silicon applied to the soil prevented membrane damage in shoots via a reduction in H<sub>2</sub>O<sub>2</sub>. Pulz *et al.* (2008) and Crusciol *et al.* (2009) found that using calcium and magnesium silicates decreased potato stem lodging (weak lower stems) in drought conditions. According to Shen *et al.* (2010), silicon reduced osmolyte leakage and lipid peroxidation.

#### Effect of silicon on water use

Gong *et al.* (2003) found that 7.14 mmol Na<sub>2</sub>SiO<sub>3</sub> per 8 kg of soil supplied to the soil resulted in an increase in leaf relative water content of 2.7% and an increase in leaf water potential of 0.4 MPa of wheat in drought conditions. Ma (2004) reports that silicon

reduces cuticular transpiration in drought-stressed rice. In Rhodes grass and sorghum subjected to drought stress in sand, Eneji *et al.* (2005) state that plant water demand decreased “linearly” with increasing calcium silicate application from 1 to 6 Mg ha<sup>-1</sup>. Gong *et al.* (2005) found that the addition of 2.11 mM Na<sub>2</sub>SiO<sub>3</sub> increased leaf water potential by ~0.2 MPa under drought conditions in potted wheat. They also observed that silicon did not decrease stomatal conductance under drought conditions. Hattori *et al.* (2005) observed an increase in stomatal conductance in sorghum supplied with 1.66 mM silicon and subjected to drought stress in sand. They also report no difference in water use efficiency due to silicon supply. Gao *et al.* (2006) found that “silicon application of 2 mmol L<sup>-1</sup> significantly decreased transpiration rate and conductance for both adaxial and abaxial leaf surface, but had no effect on transpiration rate and conductance from the cuticle” in corn subjected to polyethylene glycol osmotic stress in solution culture. This finding may have been due to an excessive silicon supply, causing the formation of silicon polymers on root surfaces. Kaya *et al.* (2006) showed that 2 mM Na<sub>2</sub>SiO<sub>3</sub> increased leaf relative water content by 26.5% in water-stressed corn (50% of FC). Romero-Aranda *et al.* (2006) report that the application of 2.5 mM Si resulted in an increase in “tomato plant water storage and plant water use efficiency” in tomato plants treated with 80 mM NaCl. They also report an increase in leaf turgor, a decrease in leaf water potential, and a decrease in percent dry mass associated with the overall water use efficiency effect. According to Gunes *et al.* (2008a), silicon applied to the soil increased sunflower leaf relative water content. According to Farooq *et al.* (2009), “Silicon has also improved drought resistance in rice by silicification of the root endodermis and improving [sic] water uptake.” Sonobe *et al.* (2009) found that 50 ppm silicon increased

stomatal conductance, increased transpiration, and ameliorated water uptake reduction in hydroponic sorghum exposed to polyethylene glycol water stress. They explained the stomatal conductance and transpiration increases by concluding that there was a “silicon-induced improvement in hydraulic conductance” in the leaves. In other words, there was a delay in induction of osmotic adjustment at the same leaf water potential. Chen *et al.* (2010) found that applying 1.5 mM silicon to drought-stressed rice increased transpiration rate from 19% in a drought-susceptible line and 53% in a drought-resistant line. According to Shen *et al.* (2010), 1.7 mM silicon increased leaf relative water content from 62.3% to 80.7%, and transpiration by 29% in hydroponic soybean seedlings subjected to polyethylene glycol stress. According to Sonobe *et al.* (2010), 1.78 mM Si (SiO<sub>2</sub>) in a 15% PEG 6000 (v/v) solution (to create -0.6 MPa) at 23 days increased root water uptake and root water content, even with decreased osmotic potential of roots. Ahmed *et al.* (2011) conclude that “silicon application may be useful to improve the drought tolerance of sorghum through the enhancement of water uptake ability.”

#### Effect of silicon on biochemistry

Ahmad *et al.* (1992) report that 0.33 mM silicon supplied to salt-stressed wheat reduced leaf sodium content, but had no effect on chlorophyll content. Lux *et al.* (2002) found high root endodermal silicification in a drought-tolerant sorghum cultivar, and proposed that this silicification may be related to the drought tolerance. In roots of salt-stressed barley, Liang *et al.* (2003) found that silicon-treated plants had decreased membrane lipid peroxidation, leading to greater membrane stability under stress. Liang *et al.* (2003) also “strongly suggest that Si may be involved in the metabolic or

physiological and/or structural activity in higher plants exposed to abiotic and biotic stresses.” Gong *et al.* (2005) found that the addition of 2.11 mmol Na<sub>2</sub>SiO<sub>3</sub> increased “antioxidant defense activities,” alleviated “oxidative damage,” and maintained “many physiological processes such as photosynthesis under drought.” Kaya *et al.* (2006) found that 2 mM Na<sub>2</sub>SiO<sub>3</sub> increased chlorophyll content by 125 mg mL<sup>-1</sup>, root Ca content by 250 mM kg<sup>-1</sup> DM greater than well-watered plants, and decreased proline content by 43%. Eneji *et al.* (2005) state that potassium and calcium silicate fertilization in sand increased the tissue concentration of both N and K in four grasses. According to Gunes *et al.* (2008a), silicon applied to the soil reduced sunflower tissue H<sub>2</sub>O<sub>2</sub>. They also state that the “application of Si under drought stress significantly improved Si, K, S, Mg, Fe, Cu, Mn, Na, Cl, V, Al, Sr, Rb, Ti, Cr, and Ba uptake whereas Zn, Mo, Ni, and Br uptake were not affected,” in most of the 12 sunflower cultivars analyzed. Pulz *et al.* (2008) report that calcium and magnesium silicate fertilization increased drought-stressed potato P and Mn content, but reduced N content. Crusciol *et al.* (2009) report that the same silicates increased potato proline concentration and decreased tuber quality. Chen *et al.* (2010) found that applying 1.5 mM silicon to drought-stressed rice reduced leaf tissue concentrations of K, Na, Ca, Mg, and Fe, but increased chlorophyll concentration. Hamayun *et al.* (2010) claim that the “adverse effects of NaCl and PEG on plant growth were alleviated by adding 100 mg L<sup>-1</sup> and 200 mg L<sup>-1</sup> Si to salt and drought stressed treatments.” According to Lee *et al.* (2010), the addition of 2.5 mM Si to hydroponically grown soybean plants increased chlorophyll content, decreased abscisic acid and proline, and had no effect on gibberellins under salt stress (-0.4 MPa) conditions. Shen *et al.* (2010) also saw a decrease in proline in soybean plants subjected to PEG stress (-0.5

MPa). Furthermore, they report that silicon slightly decreased antioxidant activity and had no effect on chlorophyll content. According to Sonobe *et al.* (2010), 1.78 mM Si ( $\text{SiO}_2$ ) in a 15% PEG 6000 (v/v) solution (to create -0.6 MPa) at 23 days increased sorghum root amino acid content, but decreased tissue Ca content.

### Silicon and corn

According to Lanning *et al.* (1980), the silica content of mature corn plants in Kansas was “highest in the leaf blades (up to 16.6 and 10.9 per cent, respectively) followed by the leaf sheath, tassel, roots, stem epidermis and pith, and ear husk. The percentage of ash as silica was also highest in the leaves.” Coors (1987) found a correlation between silicon supply and plant resistance to a European corn borer. The application of silicon to soil and leaves of corn appears to have deterred corn aphids from the usual degree of feeding in a greenhouse (Moraes *et al.* 2005). In a study in which polyethylene glycol drought stress was applied to corn plants grown in hydroponic solution, Gao *et al.* (2006) found that 2 mM  $\text{Si(OH)}_4$  reduced transpiration rate in drought-stressed plants. The authors concluded that “the role of Si in decreasing transpiration rate must be largely attributed to the reduction in transpiration rate from stomata rather than cuticula.” Fertilization of container-grown drought-stressed corn with 1 mM and 2 mM  $\text{Na}_2\text{SiO}_3$  in nutrient solution increased plant mass, foliar calcium content, foliar potassium content, root calcium content, and decreased electrolyte leakage (Kaya *et al.* 2006). Bakhat *et al.* (2009) found that 0.8 mM  $\text{Na}_2\text{SiO}_3$  supplied to corn grown in hydroponic solution increased plant height, dry mass, and leaf area. Bakhat *et*

*al.* (2009) also found that 3 mM Na<sub>2</sub>SiO<sub>3</sub> similarly supplied in hydroponic solution reduced leaf area and caused no significant increase in dry mass.

## CHAPTER 3

## MATERIALS AND METHODS

## Hydroponic technique

Trials were conducted in a greenhouse using the hydroponic solution culture method (Figure 3 – 1). Seeds were germinated and seedlings were transferred to six 50-L containers. All solutions were aerated with house air. The space between the plant stem and the bottle was sealed with open-cell foam to minimize the escape of solution culture vapor from the bottle. Solution cultures were maintained at the maximum possible volume, accommodating root growth and aeration disturbance. In each trial, NaCl was

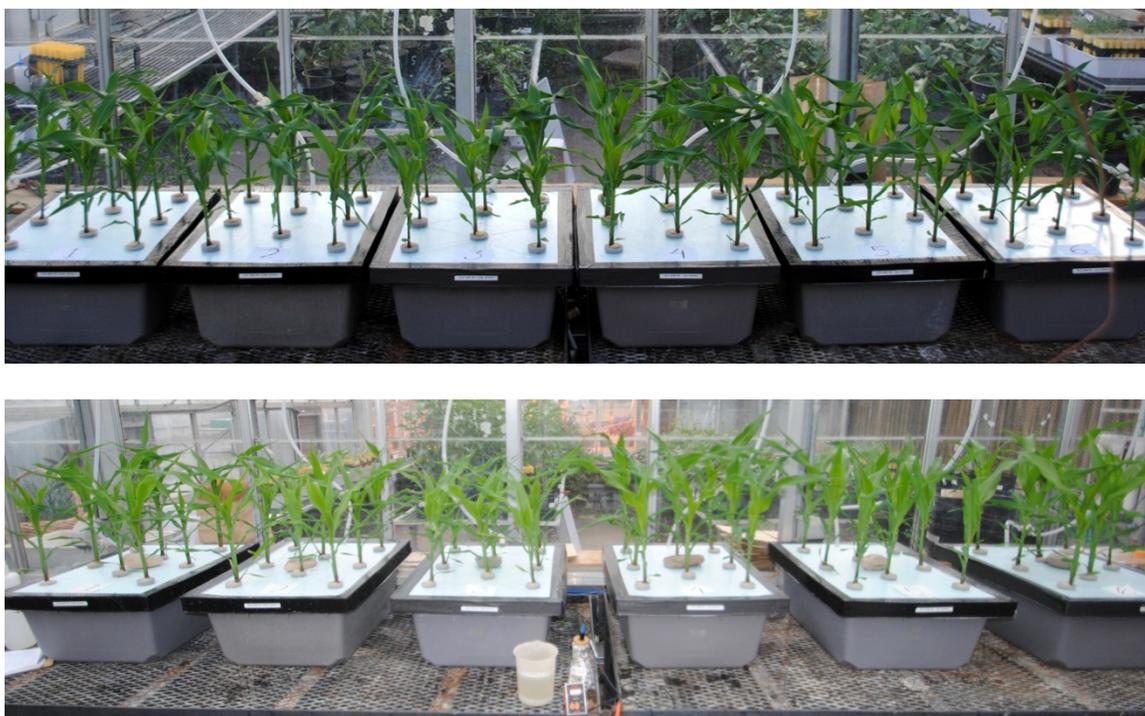


Figure 3 – 1. Corn cultivated in 6 containers of hydroponic solution.

added to the solution culture of plants intended for salt stress treatment (Appendix E). To maintain accurate solute potential of the solution, an electrical conductivity (EC) meter was used to verify potential by calculation from EC to solute potential, and NaCl was

either diluted or added as necessary (Appendix A). A modified half-strength Hoagland solution was used (Appendix F).

Daily transpiration was measured by the difference in solution volume between measurements over the difference in time. Dry mass was determined after harvesting all plant biomass. All plant biomass was dried for 48 hours in a forced-air drying oven set to 80°C. Greenhouse conditions during the two trials are presented in table 3 – 1.

Table 3 – 1. Average greenhouse conditions during two hydroponic trials

Average daytime temperature (°C)	Average nighttime temperature (°C)	Photoperiod (hours)	Average daytime relative humidity (%)	Average daily PPF ( $\text{mol m}^{-2} \text{d}^{-1}$ )
26	21	15	36	27.9

#### Lysimeter technique

$\text{Si(OH)}_4$ , or silicic acid, is the bioavailable form of silicon. Corn (*Zea mays* cv. Syngenta CB/LL 8562) was grown in 16, 21-L containers of sphagnum peat growing medium amended with 6 g L<sup>-1</sup> of either PlantTuff AgLime and soil conditioner (Edw. C. Levy Corporation, Dearborn, MI) or Dolomite 65 AGRIC.

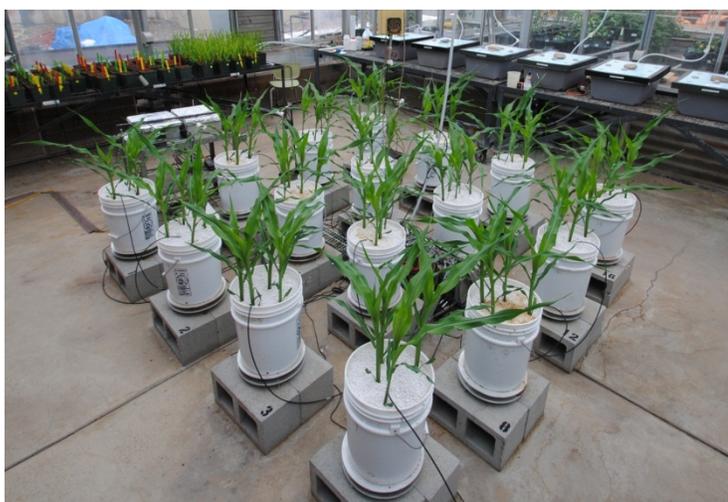


Figure 3 – 2. Corn in containers at 19 days after planting on weighing lysimeters.

(Chemical Lime, Salinas, CA) for 46 days after planting (DAP) (Figure 3 – 2). Previous

studies indicate that  $3 \text{ g L}^{-1}$  Dolomite 65 AGRIC. in peat releases  $0.30 \pm 0.03 \text{ mM}$   $\text{Si(OH)}_4$  while  $6 \text{ g L}^{-1}$  PlantTuff in peat releases  $1.25 \pm 0.23 \text{ mM Si(OH)}_4$  (Figure 3 – 3). In Trial 2, only  $3 \text{ g L}^{-1}$  Dolomite 65 AGRIC. was used. Peat growing medium was also amended with  $45 \text{ g Polyon 16-6-13 1-2}$  month slow-release fertilizer. Previous studies indicate that Canadian sphagnum peat moss releases approximately  $0.02 \text{ mM Si(OH)}_4$  in solutions of de-ionized water.

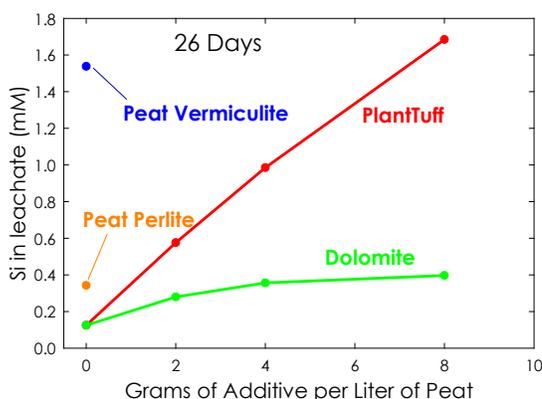


Figure 3 – 3. Concentration of silicon in leachate from containers of peat with varying amendments.

Containers were placed on balances connected to a monitoring and control system to precisely irrigate and measure transpiration in each container in a greenhouse (Appendix A). Water distribution within the containers is expected to have been generally concentrated near the drippers (see Appendix B). A verage greenhouse

conditions during the two trials were  $26/22^\circ\text{C}$  (day/night), 15 hour photoperiod, 25% relative humidity (day), and  $30\text{-}40 \text{ mol m}^{-2} \text{ d}^{-1}$  PPF.

Eight of the 16 containers were subjected to one prolonged drought stress episode and two subsequent stress episodes. Containers subjected to prolonged drought stress were identified as the “chronic” treatment. The other eight containers were subjected to two brief drought stress episodes. This treatment was termed “acute.” Transpiration was measured every 15 seconds, averaged every 30 minutes, and plotted on a 30-minute as well as daily basis. Plants were periodically harvested and analyzed for mass and

nutrient content. After the final harvest of plants, all containers were leached with tap water to determine the concentration of  $\text{Si(OH)}_4$  that was available to plants during the Trials.

### 2-L container technique

Two trials were conducted on a greenhouse bench with crops grown in 2 L of peat growing medium (Figure 3 – 4). Four crops were grown: corn (*Zea mays* cv. Syngenta LL/CB 8562 drought-tolerant), wheat (*Triticum aestivum* cv. Apogee), soybean (*Glycine max* cv. Hoyt), and rice (*Oryza sativa* cv. Ai-nan-tSao). Seeds were sown directly in 2 liters of peat growing medium and were thereafter watered to excess once daily.

Watering was accomplished using two nutrient solutions. One half of the containers were watered with a dilute nutrient solution supplemented with 0.8 mM  $\text{Na}_2\text{SiO}_3$ , while the other half were watered with a dilute nutrient solution supplemented with 1.6 mM NaCl to equalize the Na. Drought stress was applied to 50% of the containers watered with silicon and 50% of the containers watered without silicon, by reduction in watering frequency. Drought stress was relieved when leaf wilting of the plants was observed, and then reapplied on the following day. At occurrences of leaf wilting, photographs were taken of sample containers. All aboveground biomass was harvested and analyzed for fresh mass, dry mass, and foliar nutrient concentrations. Foliar nutrient concentrations were measured using ICP-AES (inductively coupled plasma atomic emission spectroscopy). Data from previous studies indicate that peat growing medium can release up to 0.2 mM Si in water, and that leaching with a 0.8 mM Si solution through the peat produces approximately 0.8 mM available Si (Figure 3 – 3).

Greenhouse conditions during the two trials are presented in table 3 – 2.



Figure 3 – 4. Four groups of 24 2-L containers per species. From left to right: wheat, corn, soybean, rice.

Table 3 – 2. Average greenhouse conditions during two 2-L container trials.

Average daytime temperature (°C)	Average nighttime temperature (°C)	Photoperiod (hours)	Average daytime relative humidity (%)	Average daily PPF ( $\text{mol m}^{-2} \text{d}^{-1}$ )
26	21	15	35	26.1

## CHAPTER 4

## RESULTS

## Hydroponic technique

The addition of 0.4 mM silicon increased corn dry mass, compared with plants that received no silicon (Figure 4 – 1). Independent of salt stress, the addition of 0.8 mM silicon slightly decreased corn dry mass, compared with plants receiving 0.4 mM Si, but this effect was not statistically significant. Salt stress significantly decreased corn dry mass ( $p < 0.001$ ).

Results of a three-factor analysis of variance are found in Table 4 – 1. Considering dry mass on a per-container basis rather than a per-plant basis, results in

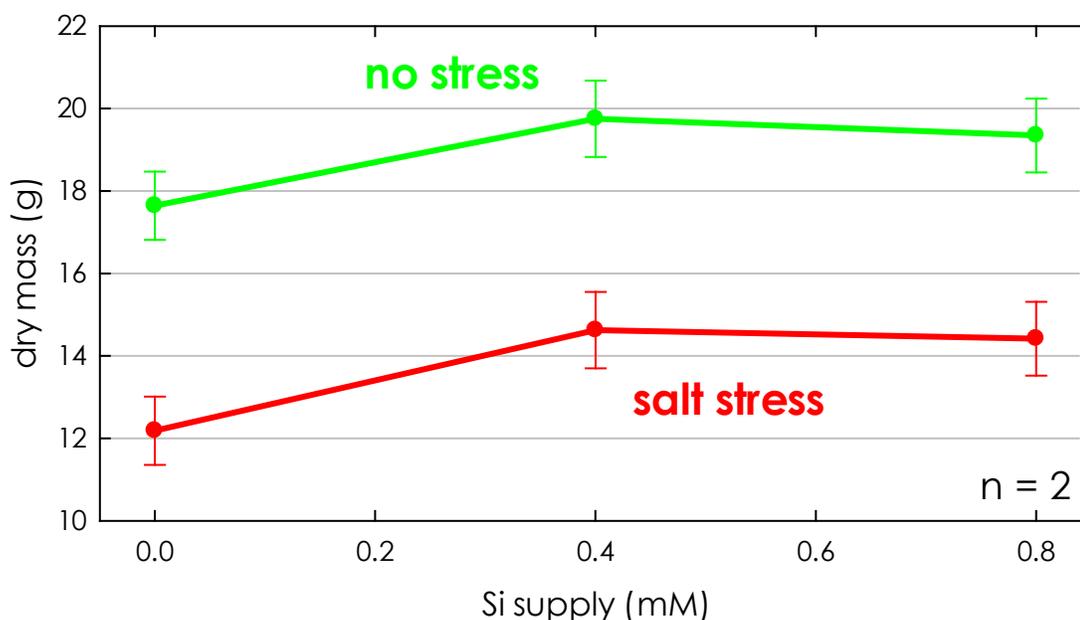


Figure 4 – 1. Whole-plant dry mass from two blocks of 4 replicate hydroponic plants. Based on a 3-factor ANOVA, mean difference in silicon level was not quite significant ( $p = 0.059$ ). Differences in stress type and block were significant ( $p < 0.05$ ). Error bars represent least square mean standard error for silicon level.

greater statistical significance of the effect of silicon (Table 4 – 2). Plants grown in one container are often not considered replicates, because they may not independently vary from one another. For example, the addition of a given nutrient to a container of plants affects all plants contained therein.

