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Approaches to Supplementing Silicon in Soilless Media and the Value of Silicon in the Mitigation of Drought Stress

Mackenzie Grace Dey
Utah State University

APPROACHES TO SUPPLEMENTING SILICON IN SOILLESS MEDIA AND THE VALUE OF SILICON IN THE MITIGATION OF DROUGHT STRESS

by

Mackenzie Grace Dey

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Approved:

Bruce Bugbee, Ph.D. Major Professor	Jennifer Boldt, Ph.D. Committee Member
Youping Sun, Ph.D. Committee Member	D. Richard Cutler, Ph.D. Vice Provost of Graduate Studies

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ABSTRACT

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Mackenzie Grace Dey, Master of Science

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

Silicon (Si) is a beneficial nutrient and it is thought to be especially beneficial to plants during stress. Although abundant in most soils, Si is minimally bioavailable in soilless media. The addition of media amendments that steadily release Si over a crop lifecycle would be beneficial. The dissolution of Si from ten substrates and media additives were quantified in water. Typical media substrates like coconut coir, peat moss, perlite, rock wool, sand, and vermiculite released less than 0.03 mmol $Si{\cdot}L^{-1}$ $substrate{\cdot}day^{-1}$ for less than 60 days. Steel slag released inconsistently and caused an alkaline pH. Diatomaceous earth released steadily, but is expensive compared to other Si additives. Wollastonite, a calcium silicate mineral, and rice hulls steadily released Si in water and were selected for further study in peat-based media. Rice hulls at 12% by volume released an average of 1.5 mmol $Si{\cdot}L^{-1}$ $media{\cdot}leaching\ event^{-1}$ over 120 days. Wollastonite (1 g $wollastonite{\cdot}L^{-1}$ peat) release quickly reached a maximum of 2.1 mmol $Si{\cdot}L^{-1}$ $media{\cdot}leaching\ event^{-1}$, then gradually decreased over

120 days averaging 1.4 mmol Si·L^{-1} media·leaching $event^{-1}$. In peat-based media over 120 days, rice hulls gradually increased media pH by 1.0 unit and wollastonite increased pH by 0.8 units. In coconut coir-based media, both amendments increased media pH up to 1.3 units, but varied based on leaching volume. Rice hulls had a slower release than wollastonite and would be valuable for crops with lifecycles longer than four months in the same media. Wollastonite would be better suited for crops with lifecycles under four months. In a separate study, wollastonite was incorporated into peat-based media to supplement *Cannabis* (*Cannabis sativa* L. 'Trump') grown under precision drought stress. Precision drought stress, sometimes referred to as "eustress," can reduce stem and petiole elongation with a minimal reduction in photosynthetic rate. Silicon supplementation did not have a statistically significant effect on dry mass or height during drought stress, but it significantly reduced the incidence of powdery mildew (*Golovinomyces* sp.). Precision drought stress warrants further study as a method to reduce stem and petiole elongation in high-value crops such as *Cannabis.* Silicon can decrease powdery mildew, but warrants further investigation during drought stress.

(98 pages)

PUBLIC ABSTRACT

Approaches to supplementing silicon in soilless media and the value of silicon in the mitigation of drought stress

Mackenzie Grace Dey

Silicon (Si) is not considered an essential element for plants to complete their lifecycle, but is known to be beneficial for plants under environmental stress such as drought. Unlike natural soils, Si is minimally bioavailable in soilless media. With indoor agriculture and greenhouse production increasing, the benefits of Si have been seen and Si should be supplemented. This work aimed to characterize and quantify the dissolution of Si from media substrates and additives to achieve a steady-state release of Si in soilless media. Typical media components such as coconut coir, peat moss, perlite, rock wool, sand, and vermiculite minimally released Si and depleted within 60 days. Wollastonite, a calcium silicate mineral, and rice hulls steadily released Si in water. The dissolution of wollastonite rapidly released bioavailable Si in peat-based media, but decreased over 120 days while rice hulls gradually released. Both amendments increased coconut coir and peat-based media pH. Either would provide bioavailable Si over the lifecycle of many crop species. To test the dissolution of Si, wollastonite was used to amend peat-based media to grow *Cannabis sativa* L. under precision drought stress. Precision drought stress can be a useful method for growers to maintain plant size and photosynthetic rates, but it is a

sensitive system to balance. We hypothesized that Si would increase the resiliency of *Cannabis* in this system under drought stress. Although Si supplementation did not increase tolerance of *Cannabis* to drought stress, Si supplementation inhibited powdery mildew (*Golovinomyces* sp.), a common fungal disease in outdoor and indoor production, in all three trials compared to Si non-supplemented treatments. Silicon is a beneficial nutrient for crops that should be supplemented in soilless media to increase tolerance to environmental stress such as drought stress and biotic stress such as fungal disease, but requires further study.

DEDICATION

To my dear husband, Cameron Dey, for his love, support, and patience as I worked my way through my graduate degree. Cam, you help me feel 1,000 feet tall and capable of anything I set my mind to.

To my wonderful family, thank you Ma and Pa for your support and also rallying behind me. You have always seen my potential, been my biggest cheerleaders, and have pushed me to succeed. I wish you both could have seen me push through to the end, but I love and miss you both dearly. Thank you to my siblings for their support and for teasing me through to the end.

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CHAPTER I

LITERATURE REVIEW

Introduction

The earth's crust is comprised of approximately 29.5% silicon (Si) by weight, which is the second most abundant element in soils behind oxygen at 52.7% (Strawn et al., 2015). Although abundant, Si is not considered an essential element for plants since most plants can complete their lifecycles without it (Epstein, 1999). This limited interest in studying the effect of Si on plant growth. In recent years, multiple studies have demonstrated the value of Si for plant growth (Coskun et al., 2019). These studies have been so influential that some argue for a redefinition of "essentiality" because Si accumulates in higher concentrations than many micronutrients and, in some cases, macronutrients in some plant species (Brown et al., 2021; Epstein, 1994). Silicon has been well studied for being advantageous for plants under stress conditions such as: disease suppression, pest mitigation, and tolerance to water stress (Coskun et al., 2019; Zargar et al., 2019). However, much remains unknown or not well-understood. This introduction will review Si availability in soils, how plants uptake the element, how Si is used throughout the plant, the effects of Si on various aspects of plant health, and how Si has the potential to be advantageous in precision drought stress.

Silicon forms and availability in soil

Silicon is an integral element in the structure of many minerals. Silicon is a base element in tetrahedral sheets that can be exchanged with aluminum. Silicon, being a semi-metal, can be found in many forms, but the most abundant form is silicon dioxide (SiO_2), often referred to as silica. Many minerals contain crystalline silica structures such as quartz, keatite, and tridymite (Strawn et al., 2015). Moreover, primary minerals commonly found in most soils are Si-rich. As these primary minerals weather, Si is leached over time creating secondary minerals like kaolinite, smectite, and vermiculite (Strawn et al., 2015). However, just as calcium, magnesium, and other essential plant nutrients, Si inevitably leaches out of soil systems (Strawn et al., 2015). Crystalline Si dissolves slowly in water, but solubility increases at an alkaline pH (Fatzinger & Bugbee, 2021). Due to the unique traits of Si as a semi-metal, Si can also be found as amorphous silica ($SiO_2 \cdot nH_2O$) creating opal. This hydrophilic form of Si is easily soluble in water (Alexander et al., 1954) and is commonly used as desiccants for food preservation.

Although abundant in soils as SiO_2, the bioavailable form of Si in plants is orthosilicic acid, a form of mono-silicic acid [$Si(OH)_4$ or H_4SiO_4]. Moreover, mono-silicic acids are pH sensitive. As mentioned, Si becomes unstable and precipitates in a solution pH of 4-8, so alkaline or acidic solutions are made and then brought to a neutral, plant-friendly pH. Some of these solutions require stabilizers like glycerin or polyethylene glycol (Preari et al., 2014). Polymerization of such mono-silicic acids can influence the concertation of bioavailable Si in solution (Preari et al., 2014).

Epstein (1994) reported the concentration of bioavailable Si in soil solutions ranged from 0.1 to 0.6 mmol Si·L^{-1} (mM Si), which is higher in concentration than some macronutrients like nitrogen. Even if deemed nonessential, all plants take up Si and incorporate the element in their tissues (Boldt et al., 2018; Boldt & Altland, 2021; Epstein, 1994).

Role of silicon in plants

All plants accumulate Si, ranging on average from 0.1 to 10%, but vary in their accumulation (Epstein, 1994, 1999). Silicon accumulating species are defined by having greater than 10,000 mg·kg^{-1} (≥1%) foliar Si in the dry mass of tissue (Epstein, 1994; Ma et al., 2001). Several important species to horticulture and agronomy in Poaceae [wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and sugarcane (*Saccharum officinarum* L.)], Cucurbitaceae [cucumber (*Cucumis sativa* L.), pumpkin (*Cucurbita* pepo L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)], Asteraceae [sunflower (*Helianthus annuus* L.) and zinnia (*Zinnia elegans* Jacq.), and Cannabaceae [cannabis (*Cannabis sativa* L.)] are Si accumulators (Epstein, 1999; Frantz et al., 2010). Non-accumulating, or excluding, species have less than 10,000 mg·kg^{-1} (≤1%) foliar Si (Epstein, 1994; Ma et al., 2001), which includes several ornamental and horticultural species (Epstein, 1999; Boldt & Altland, 2021). Foliar Si is defined as Si concentrations in the aerial shoot because some species accumulate Si in their roots. Boldt and Altland (2021) found that petunia (*Petunia ×hybrida* E. Vilm.), an excluding species, accumulated Si in the roots when excess Si was bioavailable.

Uptake of Si can be either passive or active depending on whether the plant is an accumulating or excluding species (Ma & Yamaji, 2006). Some accumulating species have active or passive uptake of Si. Rice actively uptakes Si with influx (Lsi1) and efflux (Lsi2) transporters in the roots that transfer mono-silicic acid to the xylem then a Si influx transporter (Lsi6) assists with xylem unloading to the aerial shoots. Researching this topic further is crucial to better understand these mechanisms in more species (Farooq & Dietz, 2015; Ma et al., 2006, 2007). Silicon excluders passively uptake Si through mass flow following the transpiration stream (Kaur & Greger, 2019; Ma et al., 2007; Yamaji & Ma, 2007; Yamaji et al., 2008).

Once introduced to the xylem, orthosilicic acid polymerizes to amorphous silica gel and is mobile throughout the plant body (Kaur & Greger, 2019; Yamaji et al., 2008). Once transported, the amorphous gel polymerizes back to silicon dioxide depositing Si cells or aggregates known as phytoliths (Piperno, 1986, 1988, 2006). These immobile phytoliths are found throughout the plant body from roots to shoots to floral structures and fruits to add protection and rigidity until plant death, when they are introduced back into the soil to be recycled by new plant generations (Greger et al., 2018; Jarvis, 1987; Matoh et al., 1991). Silicon is polymerized underneath the cuticle layer, adding overall rigidity and creating a barrier to the epidermal cells, thus minimizing access to the valuable vascular tissue (Coskun et al., 2016; Mitani et al., 2005). Silicon also becomes incorporated in plant cell walls by binding with hemicellulose, adding mechanical support to cell walls and preventing lodging (Guerriero et al., 2016; Hattori et al., 2003; Ma & Yamaji, 2006; Zhao et al., 2013) while fortifying vascular tissues like sclerenchyma cells in species like date palm (*Palma dactylifera* (L.) Mill.) (Bokor et al., 2019; Mitani et al., 2005; Zhao et al., 2013). In the roots, Si aggregates form in the Casparian strip, which these aggregates are proposed to regulate the intake and uptake of various elements and compounds to the vascular system (Fleck et al., 2015; Sangster & Parry, 1976). Silicon has been linked with promoting the creation and maintenance of aquaporins to support water intake (Coskun et al., 2016; Kumawat et al., 2021; Liu et al., 2015). Many plants have trichomes that contain silicon dioxide and are utilized as leaf protective organs to ward off herbivores and other pests (Frantz et al., 2008; Ranger et al., 2009)

Benefits of silicon

Biotic

Many studies have shown the benefit of Si from the aforementioned uses of Si once in the plant body, especially during situations of stress. Both indoor and outdoor agricultural settings struggle with biotic stressors such as pests and disease, which can be alleviated by Si. As stated previously, Si can affect trichome production resulting is decreased predation from herbivores (Frantz et al., 2008; Massey et al., 2006; Pullin & Gilbert, 1989; Reynolds et al., 2009). Some plants, like stinging nettle (*Urtica dioica* L.), have evolved to develop trichomes with irritating solutions that aggravate herbivore tissue that comes in contact with (Pullin & Gilbert, 1989). Plants also deposit silica under the cuticle layer, creating a physical barrier. Not only does this barrier add rigidity to individual cells and the overall plant body, but this barrier also inhibits pests such as aphids and mites from gaining access to photosynthates in tissues (Frantz et al., 2008; Liu et al., 2020; Mitani et al., 2005; Ranger et al., 2009). As pests masticate through the silica layer, the glass-like barrier wears down pests' teeth and jaws, rendering them useless (Frantz et al., 2008). Moreover, this barrier reduces bacterial infection and fungal spore proliferation due to the decreased surface area of accessible tissue (Hayasaka et al., 2008; Kim et al., 2002).

Many economically important crops in both indoor and outdoor agriculture are affected by fungal infections. Li et al. (2019) inoculated pumpkin (*Cucurbita pepo* var. *pepo* L.) with powdery mildew (*Podosphaera xanthii* (Schltdl.) U. Braun & S. Takam. and *Erysiphe cichoracearum* DC.) in plots with and without wollastonite, a calcium silicate, to amend for Si. Silicon supplementation mitigated the effects of the powdery mildew by significantly decreasing colonization on the pumpkin shoots. Other studies have displayed similar benefits of Si for powdery mildew in several species like wheat (Belanger et al., 2003; Guevel et al., 2007; Remus-Borel et al., 2005)], muskmelon [*Cucumis melo* L. (Buttaro et al., 2009), *Arabidopsis* spp. Heynh. in Holl & Heynh. (Fauteux et al., 2006; Vivancos et al., 2015), cucumber (Liang et al., 2005; Schuerger & Hammer, 2003), strawberry [*Fragaria ×ananassa* Duchesne (Liu et al., 2020)], rose [*Rosa* sp. L. (Shetty et al., 2012)], and zucchini [*Cucurbita pepo* Mill. (Savvas et al., 2009)].

Similarly, Si supplementation significantly reduced the infection of rice blast [*Magnaporthe oryzae* (T.T. Hebert) M.E. Barr (Brunings et al., 2009; Hayasaka et al., 2008; Rodrigues et al., 2003)] and wheat blast [*Pyricularia grisea* Sacc. (Filha et al., 2011; Oliveria et al., 2019)] in both of these economically and agriculturally important species. As in aerial shoots, soil studies have suggested that roots are also protected from infections like root rot (*Phytophthora* spp. de Bary) in Si accumulating and non-accumulating species like strawberry (Abd-El-Kareem et al., 2019)], avocado [*Persea americana* Mill. (Bekker et al., 2006), tomato [*Solanum lycopersicum* L. (Huang et al., 2001)], cucumber (Liang et al., 2005), ginseng [*Panax ginseng* Meyer (Abbai et al., 2019)], and rubber tree [*Hevea brasiliensis* Müll.Arg. (Shabbir et al., 2021)]. Many of these studies concluded that the polymerization of Si under the cuticle layer in roots helped decrease disease proliferation.

Abiotic

Just as Si increases biotic stress tolerance, abiotic stressors that are becoming more frequent and intense with the changing climate can be ameliorated with Si supplementation. With strict water budgets and hotter average temperatures, drought stress is devastating crops world-wide. Hattori et al. (2005) grew two sorghum (*Sorghum bicolor* (L.) Moench) cultivars (drought tolerant and drought sensitive) in sand-based media supplemented with and without potassium silicate (K_2SiO_3). Both cultivars with and without Si supplementation were not affected by Si when supplied with ample water, but after a 22-day dry period, the dry mass of both cultivars decreased only 24-26% when supplemented with Si whereas the non-supplemented sorghum cultivars were 77-80% less compared to the well-watered controls. Hattori et al. (2005) concluded that the Si supplemented sorghum cultivars were able to extract more water from the media, regulate transpiration better, and maintain a higher stomatal conductance resulting in increased water use efficiency versus the non-amended treatments. Other studies have shown similar advantages of Si supplementation with drought (Ahmed et al. 2011; Chen et al. 2011; Gao et al., 2004, 2006; Gong et al., 2003, 2005; Gong & Chen, 2012; Hattori et al., 2008; Lobato et al., 2009).