Table 4 – 1. 3-factor ANOVA table from 2 blocks (replicates in time) of average container dry mass per plant compiled.

Source of Variation	DF	SS	MS	F	P
Block (harvest)	1	81.311	81.311	153.793	0.006
Si	2	16.823	8.412	15.910	0.059
Salt	1	72.324	72.324	136.796	0.007
Residual	2	1.057	0.529		
Total	11	173.712	15.792		

Table 4 – 2. 2-factor ANOVA table from 2 blocks (replicates in time) of 4 replicate plants dry mass per plant compiled.

Source of Variation	DF	SS	MS	F	P
Block (harvest)	1	285.579	285.579	25.977	<0.001
Si	2	62.572	31.286	2.846	0.073*
Salt	1	254.017	254.017	23.106	<0.001
Block x Si	2	4.784	2.392	0.218	0.806
Block x Salt	1	0.959	0.959	0.0872	0.770
Si x Salt	2	2.167	1.083	0.0985	0.906
Block x Si	2	3.947	1.973	0.179	0.837
Residual	32	351.799	10.994		
Total	43	1010.209	23.493		

\*Pooling all the insignificant error terms in table 2–3 (all the interactions) results in a significant p-value of 0.0452 ( $F_{3,355} > F_{(0.05)_{2,39}} 3.238$ ).

In salt-stressed plants, silicon decreased corn percent dry mass. In unstressed plants, silicon slightly increased percent dry mass (Figure 4 – 2). Salt stress generally increased corn percent dry mass significantly ( $p = 0.044$ ), although the difference decreased with increasing silicon supply (Table 4 – 3).

Results of a 3-factor analysis of variance are found in table 4 – 3.

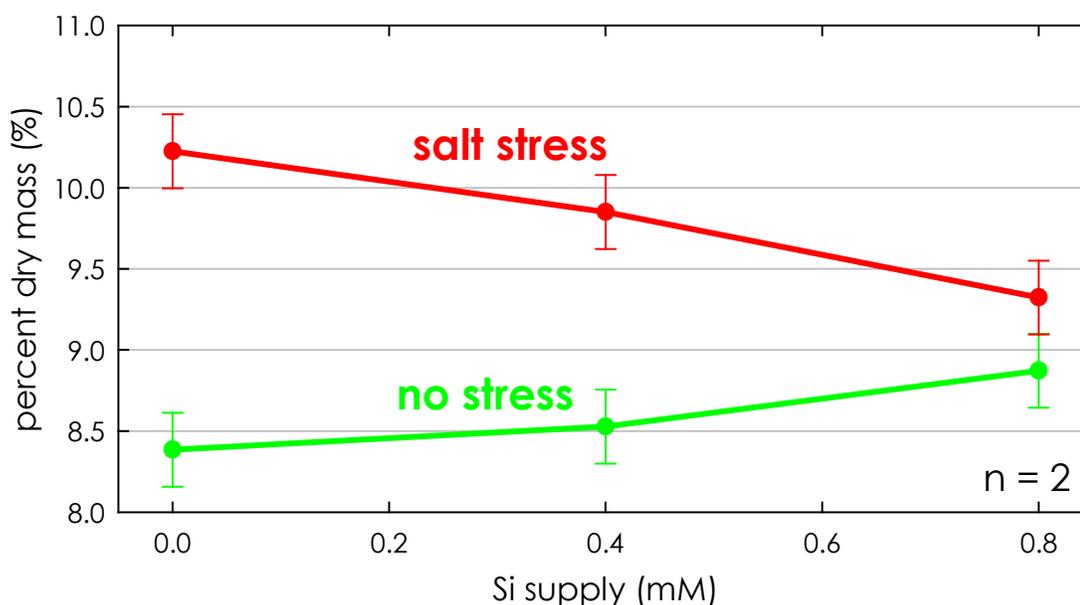


Figure 4 – 2. Whole-plant percent dry mass from two blocks of 4 replicate hydroponic plants. Based on a 3-factor ANOVA, mean differences in silicon level and harvest were not significant ( $p=0.828$  and  $p=0.695$ , respectively). Mean differences in stress type were significant ( $p=0.044$ ). Error bars represent least square mean standard error for silicon level.

Table 4 – 3. 3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers percent dry mass.

Source of Variation	DF	SS	MS	F	P
Block (harvest)	1	0.0426	0.0426	0.205	0.695
Si	2	0.0863	0.0431	0.208	0.828
Stress	1	4.358	4.358	21.027	0.044
Residual	2	0.415	0.207		
Total	11	6.962	0.633		

In unstressed plants, silicon decreased corn root percent. In salt-stressed plants, silicon had a varied effect on root percent. Salt stress generally increased corn root percent when plants were supplied with either 0.8 mM Si (Figure 4 – 3).

Results of a three-factor analysis of variance are found in Table 4 – 4.

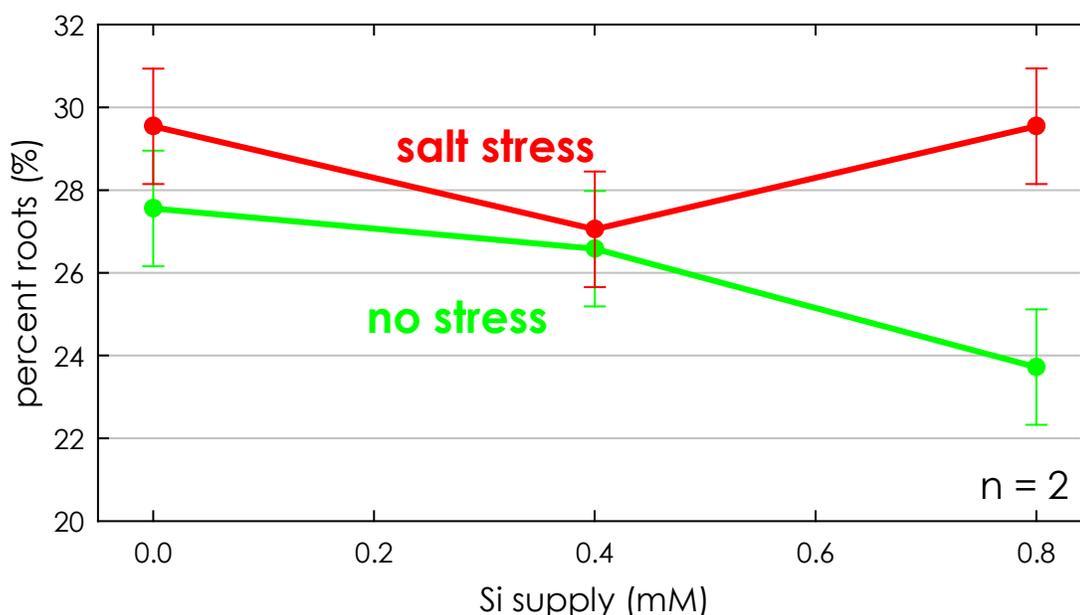


Figure 4 – 3. Percent root mass from two blocks of 4 replicate hydroponic plants. Based on a 3-factor ANOVA, mean differences in silicon level were not significant ( $p=0.635$ ). Mean differences in stress type were also not significant ( $p=0.229$ ). Error bars represent least square mean standard error for silicon level.

Table 4 – 4. 3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers percent roots.

Source of Variation	DF	SS	MS	F	P
Block (harvest)	1	154.462	154.462	19.813	0.047
Si	2	8.948	4.474	0.574	0.635
Stress	1	22.866	22.866	2.933	0.229
Residual	2	15.592	7.796		
Total	11	248.775	22.616		

In unstressed plants, silicon increased water use efficiency when supplied at 0.4 mM Si compared to plants supplied with 0.8 mM or no silicon. In salt-stressed plants, silicon had a varied and very slight effect on water use efficiency. In plants supplied with 0.4 mM Si, salt stress decreased water use efficiency (Figure 4 – 4).

Results of a three-factor analysis of variance are found in Table 4 – 5.

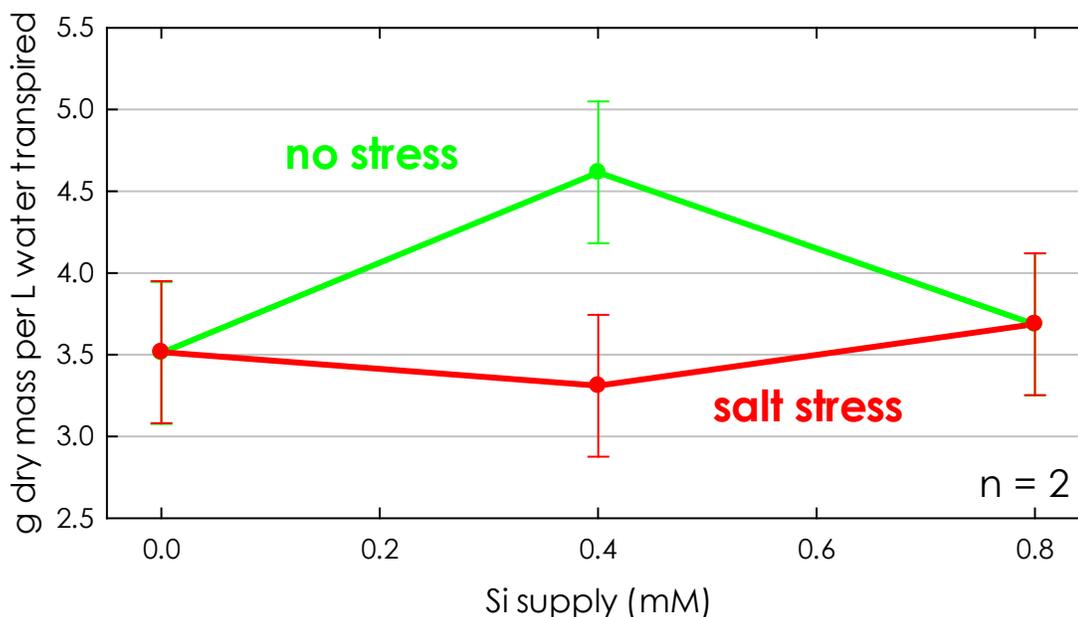


Figure 4 – 4. Water use efficiency (g dry mass per kg of water transpired) from two blocks of 4 replicate hydroponic plants. Based on a 3-factor ANOVA, mean differences in silicon level and stress were not significant ( $p=0.785$  and  $p=0.478$ , respectively). Error bars represent least square mean standard error for silicon level.

Table 4 – 5. 3-factor ANOVA table from 2 blocks (replicates in time) of 2 replicate containers water use efficiency.

Source of Variation	DF	SS	MS	F	P
Block (harvest)	1	6.058	6.058	8.058	0.105
Si	2	0.411	0.206	0.273	0.785
Stress	1	0.562	0.562	0.748	0.478
Residual	2	1.504	0.752		
Total	11	10.741	0.976		

In trial 1, silicon supply from 0.0 mM to 0.4 mM in the culture solution increased plant tissue silicon concentration to approximately 3% of total dry mass. From 0.4 mM to 0.8 mM in the culture solution, there was neither a trend of increase nor of decrease in plant tissue silicon. Salt stress appears to have increased the plant tissue silicon concentration further. Salt-stressed older corn leaves accumulated up to 5% silicon when supplied with 0.8 mM Si in the culture solution (Figure 4 – 5). Nutrient concentration in the oldest leaves of plants harvested at 27 DAP in Trial 1 varied by nutrient, salt stress,

and silicon level (Figure 4 – 6). Increased silicon had a varied effect on the foliar concentration of other nutrients in salt stress and unstressed conditions. Silicon reduced foliar calcium, iron, manganese, and copper concentrations in unstressed plants. The same nutrients were unaffected by silicon level in salt-stressed plants.

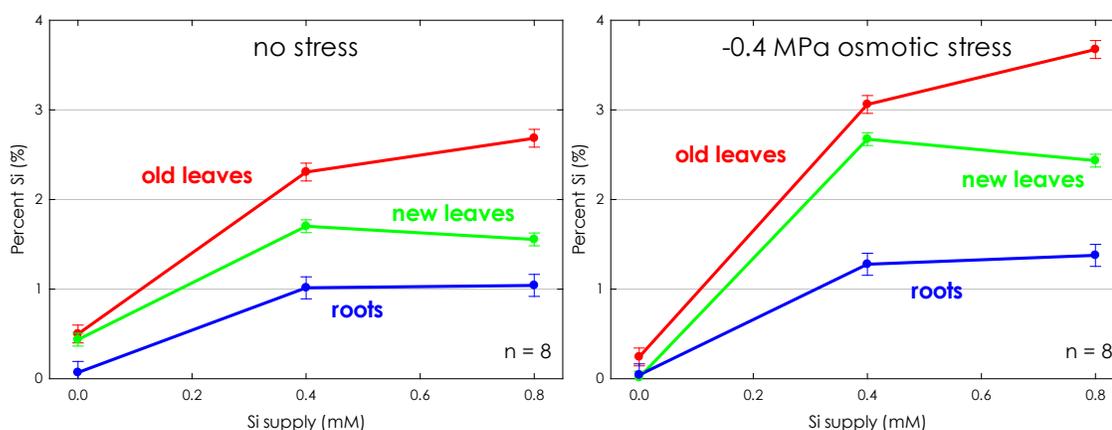


Figure 4 – 5. Silicon concentration in the oldest leaves, newest leaves, and roots of hydroponic plants harvested from both trials and subjected to no stress or -0.40 MPa osmotic (salt) stress. Error bars represent standard error of the least square means for silicon level.

#### Lysimeter technique

Leaves of plants supplied with PlantTuff accumulated approximately 3 to 4 times as much silicon as leaves of plants supplied with Dolomite (Figure 4 – 7). Leaves also appear to have accumulated more silicon during Trial 1 than during Trial 2. Drought stress effects were visibly apparent in both Trials (Figure 4 – 8).

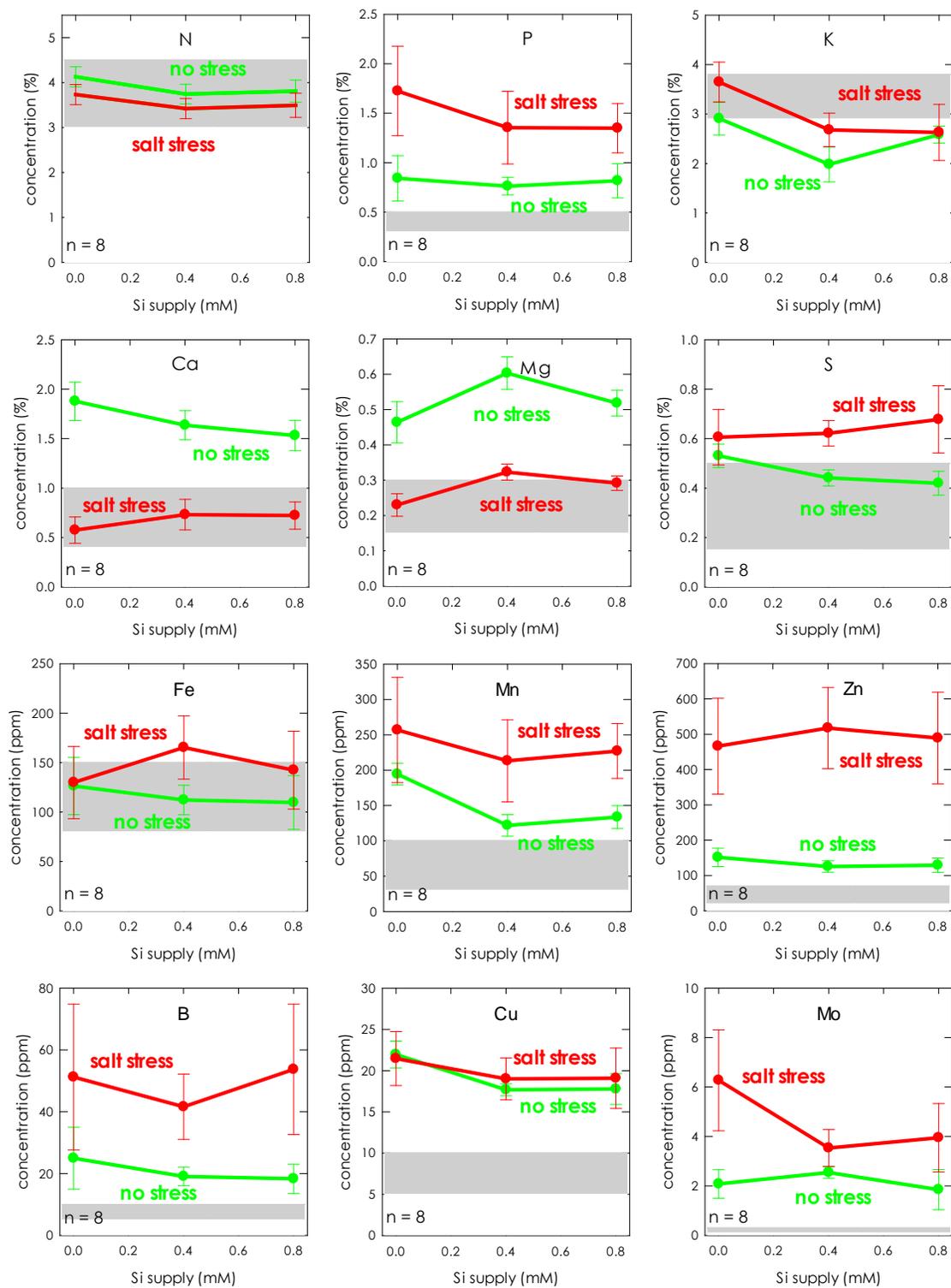


Figure 4 – 6. Corn nutrient concentration in the oldest leaves of plants from both hydroponic trials. Shaded areas indicate optimal nutrient range, based on Marschner (2012).

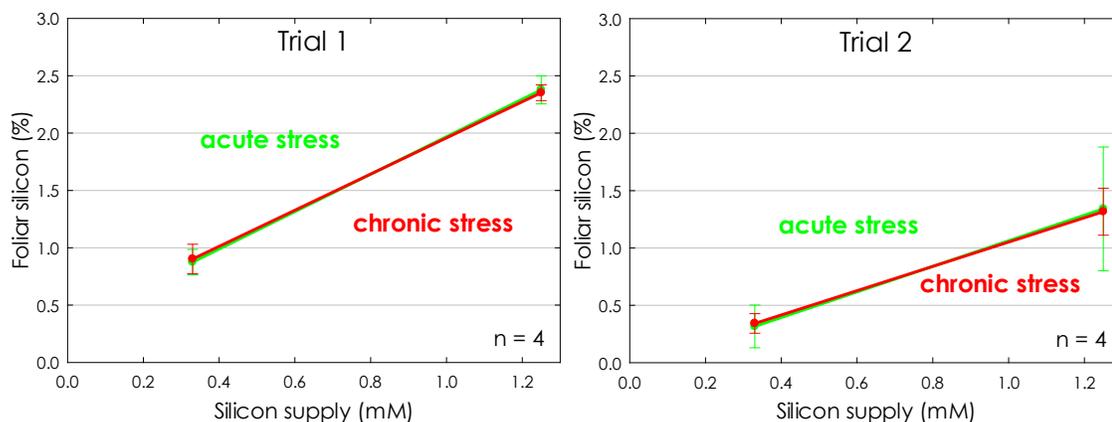


Figure 4 – 7. Silicon concentration in older leaves of corn grown in Trial 1 and Trial 2. Silicon supply estimates are from supplemental studies of Dolomite and PlantTuff in peat. Error bars represent standard deviation from the mean.



Figure 4 – 8. Corn in Trial 1 at 43 days after planting. Drought-stressed plants are on the left. Well-watered plants are on the right. Note the leaf rolling of drought-stressed plants.