Not only has Si been linked to regulated transpiration and stomatal conductance, but has also been suggested to give mechanical support to cells, which prevents lodging, fortifies fine root hairs, and helps maintain turgor pressure (He et al., 2015; Ma & Yamaji, 2006; Savant et al., 1999; Zhao et al., 2013). The fortification of fine root hairs allows the roots to survive drought conditions longer and helps the plant obtain water to sustain photosynthesis and cell elongation. Silicon has been proposed to also promote the creation of aquaporins that distribute the acquired water more efficiently (Coskun et al., 2016; Kumawat et al., 2021; Liu et al., 2015). Similar mechanisms are used to increase tolerance of crop species to salinity stress.

Oxidative stress is a key factor to both drought and salinity stress damage. Silicon has been linked with oxidative stress management within the plant body (Coskun et al., 2016; Farooq et al., 2013; Hossam et al., 2020). Many studies associate Si with increased antioxidant activity and concentrations of oxidative-stress induced compounds, but some argue that Si mitigates stress-induced strains (Coskun et al., 2016). For example, Si aggregates strengthening the Casparian strip is proposed to increase regulation of water and beneficial nutrient uptake, limiting the amount of salts, or other undesirable ions, that enter the vascular tissue and are retained in the root tissue (Coskun et al., 2016; Li et al., 2008; Liang et al., 2007).

Heavy metal toxicity is also alleviated by Si (Wu et al., 2013). Just as in salinity stress, Si has been linked to retaining heavy metals in the root tissue and preventing their entry to the vascular tissue and therefore the aerial shoots, thereby reducing oxidative stress (Liang et al., 2007; Wu et al., 2013). This effect has been noted with metals such as aluminum (Puntigo et al., 2017; Yunxia et al., 2004), cadmium (Farooq et al., 2013; Farooq et al., 2016), copper (Bosnic et al., 2019; El-Beltagi et al., 2020; Filho & Monterio, 2020; Flora et al., 2019; Frantz et al., 2011; Li et al., 2008), lead (Bharwana et al., 2013; Tripathi et al., 2016), manganese (Doncheva et al., 2009; Iwasaki & Matsumura, 1999), and zinc (Gu et al., 2012; Mehrabanjoubani et al., 2015; Song et al., 2011). Some proposed mechanisms are as previously stated, with Si "selecting" certain ions to be introduced into the vascular tissue or remain in the root tissue, while some argue that Si alters soil composition by changing pH, making certain heavy metals biologically unavailable (Liang et al., 2007; Matichenkov & Bocharnikova, 2001).

Although the benefits of Si during abiotic stress are well-known and accepted, many of the aforementioned mechanisms are proposed and require further investigation (Coskun et al., 2019). Furthermore, some studies have conflicting, inconclusive, and/or insignificant results on the suggested benefits of Si during drought stress, thus highlighting the importance of conducting further research (Coskun et al., 2019; Janislampi, 2012; Tibbitts, 2018).

Role of silicon in nutrient availability

Silicon has been shown to mitigate heavy metal toxicity, but macronutrients such as nitrogen (Cuong et al., 2017; Shen et al., 2009), phosphorus (Cuong et al., 2017; Shen et al., 2009), potassium (Cuong et al., 2017; Shen et al., 2009), magnesium (Greger et al., 2018), and calcium (Greger et al., 2018) have been found to increase with Si supplementation. Silicon is proposed to increase availability of the nutrients in the soil solution and/or nutrient partitioning within the plant tissue with and without stress. Greger et al. (2018) found that Si decreased nitrogen and potassium uptake, but increased phosphorus availability in maize (*Zea mays* L.), lettuce (*Lactuca sativa* L.), carrot (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens), wheat, and pea (*Pisum sativum* L.). However, this contradicts what Shen et al. (2009) found when soybean (*Glycine max* (L.) Merr.) supplemented with Si showed increased nitrogen, phosphorus, and potassium uptake under UV-B radiation in hydroponics and Cuong et al. (2017), who found similar results with rice grown in soil. These differences can be from media type, species type, and differences of Si application.

Silicon has been well documented to increase phosphorous availability in soils, thus reducing phosphorus deficiency in multiple crop species (Greger et al., 2018; Koski- Vähälä, 2001; Kostic et al., 2017; Ma & Takahashi, 1990; Owino-Gerroh & Gascho, 2005;). Kostic et al. (2017) grew winter wheat 'Pobeda' in acidic (pH 4.0) sandy loam textured soil with either low phosphorous concentrations with Si supplementation (Na_2SiO_3), no pH adjustment to the soil with ample phosphorus, or low phosphorus with calcium carbonate ($CaCO_3$) supplementation. After four weeks, soil samples and plant tissue samples indicated that the Si and calcium carbonate treatments significantly increased the biomass of the wheat. The addition of Si also increased the uptake of phosphorus and the total concentration of phosphorus in the wheat comparatively to the addition of phosphorus alone. Moreover, the Si supplemented wheat took up more phosphorus from the soil than the phosphorous-fertilized wheat, leading to more efficient use of the phosphorus. This has larger implications for the reduction of phosphorus run-off to waterways and would decrease the potential of eutrophication. Also, the increase of soil pH reduced the availability of aluminum.

Supplemental Si increased the uptake of some micronutrients, such as iron (Bityutskii et al, 2014; Greger et al., 2018; Pavloviv et al., 2013, 2016), and boron (Greger et al., 2018). Although the literature suggests positive, and some negative, relationships of Si on essential nutrient uptake, more research must be done to better understand these mechanisms in controlled environments and in the field.

Supplementing silicon in soilless media

Many nursery and greenhouse growers do not supplement their media with Si and it is minimally bioavailable in soilless media. However, Si is beneficial as previously highlighted. Silicon can be supplemented through liquid fertilization, but it is difficult to maintain the solubility of Si in higher concentrations unless the solution is maintained at an alkaline pH (pH=11.3) (Fatzinger & Bugbee, 2021). Foliar applications can be used, but the Si does not become incorporated in the plant tissue. Rather a film is created on the surface of the cuticle layer for pest management (Asgharipour & Mosapour, 2016; Menzies et al., 1992; Oliveira et al., 2019). Studies have supported the mentioned findings and have suggested that root-available Si is the most effective mechanism for Si accumulation, while foliar sprays are effective for biotic stress, but do not have much of an effect on growth or yield (Guével et al., 2007; Laane, 2018; Liang & Sun, 2005; Suriyaprabha et al., 2014). Media amendments provide a simple way to supplement Si to the root-zone solution (Dallagnol et al., 2012; Haque et al., 2019; Liang et al., 2005; Pilon et al., 2013).

Components sold for this purpose include parboiled rice hulls, steel slag, diatomaceous earth, and wollastonite. Although many studies have reported results of plants grown with various Si-containing additives, the release rate of bioavailable Si is poorly characterized.

Silicon is known to be beneficial, but is minimally available in soilless media and some soil types. Supplementation is crucial to achieve the aforementioned effects of the nutrient.

Objectives and Hypotheses

Objective 1: The objective of this research is to characterize the rate of bioavailable silicon from dissolution of ten media components in soilless media.

Hypothesis 1: The dissolution of wollastonite will be more consistent than other media additives.

Objective 2: The objective of this research is to compare colorimetric analysis to inductively coupled plasma – optical emission spectrometry for the analysis of bioavailable silicon.

Hypothesis 2: Colorimetric measurements are as accurate as inductively coupled plasma optical emissions spectroscopy for bioavailable silicon analysis

Objective 3: The objective of this research is to investigate the value of supplementing silicon using wollastonite to increase the resiliency of *Cannabis* to drought stress.

Hypothesis 3: The addition of silicon to soilless media will reduce the detrimental effects of water stress on dry mass and plant height compared to treatments without silicon

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CHAPTER II

BIOAVAILABLE SILICON: DISSOLUTION FROM SUBSTRATES AND MEDIA ADDITIVES

Abstract. Silicon (Si) is beneficial for plants but is minimally bioavailable in soilless media. Several Si amendments are commercially available, but the dissolution of Si is not well characterized. The ideal additive would have a steady release of bioavailable Si over time. Then dissolution of Si from media additives and substrates in water and in peat-based media were characterized. Perlite, sphagnum peat moss, vermiculite, and coconut coir minimally released Si ($\leq$ 0.03 mmol Si·L^{-1} substrate·day^{-1}) in shaken water containers. The dissolution of Si from rice hulls averaged 0.21 mmol Si·L^{-1} rice hulls·day^{-1} for 60 days in water. The dissolution of wollastonite (VanSil® W-10), a calcium-silicate, released an average of 4 mmol Si·L^{-1} wollastonite·day^{-1} for 160 days. Studies showed that a peat-based media amended with wollastonite (1 g wollastonite·L^{-1} peat) and leached with 150 mL nutrient solution twice a week peaked with a release of 2.1 mmol Si·L media^{-1}·leaching event^{-1}, then gradually decreased over 120 days. The release of Si from rice hulls in peat-based media (12% rice hulls by volume), however, gradually increased over time. After 120 days, pH of the wollastonite-amended peat-based media increased by 0.8 pH units, whereas the rice hull-amended media increased by 1.0 pH unit. Both amendments increased coconut coir-based media in 30 days, but varied based on leachate volume. These results suggest that supplementing soilless media with rice hulls to increase Si bioavailability

would be beneficial for long-term, container-grown crops and wollastonite would be beneficial for crops in greenhouse production with shorter lifecycles.

Introduction

Silicon (Si) is the second most abundant element in soils after oxygen (Epstein, 1999). While predominately found as silicon dioxide (SiO_2), the bioavailable form for plants is known as mono-silicic acid, ortho-silicic acid, or silicic acid (H_4SiO_4 or $Si(OH)_4$). Ortho-silicic acid and silicic acids are forms of mono-silicic acid, but these terms are used interchangeably (Laane, 2018). Although abundant in natural soils, Si is not considered an essential nutrient for plants because many species do not need it to complete their lifecycle (Epstein, 1994). Some argue for Si to be redefined as essential because many studies have shown the benefits of Si, such as reducing biotic and abiotic stress, in outdoor and indoor agriculture (Brown et al., 2021; Epstein, 1994; Verma et al., 2021). Natural soils consist of 31% Si on average (Epstein, 1999), but soilless media, often composed of sphagnum peat moss, pine bark, and/or perlite, has minimal bioavailable Si (Frantz et al., 2010). Supplementing Si in soilless media is becoming more important with increasing greenhouse and indoor agriculture production (Voogt & Sonneveld, 2001).

Recent studies have highlighted the benefit of supplying Si in soilless media on plant morphology and biotic resistance. Silicon supplementation has improved floral traits such as flower diameter, stem height, stem straightness, and stem thickness in gerbera [*Gerbera jamesonii* Bolus ex. Hooker f. (Kamenidou et al., 2010)], sunflower [*Helianthus annuus* L. (Kamenidou et al., 2008)], and zinnia [*Zinnia elegans* Jacq. (Kamenidou et al., 2009)]. Mattson and Leatherwood (2010) reported that some ornamental crops petunia (*Petunia ×hybrida* (Sweet) D. Don ex W. H. Baxter) and argyranthemum (*Argyranthemum* sp. Webb ex Sch.Bip.) increased in height, diameter, dry weight, and/or flower diameter with Si accumulation, but these results were species dependent. Shi et al. (2014) found that Si alleviated oxidative stress of tomato (*Solanum lycopersicum* L.) seedlings and improved seed germination during drought stress. Heine et al. (2007) found that Si supplementation in peat-based media increased resistance of tomato and bitter gourd (*Mormodica charantia* L.) roots to *Pythium aphanidermatum* (Edson) Fitzp. Jeong et al. (2012) reported that potassium silicate drenches on chrysanthemum (*Chrysanthemum ×morifolium* (Ramat.) Hemsl.) reduced aphid (*Macrosiphoniella sanborni* Gillette) colonies by 40-57% compared to non-Si-supplemented controls. Others found decreased susceptibility to common diseases like powdery mildew (*Golovinomyces* spp. (U.Braun) V.P.Heluta) with increasing Si bioavailability (Savvas et al., 2009; Shetty et al., 2012; Tibbitts, 2018).

There are several methods to supply Si, including foliar sprays (Asgharipour & Mosapour, 2016; Mantovani et al., 2018; Menzies et al., 1992), fertigation (Boldt et al., 2021; Buck et al., 2010; Nikpay & Soleyman-Nejadian, 2014), and media amendments (Altland et al., 2016; Boldt et al., 2018; Jayawardena et al., 2016; Sistani et al., 1997; Somapala et al., 2016). Foliar sprays can be useful, and are commonly used for pest or disease control as it creates an effective film over the cuticle, but the

Si does not become incorporated in the plant tissue (Li et al., 2020). Some studies suggest that foliar sprays are not effective compared to soil or substrate-applied Si if accumulation is the goal (Dallagnol et al., 2012; Liang et al., 2005; Pilon et al., 2013). A common way to supply Si to the root-zone in containers is by fertigation, but Si solubility can be difficult to maintain at higher concentrations in stock solutions. Some Si forms, like potassium silicates, can become insoluble and precipitate when pH decreases below 11.3 (Fatzinger & Bugbee, 2021). This can abrade dosing injectors over time and clog drip emitters if pH is not monitored and corrected. Media amendments can be a simpler way to supplement Si in the root-zone (Haque et al., 2019).

Commercially available Si additives include steel slag, rice hulls, diatomaceous earth, and wollastonite. Steel slag, a byproduct of the metal industry, has been well investigated as a Si supplement; it also provides magnesium and calcium (Das et al., 2020; Ito et al., 2015; Zhang et al., 2017). Frantz et al. (2010) found that steel slag had a lower release rate in water than rice hulls.

Rice hulls are well-studied and are commonly used to supplement Si (Boldt et al., 2018; Frantz et al., 2010; Jayawardena et al., 2016; Sistani et al., 1997; Somapala et al., 2016). Diatomaceous earth has been used as a form of pest control but is less studied (Mills-Ibibofori et al., 2019; Pati et al., 2016). Wollastonite is a naturally occurring calcium silicate mineral ($CaSiO_3$) that is about 51% SiO_2.Wollastonite has shown promise as a Si amendment but is not as well studied (Haque et al., 2019, 2020). Frantz et al. (2010) tested several steel slags, wollastonite, and parboiled rice hulls added to a peat-based media for 21 days and found that rice hulls released the highest amount of Si per gram of material tested, steel slag released the second, and wollastonite released the least. However, Si release rates from these products over time are not well characterized.

Therefore, the dissolution of bioavailable Si over time from diverse media components and additives were characterized.

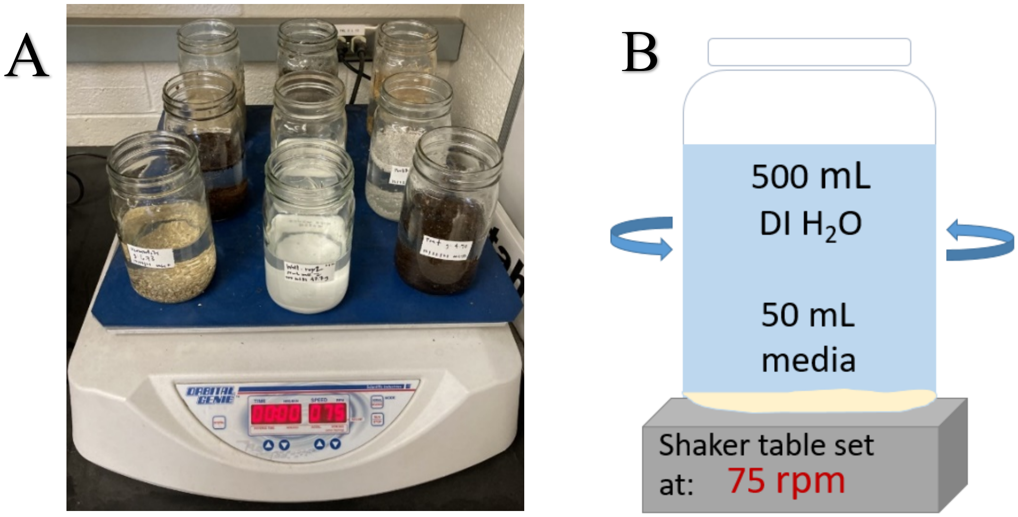
Materials and Methods

Release rate in water

Dissolution of Si from sphagnum peat moss (fibrous blond, ProMoss® III TBK; PRO-MIX, Quebec, Canada), coconut coir (Black Gold®; Sun Gro Horticulture, Agawam, MA), expanded coarse perlite (Hess®, Hess Perlite, Malad City, ID), vermiculite (Horticultural coarse grade; Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH), coarse diatomaceous earth (AxisDE®; EP Minerals, LLC, Reno, NV), powdered diatomaceous earth (Natural DE®; EP Minerals), parboiled rice hulls (PBH; Riceland Foods, Inc., Stuttgart, AK), steel slag (Plant Tuff®; Levy Corp., Dearborn, MI), Ottawa sand (30-40 mesh particle size; VWR Chemicals BDH®, Ottawa, Canada), play sand (Sandtastik® Sparkling White Play Sand; Sandtastik Products Ltd., Port Colborne, Ontario, Canada), rock wool (GRO-BLOCK™, Grodan®, Roermond, The Netherlands) and wollastonite (VanSil® W-10; Vanderbilt Minerals, LLC, Norwalk, CT) were quantified. Plant Tuff® had four replicate jars, wollastonite three replicates, and rice hulls three replicates to ensure repeatability of the release rates. The mass of 50 mL of each media component was measured, added to a quart-sized jar, then filled to 500 mL with deionized (DI) water (Figures 2.1A-B). These glass jars minimally leached Si and did not affect release rate concentrations (data not shown). Filled jars were placed uncovered on an orbital shaker (Orbital-Genie™ Benchtop Orbital Shaker; Scientific Industries, Inc., Bohemia, NY) at 75 rpm on a lab bench (air temperature 25 ± 2 C). Every week jars were hand mixed by swirling prior to sampling and then filtered with Whatman® grade 1 (11 µm) cellulose filter paper (Cytiva, Marlborough, MA). Solution Si concentrations were then measured by using a colorimeter (LaMotte® SMART 3 colorimeter; La Motte, Chestertown, MD). The remaining solution in each jar was decanted, and the water was replaced with 450 mL of deionized (DI) water for a total volume of 500 mL. Measurements of media components with steady release rates of Si were terminated after 60 days, and those without were continued for an additional 70-100 days.