PlantTuff significantly increased dry mass ( $p,0.05$ ) in Trial 1 in plants subjected to chronic drought stress (Figure 4 – 9; Table 4 – 6; Table 4 – 7). There was no significant difference in dry mass in Trial 2 (Figure 4 – 10), according to a t-test ( $p =$

0.97) or a two-factor ANOVA ( $p = 0.972$ ). In Trial 1 and Trial 2, the percent dry mass increased with plant age. The treatment effects on percent dry mass varied, but plants supplied with PlantTuff and subjected to acute stress tended to have a slightly greater relative water content (Figure 4 – 11 and Figure 4 – 12). The transpiration varied between Trial 1 and Trial 2 (Figure 4 – 13 and Figure 4 – 15). There was an inconsistent effect on water use efficiency (WUE) (g aboveground dry mass per kg water transpired) (Figure 4 – 17). Mass data are shown to provide graphic detail of stress treatments and water use efficiency calculations (Figure 4 – 14 and Figure 4 – 16).

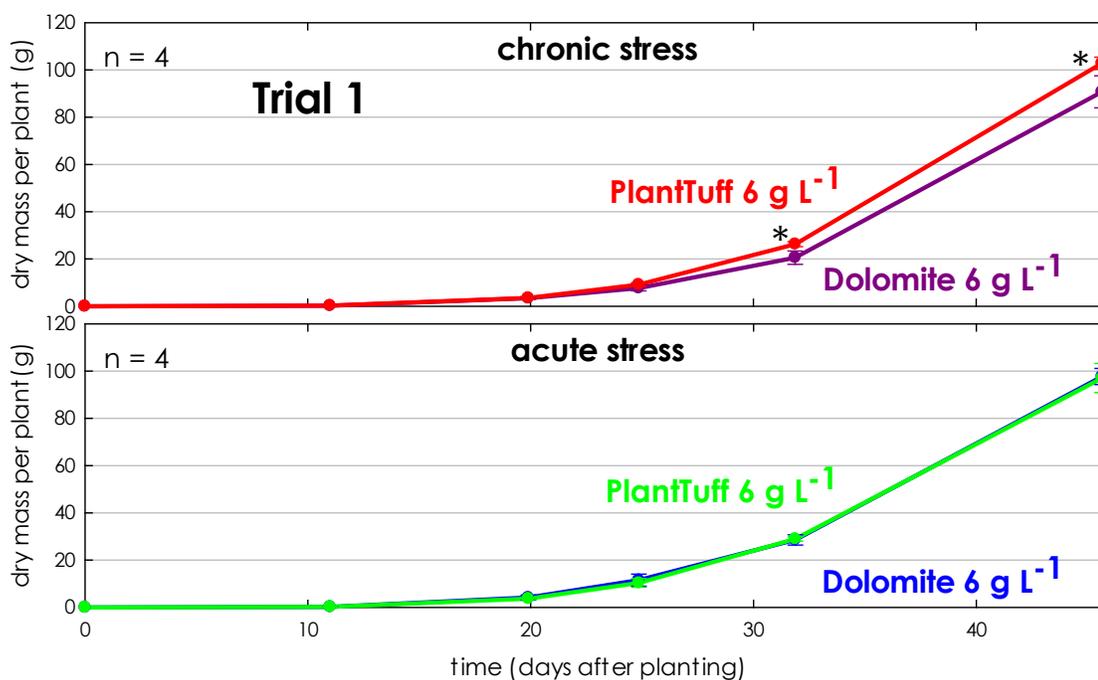


Figure 4 – 9. Corn dry mass per plant from 5 harvests in Trial 1. Asterisks indicate significant difference between mean ( $p < 0.05$ ), according to a two-tailed t-test.

Table 4 – 6. ANOVA table from Trial 1 dry mass data at 4<sup>th</sup> harvest.

Source of Variation	DF	SS	MS	F	P
Amendment	1	37.119	37.119	10.132	0.008
Stress	1	114.651	114.651	31.295	<0.001
Amendment x Stress	1	28.436	28.436	7.762	0.016
Residual	12	43.963	3.664		
Total	15	224.167	14.944		

Table 4 – 7. ANOVA table from Trial 1 dry mass data at 5<sup>th</sup> (final) harvest.

Source of Variation	DF	SS	MS	F	P
Amendment	1	131.676	131.676	5.158	0.042
Stress	1	1.381	1.381	0.0541	0.820
Amendment x Stress	1	161.417	161.417	6.323	0.027
Residual	12	306.325	25.527		
Total	15	600.798	40.053		

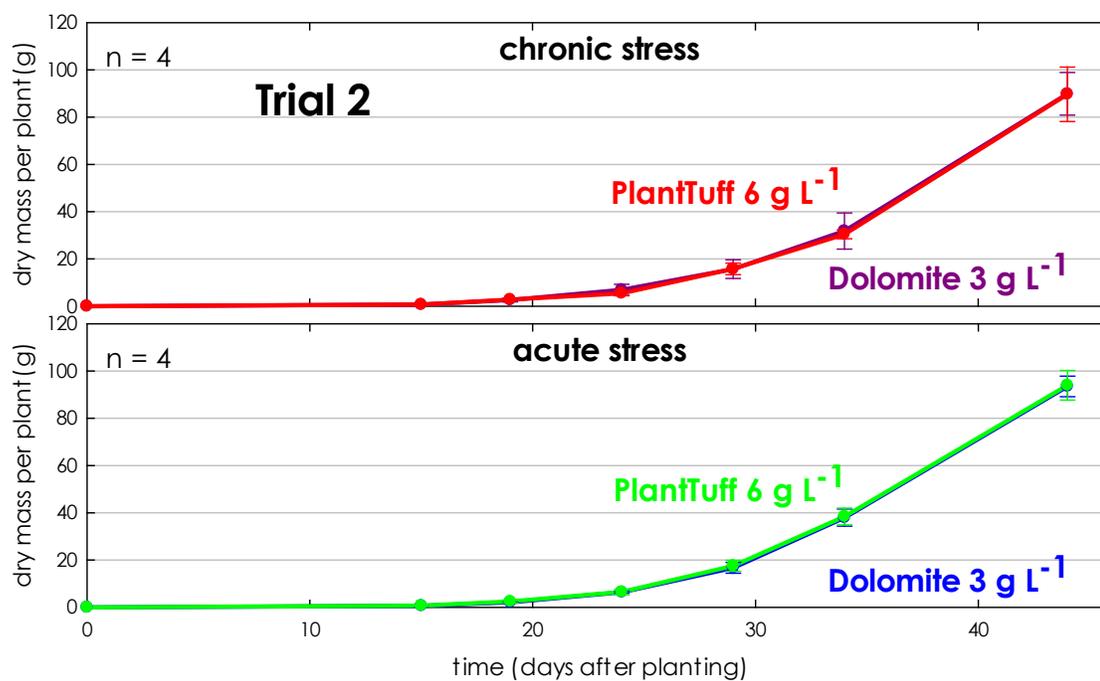


Figure 4 – 10. Corn dry mass per plant from 6 harvests in Trial 2.

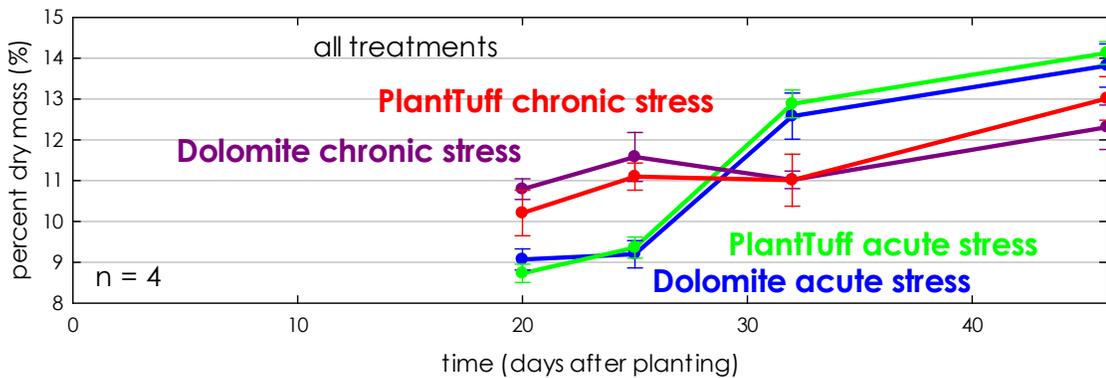


Figure 4 – 11. Percent dry mass from 6 harvests in Trial 1.

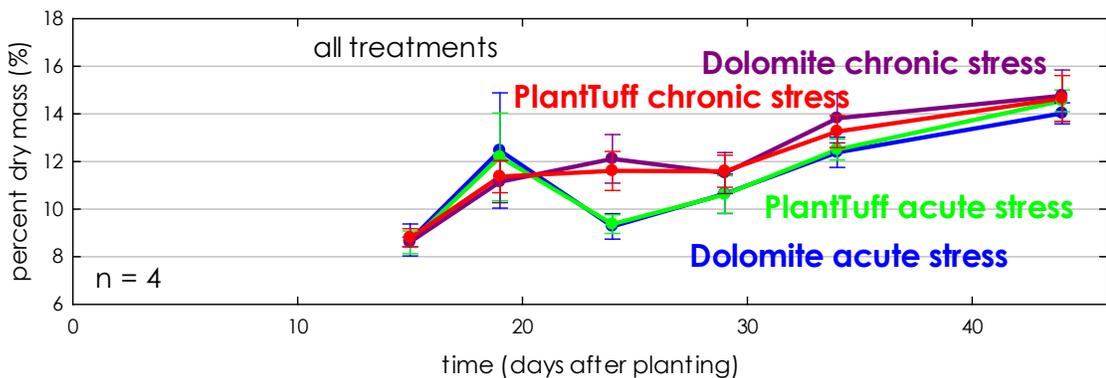


Figure 4 – 12. Percent dry mass from 6 harvests in Trial 2.

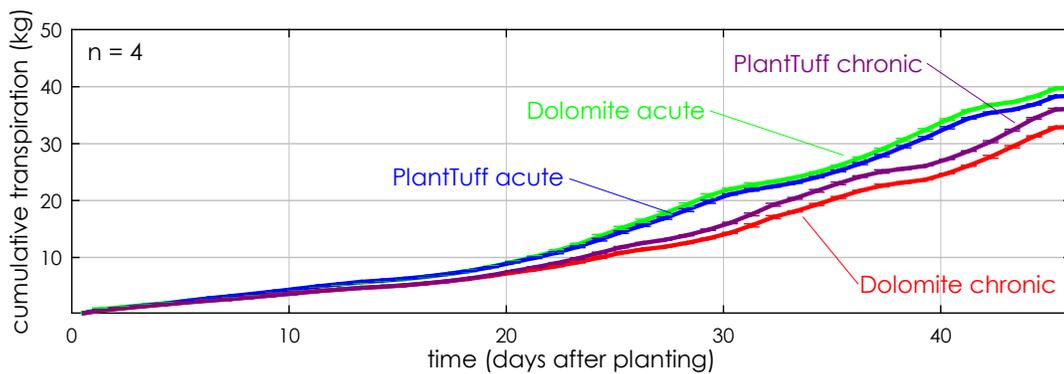


Figure 4 – 13. Cumulative transpiration of 4 replicate containers per treatment in Trial 1.

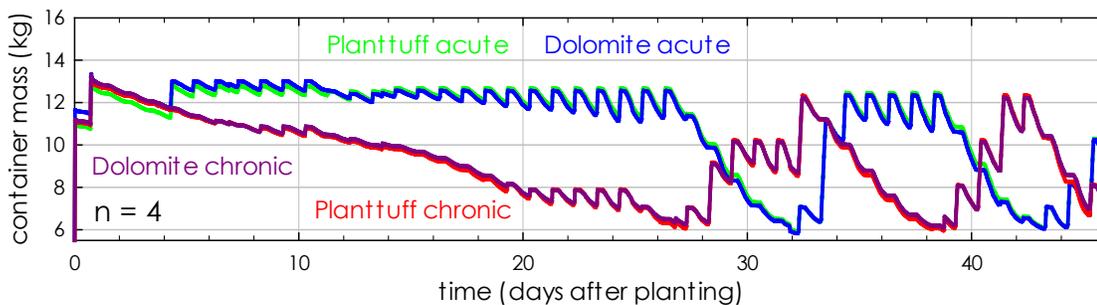


Figure 4 – 14. Mass of 4 replicate containers per treatment in Trial 1.

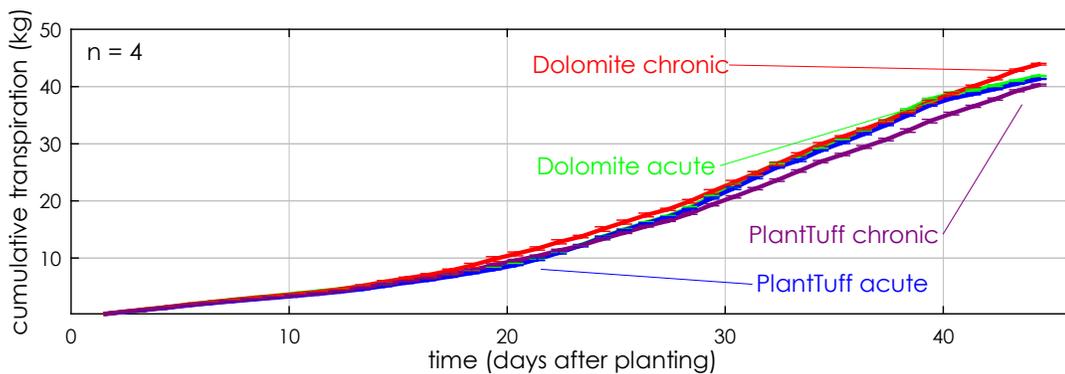


Figure 4 – 15. Cumulative transpiration of 4 replicate containers per treatment in Trial 2.

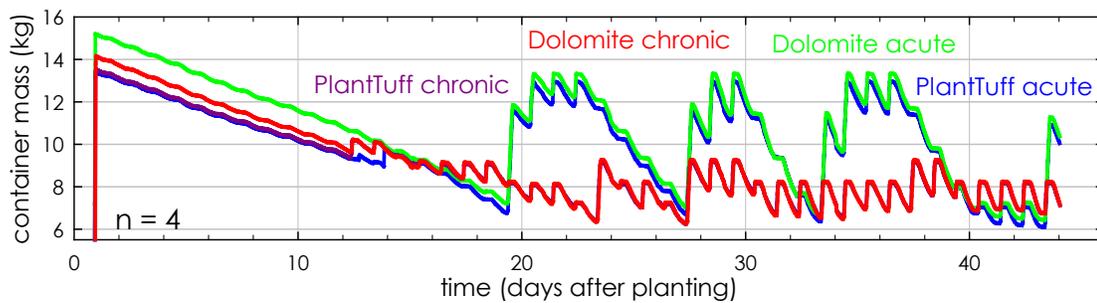


Figure 4 – 16. Mass of 4 replicate containers per treatment in Trial 2.

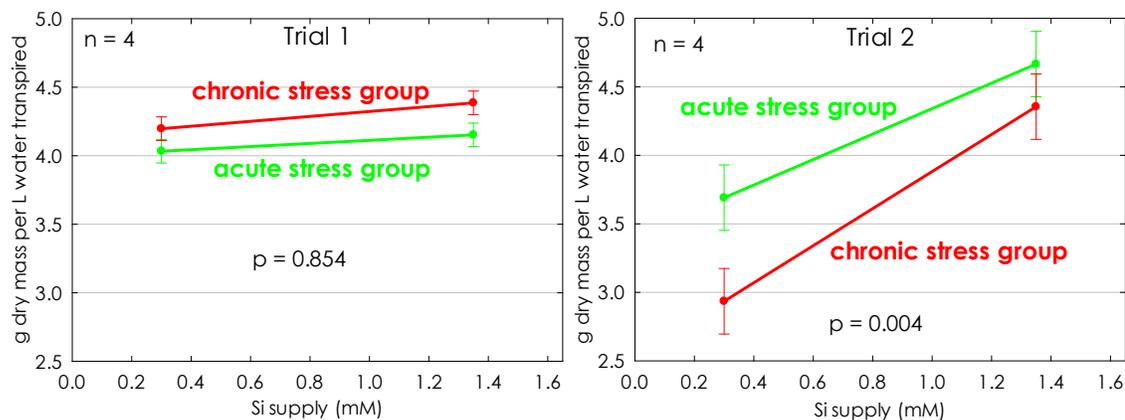


Figure 4 – 17. Corn water use efficiency (g aboveground dry mass per kg of water transpired) by treatment consisting of 4 replicate containers in Trial 1 (left) and Trial 2 (right). Error bars represent standard error of least square means for amendment (PlantTuff or Dolomite). P-values are from a 2-factor ANOVA.

Table 4 – 8. ANOVA table from Trial 1 water use efficiency data.

Source of Variation	DF	SS	MS	F	P
Stress group	1	0.00207	0.00207	0.0352	0.854
Amendment	1	0.00207	0.00207	0.0352	0.854
Stress x Amendment	1	0.0805	0.0805	1.367	0.265
Residual	12	0.706	0.0589		
Total	15	0.791	0.0527		

Table 4 – 9. ANOVA table from Trial 2 water use efficiency data.

Source of Variation	DF	SS	MS	F	P
Stress group	1	1.137	1.137	2.482	0.141
Amendment	1	5.738	5.738	12.522	0.004
Stress x Amendment	1	0.198	0.198	0.433	0.523
Residual	12	5.499	0.458		
Total	15	12.572	0.838		

All containers were leached after final harvest to determine  $\text{Si}(\text{OH})_4$  concentration available to plants during the Trials. Leachate results confirm that previous studies were an accurate indicator of silicon supply and solution pH (Figure 4 – 18).

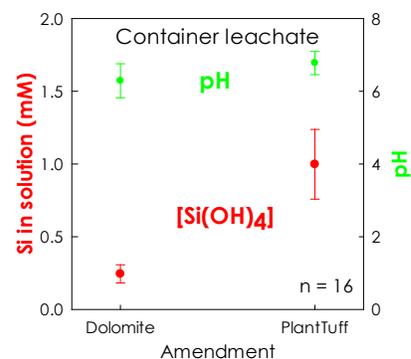


Figure 4 – 18. Silicon concentration and solution pH in leachate from post-harvest containers.

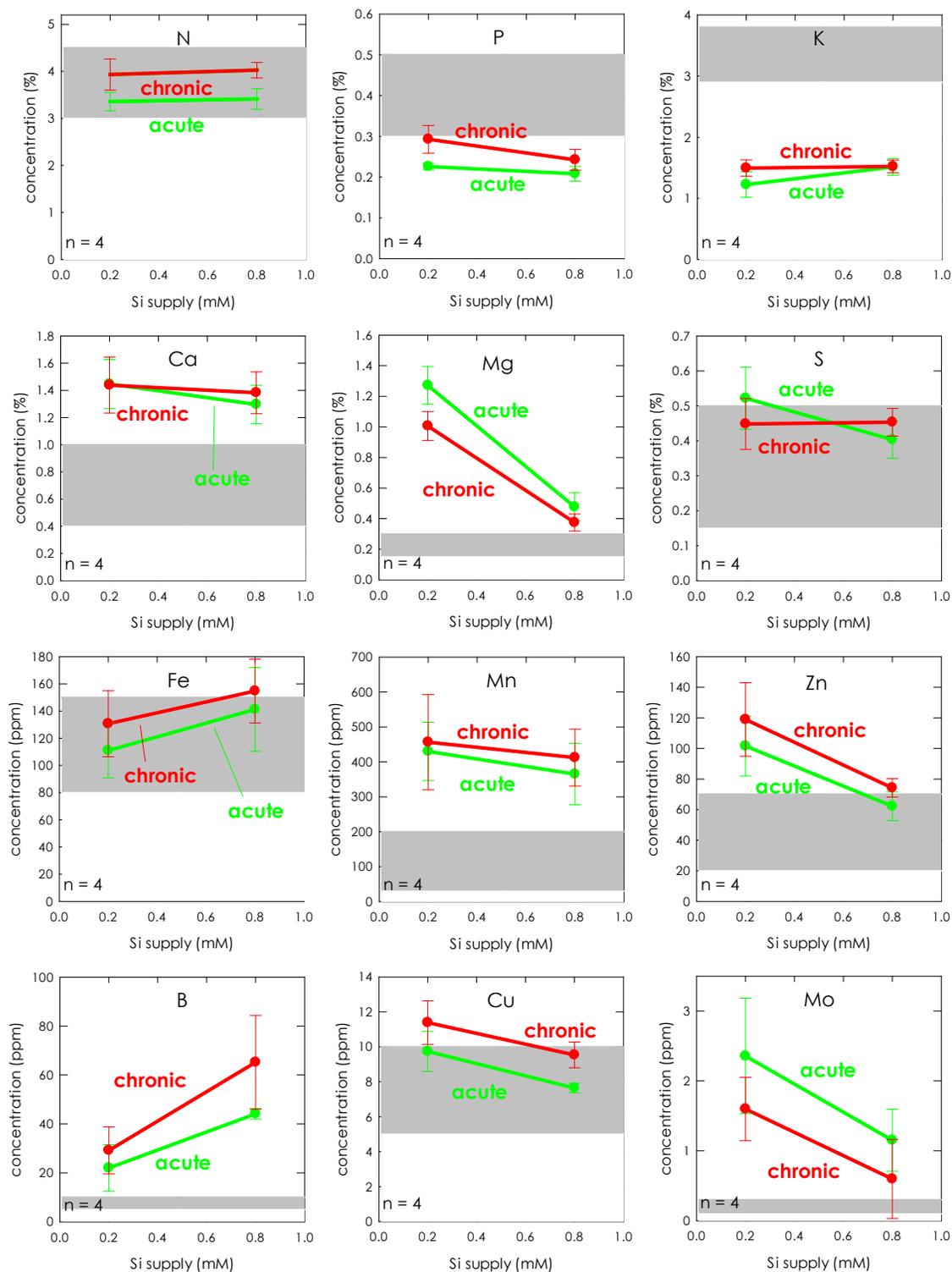


Figure 4 – 19. Nutrient concentration in the oldest leaves of corn harvested at 46 days after planting in Trial 1. Shaded areas indicate optimal nutrient range, based on Marschner (2012). Data are from weighing lysimeter studies.

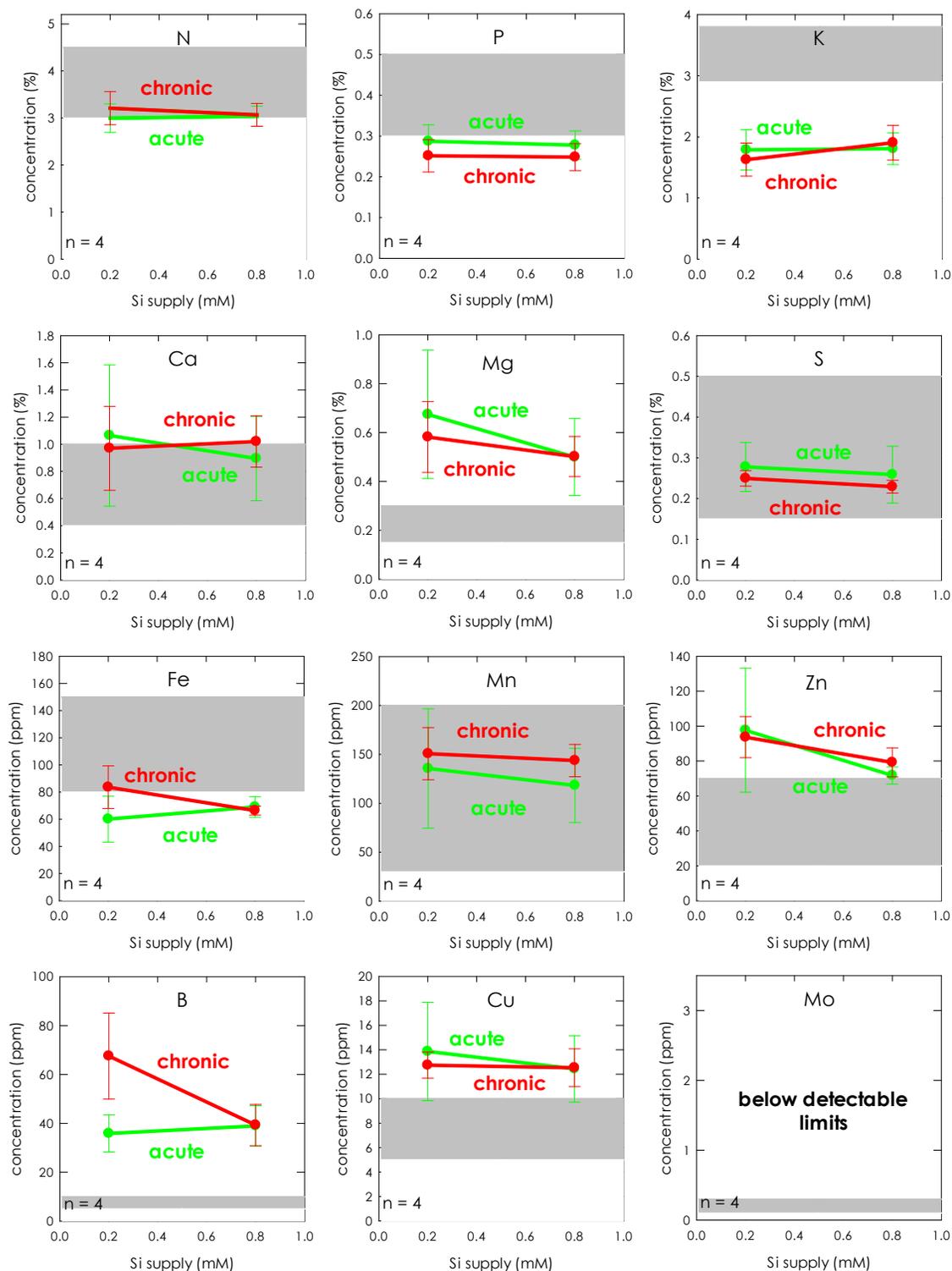


Figure 4 – 20. Nutrient concentration in the oldest leaves of corn harvested at 44 days after planting in Trial 2. Shaded areas indicate optimal nutrient range, based on Marschner (2012). Data are from weighing lysimeter studies.

The essential nutrient content of all plants did not differ by treatment or Trial (Figure 4 – 19 and Figure 4 – 20).

## 2-L container technique

Increasing the silicon from 0.2 to 0.8 mM increased tissue silicon concentration (Figure 4 – 21). Older leaves accumulated more silicon than newer leaves and leaves accumulated more silicon than seed heads in wheat. Rice accumulated more silicon than all other species. Soybean accumulated the least silicon.

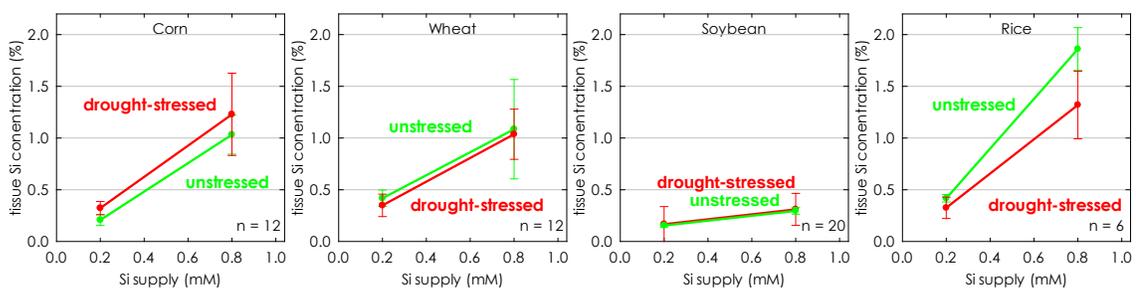


Figure 4 – 21. Silicon concentration in plant tissues. Corn, wheat, and soybean samples were from unstressed and stressed plants in two trials. Rice samples were from Trial 2.

Corn dry mass increased with the addition of silicon, independent of stress (Table 4 – 10). In plants not subjected to drought stress, silicon increased dry mass in Trial 1, but not in Trial 2 (Figure 4 – 22). In plants subjected to drought stress, silicon significantly increased ( $p < 0.05$ ) dry mass (Table 4 – 11). At times during each trial, a visible difference between plants supplied with 0.8 mM and plants supplied with 0.2 mM silicon was detectable (Figure 4 – 23), although this phenomenon was not consistent.

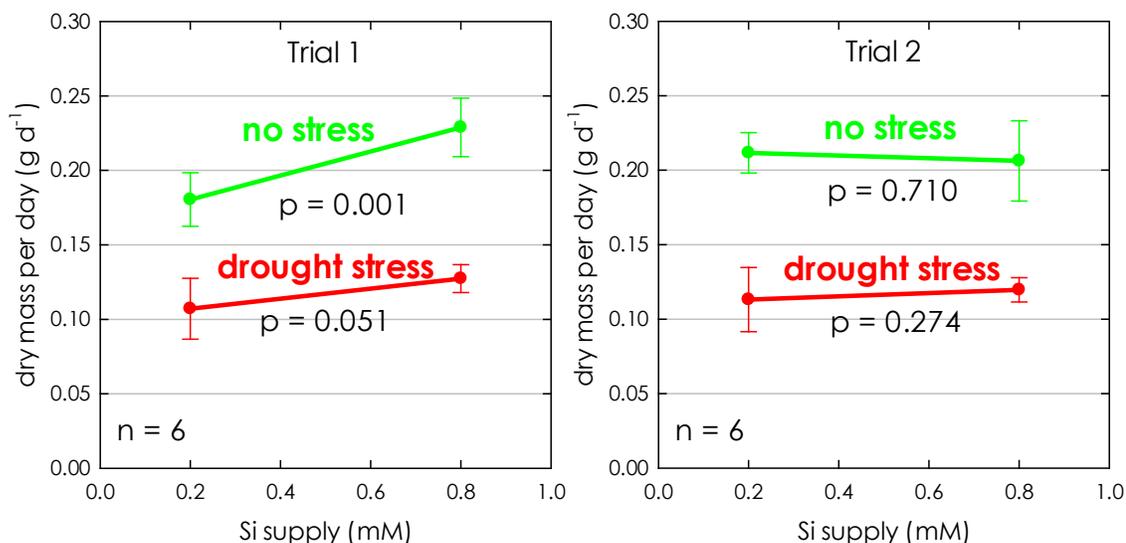


Figure 4 – 22. Aboveground corn dry mass from two trials in peat. Unstressed plants in Trial 1 were harvested at 26 DAP. Drought-stressed plants were harvested at 30 DAP. Unstressed plants in Trial 2 were harvested at 23 DAP. Drought-stressed plants were harvested at 32 DAP. P-values reported are from a 2-tailed t-test. Trial 2 data were normalized to account for side-lighting differences.

Table 4 – 10. 3-factor ANOVA table from corn dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.0000364	0.0000364	0.112	0.740
Si level	1	0.00365	0.00365	11.219	0.002
Stress	1	0.0971	0.0971	298.293	<0.001
Trial x Si level	1	0.00342	0.00342	10.513	0.002
Trial x Stress	1	0.0000792	0.0000792	0.243	0.625
Si level x Stress	1	0.000196	0.000196	0.602	0.442
Trial x Si level x Stress	1	0.00120	0.00120	3.698	0.062
Residual	40	0.0130	0.000326		
Total	47	0.119	0.00253		

Table 4 – 11. 2-factor ANOVA table from drought-stressed corn dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.00000411	0.00000411	0.0237	0.879
Si level	1	0.00108	0.00108	6.206	0.022
Trial x Si level	1	0.000283	0.000283	1.632	0.216
Residual	20	0.00347	0.000174		
Total	23	0.00484	0.000210		



Figure 4 – 23. Drought-stressed corn plants supplied with 0.2 and 0.8 mM silicon, at 23 days after planting during Trial 1.

Drought stress decreased visible turgor and stature of corn throughout both trials. Silicon visibly mitigated this decrease at times during both trials, as evidenced by the greater stature and turgor of plants supplied with silicon and subjected to drought stress (Figure 4 – 23). Increased silicon supply had variable effects on the concentration of other nutrients (Figure 4 – 24). While not statistically significant, silicon appears to have increased the foliar P, K, Fe, and Mo concentration. Silicon also appears to have decreased the concentration of Mg. Drought stress had variable effects on nutrient concentrations. A silicon-mediated decrease in Mn concentration was not observed in the two trials with corn grown in 2-L containers for up to 32 days. All nutrients were within or near the optimal range.

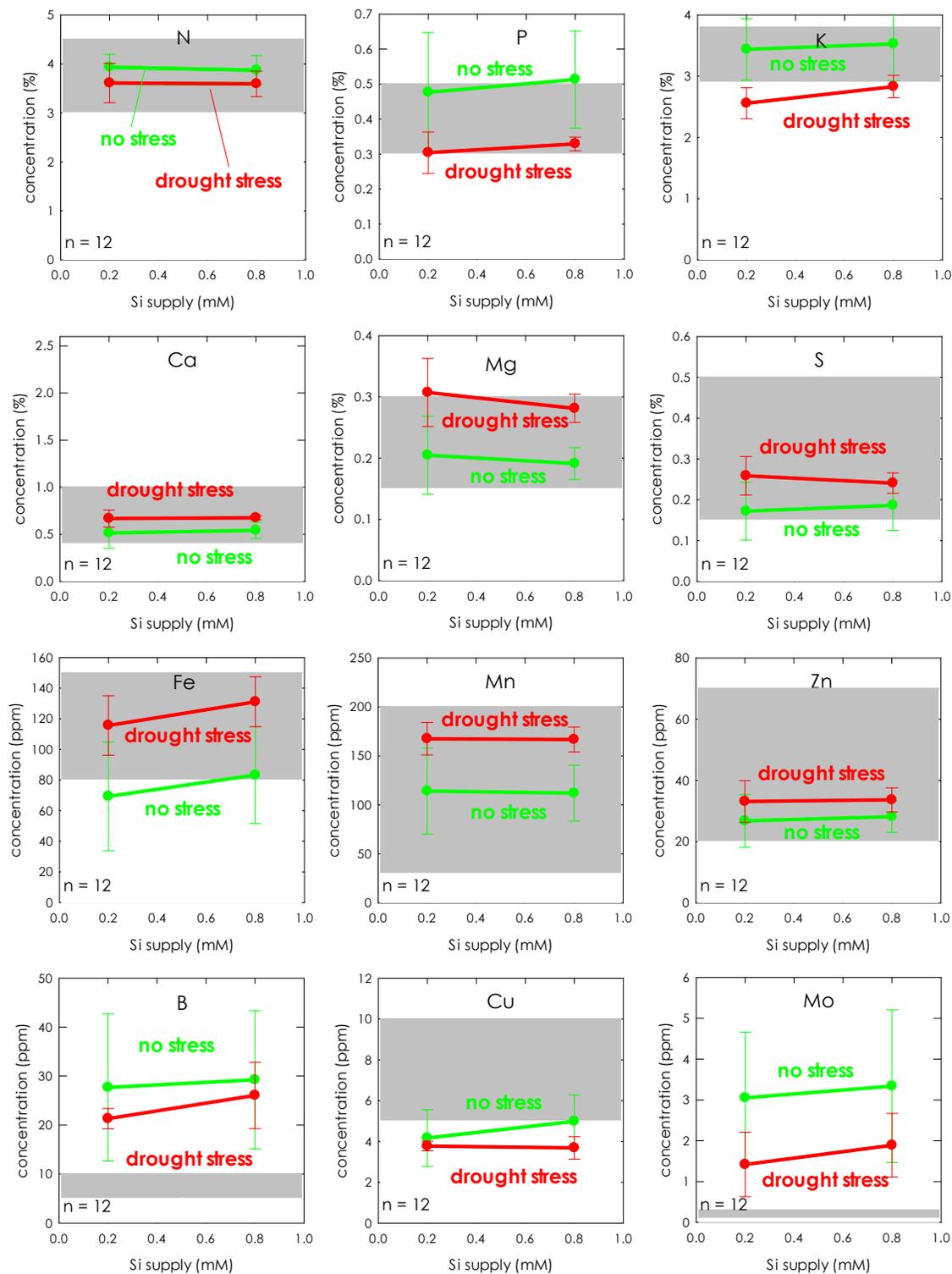


Figure 4 – 24. Nutrient concentration in the oldest fully-expanded leaves of corn plants in Trial 1 and Trial 2. Shaded areas indicate optimal nutrient range, based on Marschner (2012).

Wheat dry mass increased with the addition of 0.8 mM silicon to the growing medium (Figure 4 – 25), independent of the presence or absence of drought stress. Drought stress decreased wheat dry mass independent of silicon supply. The silicon-mediated increase in dry mass was statistically significant when both trials are taken into consideration (Table 4 – 12; and Table 4 – 13). The difference in drought-stressed dry mass between the two trials is likely due to an increase in light intensity and temperature during Trial 2. At times during each trial, a visible difference between plants supplied with 0.8 mM and plants supplied with 0.2 mM silicon was detectable (Figure 4 – 26), although this phenomenon was not perfectly consistent.

The mean difference in seed head dry mass was not detectable in either of the two trials with wheat. Increased silicon supply generally decreased the foliar concentration of other nutrients slightly (Figure 4 – 27).

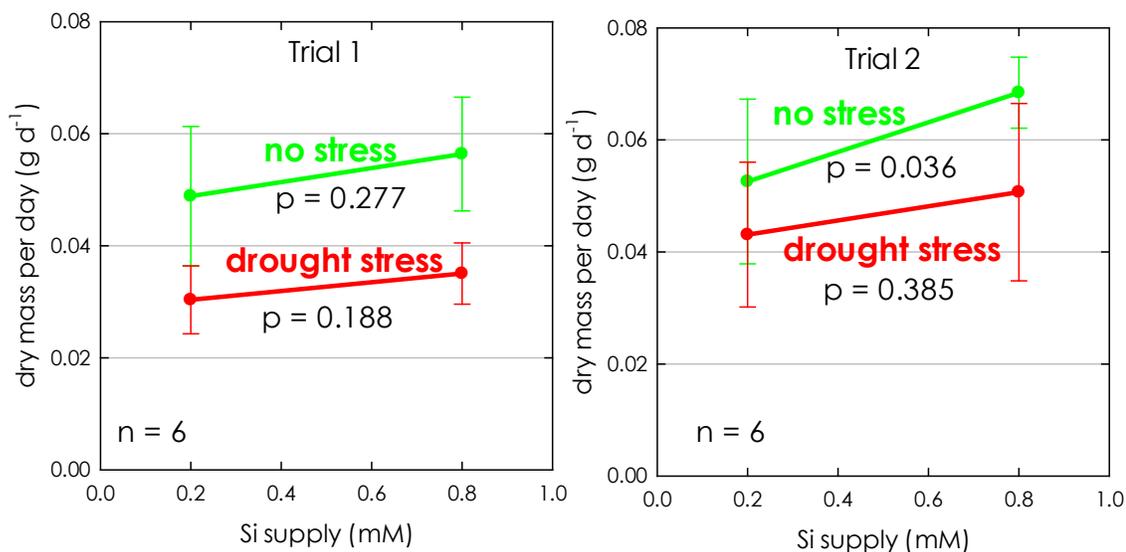


Figure 4 – 25. Aboveground wheat dry mass from two trials in peat. Unstressed plants in Trial 1 were harvested at 38 DAP. Drought-stressed plants were harvested at 54 DAP. Unstressed plants in Trial 2 were harvested at 31 DAP. Drought-stressed plants were harvested at 49 DAP. P-values reported are from a two-tailed t-test.

Table 4 – 12. 3-factor ANOVA table from wheat dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.499	0.499	2.199	0.146
Si level	1	1.477	1.477	6.511	0.015
Stress	1	0.106	0.106	0.466	0.499
Trial x Si level	1	0.0784	0.0784	0.346	0.560
Trial x Stress	1	1.289	1.289	5.683	0.022
Si level x Stress	1	0.0174	0.0174	0.0766	0.783
Trial x Si level x Stress	1	0.00578	0.00578	0.0255	0.874
Residual	40	9.075	0.227		
Total	47	12.547	0.267		

Table 4 – 13. 2-factor ANOVA table from drought-stressed wheat data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.000254	0.000254	2.395	0.137
Si level	1	0.000218	0.000218	2.057	0.167
Trial x Si level	1	0.00000434	0.00000434	0.0409	0.842
Residual	20	0.00212	0.000106		
Total	23	0.00260	0.000113		

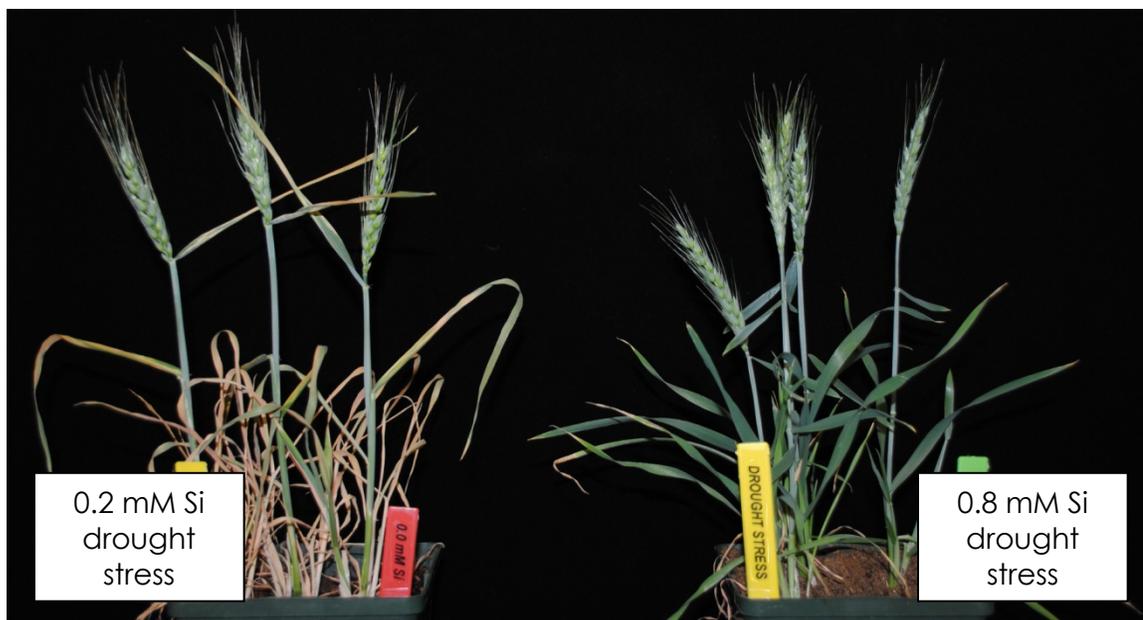


Figure 4 – 26. Drought-stressed wheat plants supplied with 0.2 and 0.8 mM silicon, at 48 days after planting during Trial 1.