Figure 2.1

The Method of the Dissolution of Silicon from Substrates and Media Additives in Water Method



Note. Dissolution of silicon (Si) from substrates and media additives in water were measured in glass jars that minimally leached Si. Method shown by photo (A) and diagram (B).

Release rate from soilless media

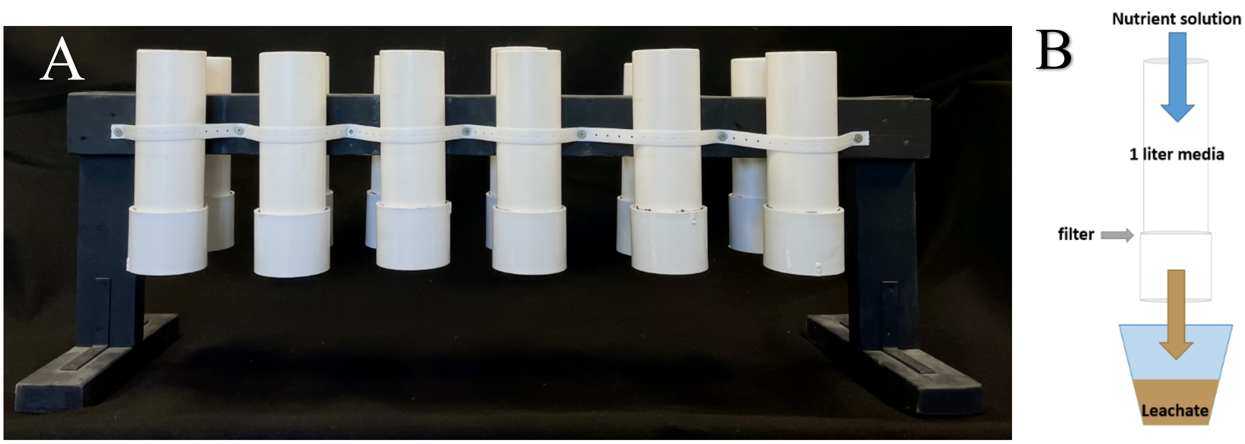
Wollastonite and rice hulls were further studied in soilless media. Each column was constructed out of 3-inch diameter (7.62 cm), 10-inch (25.4 cm) tall polyvinylchloride (PVC) piping fit with a filter of landscape fabric at the bottom and had a total media volume of 1 L (Figures 2.2A-B). Wollastonite-amended columns included 1 gram wollastonite, 0.75 grams wetting agent (AquaGro® 2000 G; Aquatrols, Paulsboro, NJ), and 1.23 grams of hydrated lime (to adjust pH to 6.0; Chemstar® Type S lime; Chemstar Products, Minneapolis, MN) per liter of sphagnum peat moss. Rice hull-amended columns included 12% rice hulls by volume, 0.75 grams wetting agent, and 1.5 grams hydrated lime per L of sphagnum peat moss to adjust pH to 6.0.

Rice hulls and wollastonite were also added to coconut coir to study the effect of these additives on media pH over time. Rice hull-amended columns were comprised of 12% rice hulls by volume and 0.75 g wetting agent per L coconut coir. Wollastonite-amended columns were comprised of 1 g wollastonite and 0.75 g wetting agent per L coconut coir. Coconut coir pH started at 6.5 $\pm$ 0.08 pH units, thus no pH adjustment was made. Total media volume in each column was 1 L.

All columns were initially flushed with 600 mL of tap water (Logan, UT) to compact the soilless media. The columns were subsequently leached using the pour-through method (Yeager et al., 1983) twice weekly (2 to 5 days between flushes) with 150, 300, or 600 mL of nutrient solution. Each treatment had two replicates. The nutrient solution contained 1.5 $mmol \cdot L^{-1}$ (mM) $Ca(NO_3)_2$, 2.25 mM NH_4Cl, 2 mM KNO_3, 0.8 mM $MgSO_4$, 0.35 mM HNO_3, 3 µM $MnCl_2$, 3 µM $ZnCl_2$, 40 µM H_3BO_3, 4 µM Cu-EDTA, 0.1 µM Na_2MoO_4, and 0.1 µM $NiCl_2$ (pH: 6.0 $\pm$ 0.1; electrical conductivity (EC): 1.2 $\pm$ 0.06 $mS \cdot cm^{-1}$). Nutrient solution was made with reverse osmosis water to reduce the addition of Si and was titrated with potassium hydroxide (KOH) or hydrochloric acid (HCl) before flushing to maintain pH at 6.0 $\pm$ 0.1. Phosphorous (P) and iron (Fe) were excluded from the nutrient solution due to interferences with the colorimeter analysis. If P and Fe had been included, inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis would have been required. Leachate was collected in plastic containers until there was no residual dripping (≈60 min.). Leachate pH (Oakton® pH electrode, Oakton Instruments, Vernon Hills, IL; Hanna HI2209 Benchtop pH Meter, Hanna Instruments, Smithfield, RI) was immediately measured. Silicon concentrations were measured by colorimeter.

Figure 2.2

The Method of the Dissolution of Silicon from Substrates and Media Additives from Media



Note. A photograph of the 12 columns that were used to quantify release rate of silicon (Si) in media (A) and a diagram to show leaching events (B).

Wollastonite pH

The dissolution of wollastonite releases two molecules of hydroxide for every molecule of Si (Equation 1).

$$CaSiO_3 + 3H_2O \rightarrow H_4SiO_4 + 2OH^- \quad (1)$$

Colorimetric analysis

Silicon concentrations of leachates were quantified colorimetrically using the heteropoly blue method (Eaton et al., 2005; LaMotte® silica low range test kit 3664-

SC, La Motte, Chestertown, MD). The silica low range test was used to have higher resolution of Si in solution. Samples were blanked with DI water to account for color added by reagents and operator effect. The concentration of the blank was subtracted from the concentration of each leachate sample. A blank was made for each treatment solution every time leachates were tested. Samples were then analyzed by ICP-OES at the Utah State University Analytical Laboratory (USUAL; Logan, UT) to test the accuracy of colorimetric analysis.

Heavy metal analysis

Basil (*Ocimum basilicum* 'Genovese') and sunflower 'Pacino Gold' were grown for six weeks in soilless media amended with wollastonite, steel slag, or no Si additive for heavy metal uptake analysis. Pots (11.4-cm diameter) were filled with a base substrate of 85 sphagnum peat moss : 15 coarse grade perlite (v:v; SunGro Horticulture) and 0.196 $L \cdot m^{-3}$ of a wetting agent (SOAX, Oasis Grower Solutions, Kent, OH). It was amended with 1.17 $g \cdot L^{-1}$ wollastonite (VanSil® W-10) and 1.78 $kg \cdot m^{-3}$ dolomitic limestone (ECOpHRST; National Lime and Stone Co., Findlay, OH), with 7.12 $g \cdot L^{-1}$ steel slag (PlantTuff®) and 1.48 $kg \cdot m^{-3}$ lime, or only with 2.67 $kg \cdot m^{-3}$ lime. This provided ≈170 g Si per pot to the Si-amended treatments and adjusted initial media pH to 6.0 for all treatments. Each treatment contained three replicates per species.

Plants were grown in a glass-glazed greenhouse (Toledo, OH) from 24 January to 7 March 2020. Supplemental irradiance was provided by 1000-W high pressure sodium lamps between 0600 – 2000 HR when benchtop ambient irradiance was less than 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD). Air temperature and PPFD were measured with aspirated thermocouples and quantum sensors (MQ-200; Apogee Instruments, Logan, UT), respectively, and recorded every 15 min using a Campbell Scientific datalogger (CR10X; Campbell Scientific, Logan, UT). Mean air temperatures were 25.2 ± 1.2 °C day/21.0 ± 1.9 °C night, and mean daily light integral (DLI) was 11.4 ± 1.3 $mol \cdot m^{-2} \cdot d^{-1}$.

Plants were irrigated as needed with 15N-2.2P-12.4K (Jacks 15-5-15, JR Peters, Inc., Allentown, PA) at a concentration of 150 $mg \cdot L^{-1}$ N. At two and four weeks after transplant, plants were provided 150 mL of magnesium sulfate (1.06 $g \cdot L^{-1}$ magnesium sulfate heptahydrate; Magriculture, Giles Chemical, Waynesville, NC).

After six weeks, aboveground tissue was separated into leaves and stems (including stems and inflorescences). They were dipped in acidified water (0.1 M HCl), rinsed in 18 MΩ water, placed in paper bags, dried in a forced-air oven at 60 °C for a minimum of five days, and weighed for dry mass. Leaves were ground into a fine powder using a mortar and pestle for elemental analysis. Tissue samples were digested in nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) for heavy metal analysis with ICP-OES at USUAL.

Results

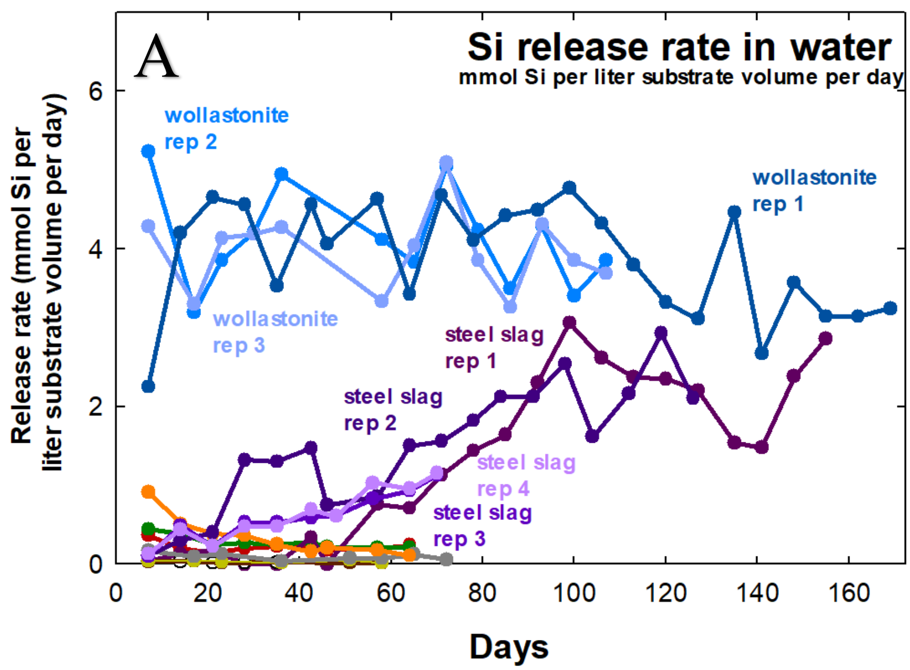
Release rate in water

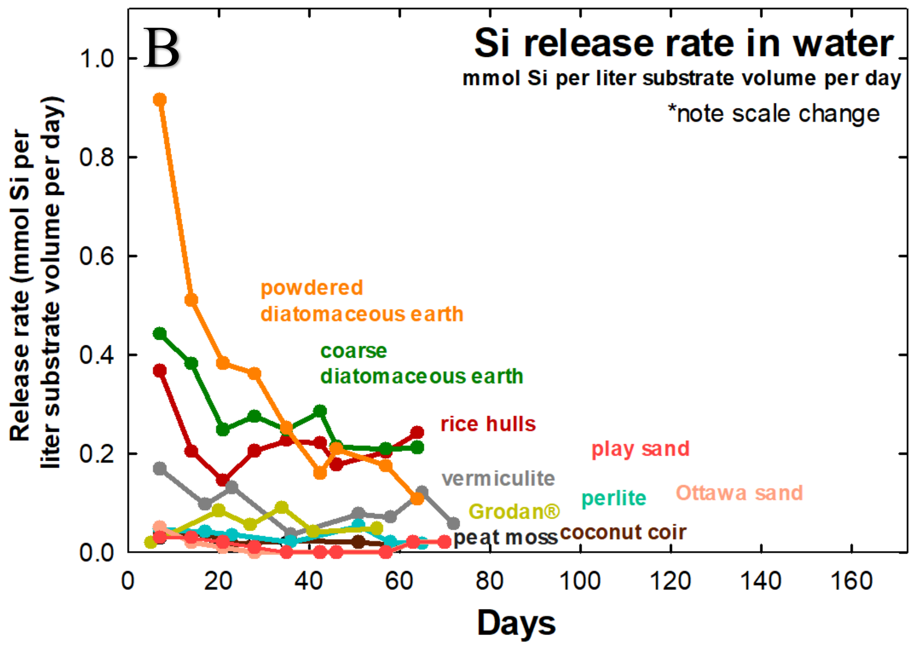
The dissolution of Si from wollastonite had the highest release rate in water (4.03 $\pm$ 0.65 mmol $Si \cdot L^{-1}$ $wollastonite \cdot day^{-1}$) over 150 days (Figure 2.3A; Table 2.1).

Peat moss, coconut coir, perlite, and sand released the least Si (< 0.03 mmol Si·L^{-1} substrate·day^{-1}; Figure 2.3B).

Figure 2.3

Dissolution of Silicon from Media Components in Water





Note. Wollastonite (Vansil® W-10) released the most silicon (Si) over 160 days (A). Release rates without wollastonite and steel slag (Levy Plant Tuff®) are graphed (B). Note the scale change. Media components minimally released Si and were depleted before day 60.

Release rates from Figure 2.3A-B are given per volume and mass listed in Table 2.1.