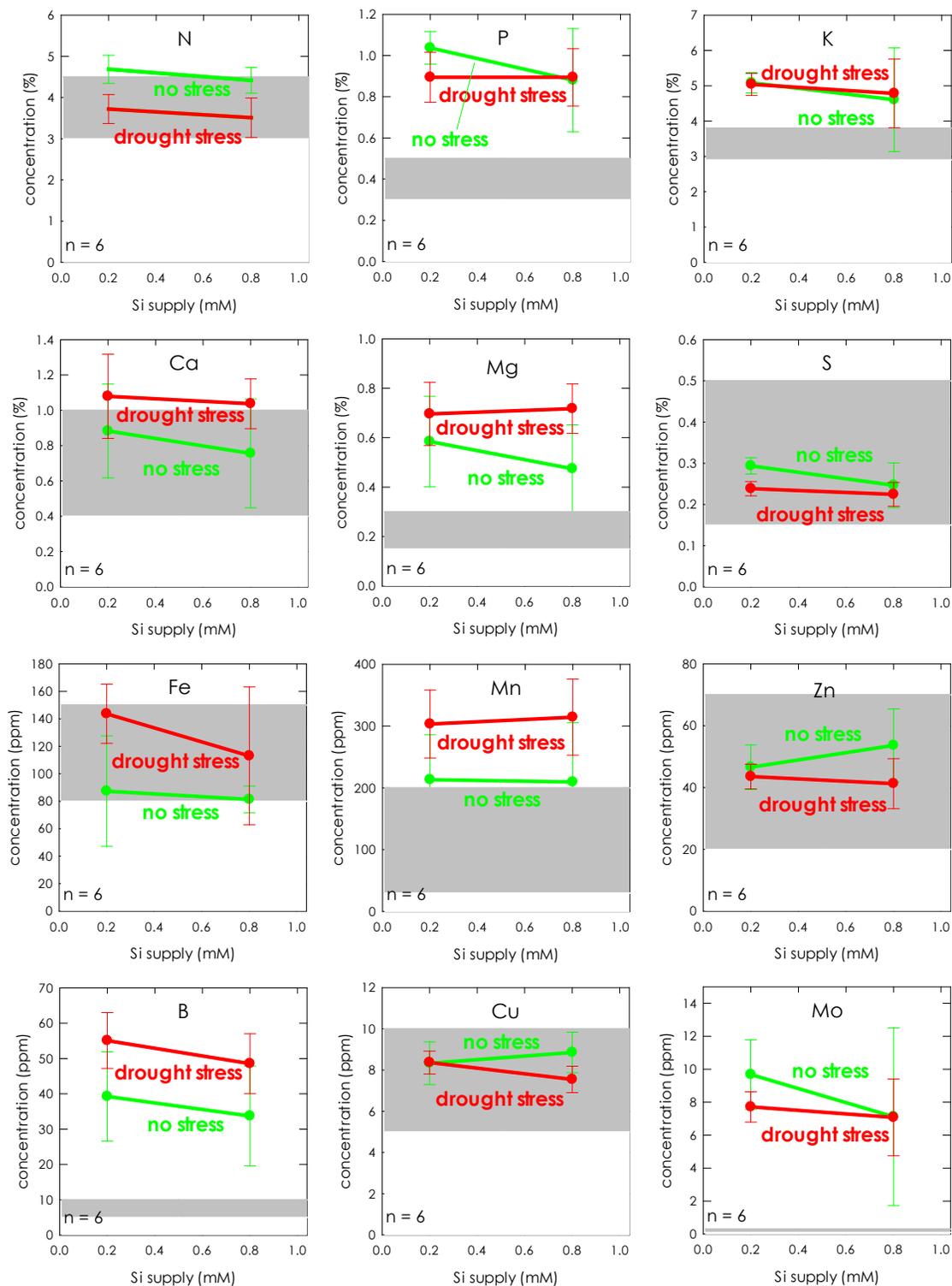


Figure 4 – 27. Nutrient concentration in the oldest fully-expanded leaves of wheat plants in Trial 1. Shaded areas indicate optimal nutrient range, based on Marschner (2012).

Soybean dry mass did not significantly ( $p < 0.05$ ) increase with the addition of silicon to the growing medium, when subjected to drought stress (Figure 4 – 28). Silicon effect on dry mass was not statistically significant when considering all data (Table 4 – 14) or drought-stressed plant data alone (Table 4 – 15). Soybean dry mass significantly decreased with the addition of silicon when plants were not subjected to drought stress in Trial 2 ( $p = 0.005$ ). Drought stress decreased dry mass independent of silicon supply. At times during each trial, a visible difference between plants supplied with 0.8 mM and plants supplied with 0.2 mM silicon was detectable (Figure 4 – 29), although this phenomenon was not perfectly consistent. Increased silicon had a varied effect on the foliar concentration of other nutrients in drought stress and unstressed conditions (Figure 4 – 30).

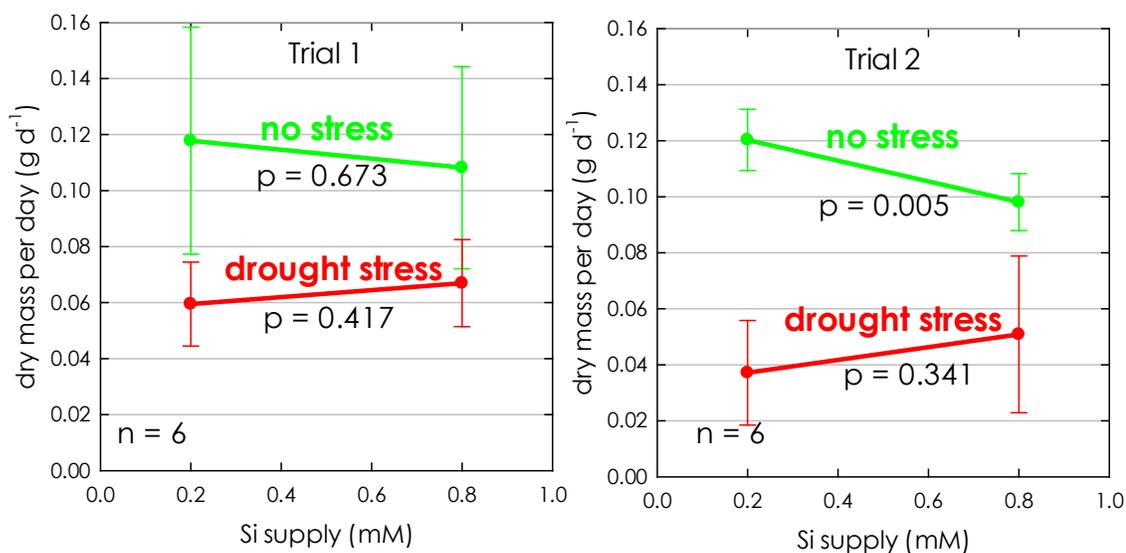


Figure 4 – 28. Aboveground soybean dry mass from two trials in peat. Unstressed plants in trial 1 were harvested at 23 DAP. Drought-stressed plants were harvested at 27 DAP. Unstressed plants in trial 2 were harvested at 25 DAP. Drought-stressed plants were harvested at 52 DAP. P-values reported are from a two-tailed t-test.

Table 4 – 14. 3-factor ANOVA table from soybean dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	1.527	1.527	2.398	0.129
Si level	1	0.0144	0.0144	0.0225	0.881
Stress	1	5.320	5.320	8.358	0.006
Trial x Si level	1	0.0239	0.0239	0.0375	0.847
Trial x Stress	1	0.612	0.612	0.961	0.333
Si level x Stress	1	2.146	2.146	3.372	0.074
Trial x Si level x Stress	1	0.536	0.536	0.841	0.365
Residual	40	25.462	0.637		
Total	47	35.641	0.758		

Table 4 – 15. 2-factor ANOVA Table from drought-stressed soybean dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.00221	0.00221	5.541	0.029
Si level	1	0.000673	0.000673	1.687	0.209
Trial x Si level	1	0.0000586	0.0000586	0.147	0.706
Residual	20	0.00798	0.000399		
Total	23	0.0109	0.000475		

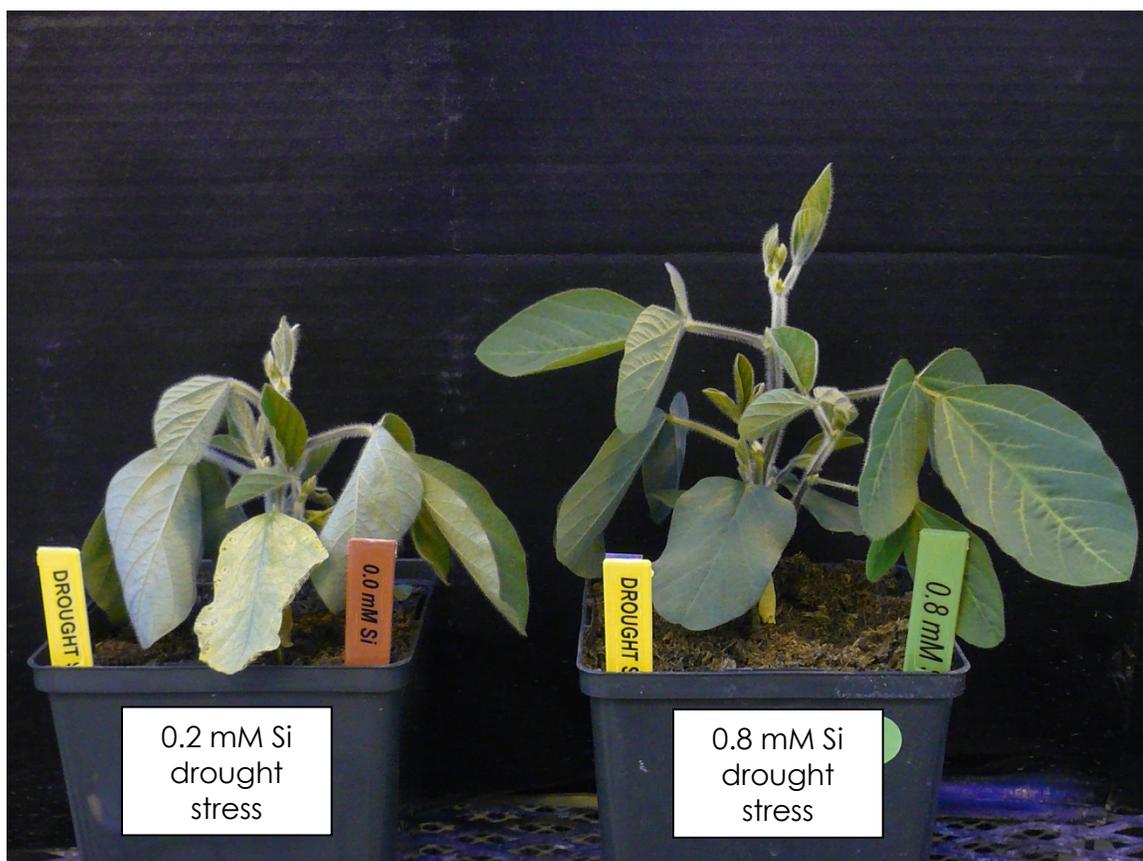


Figure 4 – 29. Drought-stressed soybean plants supplied with 0.2 and 0.8 mM silicon, at 27 days after planting, during Trial 1.

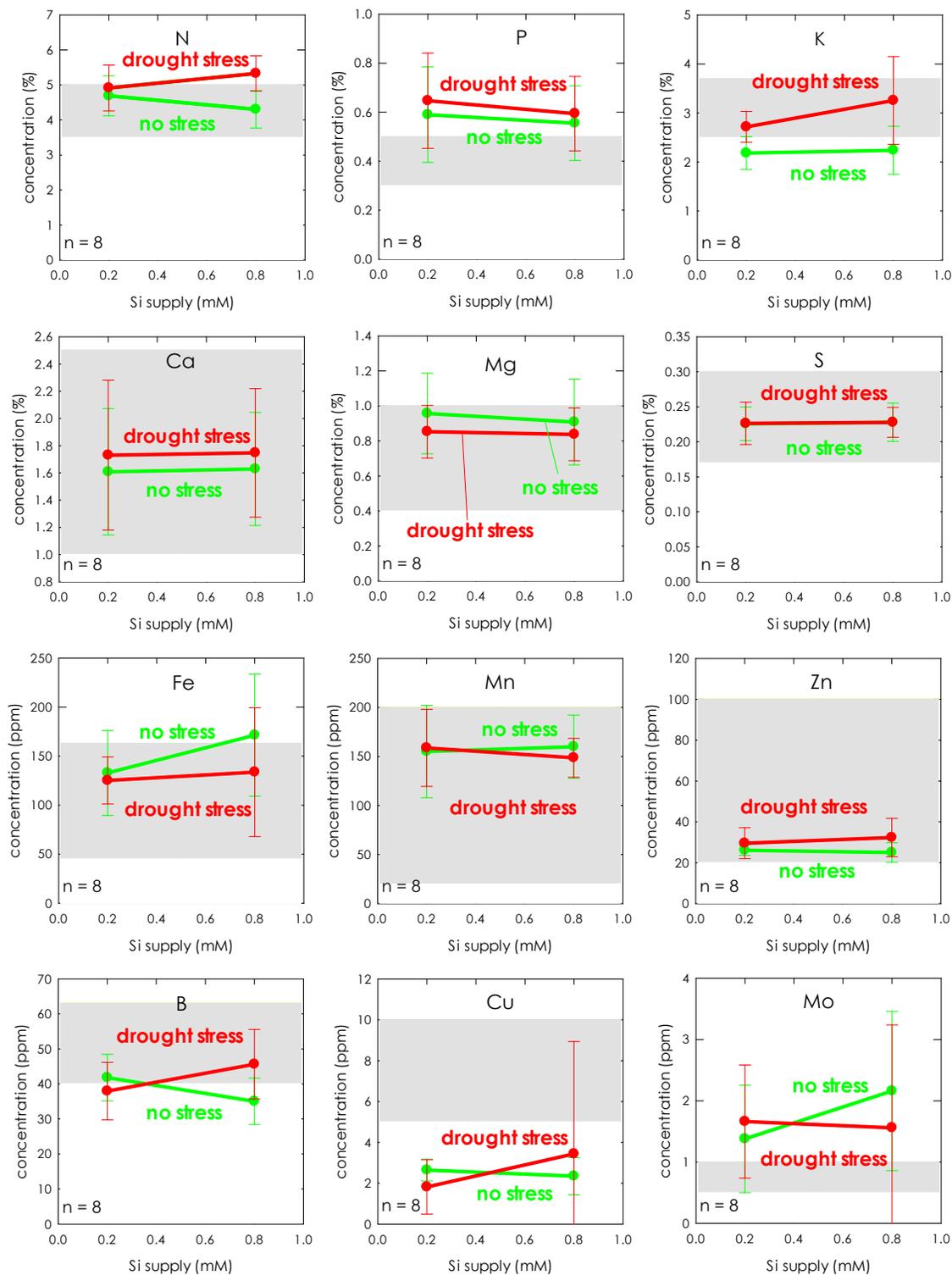


Figure 4 – 30. Nutrient concentration in the oldest fully-expanded leaves of soybean plants in both trials. Shaded areas indicate optimal nutrient range, based on Chapman (1966), Barker & Pilbeam (2007), and Marschner (2012).

Rice dry mass did not significantly ( $p < 0.05$ ) increase (Table 4 – 16) with the addition of silicon to the growing medium (Figure 4 – 31). When subjected to drought stress, silicon significantly increased rice dry mass at the 0.1 level (Table 4 – 17). Rice dry mass was mostly unaffected by the addition of silicon when plants were not subjected to drought stress. There is roughly a 70% chance that the addition of silicon decreased dry mass of unstressed plants in the second trial, based on a two-tailed t-test. At many times during each trial, a visible difference between plants supplied with 0.8 mM and plants supplied with 0.2 mM silicon was detectable (Figure 4 – 32), although this phenomenon was not perfectly consistent.

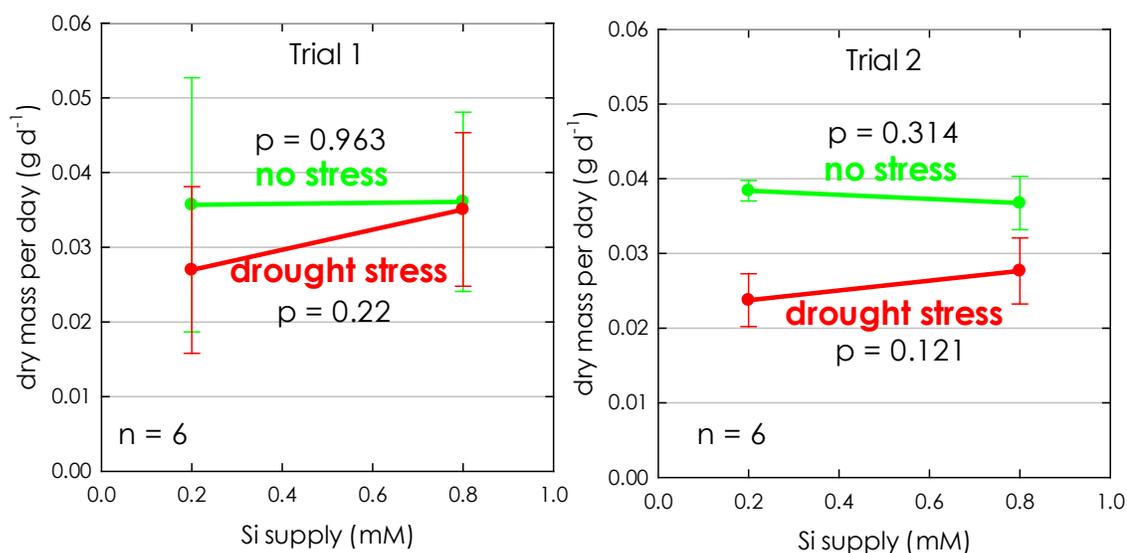


Figure 4 – 31. Aboveground rice dry mass from two trials in peat. Unstressed plants in trial 1 were harvested at 39 DAP. Drought-stressed plants were harvested at 53 DAP. Unstressed plants in trial 2 were harvested at 32 DAP. Drought-stressed plants were harvested at 45 DAP. P-values reported are from a 2-tailed t-test.

Table 4 – 16. 3-factor ANOVA table from rice dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.615	0.615	3.477	0.070
Si level	1	0.273	0.273	1.546	0.221
Stress	1	0.554	0.554	3.134	0.084
Trial x Si level	1	0.0618	0.0618	0.350	0.558
Trial x Stress	1	0.0104	0.0104	0.0590	0.809
Si level x Stress	1	0.345	0.345	1.949	0.170
Trial x Si level x Stress	1	0.0168	0.0168	0.0949	0.760
Residual	40	7.073	0.177		
Total	47	8.949	0.190		

Table 4 – 17. 2-factor ANOVA table from drought-stressed rice dry mass data from both trials.

Source of Variation	DF	SS	MS	F	P
Trial	1	0.000169	0.000169	2.583	0.124
Si level	1	0.000217	0.000217	3.305	0.084
Trial x Si level	1	0.0000264	0.0000264	0.402	0.533
Residual	20	0.00131	0.0000655		
Total	23	0.00172	0.0000749		

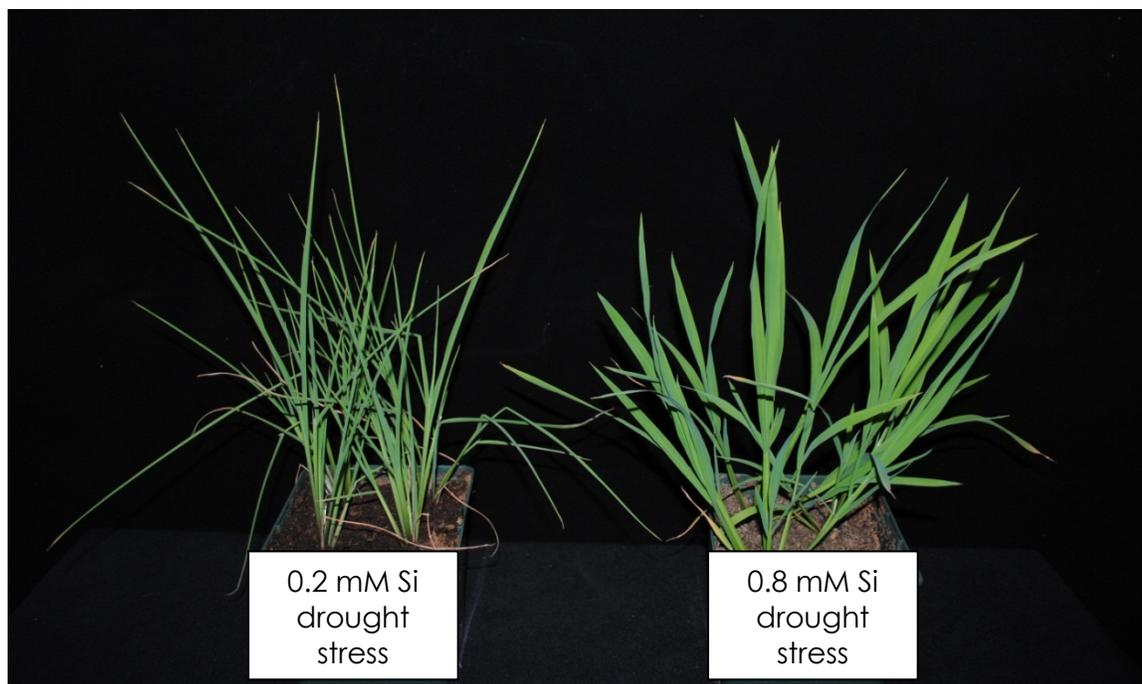


Figure 4 – 32. Drought-stressed rice plants supplied with 0.2 and 0.8 mM silicon, at 27 days after planting during Trial 1.

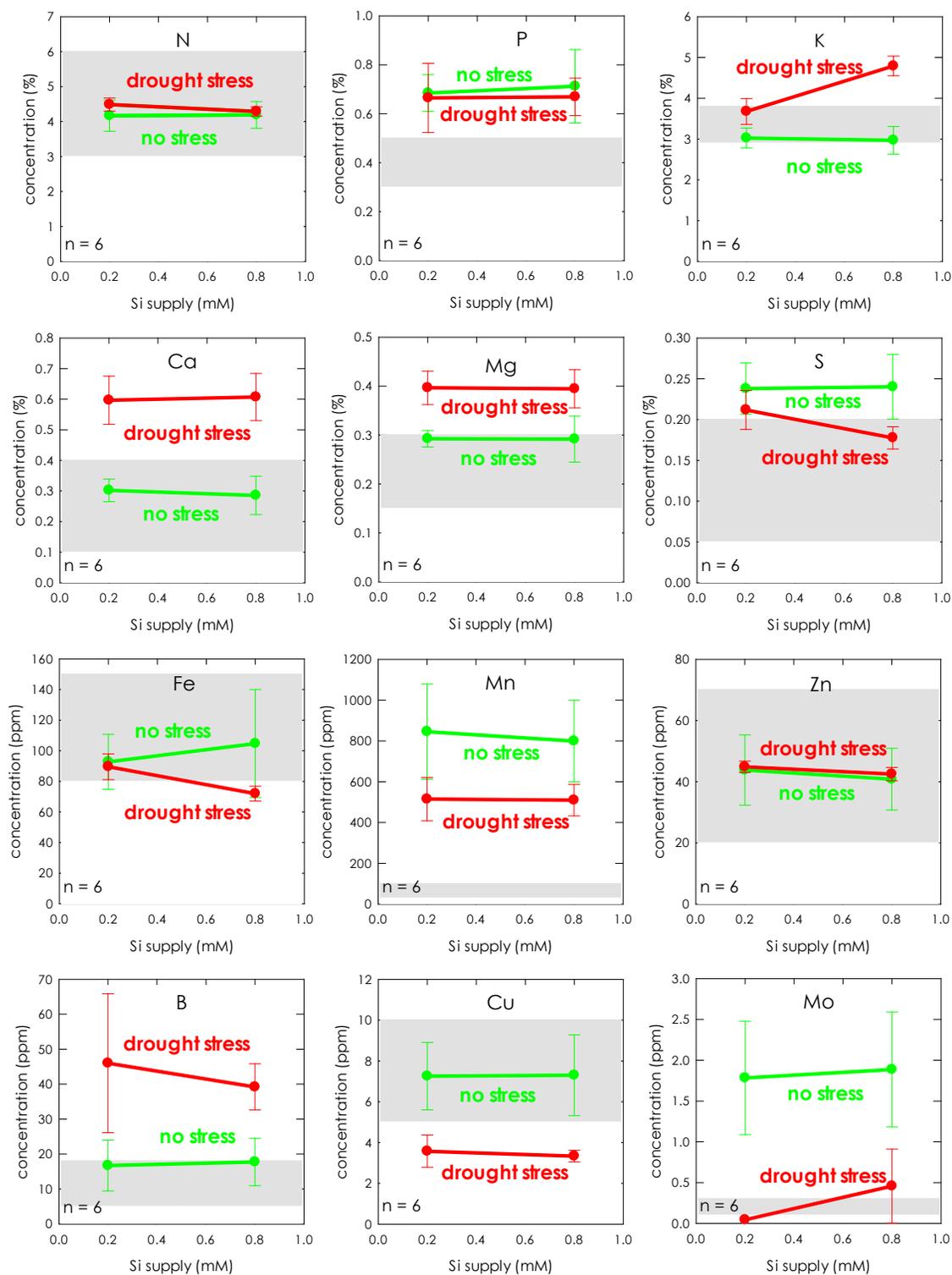


Figure 4 – 33. Nutrient concentration in the fully-expanded leaves of rice plants in both trials. U nstressed plants were harvested at 39 DAP. D rought-stressed plants were harvested at 45 DAP. Shaded areas indicate optimal nutrient range, based on Chapman (1966), Barker & Pilbeam (2007), and Marschner (2012).

Increased silicon had a varied effect on the foliar concentration of other nutrients in drought stress and unstressed conditions (Figure 2 – 37). The effect of drought stress on foliar manganese concentration is peculiar, as >800 ppm Mn approaches toxic levels. The findings here suggest possible evidence for active uptake of manganese by rice roots under unstressed conditions, although the observed phenomenon here must be replicated before any such claims may properly be with any confidence.

## CHAPTER 5

## DISCUSSION AND CONCLUSIONS

## Hydroponic studies

The addition of 0.4 mM silicon significantly increased ( $p = 0.059$ ) corn growth and the effect was similar in both the stressed and unstressed treatments. Silicon decreased percent dry mass in salt-stressed plants, but the effect was not significant. Silicon decreased root percent mass in unstressed plants; the decrease was not statistically significant. While water use efficiency was only affected in unstressed plants supplied with 0.4 mM Si, this may provide some evidence for a beneficial silicon effect in hydroponic corn.

Salt-stressed plants accumulated more silicon in leaf and root tissue. This may be due to a reaction between NaCl and Na<sub>2</sub>SiO<sub>3</sub> in the culture solution, or a charge balance reaction between Na and Si in plant tissues. The accumulation of sodium in plant tissues may also have produced an osmotic potential change between the culture solution and the plant symplast, increasing the uptake of some nutrients.

The effect of silicon and salt stress on the foliar nutrient concentrations was statistically significant in some cases, but the effect may not be biologically important. The foliar nutrient analysis provides evidence that the plants in this study were well-nourished. The effects of silicon were not likely due to nutrient deficiency or toxicity.

There appeared to be a silicon-induced reduction in tissue nitrogen concentration. In this study, nitrate (supplied as Ca(NO<sub>3</sub>)<sub>2</sub> and K(NO<sub>3</sub>)) was the sole nitrogen source. Silicon may have polymerized at root surfaces, reducing the uptake of NO<sub>3</sub><sup>-</sup>. There also

appeared to be some effect of silicon on tissue iron concentration. This may be caused by the formation and precipitation of silicon-iron complexes in the hydroponic solution (Grossl P., personal communication).

#### 20-liter container studies on weighing lysimeters

In Trial 1, containers were amended with  $6 \text{ g L}^{-1}$  Dolomite; in Trial 2, containers were amended with  $3 \text{ g L}^{-1}$ , but it is unlikely that the difference in dry mass between Trial 1 and Trial 2 was due to the difference in amendment application rate. The difference in duration and frequency of the stress is the most probable cause of the dry mass difference between Trial 1 and Trial 2. In Trial 1, transpiration decreased rapidly in response to the withholding of water. In Trial 2, transpiration did not decrease rapidly to either type of stress. This is not likely due to a dolomite concentration difference. The prolonged chronic stress applied in Trial 2 may have been sufficiently severe as to damage plants supplied with both amendments. Furthermore, being subjected to drought stress earlier and to a greater degree may have precluded the uptake of soluble silicon from the growing medium solution. In other words, plants may have received less silicon in Trial 2 because of being supplied with less water; the water is expected to have contained available silicon.

Foliar silicon concentration data suggest that more silicon may have been available to plants in Trial 1. Assuming no analytical error, leaf tissue concentrations indicate that the silicon treatment in Trial 2 received approximately the same amount of silicon as the control group in Trial 1. This may explain the greater stress tolerance

response of plants in Trial 1, in conjunction with the possibility that plants in Trial 2 also received less water, being subjected to drought stress earlier.

Despite variable dry mass results, the water use efficiency results are promising. In the second Trial, silicon supply increased water use efficiency by approximately 13% g dry mass per kg of water transpired. This is about 7 times greater than the increase in Trial 1. There may be something about the type and duration of stress applied in Trial 2 that revealed more clearly the effect of silicon on water use efficiency. The silicon supply in Trial 2 may also have been closer to the optimum for greatest water use efficiency. The mean relative humidity during Trial 2 was 31%. The mean relative humidity during Trial 1 was 29%. It is not likely that the additional water vapor in the greenhouse during Trial 2 could account for such a great water use efficiency difference, especially since the overall water use efficiency was not greater in Trial 2.

Results from dry mass and water use efficiency measurements of corn grown in containers of peat and supplied with PlantTuff or Dolomite indicate that PlantTuff may improve the drought stress tolerance of corn. This improvement is most likely attributed to PlantTuff's release of silicon and/or calcium, as evidenced by the results of foliar nutrient analysis. Additional study of the effects of PlantTuff it is recommended, including experimentation with drought stress frequency, duration, and severity.

#### Two-liter container studies

The difference in dry mass between the two trials with corn and wheat is likely due to the difference in growing times and conditions. During the second trial, air temperatures and light intensity were slightly greater. Although there appears to be a

consistent trend in silicon effect in plants subjected to drought stress, the effect on dry mass is slight. There also appears to be a slight beneficial dry mass effect in unstressed plants. More noteworthy is the visible effect in wilting sometimes observed during the two trials. The results of additional studies will likely remove error and produce better evidence for the quantification of a silicon effect. In corn Trial 2, the plants subjected to drought stress were spaced at a greater distance from one another. This spacing difference and the resulting increase in light exposure is likely the cause of the dry mass difference compared to unstressed plants that were harvested 9 days earlier.

There appears to be a difference in silicon effect between soybean and rice plants subjected to drought stress and unstressed plants. Results from two trials with soybean and rice indicate that increasing silicon supply to 0.8 mM increases plant dry mass under drought conditions. Results indicate the opposite in unstressed plants. Photographs of drought-stressed plants generally confirm the effect in drought-stressed plants, but there was no obvious visible difference in unstressed plants.

In consideration of the dry mass results from any study, it is important to recall that larger plants have the capacity to transpire a greater volume of water from the growing medium. In these studies, it is possible that plants that accumulated greater mass during a trial also transpired at a greater rate, thus creating a condition of more intense drought stress. This would favor the smaller plants and lead to the interpretation that smaller plants exhibited greater drought stress tolerance. Measurement of transpiration as well as mass could remove this confounding factor.

A review of the nutrient content of leaf samples leads to the conclusion that all plants were generally well-nourished. Any differences in plant biomass were most likely

not due to any essential nutrient deficiency or toxicity. While there is sometimes a statistically significant effect of silicon supply on the concentration of other nutrients, the effect is not great enough to be considered biologically significant. It is expected that a greater interaction between silicon and other nutrients would be observed in studies in which the control group was supplied with 0.0 mM Si rather than 0.2 mM Si. In contrast to the other nutrients, it appears that the accumulation of silicon in plant tissues has a biologically significant effect on the four plant species studied. The greater accumulation of silicon in leaves of rice plants as compared to those of corn, wheat, and soybean suggests the possibility of an active uptake mechanism for silicon in rice roots. On the other hand, the lesser accumulation of silicon in leaves of soybean plants as compared to those of rice, corn, and wheat may indicate a selective uptake of silicon in soybean roots. Replicate studies are warranted.

#### All techniques considered together

Considering the results of all three techniques, there is an effect of silicon fertilization on amelioration of salinity or drought stress (Table 5 – 1). This effect, however, was not always statistically significant. In a hydroponic culture solution system, there was approximately a 94% chance that the addition of silicon increased corn dry mass ( $p=0.059$ ), independent of stress. Others have observed that silicon increased plant dry mass under drought or salt stress conditions with corn (Kaya *et al.* 2006), wheat (Ahmad *et al.* 1992; Gong *et al.* 2003), soybean (Hamayun *et al.* 2010; Lee *et al.* 2010; Shen *et al.* 2010), and rice (Chen *et al.* 2010) as well as sunflower (Gunes *et al.* 2008a;

Gunes *et al.* 2008b), sorghum (Hattori *et al.* 2005; Sonobe *et al.* 2009; Sonobe *et al.* 2010; Ahmed *et al.* 2011), and some grasses (Eneji *et al.* 2008).

Table 5 – 1. Overall statistical significance of results.

	Hydroponic studies – two trials			
	dry mass 18% increase	% dry mass 2% decrease	% roots 8% decrease	WUE 13% increase
p-value (ANOVA)*	0.059	n.s.	n.s.	n.s.

\*P-values from 3-factor ANOVA were obtained from analysis of all treatments considered together (Stress, Si level, and Block). Value of “n.s.” indicates no statistically significant difference ( $p < 0.05$ ).

20-L lysimeter studies dry mass and water use efficiency (WUE)						
	acute stress dry mass	chronic stress dry mass	combine d dry mass	acute stress WUE	chronic stress WUE	combine d WUE
Trial 1	0.6% increase	13% increase	6% increase	3% increase	4% increase	4% increase
p-value (t-test)*	n.s.	0.016	0.067	n.s.	n.s.	n.s.
p-value (ANOVA)*			0.042			n.s.
Trial 2	0.5% increase	0.2% decrease	0.2% increase	26% increase	48% increase	36% increase
p-value (t-test)*	n.s.	n.s.	n.s.	0.092	0.023	0.004
p-value (ANOVA)*			n.s.			0.004

\*P-values from the t-tests were obtained from analysis of data from one treatment only, unless otherwise stated; p-values from 2-factor ANOVA were obtained from analysis of all treatments considered together (stress and Si level).

2-liter container studies – dry mass analysis				
	Corn	Wheat	Soybean	Rice
	12% increase	17% increase	22% increase	24% increase
p-value (t-test)*	0.02	0.17	0.242	0.089
p-value (2-factor ANOVA)*	0.022	0.167	0.209	0.084
p-value (3-factor ANOVA)*	<0.001	0.015	0.881	0.221

\* P-values from t-test and 2-factor ANOVA were obtained from analysis of drought-stressed data only (stress and Trial); p-values from 3-factor ANOVA were obtained from analysis of drought-stressed and unstressed data considered together (stress, Si level, and Trial).

---

In a precision drought stress lysimeter system, the addition of a high silicon additive (Plantuff) to a low-silicon medium (peat) resulted in a statistically significant increase in corn dry mass ( $p < 0.05$ ) in one trial, but there was no effect in a second trial. In the same system, silicon significantly improved water use efficiency ( $p = 0.004$ ) in one trial, but not in the other. This inconsistency may be due to a combination of the frequency and duration of drought stress. It appears that acute drought stress may enhance the ameliorative effect of silicon fertilization. In studies of sorghum (Eneji *et al.* 2005), Rhodes grass (Eneji *et al.* 2005), tomato (Romero-Aranda *et al.* 2006), and rice (Chen *et al.* 2010) subjected to drought or salt stress, it was observed that silicon improved water use efficiency. In other studies it was also observed that silicon did not improve water use efficiency (Hattori *et al.* 2005; Ahmed *et al.* 2011). Gao *et al.* (2006) found that aboveground corn tissue accumulated 0.6% Si when cultivated in nutrient solution. In the current study utilizing peat as a growing medium, corn leaves accumulated approximately 0.3% Si. Variability in results may be due to variability in the actual amount of silicon supplied to plant tissues.

In 2-liter containers with repeated episodes of acute stress, the ameliorative effect of silicon fertilization was statistically significant in corn and wheat. However, silicon increased dry mass of all four crops in the study (corn, wheat, soybean, and rice). This increase occurred in unstressed as well as stressed plants, although there was no effect on dry mass in unstressed plants (Figure 4 – 28 and Figure 4 – 31). An increase in visible

plant size was also observed at times. Hattori *et al.* (2005) appear to be the only researchers to report that silicon increased the visible size of a plant.

Overall, the effects are promising. Further testing of the effects of varying frequencies and durations of drought stress should provide more consistent results. Although only statistically significant at the 0.05 level using two of three techniques, there was a beneficial effect of silicon water use efficiency under drought conditions. While silicon fertilization may improve the growth and yield of crops in semi-arid and arid conditions, it is not a substitute for an appropriate water supply. Furthermore, silicon fertilization may improve growth and drought stress tolerance only in regions with weathered soils that are low in available silicon. The results indicate that further studies of the effects of silicon on drought tolerance are necessary before any definitive claims are made.

## LITERATURE CITED

- Agarie, S., N. Hanaoka, O. Ueno, A. Miyazaki, F. Kubota, W. Agata, and P.B. Kaufman. 1998. Effects of silicon on tolerance to water deficit and heat stress in rice plants (*Oryza sativa* L.), monitored by electrolyte leakage. *Plant Prod. Sci.* 1:96–103.
- Ahmad, R., S. Zaheer, and S. Ismail. 1992. Role of silicon in salt tolerance of wheat (*Triticum aestivum* L.). *Plant Sci.* 85:43–50.
- Ahmed, M., F. Hassen, U. Qadeer, and M.A. Aslam. 2011. Silicon application and drought tolerance mechanism of sorghum. *Afr. J. Agric. Res.* 6:594–607.
- Al-Karaki, G.N., R.B. Al-Karaki, and C.Y. Al-Karaki. 1996. Phosphorus nutrition and water stress effects on proline accumulation in sorghum and bean. *J. Plant Phys.* 148:745–751.
- Alvarez, J., G.H. Snyder, D.L. Anderson, and D.B. Jones. 1988. Economics of calcium silicate slag application in a rice-sugarcane rotation in the Everglades. *Agric. Systems* 28:179–188.
- Ashraf, M., A. Ahmad, and T. McNeilly. 2001. Growth and photosynthetic characteristics in pearl millet under water stress and different potassium supply. *Photosynthetica* 39:389–394.
- Bakhat, H.F., S. Hanstein, and S. Schubert. 2009. Optimal level of silicon for maize (*Zea mays* L. c.v. Amadeo) growth in nutrient solution under controlled conditions. *The Proceedings of the International Plant Nutrition Colloquium XVI, Davis, CA.*
- Barcelo, J., P. Guevara, and C. Poschenrieder. 1993. Silicon amelioration of aluminium toxicity in teosinte (*Zea mays* L. ssp. mexicana). *Plant Soil* 154:249–255.

- Barker, A.V., and D.J. Pilbeam. 2007. Handbook of plant nutrition. CRC Press, Boca Raton, FL.
- Bollard, E.G., and G.W. Butler. 1966. Mineral nutrition of plants. *Annu. Rev. Plant Physiol.* 17:77–112.
- Carpita, N.C. 1996. Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:445–476.
- Chapman, H.D. 1966. Diagnostic criteria for plants and soils. University of California, Division of Agricultural Sciences, Riverside.
- Chen, C.H., and J. Lewin. 1969. Silicon as a nutrient element for *Equisetum arvense*. *Can. J. Bot.* 47:125–131.
- Chen, W., X. Yao, K. Cai, and J. Chen. 2010. Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biol. Trace. Elem. Res.* Available at <http://www.springerlink.com/content/nv2685m42700841p> (accessed 6 June 2011).
- Clements HF. 1964. Interaction of factors affecting yield. *Annu. Rev. Plant Physiol.* 15:409–442.
- Clements, H.F. 1965. The roles of calcium silicate slags in sugarcane growth. *Rep Hawaiian Sugarcane Technol.* 103–126.
- Cocker, K.M., D.E. Evans, and M.J. Hodson. 1998. The amelioration of aluminum toxicity by silicon in higher plants: Solution chemistry or an *in planta* mechanism? *Physiologia Planetarium* 104:608–614.