Table 2.1

Numerical Summary of Figure 2.3

Substrate or additive	mmol Si per day per liter substrate	mmol Si per day per kilogram substrate
Wollastonite (n=3)	4.03 $\pm$ 0.65	4.64 $\pm$ 0.37
Steel slag (n=4)	0.67 $\pm$ 0.45	0.34 $\pm$ 0.14
Rice hulls (n=3)	0.22 $\pm$ 0.07	1.63 $\pm$ 0.08
Powdered diatomaceous earth	0.34	0.95
Coarse diatomaceous earth	0.28	0.64
Vermiculite	0.08	0.55
Grodan®	0.04	< 0.001
Play sand	0.03	0.02
Ottawa sand	0.03	0.02
Coconut coir	0.01	0.39
Perlite	0.02	0.14
Peat	0.01	0.08

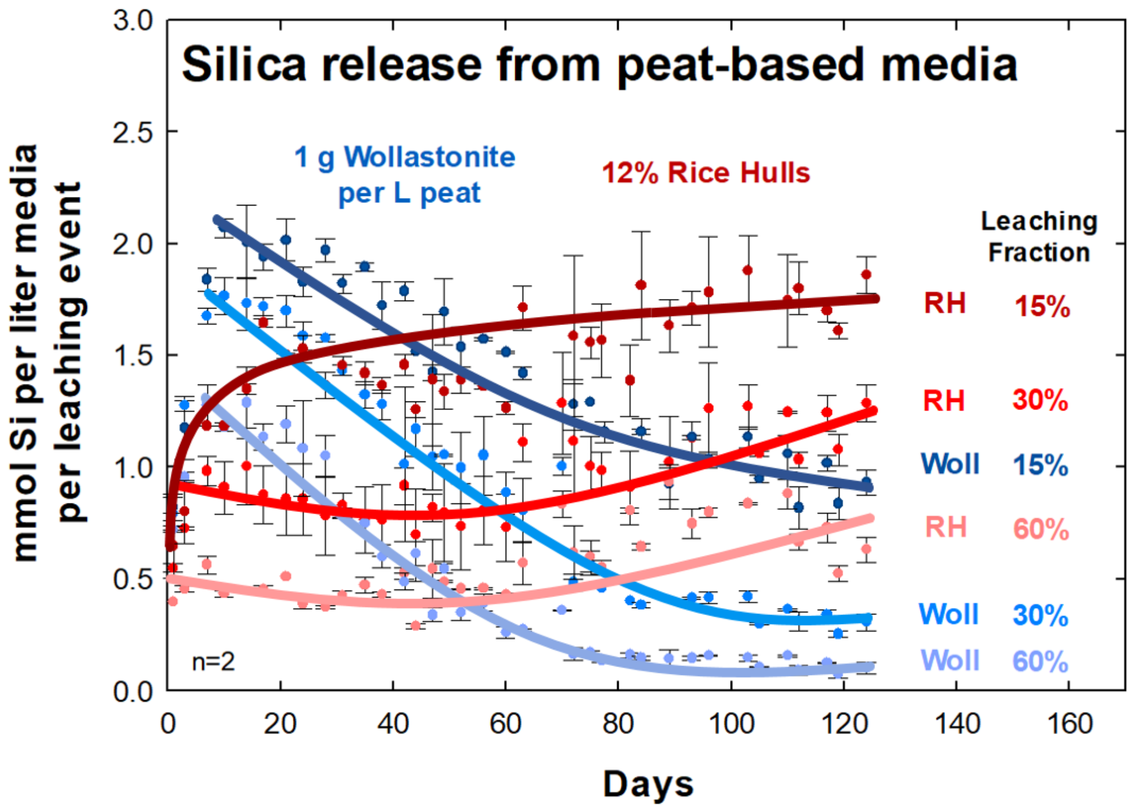
Note. This table shows the average release rates by volume and mass up to 60 days. Peat moss, coconut coir, vermiculite, Grodan®, and perlite were measured until silicon (Si) depletion (30-40 days). Where sample size is not listed, one replicate was evaluated.

Si release from soilless media

Rice hull-amended peat leached with 150 mL nutrient solution (15% leaching fraction) released an average of 1.47 $\pm$ 0.27 mmol Si·L^{-1} media·leaching $event^{-1}$, 300 mL (30% leaching fraction) released 0.95 $\pm$ 0.20 mmol Si·L^{-1} media·leaching $event^{-1}$, and 600 mL (60% leaching fraction) released 0.57 $\pm$ 0.17 mmol Si·L^{-1} media·leaching $event^{-1}$ over 120 days (Figure 2.4). Rice hulls released steadily and gradually increased over time, while wollastonite peaked at day 10, then decreased over time. Wollastonite-amended peat with a 15% leaching fraction peaked at 2.1 mmol Si·L^{-1} media·leaching $event^{-1}$ then decreased to about 0.85 mmol Si·L^{-1} media·leaching $event^{-1}$ at day 120 but averaged 1.44 $\pm$ 0.40 mmol Si·L^{-1} media·leaching $event^{-1}$ over the span of the study. Wollastonite columns with a 30% leaching fraction peaked at 1.8 mmol Si·L^{-1} media·leaching $event^{-1}$ and decreased to 0.25 mmol Si·L^{-1} media·leaching $event^{-1}$ but averaged 0.94 $\pm$ 0.51 mmol Si·L^{-1} media·leaching $event^{-1}$ over 120 days. Wollastonite columns with a 60% leaching fraction peaked at 1.3 mmol Si·L^{-1} media·leaching $event^{-1}$ then decreased to less than 0.1 mmol Si·L^{-1} media·leaching $event^{-1}$ but averaged 0.50 $\pm$ 0.41 mmol Si·L^{-1} media·leaching $event^{-1}$ over 120 days (Figure 4).

Figure 2.4

Release of Silicon from Peat Moss Amended with Wollastonite or Rice Hulls



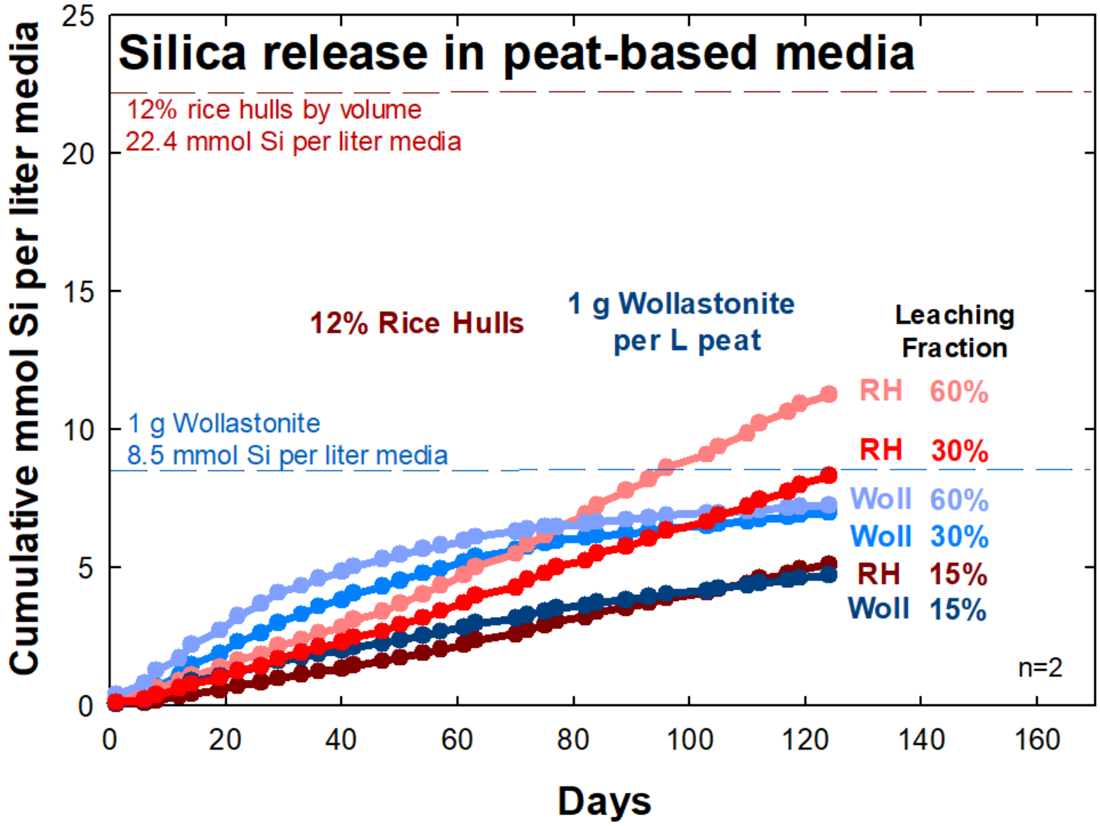
Note. Release of silicon (Si; mmol Si·L^{-1} media·leaching event^{-1}) over 120 days. Each data point represents the average of two replicate columns with error bars representing standard deviation (n=2). Leachate Si concentration correlated with leaching volume and time between leaching events. Some of the variability was associated with the time interval between leaching events, which varied from 2 to 6 days. The longer intervals tended to increase the concentration of Si in the leachate as the amendments were able to release more Si.

Leaching volume influenced the release and concentration of Si in leachates. For both amendments, leaching with 600 mL (60% leaching fraction) resulted in a consistent release of Si that quickly depleted wollastonite after 120 days (Figure 2.4).

The addition of 12% rice hulls, leached with 600 mL, surpassed the calculated maximum concentration of Si that could be released by wollastonite (8.5 mM Si) in 96 days (Figure 2.5). Wollastonite was depleted of Si for both the 300 and 600 mL treatments in about 100 days. Both amendments released similar amounts of Si when leached with 150 mL (Figure 2.5).

Figure 2.5

Cumulative Concentration of Silicon Released by Wollastonite or Rice Hulls in Peat Moss



Note. Cumulative concentration of silicon (Si) released by wollastonite (1 g·L^{-1} peat) and rice hulls (12% incorporation, by volume) over time. Each data point represents the average release from two columns (n=2).

Effect of amendment on pH

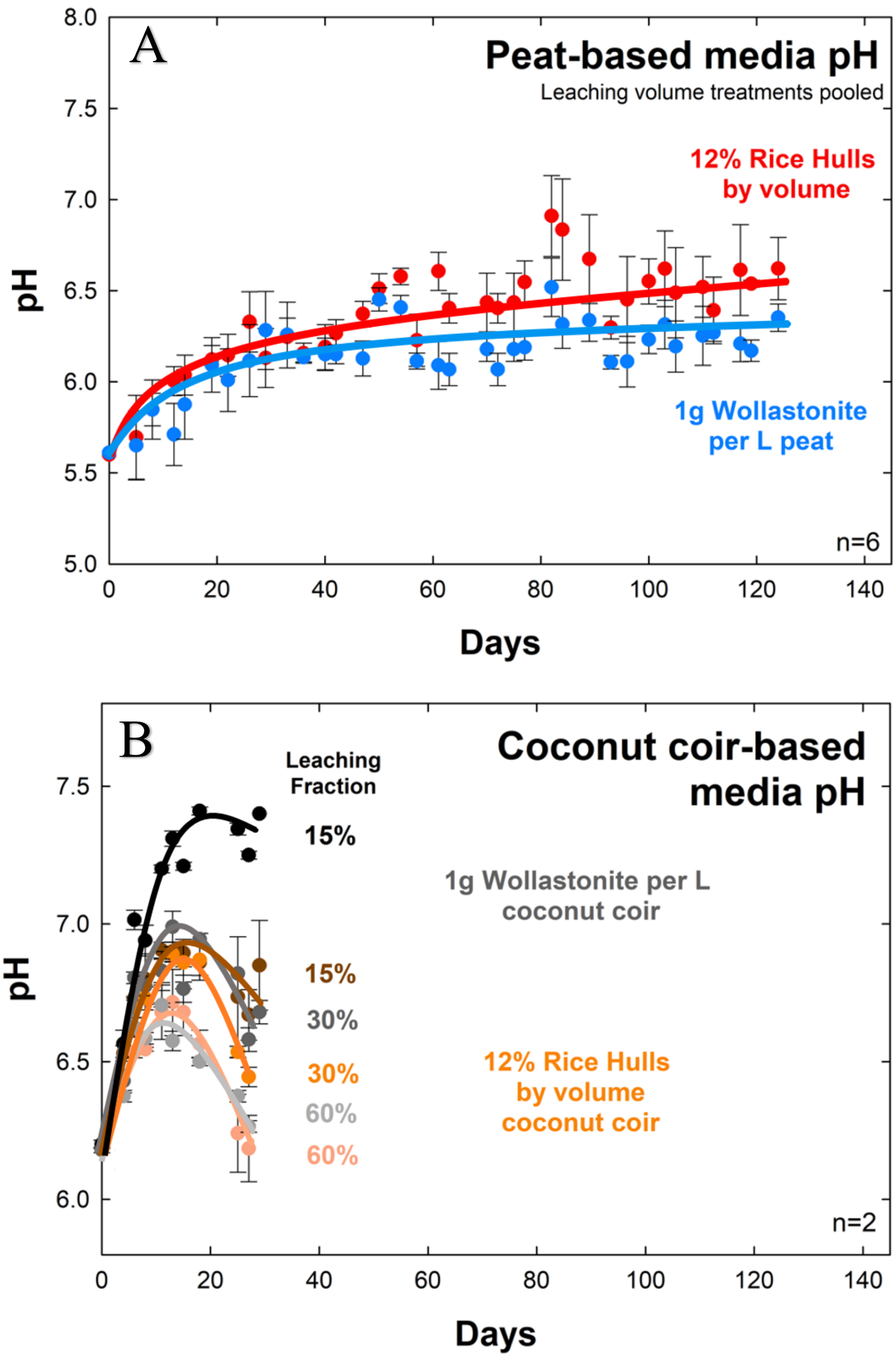
Wollastonite (1 g·L^{-1} peat) and rice hulls (12% incorporation, by volume) increased peat-based media pH over time (Figure 2.6A). Across all three leaching volume treatments, wollastonite raised media pH about 0.8 pH units while rice hulls raised media pH about 1.0 pH units over 120 days (Figure 2.6A).

Both wollastonite (1 g·L^{-1} peat) and rice hulls (12% incorporation, by volume) in coconut coir-based media increased media pH, but varied based on leaching volume (Figure 2.6B) unlike peat-based media. Wollastonite amended coconut coir based-media leached with a 15% fraction raised media pH by 1.2 units and has stabilized at around a pH of 7.35. A leaching fraction of 30% increased pH to 7.0 then decreased to 6.6 in 30 days. A leaching fraction of 60% increased media pH to 6.6 then decreased to 6.3. Rice hull amended coconut coir based-media pH increased to 6.9 then decreased to 6.75 with a 15% fraction. A 30% leaching fraction increased media pH to 6.9 then decreased to 6.45. A 60% leaching fraction increased media pH 6.65 then decreased to 6.2.

Both amendments affected media pH, but the substrate base was influenced differently (Figure 2.6A-B).

Figure 2.6

Effect of Amendment on Media pH



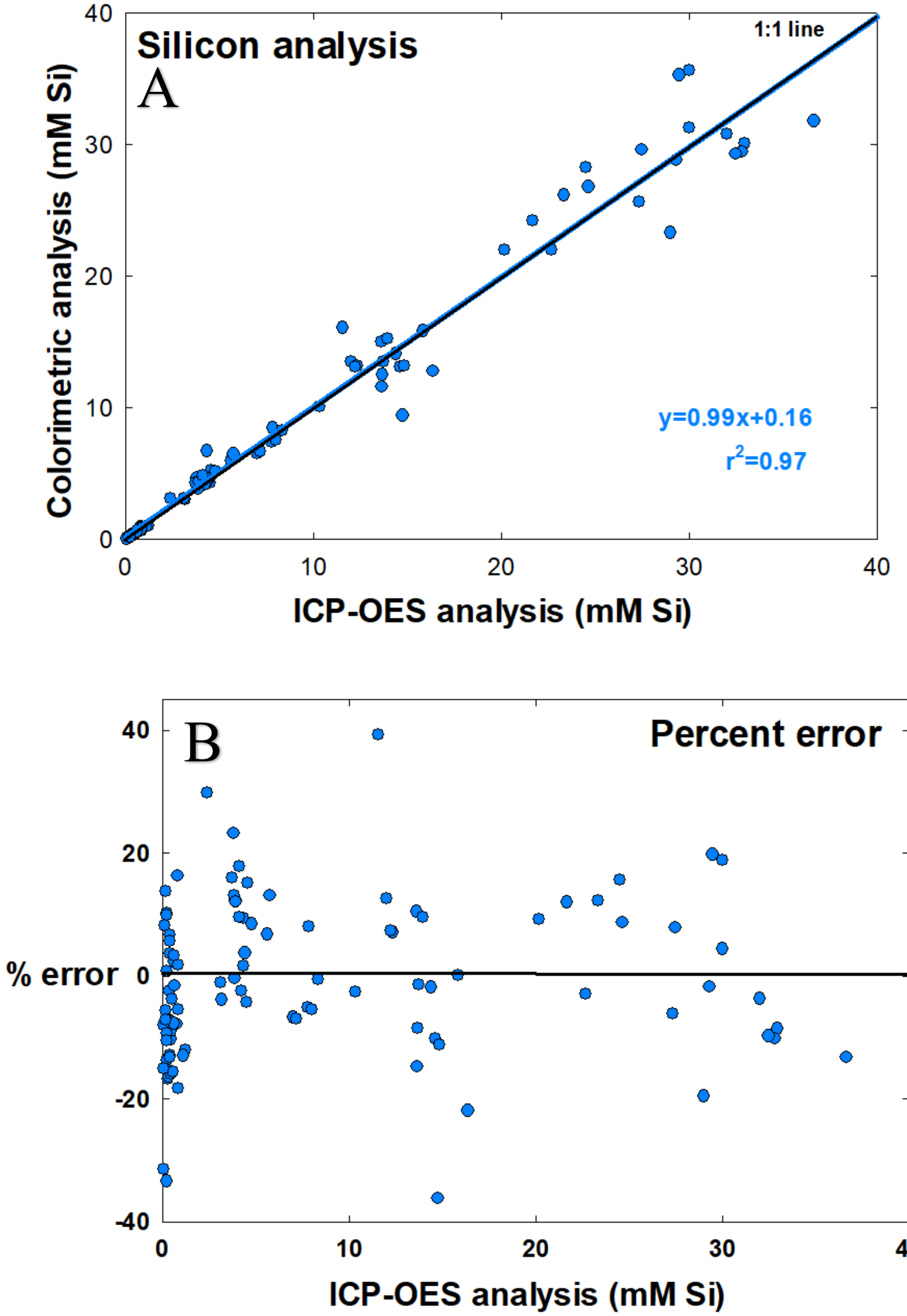
Note. pH increased over time in both wollastonite and rice hull amended peat-based (A) and coconut coir-based media (B). Nutrient solution was maintained at a pH of 5.9 $\pm$ 1.0. Data points represent the average of the treatment (A: n=6; B: n=2) and error bars represent standard deviation. Figure 2.6A: Leaching volumes were not different from one another and were pooled for peat-based media. Data over 120 days. Figure 2.6B: Note scale change. Leaching volumes were graphed separately over a 30-day period.

Colorimetric analysis

Colorimetric analysis was compared to ICP-OES analysis to ensure accurate measurements. The ICP-OES analysis explained 97% of the colorimetric analysis of Si in leachates (Figure 2.7A). Percent error varied among Si concentrations, but no obvious pattern is seen (Figure 2.7B).

Figure 2.7

Comparison of Silicon Analysis Methods



Silicon analysis
A
1:1 line
y=0.99x+0.16
r2=0.97
Colorimetric analysis (mM Si)
ICP-OES analysis (mM Si)
0
10
20
30
40
B
Percent error
% error
40
20
0
-20
-40
ICP-OES analysis (mM Si)

Note. Comparison of colorimetric to inductively coupled plasma-optical emission spectrometry (ICP-OES) silicon (Si) analysis (n=106). Figure 2.7A: Regression is shown in blue (y=0.99x+0.16; r^2 = 0.97). The 1:1 line is shown in black. Figure 2.7B: Percent error of colorimetric analysis to inductively coupled plasma-optical emission spectroscopy (ICP-OES). Differences between measurement methods may be caused by time duration between sampling, sample storage temperature, and/or dilution errors (n=106). There was no difference in deviations as Si concentration increased.

Heavy metal analysis

Nine heavy metals were analyzed in leaves of basil and sunflower grown in a non-amended or wollastonite-amended peat:perlite media. While chromium uptake was higher in sunflower than basil, the uptake of the other metals was similar between species, so they were pooled for analysis. Aluminum, barium, cadmium, and strontium concentrations were significantly higher in wollastonite amended media compared to the non-amended control (Table 2.2).

Table 2.2

Heavy Metal Analysis of Basil and Sunflower Grown with Wollastonite

Element	(-) Wollastonite (mg/kg)	(+) Wollastonite (mg/kg)	Significance (p-value)
Al	4.1 $\pm$ 1.6	7.3 $\pm$ 1.5	0.009
As	0.21 $\pm$ 0.07	0.21 $\pm$ 0.08	0.48
Ba	6.2 $\pm$ 0.6	7.6 $\pm$ 0.7	0.003
Cd	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.04
Co	0.18 $\pm$ 0.06	0.24 $\pm$ 0.08	0.14
Cr	0.27 $\pm$ 0.04	0.32 $\pm$ 0.04	0.09
Pb	*	*	*
Se	----	----	----
Sr	25 $\pm$ 2.5	33 $\pm$ 1.9	0.0004

Note. Basil (n=3) and sunflower (n=3) were grown in soilless media amended with and without wollastonite. Average and standard deviation of metal uptake is shown. Species were pooled (n=6) due to an insignificant interaction between species apart from chromium. Significance was determined using an alpha level of 0.05. Below detection limit (BDL) is represented with hyphens (--). *Lead (Pb) was not statistically analyzed due to only one out of the six replicates being above the detection limit.

Basil and sunflower were also grown in media amended with Plant Tuff®. Aluminum, arsenic, barium, chromium, and strontium were significantly higher in Plant Tuff® amended media than the non-amended control treatment (Table 2.3). However, these concentrations are not biologically important because they did not cause any notable toxicity effects.

Table 2.3

Heavy Metal Analysis of Basil and Sunflower Grown with Plant Tuff®

Element	(-) Plant Tuff® (mg/kg)	(+) Plant Tuff® (mg/kg)	Significance (p-value)
Al	4.06 ± 1.6	10.5 ± 3.1	0.002
As	0.21 ± 0.07	0.34 ± 0.11	0.02
Ba	6.22 ± 0.62	9.73 ± 0.9	0.0001
Cd	0.15 ± 0.01	0.08 ± 0.02	NA
Co	0.18 ± 0.06	0.19 ± 0.08	0.86
Cr	0.27 ± 0.04	0.32 ± 0.04	0.03
Pb	----	----	----
Se	----	----	----
Sr	25.1 ± 2.53	30 ± 2.38	0.008

Note. Sunflower (n=3) and basil (n=3) were grown in soilless media amended with and without Plant Tuff®. Species were pooled (n=6) due to an insignificant interaction between species and treatment effect. Significance was determined using an alpha level of 0.05. Below detection limit (BDL) is represented with hyphens (--). Significance was not calculated for cadmium (Cd) because the control plant tissue had higher concentrations of Cd than the Plant Tuff® supplemented plant tissue (represented with NA).

Discussion

Soilless media components minimally released Si, but media amendments can be added to supplement for Si. The ideal Si additive would have a steady-state release of Si throughout the lifecycle of the crop. Diatomaceous earth had a steady-state release of Si but is expensive and would require large quantities to supply adequate Si. Rice hulls had a comparable release rate as diatomaceous earth and they increase the volume of the media, which reduces overall media cost. Steel slag released a high concentration of Si but the release was inconsistent probably because of variable particle size. Steel slag, at the rate evaluated, also caused an alkaline pH of 11.3, which would require accurate liming and application of the additive to maintain a suitable root-zone pH for crop production. Wollastonite had the highest release of Si in water over 160 days when compared to the other substrates (Figure 2.1A), but this is inconsistent with the findings of Frantz et al. (2010). They observed that rice hulls released the most Si per gram tested while wollastonite released the least. The particle size used by Frantz was not reported so the results could be associated with different particle sizes.

Release of Si from the dissolution of rice hulls and wollastonite was further studied in soilless media. In all leaching volumes, rice hulls steadily released and slowly increased from 0.5 to 1.75 mmol $Si \cdot L^{-1}$ media·leaching $event^{-1}$ in the 15% leaching fraction over 120 days while wollastonite peaked at about 2.1 mmol $Si \cdot L^{-1}$ media·leaching $event^{-1}$, but slowly decreased and depleted over time (Figure 2.4). If a complete nutrient solution was used, microbial colonies might have been better supported, potentially helping decompose the rice hulls more rapidly and releasing more Si (Marxen et al., 2016; Oliverio et al., 2020). In this study, Vansil® W-10 wollastonite was used, which is the coarsest particle grade commercially available, and has a lower risk of inhalation than finer grades (Vanderbilt, 2015). Using an even coarser grade may increase the longevity of Si release over time in soilless substrates. The ratio of 1 g wollastonite$\cdot L^{-1}$ peat was the only application rate studied, so more or less could be applied if desired, but the addition of lime would need to be adjusted to account for the increase of pH caused by more or less wollastonite. Rice hulls released less total Si than wollastonite until about day 80 but had a higher maximum release of Si (22.4 mmol $Si\cdot L^{-1}$ peat) and a longer sustained release than wollastonite (8.5 mmol $Si\cdot L^{-1}$ peat; Figure 2.5).

Although these amendments release Si, mono-silicic acid may not remain soluble in the root-zone solution due to its instability (Schaller et al., 2021). The volume of rice hulls added to media mixes can be altered depending on cost and crop needs, but the concentration of released Si would vary. These results suggest that wollastonite would be a beneficial addition to soilless media mixes for short-term, high turn-over containerized crops with fast lifecycles. Rice hulls would be better suited to provide a steady release of Si for longer-term container-grown crops, provided the breakdown of the rice hulls over time does not negatively impact substrate physical properties. Temperature may influence the release rate of Si from either additive depending on the environmental conditions, but was not studied in this study. Regardless of additive, the longevity of release depends on watering style (e.g., irrigation frequency and volume).

Leachate volume and time between leaching influenced Si release. The greatest amount of Si was released using 600 mL (Figures 2.4 and 2.5) but quickly depleted the release of Si from wollastonite compared to other leaching volumes. The 30% leaching fraction released Si quicker than the 15% leaching fraction but extended the life of the Si additive about 10 days longer than the 60% leaching fraction (Figures 2.4 and 2.5). The lowest volume tested (i.e., 15% leaching fraction) is more akin to greenhouse and nursery growers' practices to reduce nutrient and water waste. This low leaching volume maintained a steady release of Si in both amendments while extending the life of the additives about 30 days (Figures 2.4 and 2.5). Noise in the data (Figure 2.4) corresponded with time between leaching events. Leaching style can also affect Si release. If water or nutrient solution is not applied slowly to the growing media, channeling may occur and misrepresent the release of Si from the whole container (Atland, 2021).

Silicon media additives influenced media pH. Both rice hulls and wollastonite increased peat-based media, but rice hulls raised pH more than wollastonite in peat-based media (Figure 2.6A). Rice hulls and wollastonite amended coconut coir-based media increased media pH over time (Figure 2.6B), but, wollastonite raised coconut coir-based media pH more than rice hulls. A leaching fraction of 15% increased media pH the most (Figure 2.6B). The dissolution of wollastonite releases hydroxide ions as Si is released, which raises pH (supplemental Figure 2.8; Equation 1). The amount of additive may need to be adjusted depending on the media base being used. Sphagnum peat moss is acidic (pH of 3.0-4.0; Lee et al., 2021) and may or may not require additional lime depending on the type and volume of Si amendment added. Coconut coir is less acidic than peat moss (pH 4.2-6.1; Abad et al., 2002) and may or may not need lime to adjust starting pH to a suitable range for crop production. It is important to note that media bales differ in pH and should be checked prior to mixing each media batch to adjust recipes to ensure a suitable root-zone environment.

Silicon amendments, particularly from mined products, can supply other undesired elements. The heavy metal concentrations in plant tissue were well under legal concentration limits for human and animal consumption in the United States (U.S. Food and Drug Administration, 2022), but regulations vary by country. Wollastonite purity and elemental concentration might vary based on the area mined, but other wollastonite sources were not studied.

Silicon release rates were measured primarily by colorimetric analysis. Phosphorous and Fe interfere with the analysis (Eaton et al., 2005) and so were excluded from the nutrient solution. Elemental concentrations could have been analyzed with ICP (Hansen et al., 2013), but this is expensive. Colorimetric analysis is an affordable way to measure Si in solution if interferences are not present. Although P interferes with the colorimetric Si test, the active uptake of P should reduce this interference in leachates collected from container-grown plants, but this should be kept in mind when colorimetrically analyzing nutrient solutions or leachates for Si concentrations. Results are comparable to ICP-OES analysis (Figures

2.7A-B). Testing for Si is inherently difficult because of the natural instability of mono silicic acid (Laane, 2018). Temperature and length between testing samples colorimetrically versus ICP-OES may have resulted in differing measurements (Figure 2.8) due to the reported instability of mono silicic acid (Laane, 2018).

Concentrations of bioavailable Si from leachates were estimated instead of being quantified by traditional in-plant elemental concentrations. Although plant tissue analysis gives an exact concentration, plant uptake can be calculated and estimated with water use efficiency (WUE) and desired tissue concentrations with mass balance since Si is primarily taken up passively through mass flow, unless the crop is a Si accumulator (Ma & Yamaji, 2006). Quantifying the release rates of media components provides an estimate of the concentration of Si available for uptake, and Si concentrations in plant tissue can be calculated using WUE. If WUE is known, the percent desired elemental concentration can then be calculated to approximate supplementation (Equations 2 and 3). This example uses 0.01 g Si per gram dry mass, or 1% Si on a dry mass basis, which is the threshold defined for a Si-accumulating species. Many greenhouse and nursery crops are well below this threshold, although notable exceptions include cucumber (*Cucumis sativas* L.) and other cucurbits, lantana (*Lantana camara* L.), phlox (*Phlox* spp. L.), sunflower (*Helianthus annuus* L.), verbena (*Verbena bonariensis* L.), and zinnia (*Zinnia elegans* Jacq.) (Boldt & Altland, 2018; Frantz et al., 2010).

$$WUE \times Tissue\ concentration = Solution\ concentration\ needed \quad (2)$$

$$\frac{3\ g\ dry\ mass}{L\ H_2O} \times \frac{0.01\ g\ Si}{g\ dry\ mass} = \frac{30\ mg\ Si}{L\ H_2O}\ or\ \frac{1.07\ mmol\ Si}{L\ \ H_2O} \quad (3)$$

This desired concentration in Equation 3 can be satisfied by supplementing wollastonite or rice hulls in soilless media (Figures 2.4 and 2.5). This mass balance technique is commonly used in hydroponic culture (Bugbee, 2004; Langenfeld et al., 2022) and works well if the specific element is passively taken up. Silicon is mainly taken up passively following the transpiration stream but can be active if the species is a Si accumulator (Ma & Yamaji, 2006) and/or exposed to stress. Prakash et al. (2010) found correlations between Si concentration of growing media and final Si concentration in rice (*Oryza sativa* L.) tissue. Knowing the concentration of Si supplied in the root-zone solution, at non-saturating concentrations, can give an accurate estimation of how much Si will be in plant tissue. Amending soilless media with a Si additive that steadily and continually releases Si throughout the crop lifecycle can provide the aforementioned benefits.

Conclusion

The dissolution of wollastonite and parboiled rice hulls steadily released bioavailable Si over time in peat-based media with minimum effects on media pH and heavy metal concentration in the plant tissue of two species at the incorporation rates evaluated. Silicon concentrations were analyzed by colorimeter, which is an affordable, time effective, and reliable method for quantifying Si concentrations, compared to ICP-OES, for aqueous samples that do not contain interfering elements. Wollastonite would be beneficial for container production of crops grown less than 4 months and rice hulls would be better suited for longer-term container-grown crops.

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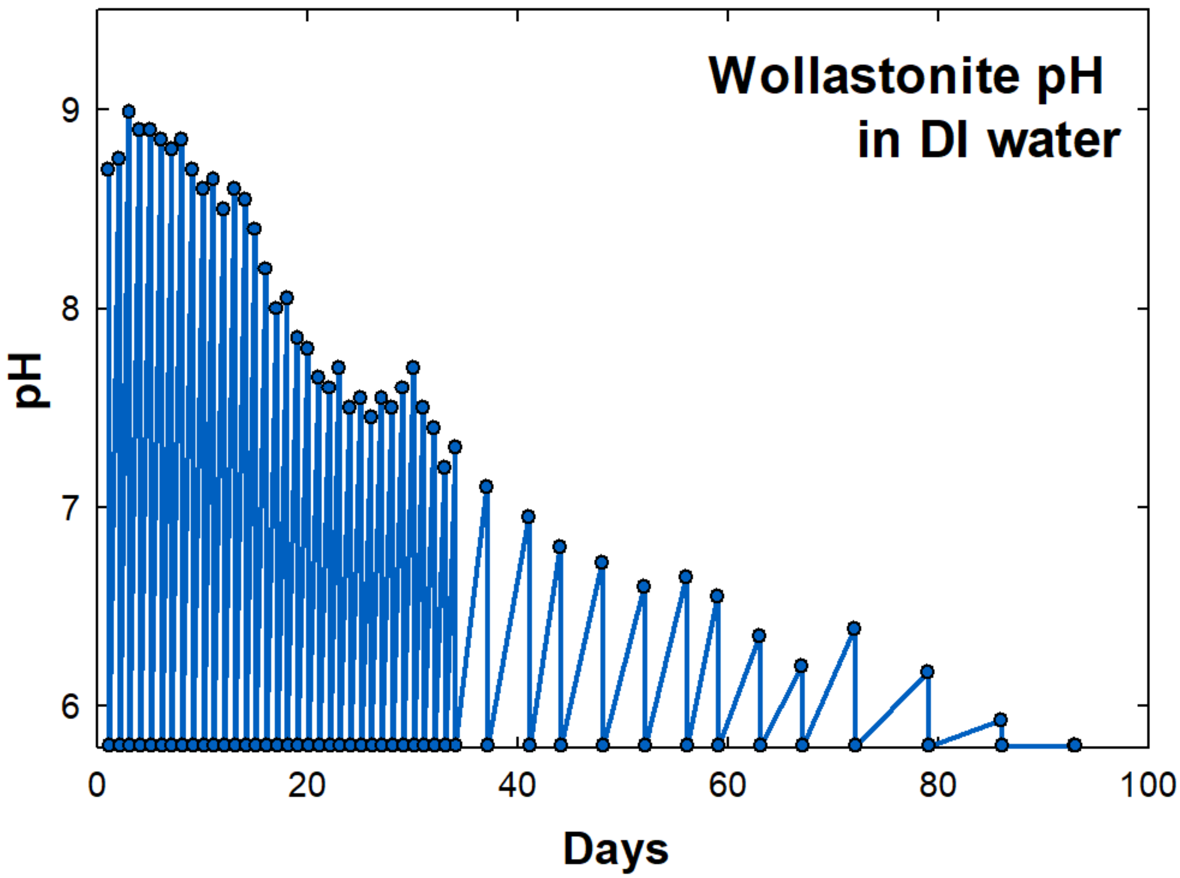
Supplemental Data

To track the change of pH and release of Si over time from wollastonite, a 1 L Erlenmeyer flask was filled with 100 grams of wollastonite and diluted to one liter with DI water. One milliliter of 1.0 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer titrated with KOH to a pH of 5.8 was added. The solution was stirred at 300 rpm on a stir plate and the pH was measured after 8 h. The solution was left overnight for the wollastonite to settle. The following morning, the water was decanted and fresh DI water with one milliliter of 1.0 M MES buffer was added to bring the total solution to one liter. This was done daily for three weeks, then was done twice a week for the next three weeks, then once a week for next three weeks, and every other week for the remainder of the 93-day study.