- Coors, J.G. 1987. Resistance to the European corn borer, *Ostrinia nubilalis* (Hubner), in maize, *Zea mays* L., as affected by soil silica, plant silica, structural carbohydrates, and lignin. p. 445–456. *In* H.W. Gabelman and B. Laughman (ed.) Genetic aspects of plant mineral nutrition. Martinus Nijhof, Dordrecht/Boston/Lancaster.
- Corn overview. 2010. Available at <http://www.ers.usda.gov/Briefing/Corn/> (accessed 17 Jan. 2012). USDA–ERS, Washington, D.C.
- Cornelis, J.T., B. Delvaux, and H. Titeux. 2010. Contrasting silicon uptakes by coniferous trees: a hydroponic experiment on young seedlings. *Plant Soil* 336:99–106.
- Crusciol, C.A.C., A.L. Pulz, L.B. Lemos, R.P. Soratto, and G.P.P. Lima. 2009. Effects of silicon and drought stress on tuber yield and leaf biochemical characteristics in potato. *Crop Sci.* 49:949–954.
- Datnoff, L.E., G.H. Snyder, and G.H. Korndörfer. 2001. Silicon in agriculture. Elsevier, New York.
- Deren, C.W. 2001. Plant genotype, silicon concentration, and silicon-related responses. p. 1–15. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) Silicon in agriculture. Elsevier, New York.
- Egilla, J.N., F.T. Davies, and M.C. Drew. 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro- and micronutrient content and root longevity. *Plant Soil* 229:213–224.

- Eneji, E., S. Inanaga, S. Muranaka, J. Li, P. An, T. Hattori, and W. Tsuji. 2005. Effect of calcium silicate on growth and dry matter yield of *Chloris gayana* and *Sorghum sudanense* under two soil water regimes. *Grass Forage Sci.* 60:393–398.
- Eneji, A.E., S. Inanaga, S. Muranaka, J. Li, T. Hattori, P. An, and W. Tsuji. 2008. Growth and nutrient use in four grasses under drought stress as mediated by silicon fertilizers. *J. Plant Nutr.* 31:355–365.
- Epstein, E. 1994. The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America* 91:11–17.
- Epstein, E. 1999. Silicon. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:641–664.
- Epstein, E. 2001. Silicon in plants: Facts vs. concepts. p. 1 – 15. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) Elsevier, New York.
- Epstein, E. 2002. Silicon in plant nutrition. p. 1 – 5. *In* Second silicon in agriculture conference; 2002 Aug 22-26. Japanese Society of Soil Science and Plant Nutrition, Tsuruoka, Japan.
- Epstein, E. and A.J. Bloom. 2005. Mineral nutrition of plants: principles and perspectives. 2nd ed. Sunderland (MA): Sinauer Associates, Sunderland, MA.
- Farooq, M., A. Wahid, D.J. Lee, O. Ito, and K.H.M. Siddique. 2009. Advances in drought resistance of rice critical reviews in plant sciences. *Crit. Rev. Plant Sci.* 28:199–217.
- Fawe, A., A.J.G. Menzies, M. Chérif, and R.B. Bélanger. 2001. Silicon and disease resistance in dicotyledons p. 1–15. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) Silicon in agriculture. Elsevier, New York.

- Fumis, T.F. and J.F. Pedras. 2002. Variação nos níveis de prolina, diamina e poliaminas em cultivares de trigo submetidas a déficits hídricos. *Pesqui. Agropecu. Bras.* 37:449–459.
- Gao, X., C. Zou, L. Wang, and F. Zhang. 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. *J. Plant Nutr.* 29:1637–1647.
- Gong, H., K. Chen, G. Chen, S. Wang, and C. Zhang. 2003. Effects of silicon on growth of wheat under drought. *J. Plant Nutr.* 26:1055–1063.
- Gong, H., X. Zhu, K. Chen, S. Wang, and C. Zhang. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.* 169:313–321.
- Gunes, A., D.J. Pilbeam, A. Inal, and S. Coban. 2008. Influence of silicon on sunflower cultivars under drought stress, I: growth, antioxidant mechanisms, and lipid peroxidation. *Comm. Soil Sci. Plant Analysis* 39:1885–1903.
- Gunes, A., Y.K. Kadioglu, D.J. Pilbeam, A. Inala, S. Cobana, and A. Aksu. 2008. Influence of silicon on sunflower cultivars under drought stress, II: essential and nonessential element uptake determined by polarized energy dispersive x-ray fluorescence. *Comm. Soil Sci. Plant Analysis* 39:1904–1927.
- Hamayun, M., E.Y. Sohn, S.A. Khan, Z.K. Shinwari, A.L. Khan, and I.J. Lee. 2010. Silicon alleviates the adverse effects of salinity and drought stress on growth and endogenous plant growth hormones of soybean (*Glycine max* L.). *Pak J. Bot.* 42:1713–1722.
- Hattori, T., S. Inanaga, H. Araki, P. An, S. Morita, M. Luxova, and A. Lux. 2005. Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *Physiologia Plantarum* 123:459–466.

- Hemmi, T. 1933. Experimental studies on the relation of environmental factors to the occurrence and severity of blast disease in rice plants. *Phytopath. Zeit.* 6:305–324.
- Hodson, M.J., A.G. Sangster, and D.W. Parry. 1985. An ultrastructural study on the developmental phases and silicification of the glumes of *Phalaris canariensis* L. *Ann. Bot.* 55:649–665.
- Hsiao, T.C. 1973. Plant responses to water stress. *Ann. Rev. Plant Physiol.* 24:519–570.
- Iler, R.K. 1978. Hydrogen-bonded complexes of silica with organic compounds. In: Bendz G, Lindquist J, editors. *Biochemistry of silicon and related problems*. New York: Plenum. p. 53–76.
- Ingri, N. 1978. Aqueous silicic acids, silicates and silicate complexes p. 3–52. In G. Bendz and J. Lindquist (ed.) *Biochemistry of silicon and related problems*. Plenum, New York.
- Jones, L.H.P. and K.A. Handreck. 1967. Silica in soils, plants, and animals p. 107–149. In A.G. Norman (ed.) *Advances in agronomy*. Vol. 19. Academic Press, New York.
- Kameli, A. and D.M. Losel. 1993. Carbohydrates and water status in wheat plants under water stress. *New Phytol.* 125:609–614.
- Kaya, C., L. Tuna, and D. Higgs. 2006. Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. *J. Plant Nutr.* 29:1469–1480.
- Ketchum, B.H. 1954. Mineral nutrition of phytoplankton. *Annu. Rev. Plant Physiol.* 5:55–64.

- Kidd, P.S., M. Llugany, C. Poschenrieder, B. Gunsé, and J. Barceló. 2001. The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminum toxicity in three varieties of maize (*Zea mays* L.). *J. Exp. Bot.* 52:1339–1352.
- Kinrade, S.D., R.J. Hamilton, Schach, and C.T.G. Knight. 2001. Aqueous hypervalent silicon complexes with aliphatic sugar acids. *J. Chem. Soc.-Dalton Tran.* 961–963.
- Kinrade, S.D., A.M.E. Gillson, and C.T.G. Knight. 2002. Silicon-29 NMR evidence of a transient hexavalent silicon complex in the diatom *Navicula pelliculosa*. *J. Chem. Soc.-Dalton Tran.* 307–309.
- Korndörfer, G.H. and I. Lepsch, 2001. Effect of silicon on plant growth and crop yield p. 133–147. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) *Silicon in agriculture*. Vol. 8 studies in plant science. Elsevier, Amsterdam.
- Lanning, F.C., T.L. Hopkins, and J.C. Loera. 1980. Silica and ash content and depositional patterns in tissues of mature *Zea mays* L. plants. *Ann. Bot.* 45:549–554.
- Lee, S.K., E.Y. Sohn, M. Hamayun, J.Y. Yoon, and I.J. Lee. 2010. Effect of silicon on growth and salinity stress of soybean plant grown under hydroponic system. *Agroforest. Syst.* 80:333–340.
- Li, M., G.X. Wang, and J.S. Lin. 2003. Application of external calcium in improving the PEG-induced water stress tolerance in liquorice cells. *Bot. Bull. Acad. Sin.* 44: 275–284.

- Liang, Y.C., Q.R. Chen, Q. Liu, W.H. Zhang, and R.X. Ding. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol.* 160:1157–1164.
- Liang, Y.C., J.W.C. Wong, and L. Wei. 2005. Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere* 58:475–483.
- Lindsay, W.L. 1979. Chemical equilibria in soils p. 51–54. John Wiley & Sons, New York.
- Lux, A., M. Luxova, T. Hattori, S. Inanaga, and Y. Sugimoto. 2002. Silicification in sorghum (*Sorghum bicolor*) cultivars with different drought tolerance. *Physiol. Plantarum* 115:87–92.
- Ma, J.F., Y. Miyaki, and E. Takahashi. 2001. Silicon as a beneficial element for crop plants p. 17 – 39. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) *Silicon in agriculture*. Elsevier, New York.
- Ma, J.F. and E. Takahashi E. 2002. Soil, fertilizer, and plant silicon research in Japan, 1st ed. Elsevier, Amsterdam.
- Ma, J.F., K. Tamai, M. Ichii, G.F. Wu. 2002. A rice mutant defective in Si uptake. *Plant Physiol.* 130:2111–2117.
- Ma, J.F. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50:2004–2002.
- Ma, J.F. and N. Yamaji. 2006. Silicon uptake and accumulation in higher plants. *Trends Plant Sci.* 11:392–397.

- Marschner, H. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, San Diego, CA.
- Mengel, K. and E.A. Kirkby. 2001 Principles of plant nutrition. Kluwer Academic Publication, Dordrecht, The Netherlands.
- Mitani, N., and J.F. Ma. 2005. Uptake system of silicon in different plant species. J. Exp. Bot. 56(414):1255–1261.
- Moraes, J.C., M.M. Goussain, G.A. Carvalho, and R.R. Costa. 2005. Não-preferência do pulgão-da-folha *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) para plantas de milho (*Zea mays* L.) tratadas com silício. Ciênc. Agrotec. 29:761–766.
- Morikawa, C.K. and M. Saigusa. 2004. Mineral composition and accumulation of silicon in tissues of blueberry (*Vaccinium corymbosus* cv. Bluecrop) cuttings. Plant Soil 258:1–8.
- Neumann, D. and U. zur Nieden. 2001. Silicon and heavy metal tolerance of higher plants. Phytochem. 56:685–692.
- Pereira, S.H., G.H. Korndörfer, A.D.A. Vidal, and M.S.D. Camargo. 2004. Silicon sources for rice crop. Sci. Agric. 61:522–528.
- Pulz, A.L., C.A.C. Crusciol, L.B. Lemos, and R.P. Soratto. 2008. Influência de silicato e calcário na nutrição, produtividade e qualidade da batata sob deficiência hídrica. Rev Bras Ciênc Solo 32:1651–1659.
- Purcell, L.C. and C.A. King. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. J. Plant Nutr 19:969–993.

- Rafi, M.M. and E. Epstein. 1999. Silicon absorption by wheat (*Triticum aestivum* L.).  
Plant Soil 211:223–230.
- Riederer, M., and L. Schreiber. 2001. Protecting against water loss: analysis of the barrier  
properties of plant cuticles. J. Exp. Bot. 52:2023–2032.
- Richmond, K.E. and M. Sussman. 2003. Got silicon? The non-essential beneficial plant  
nutrient. Curr. Opin. Plant Biol. 6:268–272.
- Rodrigues, F.A., D.J. McNally, L.E. Datnoff, J. B. Jones, C. Labbé, N. Benhamou, J.G.  
Menzies, and R.R. Bélanger. 2004. Silicon enhances the accumulation of  
diterpenoid phytoalexins in rice: a potential mechanism for blast resistance.  
Phytopathology 94:177–183.
- Romero-Aranda, M.R., O. Jurado, and J. Cuartero. 2006. Silicon alleviates the deleterious  
salt effect on tomato plant growth by improving plant water status. J. Plant Phys.  
163:847–855.
- Round, F.E., R.M. Crawford, and D.G. Mann. 1990. The diatoms: biology and  
morphology of the genera. Cambridge University Press, Cambridge, United  
Kingdom.
- Sangster, A.G., M.J. Hodson, D.W. Parry, J.A. Rees. 1983. A developmental study of  
silicification in the trichomes and associated epidermal structures of the  
inflorescence bracts of the grass, *Phalaris canariensis* L. Ann Bot 52:171–187.
- Savant, N.K., G.H. Snyder, and L.E. Datnoff. 1996. Silicon Management and Sustainable  
Rice Production. Adv Agron 58:151–199.

- Savant, N.K., L.E. Datnoff, and G.H. Snyder. 1997. Depletion of plant-available silicon in soils: a possible cause of declining rice yields. *Comm. Soil Sci. Plant Analysis* 28: 1245–1252.
- Savant, N.K., G.H. Korndörfer, L.E. Datnoff, and G.H. Snyder. 1999. Silicon nutrition and sugarcane production: a review. *J. Plant Nutr.* 22:1853–1903.
- Shen, X., Y. Zhou, L. Duan, Z. Li, A.E. Eneji, and J. Li. 2010. Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. *J. Plant Phys.* 167:1248–1252.
- Singh, D.K., and P.W.G. Sale. 2000. Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agron. J.* 92:868–874.
- Sonobe, K., T. Hattori, P. An, W. Tsuji, E. Eneji, K. Tanaka, and S. Inanaga. 2009. Diurnal variations in photosynthesis, stomatal conductance and leaf water relation in sorghum grown with or without silicon under water stress. *J. Plant Nutr.* 32:433–442.
- Sonobe, K., T. Hattori, P. An, W. Tsuji, A.E. Eneji, S. Kobayashi, Y. Kawamura, K. Tanaka, and S. Inanaga. 2010. Effect of silicon application on sorghum root responses to water stress. *J. Plant Nutr.* 34:71–82.
- Sposito, G. 1989. *The chemistry of soils*. Oxford University Press, New York.
- Taiz, L., and E. Zeiger. 2006. *Plant Physiology*. 4th ed. Sinauer Associates, Sunderland, MA.
- Tamai, K., and J.F. Ma. 2003. Characterization of silicon uptake by rice roots. *New Phytologist* 158:431–436.

- USDA Feed Grain Baseline. 2010. Available at <http://www.ers.usda.gov/Briefing/Corn/2010baseline.htm> (accessed 17 Jan. 2012). USDA-ERS, Washington, D.C.
- Valamis, J., and D.E. William. 1967. Manganese and silicon interaction in the gramineae. *Plant Soil* 27:131–140.
- Van Soest, P.J. 2006. Rice straw, the role of silica and treatments to improve quality. *Anim. Feed Sci. Technol.* 130:137–171.
- Verbruggen, N., and C. Hermans. 2008. Proline accumulation in plants: a review. *Amino Acids* 35:353–359.
- Volcani, B.E. 1978. Role of silicon in diatom metabolism and silicification. p. 177–206. *In* G. Bendz and J. Lindquist (ed.) *Biochemistry of silicon and related problems*. Plenum, New York.
- Voogt, W., and C. Sonneveld. 2001. Silicon in horticultural crops in soilless culture. p. 115–131. *In* L.E. Datnoff, G.H. Snyder, and G.H. Korndörfer (ed.) *Silicon in agriculture*. Elsevier, New York.
- Wainwright, M. 1997. The neglected microbiology of silicon: from the origin of life to an explanation for what Henry Charlton Bastian saw. *Soc. Gen. Microbiol. Quart.* 24:83–85.
- Weiss, A., and A. Herzog. 1978. Isolation and characterization of a silicon organic complex from plants p. 109–127. *In* G. Bendz and J. Lindquist (ed.) *Biochemistry of silicon and related problems*. Plenum, New York.
- Wiese, H., M. Nikolic, V. Römheld. 2007. Silicon in plant nutrition. p. 33–47. *In* B. Sattelmacher and W.J. Horst (ed.) *The apoplast of higher plants: compartment of storage, transport and reactions*. Springer, The Netherlands.

- Williams, R.F., and R.E. Shapter. 1955. A comparative study of growth and nutrition in barley and rye as affected by low-water treatment. *Australian J. Biol. Sci.* 8:435–466.
- Wilson, D.R., and P.D. Jamieson. 1985. Models on growth and water use of wheat in New Zealand p. 211–216. *In* W. Day and R.K. Atkin (ed.) *Wheat growth and modeling*. Plenum Press, New York.
- Yeo, A.R., S.A. Flowers, G. Rao, K. Welfare, N. Senanayake, and T.J. Flowers. 1999. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. *Plant Cell Env.* 22:559–565.

## APPENDICES

## Appendix A

### Conversion of electrical conductivity to osmotic potential in dilute nutrient solution

#### Introduction

Osmotic potential of a solution indicates of the osmotic adjustment required by plant roots to extract water from the solution. The osmotic adjustment can cause stress on plants, generally considered drought or salt stress. Direct measurement of osmotic potential can be expensive. However, direct measurement of solution electrical conductivity is relatively inexpensive. We sought to develop conversion factors to relate the two units.

#### Materials and Methods

The electrical conductivity (EC) of seven concentrations of NaCl (F.W. 58.44 g mol<sup>-1</sup>) from 0 to 10 g L<sup>-1</sup> in dilute nutrient solution (Peters Excel 21-5-20) was measured using an EC meter. An equation to calculate  $\Psi_w$  from molality (M) was obtained by fitting a 6<sup>th</sup> order polynomial to the results from Lang (1967).

#### Results

Table A – 1. Comparison of osmotic potential with electrical conductivity in a dilute nutrient solution. Highlighted row is from dilute nutrient solution alone.

$\Psi_w$ (MPa)	EC (mS m <sup>-1</sup> )	Multiplier ( $\Psi_w \rightarrow$ EC)	inverse multiplier (EC $\rightarrow$ $\Psi_w$ )
$-1.5 \times 10^{-4}$	0.055	-376.7	-0.0027
-0.1	28.8	-287.8	-0.0035
-0.2	50.5	-252.4	-0.0040
-0.3	71.9	-239.8	-0.0042
-0.4	93.0	-232.6	-0.0043
-0.5	113.8	-227.7	-0.0044
-0.6	134.1	-223.6	-0.0045

#### References

Lang ARG. 1967. Osmotic coefficients and water potential of sodium chloride solutions from 0 to 40°C. Aust J. of Chem 20:2017-2023.

## Appendix B

## Drought stress lysimeter system

Sixteen load cells (Transducer Techniques, Temecula, CA, model ESP-35) were interfaced with the control system in a greenhouse described in Figure B – 1.

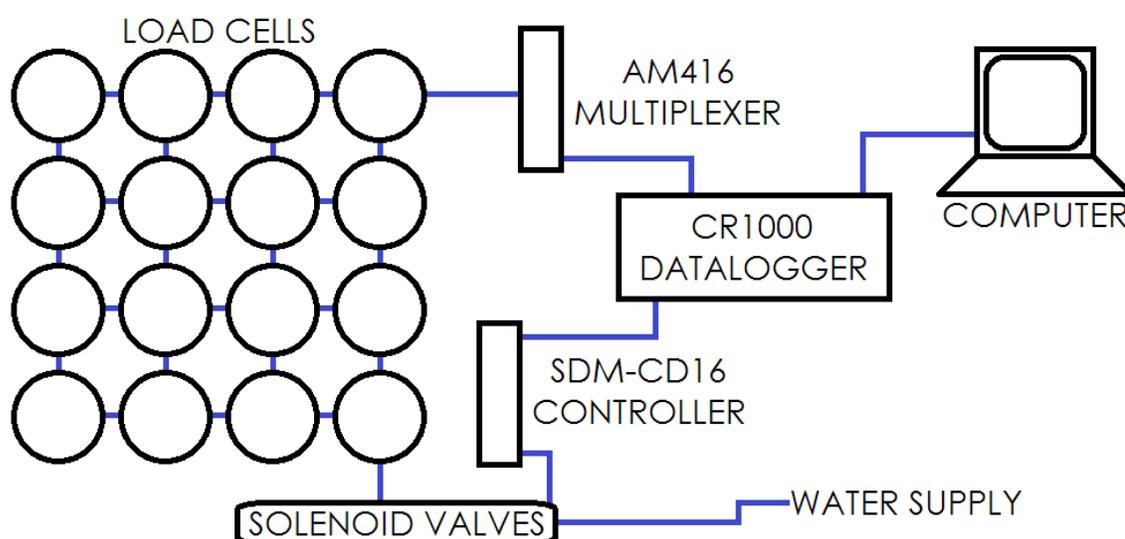


Figure B – 1. System for monitoring and control of load cell balance mass. Wiring connections are displayed in blue. Load cells were not wired to each other, but were connected individually to the multiplexer. The multiplexer is necessary to interface sixteen load cells with the datalogger. Solenoid valves were connected to individual drip emitters placed in each container separately.