Figure 2.8

Effect of Wollastonite on pH



Note. Wollastonite continuously increased solution pH until day 90, suggesting the wollastonite was depleted of silicon (Si) in the rapidly stirred water. The pH increased as Si was released because with every mole of mono-silicic acid released, two moles of hydroxide were released (Equation 1). As the pH change became smaller, it suggests that Si release rate decreased. After pH was measured after 8 hr, water was decanted and refilled with deionized water and buffer to bring pH to 5.8.

CHAPTER III

SILICON DID NOT IMPROVE TOLERANCE TO PRECISION DROUGHT STRESS IN VEGETATIVE *CANNABIS*

Abstract. Precision drought stress (PDS) has the potential to reduce stem elongation without reducing photosynthesis. Precision drought stress could help growers increase yields of *Cannabis sativa* L. by decreasing the likelihood of lodging, but PDS is a sensitive system. We aimed to stabilize PDS with silicon (Si) since Si has been shown to increase the tolerance of plants to drought stress, especially Si-accumulating species such as *Cannabis*. Drought was applied to vegetative *Cannabis* with and without Si to see if Si would strengthen *Cannabis* in this system. Plants treated with PDS with and without Si were 10% to 40% shorter and had 20% to 75% less dry mass than control treatments among three trials. Benefits from supplementing Si to *Cannabis* under drought were not consistently seen, but powdery mildew (*Golovinomyces* sp. (U.Braun) V.P.Heluta) was inhibited in every trial by Si accumulation in tissues compared to Si non-supplemented treatments. Precision drought stress has potential to reduce plant growth without impairing plant health but requires further study with Si supplementation to investigate the benefits Si has on the system. Although Si did not consistently increase resistance of *Cannabis* to drought stress, Si supplementation in soilless media increased biotic resistance of *Cannabis*.

Introduction

Drought stress is an environmental factor that hinders plant growth and development (Farooq et al., 2012), but, if controlled, can be beneficial. This is sometimes referred to as "eustress" or "precision drought stress" (PDS) and can be applied by several ways, but is usually done by manipulating matric or osmotic potential (Vázquez-Hernández et al., 2019). Precision drought stress has been shown to promote desirable defense responses in plants like increased secondary metabolite production (Isah, 2019) and maintained plant size (Boyer, 1970; Carillo et al., 2021) without hindering yields.

Some studies have shown PDS to be a successful method to increase the production of secondary metabolites in high-value crops. Caplan et al. (2019) withheld water from *Cannabis* and found a significant increase in cannabinoid (CBD) concetrations. An increase in the production of derisible terpenes or the concentration of specific terpenes has been seen in other common oil-producing species such as basil [*Ocimum basilicum* L. (Al-Huqail et al., 2020; Simon et al., 1992)], sage [*Salvia officinalis* L. (Caser et al., 2019) and *Salvia miltiorrhiza* Bunge (Liu et al., 2011)], and safflower [*Carathamus tinctorius* L. (Chavoushi et al., 2020)] (for review Akula & Ravishankar, 2011; Yadav et al., 2021). An increase in secondary metabolite production is beneficial for oil-yielding crops, but some of these crops are grown indoors with restricted space that growers wish to control.

Some of these crops are grown indoors where restricted spaces lead growers to look at compact cultivars of production strategies that can be utilized to control growth. A typical procedure to manage plant growth is to apply hormone-based synthetic or natural plant growth regulators (PGRs). These hormones, typically gibberellic acid-inhibitor or ethylene-forming compounds, reduce stem elongation. Plant growth regulators are effective but wear off at various rates and can require multiple applications for an even effect, which can be time-intensive. Instead, PDS could be applied to maintain plant size. Boyer (1970) measured photosynthetic rates and leaf enlargement while withholding water from corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and sunflower (*Helianthus annuus* L.). Boyer (1970) found that leaf enlargement decreased by 10% to 15% between -0.7 to -1.2 MPa (-7 to -12 bars) in all three species and maintained typical photosynthetic rates. Leaf expansion is usually seen as a positive use of energy for plants to maximize photon capture, but stem and petiole elongation occurs simultaneously, and this is seen as a negative use of energy in indoor agriculture (Kutschera & Niklas, 2007). This creates large plants that can take up more space in restricted environments of greenhouse and indoor agriculture production, which usually elicits the use of PGRs. Applying PDS could be an alternative method to reduce leaf expansion and stem elongation to maximize yield in these limited spaces, especially for crops intended for consumption. However, PDS is a sensitive system to balance: too much stress and photosynthetic rates rapidly decrease from stomatal closure, or not enough stress and plant size will be unaffected

(Boyer, 1970). Studies have coupled the application of drought with silicon (Si) to strengthen plants (see review Luyckx et al., 2017).

In recent years, the value of supplementing crops with Si during drought stress has become better known and understood (Verma et al., 2021). The observed benefits for crops during abiotic stress have increased interest in Si supplementation (Luyckx et al., 2016). These benefits have been most noticeable in Si-accumulating species, which have ≥ 10,000 mg $Si \cdot kg^{-1}$ dry tissue (1% total dry mass is Si) in the aerial shoot (Epstein, 1999). These species tend to require higher concentrations of Si in the root-zone, such as *Cannabis* (Guerriero et al., 2019). Supplementing Si strengthened stems in other Si-accumulating, herbaceous species (Kamenidou et al., 2008, 2009, 2010; Mattson & Leatherwood, 2010), likely by distributing Si underneath the cuticle layer and binding with hemicellulose and cellulose in cell walls that add mechanical support to plant tissue (Coskun et al., 2016; Ma & Yamaji, 2006). Silicon supplementation may be able to reduce the susceptible of *Cannabis* to lodging with large, top-heavy female inflorescences as it has in other species (Shah et al., 2019). Silicon supplementation is especially beneficial for greenhouse and indoor agriculture production systems that use soilless media, which typically lacks sufficient levels for Si accumulators (Frantz et al., 2010).

The aim of this study was to investigate the value of supplementing silicon when applying PDS to keep vegetative *Cannabis* compact by reducing stem elongation.

Materials and Methods

Plant Material

Cannabis sativa L. 'Trump' cuttings were rooted in a 1:1 ratio of peat (fibrous blond, ProMoss® III TBK; PRO-MIX, Quebec, Canada) to perlite (Hess®, Malad City, ID) media adjusted to pH 5.8 with hydrated lime (Chemstar® Type S lime; Chemstar Products, Minneapolis, MN). Cuttings were placed in humidity domes and misted under a photosynthetic photon flux density (PPFD) of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ supplemented by light-emitting diodes (LEDs) (Physiospec; Fluence Biosciences, Austin, TX) with a 18/6 hr day/night photoperiod. Temperature was maintained at 26 C.

After two weeks of rooting, cuttings were randomly assigned and transplanted into 1.7-L pots filled with their designated Si media treatment (see below). Once transplanted, cuttings were pinched at the fourth node and watered with Si supplementation (+Si) nutrient solution to ensure health for one week. Plants were selected for uniformity and transplanted to 6.7-L pots with their coordinating media treatment. Plants were watered as needed with tap water for four days to ensure no transplant shock, then were randomly selected for placement on the bench. Water treatments (drought stress and no stress) were randomly assigned within the Si supplemented (+Si) and Si non-supplemented (-Si) treatments, and they were started after the four-day transplanting period. All pots had the same quantity of 2-L per hour drip emitters. Plants were harvested 22-30 days after the start of drought stress treatments. Fresh mass (g) was collected then dry mass (g) after the tissue dried in an 80 C drying oven for four days. Trial 1 had three replicates per treatment, Trial 2 had four, and Trial 3 had five.

Environmental Conditions

Transplanted cuttings were grown in a greenhouse (Logan, UT) 8 July to 2 Nov. 2022. Ambient sunlight was supplemented by LEDs (model LUXX-200-277-88 / 80R Spectrum, LUXX Lighting Systems, Los Angeles, CA) with a PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a 16/8 hr day/night photoperiod to maintain vegetative conditions. The daily light integral (DLI) varied from 16-35 $mol \cdot m^{-2} \cdot d^{-1}$. Air temperatures were maintained at 25/20 C day/night and relative humidity was 40%/60% day/night.

Media

Cuttings were randomly selected and transplanted to either -Si or +Si media. Silicon non-supplemented media was made with 85% sphagnum peat moss by volume, 15% vermiculite by volume (Horticultural coarse grade; Industries, Inc., North Bloomfield, OH), wetting agent (AquaGro® 2000 G; Aquatrols, Paulsboro, NJ) added at a rate of 0.75 $g \cdot L^{-1}$ peat, and hydrated lime (Chemstar Products) at a rate of 1.5 $g \cdot L^{-1}$ peat to raise pH to 5.8. Silicon supplemented media was a base of the -Si media with the addition of wollastonite (Vansil® W-10; Vanderbilt Minerals, LLC, Norwalk, CT) at 1 $g \cdot L^{-1}$ peat and hydrated lime added was adjusted to 1.25 $g \cdot L^{-1}$ peat to raise pH to 5.8.

Nutrient Solution

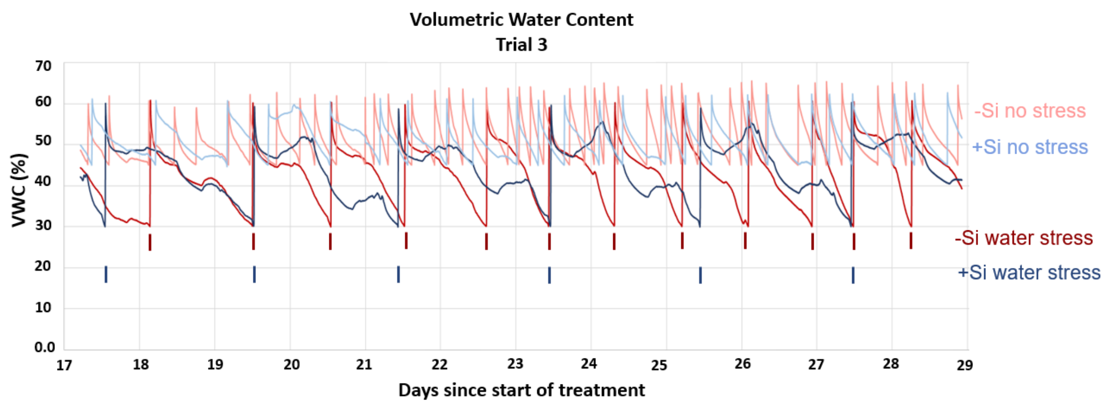
The -Si nutrient solution was made with Peters 21-5-20 Excel (JR Peters Inc., Allentown, PA) with N at 120 $mg{\cdot}L^{-1}$, and with additions to increase total P to 30 $mg{\cdot}L^{-1}$, 1 $mg{\cdot}L^{-1}$ Cu-EDTA, 0.4 $mg{\cdot}L^{-1}$ B, 1 $mg{\cdot}L^{-1}$ Fe-DTPA, 21 $mg{\cdot}L^{-1}$ S, and 183 $mg{\cdot}L^{-1}$ K. The +Si nutrient solution was identical to the -Si solution but with the addition of 17 $mg{\cdot}L^{-1}$ Si (ppm) +Si nutrient solution was made as the -Si solution but with the addition of 17 mg $Si{\cdot}L^{-1}$ (ppm) from AgSil® (K_2SiO_3; PQ Corp., Valley Forge, PA) maintained in solution with KOH (pH maintained at 11.3) in a separate concentrate tank. Both nutrient solutions were maintained at a pH of 5.9 $\pm$ 0.2 and electrical conductivity (EC) of 1.35 $\pm$ 0.1 $mS{\cdot}cm^{-1}$.

Precision Drought Stress

Precision drought stress was induced through matric potential using a data logger (CR1000x; Campbell Scientific, Logan, UT) with dielectric sensors (TEROS 12; METER, Pullman, WA) and solenoid valves to maintain desired volumetric water content (VWC). One dielectric sensor was placed in a representative pot for each treatment. Sensors were placed in the middle of the pot (10 cm from bottom) parallel to the bottom of the container in the media. When the representative pot reached the minimum VWC, dielectric sensors signaled the flow of either -Si or +Si fertigation by solenoid valves until the programmed maximum VWC was met. Treatments receiving no stress maintained a VWC between 40% to 60% while drought stress treatments would receive a watering to 60% VWC once a VWC of 20% (Trial 2), 25% (Trial 1) or 30% (Trial 3) was met (Figure 3.1).

Figure 3.1

Volumetric Water Content for Trial 3



Note. Volumetric water content (VWC) of all treatments during the last two weeks of Trial 3. One plant represented a treatment and controlled the watering of the other replicate pots. Ticks on the graph represent a watering event for either the silicon (Si) non-supplemented (-Si; dark red) or Si supplemented (+Si; dark blue) water stressed treatments. Pots of the -Si drought stress treatment were watered two times more than the +Si drought stress treatment during the last two weeks of the study. This difference in watering is most likely due to the incorrect selection of presentative pot for the treatment and/or sensor malfunction.

Data collection

Height and leaf area (for Trial 3 only) were measured and recorded weekly from start of transplanting until harvest, then fresh and dry mass (g) were collected at the end of each trial. Tissue samples were collected from young leaves and analyzed through inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis for elemental concentrations at The Utah State University Analytical Laboratories (USUAL; Logan, UT) in Trial 3 only. Silicon concentrations were analyzed by the oven-induced digestion (OID) method (Kraska & Breitenbeck, 2010) then analyzed by colorimeter (LaMotte® SMART 3 colorimeter; La Motte, Chestertown, MD) with the heteropoly blue method (Eaton et al., 2005; LaMotte® silica low range test kit 3664-SC; La Motte, Chestertown, MD).

Statistical Analysis

Data were analyzed by SAS® Studio (9.4M7; SAS Institute, Cary, NC) using a two-way analysis of variance (ANOVA) for the factorial of water stress and Si. Trials were treated as blocks, thus pooled together then analyzed. The interaction term was first tested for significance (p-value ≤ 0.05) then main effects were tested. Diagnostics and a Box-Cox analysis were checked to ensure normality of the dataset. A post-hoc test using Tukey's honest significant difference (HSD) method was used to contrast treatment effects.

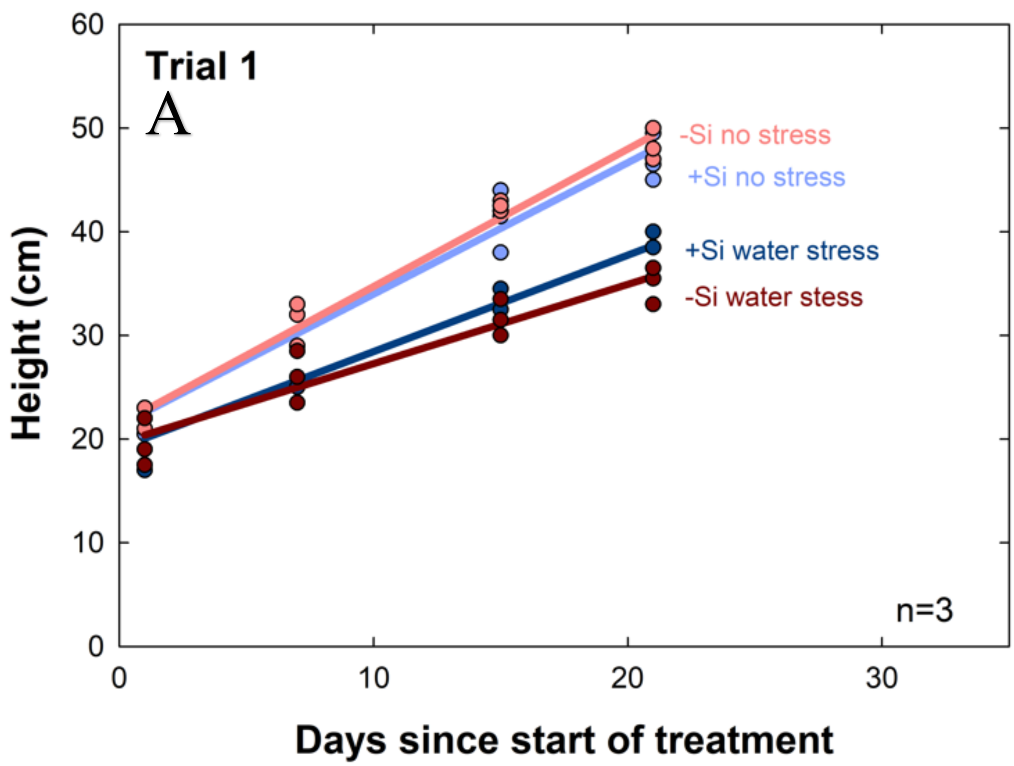
Results

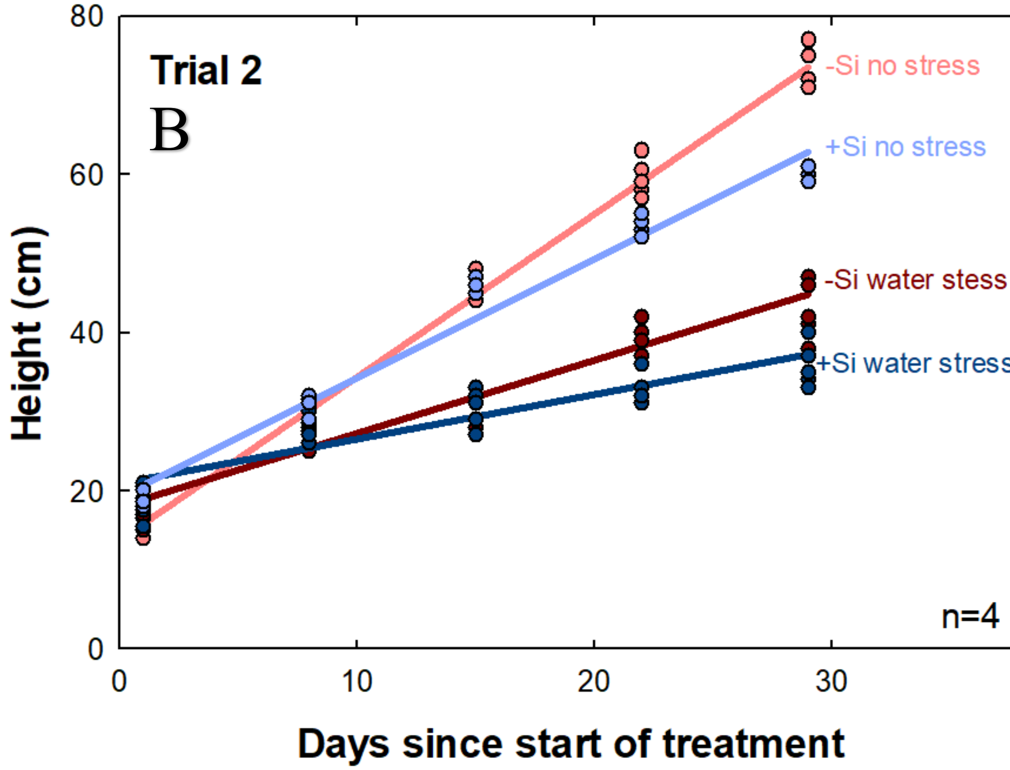
Height

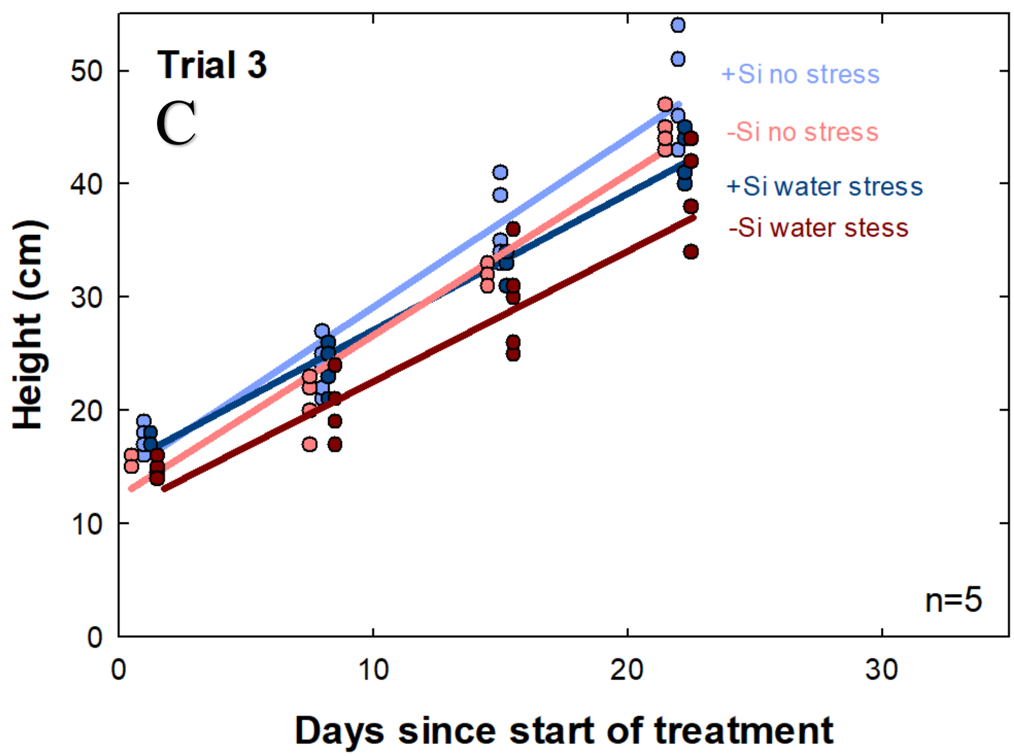
Drought stress significantly limited *Cannabis* height ($p<0.001$) in all three trials (Figures 3.2A-C), but the severity of height reduction was dependent on the amount of stress applied. Trial 2 (Figure 3.2B) was stressed the most (i.e., lowest VWC before re-watering) and Trial 3 (Figure 3.2C) was stressed the least. The +Si treatments did not consistently impact plant height under PDS.

Figure 3.2

Precision Drought Stress and Silicon on Cannabis *Height*




Trial 2
B
-Si no stress
+Si no stress
-Si water stess
+Si water stress
n=4
Height (cm)
Days since start of treatment
0
20
40
60
80
0
10
20
30


Trial 3
C
+Si no stress
-Si no stress
+Si water stress
-Si water stess
n=5
Height (cm)
Days since start of treatment
0
10
20
30
40
50
0
10
20
30

Note. Plant height of *Cannabis sativa* L. 'Trump' grown with or without precision drought stress (PDS) and with or without supplemental silicon (Si). Height was shorter reduced with PDS in all three trials, but and the amount severity of the stress correlated with the severity in reduction of height.

The main effect of +Si, pooled across drought stress, did not statistically affect plant height in these trials (Table 3.1).

Table 3.1

Significance of Precision Drought Stress and Silicon on Cannabis *Height*

Height	**No Stress**		**Drought Stress**	
Trial	**+Si**	**-Si**	**+Si**	**-Si**
1	100%	97 $\pm$ 0.04%	79 $\pm$ 0.03%	72 $\pm$ 0.03%
2	100%	122 $\pm$ 0.05%	60 $\pm$ 0.04%	71 $\pm$ 0.06%
3	100%	90 $\pm$ 0.1%	86 $\pm$ 0.08%	---
significance	---------------ns---------------		---------------ns---------------	

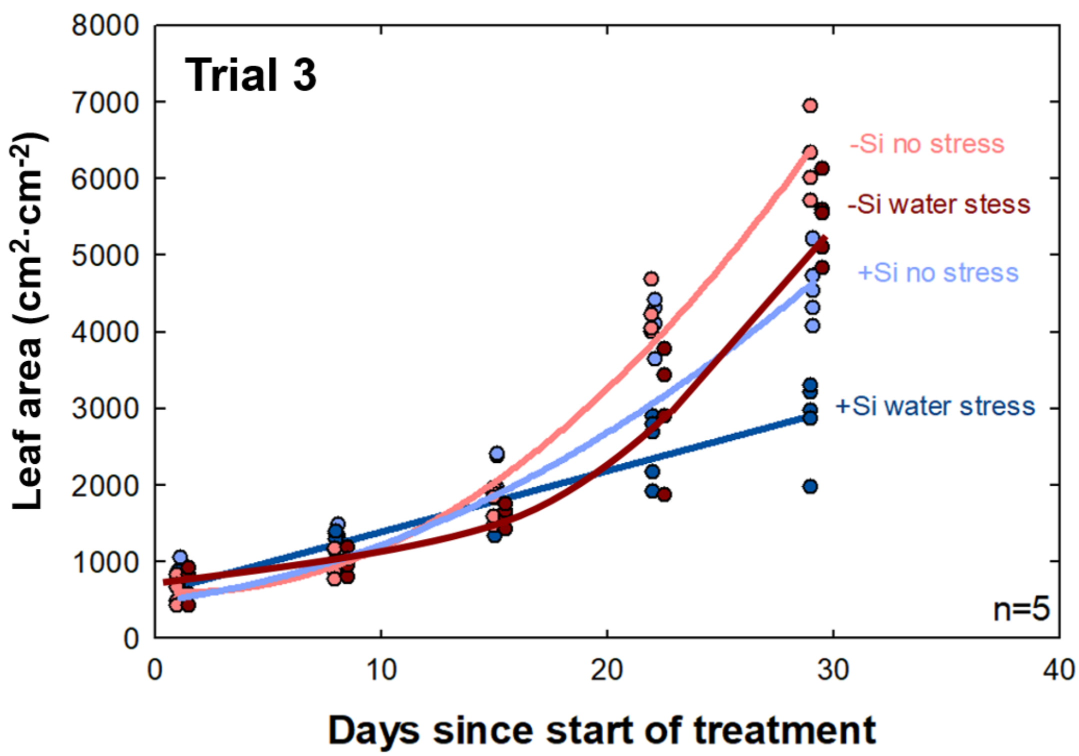
Note. Significance ($p \leq 0.05$) of supplementing silicon (+Si) with and without drought stress on plant height relative to +Si no water stress control of *Cannabis*. Trial 3 Si non-supplemented (-Si) drought stress treatment was not included due to experimental errors. "ns" represents statistical insignificance.

Leaf Area

Leaf area was significantly smaller in the +Si drought stressed treatments, but not the -Si drought stressed treatments in Trial 3 (Figure 3.3). The -Si drought stressed treatments seemed to significantly increase in leaf area after day 15 through the end of the trial, but this was experimental error caused by an increase in watering from selecting an unrepresentative pot for the treatment and/or sensor malfunction (Figure 3.1).

Figure 3.3

Precision Drought Stress and Silicon on Leaf Area



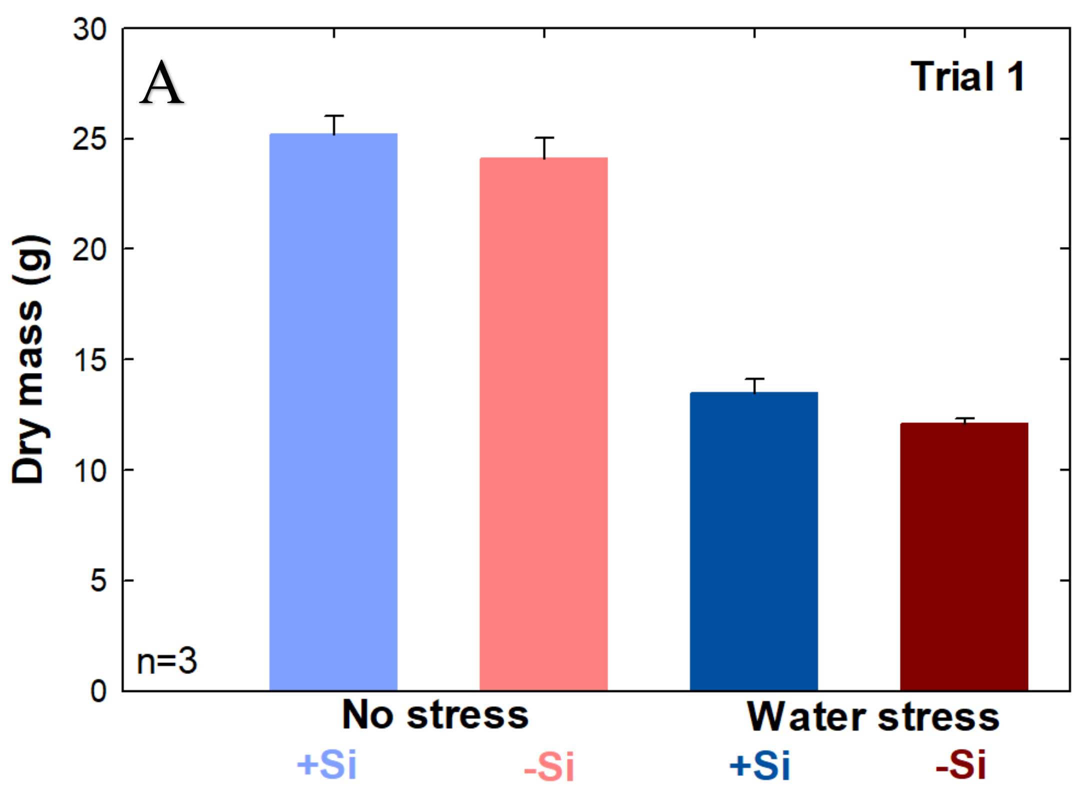
Note. Drought stress limited leaf area index ($cm^2 \cdot cm^{-2}$) in the silicon supplemented (+Si) treatments, but not the Si non-supplemented (-Si) treatments. Data only collected for Trial 3.