Load cell balances were constructed by affixing a rigid circular base and platform to each load cell as shown in Figure B – 2. Each load cell was attached to aluminum plates for use as an electronic balance (Figure 2). Conversion of the load cell mV output to mass in grams was accomplished by placing two objects of known mass on the load cell, one at a time. The resulting mV output for each of the two mass values was plotted with mass on the y-axis and mV on the x-axis. The slope and y-intercept of the linear regression line

provide the multiplier and offset, respectively, to convert mV output to mass. The multiplier and offset were added with software. Prior testing revealed that load cell output increases directly proportional to increasing weight, thus necessitating only a two-point calibration.

Temperature changes alter load cell output. Indeed, previous studies indicate that load cell output can be altered by up to 3 g per °C of load cell body temperature. The effect of temperature on load cell output was measured and appropriate corrections

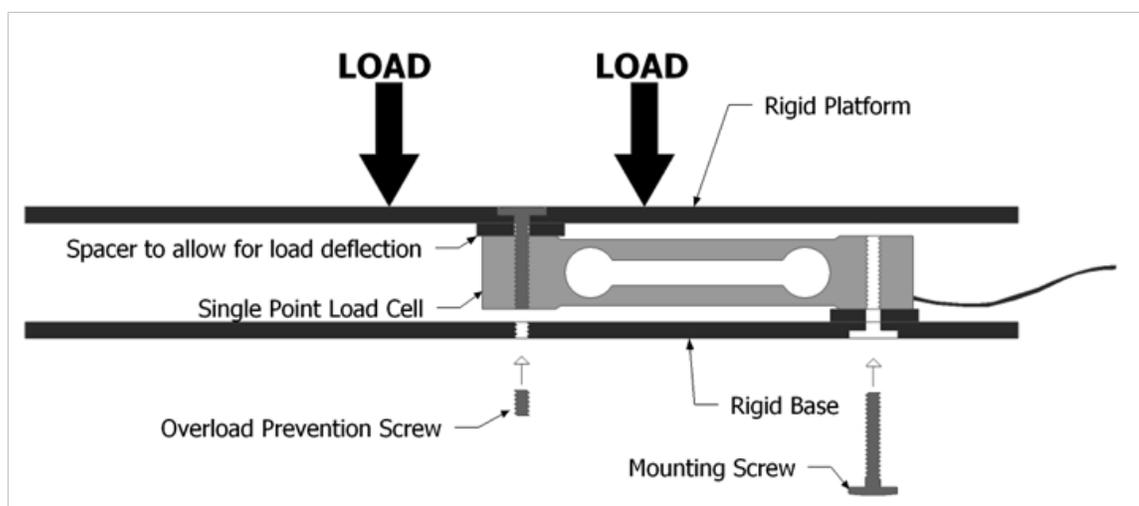


Figure B – 2. Side view diagram showing the attachment of rigid platforms to a beam sensor load cell to facilitate mass measurements. Spacers allow for deflection of the load cell. An overload prevention screw in the bottom platform prevents damage due to over-deflection of the load cell. An overload prevention screw in the bottom platform prevents damage due to over-deflection of the load cell body.

were applied in software. Three fine wire thermistors were glued with epoxy directly to three load cell bodies. Containers filled with 30 kg of wet soil were covered with tight-fitting lids and placed on all load cells. Temperature was controlled to change slowly between 15°C and 30°C daily for three days. Temperature and load cell output were measured and plotted. A simple linear regression was used to characterize the effect of

temperature on load cell output. Each load cell was assigned a correction factor based on the regression equation.

Plants were grown in 20-L containers filled with Canadian sphagnum peat moss growing medium. The growing medium in each container was amended with slow-release fertilizer (Polyon® 15-6-11, 1 to 2 month release, Pursell Industries, Sylacauga, Alabama). Two drip emitters were used to water each container at the surface. Evaporation was minimized by covering the growing medium surface with dry expanded perlite to a depth of 5 cm.

The system was programmed to maintain a constant mass on each load cell balance by nightly watering. A mass set-point was established for each load cell based on a percentage of the saturated volumetric water content (VWC). The system compared each load cell mass value every 15 seconds against that load cell's set-point, from 0:01 to 05:00 daily. If the load cell mass value was less than the set-point at any time between 0:01 and 05:00, watering would be initiated, and watering endure until the load cell mass exceeded the set-point. Watering was initiated by a signal from the datalogger to the SDM-CD16 controller, which opened the appropriate solenoid valves, allowing pressurized municipal water to enter and be emitted by the drippers.

Transpiration ( $\text{g min}^{-1}$ ) was calculated as the difference between the average of all 15-second mass values in a 30-minute period and the average of all 15-second mass values in the subsequent 30-minute period, divided by 30 (Figure B – 3). Cumulative daily transpiration was calculated as the sum of all 30-minute average transpiration values, multiplied by 48.

$$\left[ \left( \frac{1}{n} \times \sum_{i=1}^{120} x_i \right) - \left( \frac{1}{n} \times \sum_{i=121}^{241} x_i \right) \right] \times \frac{1}{30} = \text{transpiration rate (g min}^{-1}\text{)}$$

Figure B – 3. Transpiration equation.

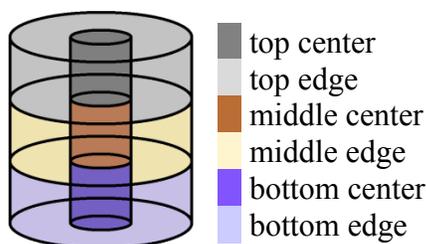
Water stress was controlled by manipulation of the watering set-points in software. Visual observation of leaf rolling and analysis of transpiration measurements determined whether water stress conditions were satisfied or not. When transpiration rates were roughly equal to 50% of the control and leaf rolling was observed, water stress was determined to occur. Since water stress recovery was of particular interest in this study, watering set-points were manipulated such that water stress was allowed to occur for at least 48 hours. Following water stress, a restoration of full watering occurred for at least 48 hours before a recurrent onset of water stress.

Dry mass was determined after harvesting all aboveground plant biomass. All aboveground plant biomass was dried for 48 hours in an oven at 80°C.

## Appendix C

## Moisture distribution in 20-L containers of growing medium

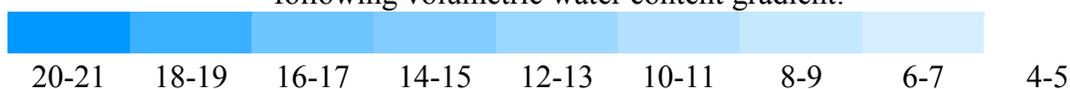
Samples were collected from the following locations within 20-L containers of soil in which soybeans were grown:



The volumetric water content of soil samples was measured and analyzed. Volumetric water content results from a study of soybeans grown in 20-L containers of soil indicate that water was not evenly distributed throughout the soil (Table C-1).

<b>morning</b>	<b>wet</b>		<b>somewhat wet</b>		<b>somewhat dry</b>		<b>dry</b>	
	center	edge	center	edge	center	edge	center	edge
top	20.5	15.3	20.8	12.1	20.9	8.6	18.6	8.1
middle	19.6	15.5	18.8	13.1	18.4	11.4	13.7	6.7
bottom	14.6	14.8	11.6	6.6	7.5	6.4	6.5	5.9
sample average	16.7		13.8		12.2		9.9	
based on mass	19.9		14.4		13.0		11.1	
<b>afternoon</b>	<b>wet</b>		<b>somewhat wet</b>		<b>somewhat dry</b>		<b>dry</b>	
	center	edge	center	edge	center	edge	center	edge
top	11.4	9.7	10.5	8.1	11.0	7.4	9.7	6.1
middle	11.2	10.3	9.8	7.1	9.3	6.2	7.7	6.1
bottom	8.9	8.1	7.7	5.9	6.2	5.8	6.3	5.3
sample average	9.9		8.2		7.7		6.9	
based on mass	13.0		10.8		10.2		9.0	

Table C-1. Mean volumetric water content (VWC or  $\theta_v$ ) of two replicate containers. Cells are highlighted in varying colors to assist in data interpretation according to the following volumetric water content gradient:



## Appendix D

## Preliminary studies using lysimeter system

The foliar silicon content in older leaves of corn grown in peat/vermiculite (50/50 v/v) growing medium reveals that the growing medium with or without the addition of silicon supplied plants with a detectable amount of silicon (Figure D-1). Approximate silicon concentration of  $K_2SiO_3$  was 2.0 mM.

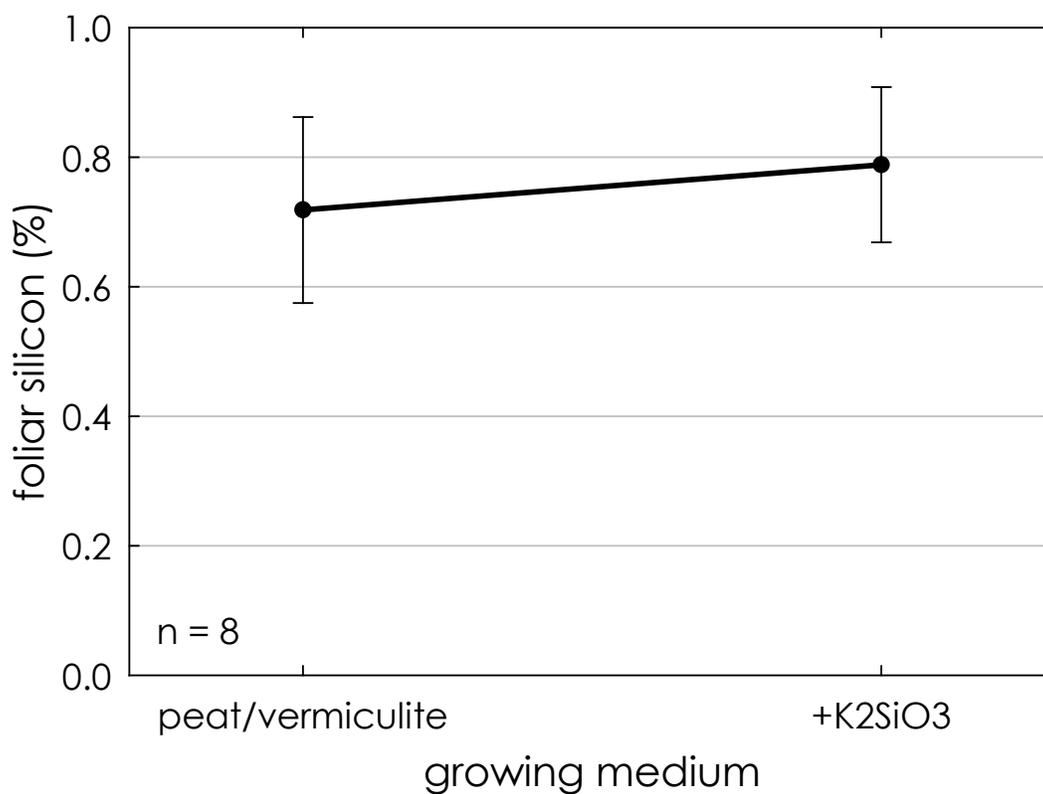


Figure D-1. Foliar silicon content in older leaves of plants grown in a preliminary trial in peat/vermiculite.

## Appendix E

### Initial research on silicon as a beneficial plant nutrient

#### **Silicon and plant nutrition Literature Review**

**Kärlek W. Jänislampi**

Plant Nutrition PSC 6430

28 September 2010

#### **Introduction**

Silicon has not generally been considered “essential” for plant survival or growth. This essentiality consideration disregards the fact that most plants take up large quantities of silica. However, recent research indicates that for some plants, silicon has the potential to enhance growth and yield. In the words of Epstein (1999), “in the real world of plant life, Si matters.” As pertaining to crop plants, in particular, this review has been conducted to discover if growth and yield of crop plants are improved by fertilization of soils with silica.

#### **Literature review**

The beneficial effects of silicon fertilization on crop plants include increased drought tolerance (Gong *et al.* 2008), yield (Alvarez *et al.* 1988, Epstein 2001), cold hardiness, mechanical strength, and resistance to salinity stress, disease, herbivory, and metal toxicity (Epstein 1999, 2001). Silicon fertilization seems to provide at least some benefit to most monocotyledon crop plants (Epstein 1999). Some species of dicots (e.g. cucumber and grape) seem to also be benefited by silicon fertilization (Epstein 1999, Wiese *et al.* 2007). The effects of temperature and humidity on the efficacy of silicon

fertilization have not been studied, insofar as I was able to detect. I was also unable to locate any studies on the effect of soil moisture on silicon fertilization efficacy. Plants grown in soils with higher silica content (i.e. volcanic soils) tend to take up higher amounts of silica (Korndörfer and Lepsch 2001). Crops fertilized with silicon and grown in tropical or highly-leached soils may respond positively in terms of growth and yield (Alvarez *et al.* 1988, Korndörfer and Lepsch 2001).

### **Conclusion**

Despite all the alleged and demonstrated benefits, the effects of silica fertilization on growth and yield seem to be mostly limited to monocotyledons. The beneficial effects are mostly through indirect means such as silicon-induced disease and other stress resistance. Sugarcane (Alvarez *et al.* 1988, Epstein 1999) and rice (Alvarez *et al.* 1988, Korndörfer and Lepsch 2001, Ma and Yamaji 2006) have been confirmed to benefit the most from silica fertilization. Wheat, barley, corn, and most other crops are benefited to varying small degrees, except in the case of silica-deficient soils. Information available for silicon-induced yield increases of cucumber (Xiyan *et al.* 2007), tomato, potato, and beans was sparse and mostly limited to obscure or inaccessible journals.

### **References**

- Alvarez J, Snyder GH, Anderson DL and Jones DB. 1988. Economics of calcium silicate slag application in a rice-sugarcane rotation in the Everglades. *Ag. Systems.* 28(3): 179-188.
- Epstein E. 1999. Silicon. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 641-644.

- Epstein E. 2001. Silicon in plants: facts vs. concepts In: Datnoff LE, Snyder GH, Korndöfer GH, editors. Silicon in agriculture. Amsterdam: Elsevier. p. 1-16.
- Gong H, Chen K, Zhao Z, Chen G, Zhou W. 2008. Effects of silicon on defense of wheat against oxidative stress under drought at different developmental stages. *Biologia Plantarum*. 52(3): 592-596.
- Korndörfer GH, Lepsch I, 2001. Effect of silicon on plant growth and crop yield. In: Datnoff LE, Snyder GH, and Korndorfer GH, editors. Silicon in Agriculture. Vol. 8 Studies in plant science. Amsterdam: Elsevier. p. 133-147.
- Ma JF, Yamaji N. 2006. Silicon uptake and accumulation in higher plants. *Trends in Plant Sci*. 11: 329-397.
- Wiese H, Nikolic M, Römheld V. 2007. Silicon in plant nutrition. In: Sattelmacher B, Horst WJ, editors. *The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions*. Netherlands: Springer. p. 33-47.
- Xiyan W, Yulong Z, Na Y, Qinglong M, Jun L. 2007. Effect of silicon on cucumber photosynthesis and yield in protected field. *Jour. Changjiang Veg*. 2:50-52.

## Appendix F

## Nutrient solutions used in hydroponic studies

STARTER SOLUTION				0.0 mM Si REFILL SOLUTION			
SALT	STOCK CONC	mL or g PER 100 L	FINAL CONC	SALT	STOCK CONC	mL or g PER 100 L	FINAL CONC
<b>Ca(NO<sub>3</sub>)<sub>2</sub></b>	1 M	100	1 mM	<b>Ca(NO<sub>3</sub>)<sub>2</sub></b>	1 M	100	1 mM
<b>K(NO<sub>3</sub>)</b>	2 M	50	4 mM	<b>K(NO<sub>3</sub>)</b>	2 M	200	4 mM
<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.5 M	10	0.05 mM	<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.5 M	100	0.5 mM
<b>MgSO<sub>4</sub></b>	1 M	50	0.5 mM	<b>MgSO<sub>4</sub></b>	1 M	50	0.5 mM
<b>K<sub>2</sub>SiO<sub>3</sub></b>	0.1 M	0	0 mM	<b>MnCl<sub>2</sub></b>	60 mM	5	3 μM
<b>K<sub>2</sub>SO<sub>4</sub></b>	0.5 M	0	0 mM	<b>ZnCl<sub>2</sub></b>	20 mM	30	6 μM
<b>FeCl<sub>3</sub></b>	50 mM	40	20 μM	<b>H<sub>3</sub>BO<sub>3</sub></b>	40 mM	5	2 μM
<b>Fe-HEEDTA</b>	100 mM	50	10 μM	<b>CuCl<sub>2</sub></b>	20 mM	15	3 μM
<b>MnCl<sub>2</sub></b>	60 mM	5	3 μM	<b>Na<sub>2</sub>MoO<sub>4</sub></b>	1 mM	10	0.1 μM
<b>ZnCl<sub>2</sub></b>	20 mM	30	6 μM	<b>NaCl</b>	58.44 g/mol	9.35	1.6 mM
<b>H<sub>3</sub>BO<sub>3</sub></b>	40 mM	5	2 μM	<b>Na<sub>2</sub>SiO<sub>3</sub></b>	284.2 g/mol	0	0.0 mM
<b>CuCl<sub>2</sub></b>	20 mM	15	3 μM	<b>acidify to pH 5.0 before adding Iron</b>			
<b>Na<sub>2</sub>MoO<sub>4</sub></b>	1 mM	10	0.1 μM	<b>HNO<sub>3</sub>/HCl</b>	___ M	—	X
<b>HNO<sub>3</sub>/HCl</b>	___ M	—	X	<b>FeCl<sub>3</sub></b>	50 mM	20	10 μM
<b>KOH</b>	___ M	—	X	<b>Fe-HEEDTA</b>	100 mM	25	5 μM

0.4 Mm Si REFILL SOLUTION				0.8 mM Si REFILL SOLUTION			
SALT	STOCK CON C.	mL or g PER 100 L	FINAL CON C.	SALT	STOCK CON C.	mL or g PER 100 L	FINAL CON C.
<b>Ca(NO<sub>3</sub>)<sub>2</sub></b>	1 M	100	1 mM	<b>Ca(NO<sub>3</sub>)<sub>2</sub></b>	1 M	100	1 mM
<b>K(NO<sub>3</sub>)</b>	2 M	200	4 mM	<b>K(NO<sub>3</sub>)</b>	2 M	200	4 mM
<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.5 M	100	0.5 mM	<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.5 M	100	0.5 mM
<b>MgSO<sub>4</sub></b>	1 M	50	0.5 mM	<b>MgSO<sub>4</sub></b>	1 M	50	0.5 mM
<b>MnCl<sub>2</sub></b>	60 mM	5	3 μM	<b>MnCl<sub>2</sub></b>	60 mM	5	3 μM
<b>ZnCl<sub>2</sub></b>	20 mM	30	6 μM	<b>ZnCl<sub>2</sub></b>	20 mM	30	6 μM
<b>H<sub>3</sub>BO<sub>3</sub></b>	40 mM	5	2 μM	<b>H<sub>3</sub>BO<sub>3</sub></b>	40 mM	5	2 μM
<b>CuCl<sub>2</sub></b>	20 mM	15	3 μM	<b>CuCl<sub>2</sub></b>	20 mM	15	3 μM
<b>Na<sub>2</sub>MoO<sub>4</sub></b>	1 mM	10	0.1 μM	<b>Na<sub>2</sub>MoO<sub>4</sub></b>	1 mM	10	0.1 μM
<b>NaCl</b>	58.44 g/mol	4.675	0.8 mM	<b>NaCl</b>	58.44 g/mol	0	0.0 mM
<b>Na<sub>2</sub>SiO<sub>3</sub></b>	284.2 g/mol	11.368	0.4 mM	<b>Na<sub>2</sub>SiO<sub>3</sub></b>	284.2 g/mol	22.736	0.8 mM
<b>acidify to pH 5.0 before adding Iron</b>				<b>acidify to pH 5.0 before adding Iron</b>			
<b>HNO<sub>3</sub>/HCl</b>	— M	—	X	<b>HNO<sub>3</sub>/HCl</b>	— M	—	X
<b>FeCl<sub>3</sub></b>	50 mM	20	10 μM	<b>FeCl<sub>3</sub></b>	50 mM	20	10 μM
<b>Fe-HEEDTA</b>	100 mM	25	5 μM	<b>Fe-HEEDTA</b>	100 mM	25	5 μM