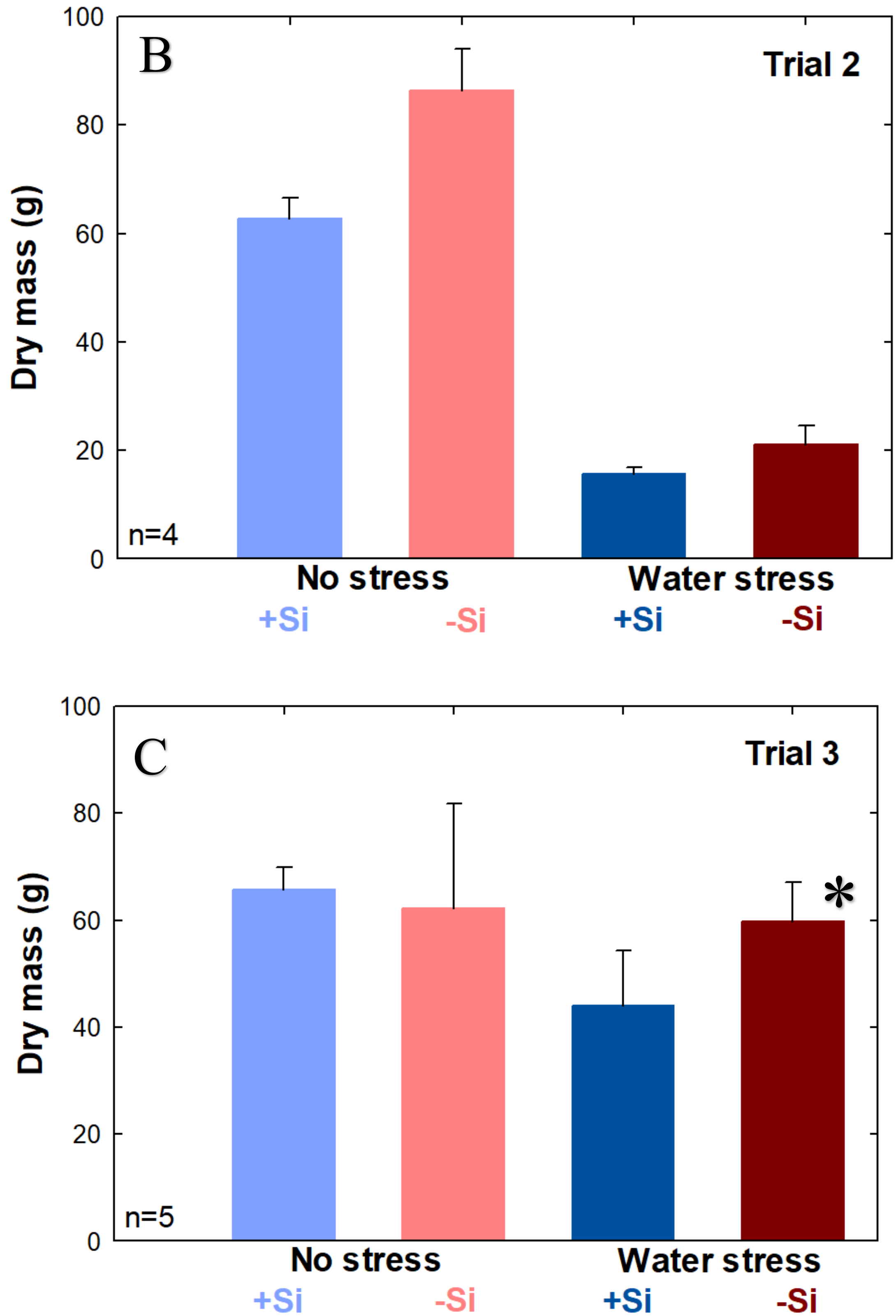
Dry mass

Dry mass was significantly lower with drought stress in all three trials (p<0.001), but not as severely in Trial 3 due to selecting the incorrect representative pot for the treatment that effected the watering treatments (Figure 3.1). Dry mass was higher in the +Si no PDS treatment compared to -Si no PDS by 4.4% in Trial 1 and 5.1% in Trial 3, but not significantly (Figure 3.4 and Table 3.2). Silicon supplementation did not increase dry mass for drought stress treatments in Trial 2 or 3 but did in Trial 1 by 10.5% although this was not statistically significant.

Figure 3.4

Precision Drought Stress and Silicon on Dry Mass




B
Trial 2
Dry mass (g)
100
80
60
40
20
0
n=4
No stress
Water stress
+Si
-Si
+Si
-Si
C
Trial 3
Dry mass (g)
100
80
60
40
20
0
n=5
*
No stress
Water stress
+Si
-Si
+Si
-Si

Note. Dry mass (g) was reduced with drought stress. Silicon supplementation (+Si) did not increase biomass accumulation in Trial 2 but did in Trial 1 and 3 compared to Si non-supplemented treatments. Note scale change among trials. "*" represents experimental error with the -Si water stress treatment.

Dry masses were compared to +Si no stress treatments. Silicon supplementation did not make a difference on growth with and without drought stress among the three trials (Table 3.2).

Table 3.2

Significance of Precision Drought Stress and Silicon on Dry Mass

Dry Mass	**No Stress**		**Drought Stress**	
Trial	**+Si**	**-Si**	**+Si**	**-Si**
1	100%	96 $\pm$ 0.04%	54 $\pm$ 0.03%	48 $\pm$ 0.01%
2	100%	138 $\pm$ 0.12%	25 $\pm$ 0.02%	34 $\pm$ 0.05%
3	100%	95 $\pm$ 0.3%	74 $\pm$ 0.03%	---
significance	---------------ns---------------		---------------ns---------------	

Note. Significance ($p \leq 0.05$) of supplementing silicon (+Si) with and without drought stress on *Cannabis* relative dry mass to +Si non stress treatment. "ns" represents not significant.

Treatments +Si tended to have less dry biomass partitioned to leaves and heavier stems, regardless of water treatment (Table 3.3). Percent dry biomass increased in +Si water treatments compared to -Si treatments (Table 3.3).

Table 3.3

Fresh and Dry Mass of Trial 3

Trial 3		**Fresh mass (g)**				**Dry mass (g)**				
		leaves	**stem**	**total**	**% leaves**	**leaves**	**stem**	**total**	**% leaves**	**% bio-mass**
+Si	**no stress**	124.3	85.2	209.5	59%	42.12	22.87	64.99	65%	31%
-Si	**no stress**	165.9	89	254.9	65%	46.45	21.33	67.78	69%	27%
+Si	**stress**	89.2	56.1	145.3	61%	29.82	17.16	46.98	64%	32%
-Si	**stress**	160.6	102.5	263.1	61%	38.9	18.98	57.88	67%	22%

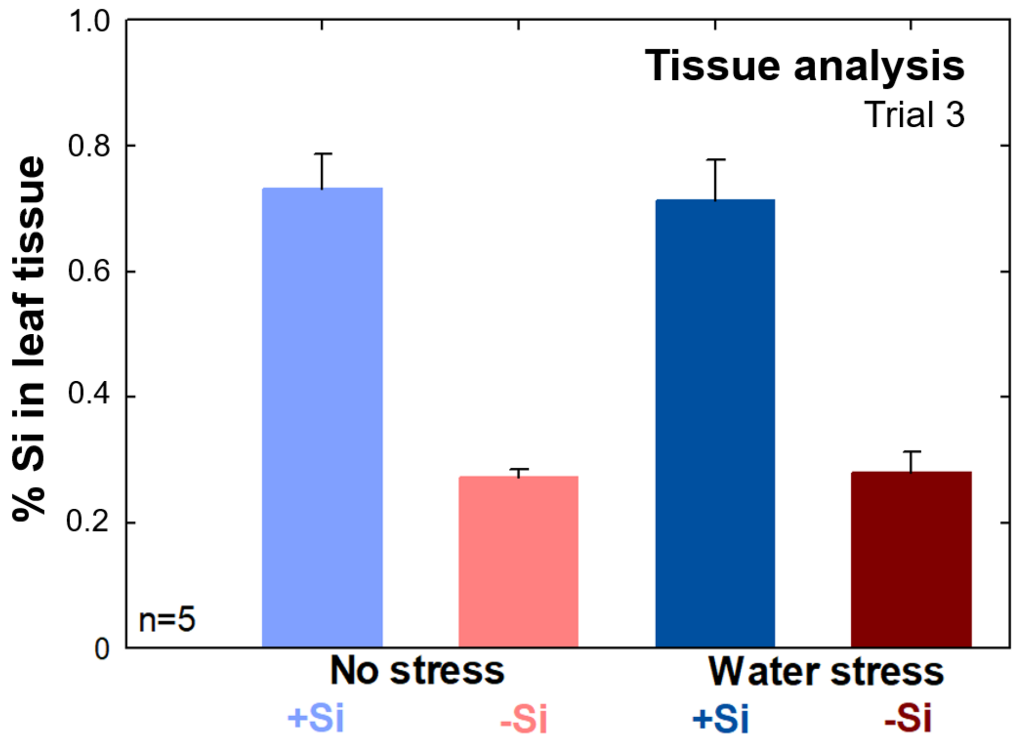
Note. Dry mass (g) of leaves and stems from one replicate in each treatment from Trial 3. Silicon supplementation is denoted by "+Si" and silicon non-supplemented by "-Si".

Silicon Accumulation

Treatments +Si no stress and +Si drought stress accumulated similar concentrations of Si, as did the -Si no stress and drought stress treatments (Figure 3.5). Treatments +Si accumulated 3.7 times more Si than -Si treatments.

Figure 3.5

Silicon Concentrations of Cannabis *Leaf Tissue*



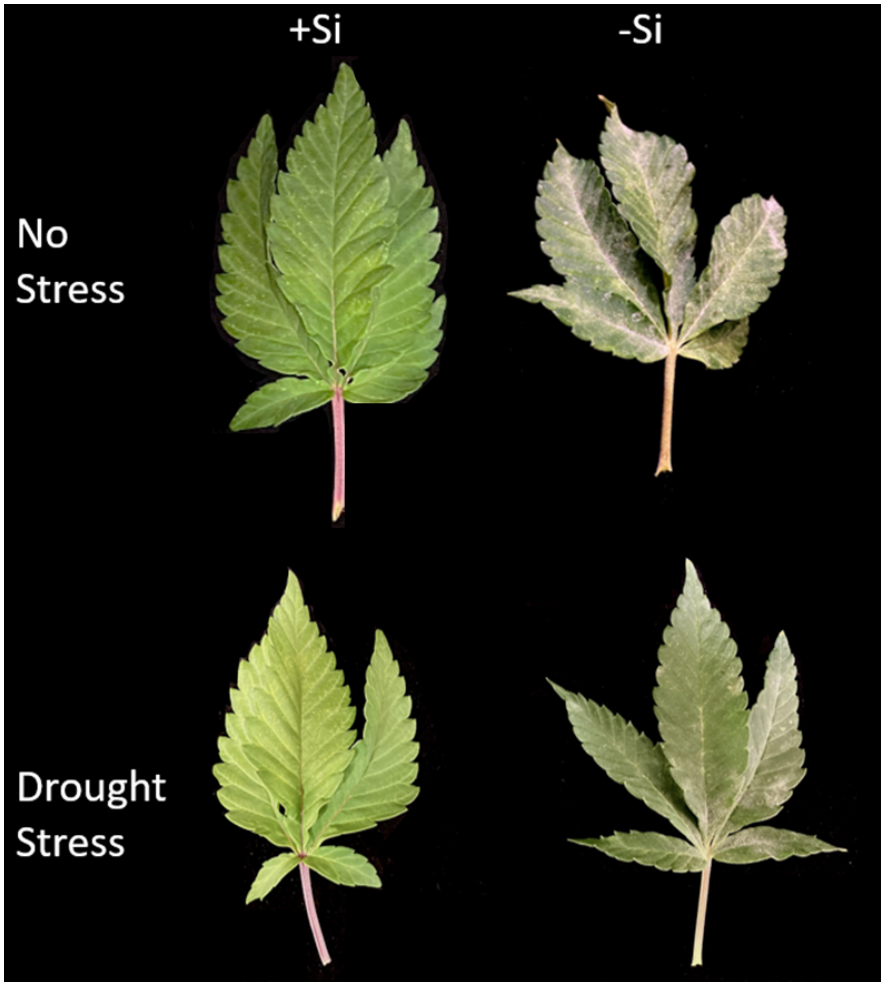
Note. Tissue analysis of silicon (Si) concentrations from dried, young leaves of *Cannabis*. Silicon supplementation is denoted by "+Si" and Si non-supplemented by "-Si". Only data from Trial 3 was collected and shown.

Biotic Resistance

In all three trials, both +Si drought stressed and +Si not stressed treatments reduced powdery mildew infections (Figure 3.6) due to Si accumulation in plant tissue compared to –Si treatments (Figure 3.5).

Figure 3.6

Effect of Silicon on Powdery Mildew Resistance in Cannabis



Note. Silicon supplemented (+Si) treatments had increased resistance to powdery mildew (*Golovinomyces sp.)* compared to Si non-supplemented (-Si) treatments from Si accumulation in tissues. Pictures are from Trial 3, but similar effects were observed in all trials.

Discussion

Precision drought stress reduced stem elongation (Figures 3.2A-C) and dry mass accumulation (Figures 3.4A-C) in all three trials as expected, but Si was not shown to be beneficial. Unexpected results could be due to experimental errors including a lack of uniformity of replicates within and among treatments or sensor malfunction. One replicate container had the dielectric sensor that represented the treatment to control watering, which is realistic to how growers would apply PDS. However, some representative plants had accelerated, or decelerated, growth rates even though they appeared to be an "average" in the beginning of the trial. This influenced the watering for every pot in the treatment as seen in the -Si drought stress containers from Trial 3 (Figure 3.1). The representative pot was the largest plant of the treatment (data not shown) that grew rapidly after day 15 and went through water faster than the other replicates (Figure 3.1). The sensor could have been moved to an average of the treatment, but, in the future, recording leaf area to understand the relative growth rate of each replicate would be beneficial in selecting for the true average in each treatment. This increase in water meant this treatment was no longer drought stressed and as a result height (Figures 3.2A-C), leaf area (Figure 3.3), and dry mass (Figures 3.4A-C) rapidly increased across all replicates in Trial 3. This could be avoided by changing the method for selecting uniformity or by controlling each replicate with an individual dielectric sensor to avoid selecting for uniformity. However, this would be costly and unrealistic for growers to implement.

Precision drought stress was difficult to apply uniformly among replicates within a treatment and to replicate. Trial 1 seemed to have an adequate amount of drought applied where +Si drought stress were taller and had greater dry mass compared to -Si drought stress plants (Figure 3.2A) but neither was statistically significant. More drought stress was applied in Trial 2, but it was too much stress

(Figure 3.2B), then Trial 3 did not have enough (Figures 3.1 and 3.2C). An adequate amount of stress could potentially show the benefit of Si as seen in Trial 1 (Figures 3.2A and 3.4A). Hattori et al. (2005) saw benefit of Si supplementation with sorghum (*Sorghum bicolor* (L.) Moench) under drought stress as have other studies (Ahmed et al., 2016; Coskun et al., 2016; Hajiboalnd et al., 2017; Ma et al., 2004), but it is difficult to reproduce. Janislampi (2012) and Tibbitts (2018) observed benefits from Si supplementation on plants under drought stress, but their results were statistically insignificant and inconclusive. Increased drought stress might have built upon the promising trends observed in Trial 1 of this study, but too much stress reduces stomatal conductance, transpiration and photosynthetic rates (Chen et al., 2011), as seen in Trial 2. There is a fine line when applying PDS. Boyd (1970) demonstrated that the difference between -1.2 to -1.6 MPa decreased photosynthetic rates by 50% to 75% in corn, soybean, and sunflower.

Although Si did not increase tolerance of *Cannabis* to PDS in all trials, biotic stress tolerance increased compared to -Si treatments. All +Si treatments were resilient to powdery mildew (*Golovinomyces* sp. (U.Braun) V.P.Heluta) infection in all three trials (Figure 3.6). Similar results have been observed by Dixon et al. (2022) in *Cannabis* and in other Si-accumulating species such as cucumber [*Cucumis sativus* L. (Guével et al., 2007; Liang et al., 2005)], bitter gourd [*Momordica charantia* L. (Dallagnol et al., 2012)], muskmelon [*Cucumis melo* L. (Guével et al., 2007)], pumpkin [*Cucurbita pepo var. pepo* L. (Li et al., 2019)], wheat [(Guével et al., 2007; Tibbitts, 2018)], and zucchini [*Cucurbita pepo* L. (Savvas et al., 2009)]. Tolerance has been linked to Si polymerization under the cuticle layer (Hattori et al., 2003; Ma & Yamaji, 2006), as well as binding with cellulose, hemicellulose, and lignin in cell walls (Guerriero et al., 2016; Ma & Yamaji, 2006; Zhao et al., 2013) to strengthen plant tissues.

Conclusions

Precision drought stress reduced height and dry mass in *Cannabis* compared to well-watered treatments. Silicon (Si) supplementation did not benefit *Cannabis* under drought stress in these studies but reduced powdery mildew infection of Si supplemented treatments on the aerial shoots due to the accumulation of Si in tissues. Precision drought stress has shown potential but requires further study in *Cannabis*.

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CHAPTER IV

CONCLUSIONS

Silicon (Si) is a beneficial element that can easily be supplemented in soilless media with amendments. Wollastonite and rice hulls were found to steadily release Si over time and minimally affected pH in peat-based media mixes. Wollastonite had a rapid, quick release of Si that would make it a valuable addition to greenhouse media mixes for crops with a lifecycle less than four months. Rice hulls released slower than wollastonite, but had a higher theoretical maximum of Si that could be released, which would be valuable in nursery production with long-term containerized crops. Other methods to add Si to soilless media are available, but media amendments are a simple way growers can supplement the nutrient.

Supplementing Si in soilless media can provide increased biotic and abiotic stress tolerance as shown in the literature and seen in this study. We hypothesized that the addition of Si with precision drought stress would stabilize this sensitive system by strengthening vegetative *Cannabis* to reduce stem elongation. Our results suggest that Si may be beneficial during drought stress, but the severity of drought needs to be further investigated: too much drought and the beneficial threshold of Si is exceeded and not enough stress and there is no difference made by Si supplementation. In general, it is difficult to show the benefits of Si during drought stress. Benefits can be observed, but may not be statistically significant or consistent. However, many studies have seen increased resistance to common fungal diseases, such as powdery mildew, which was seen in this study.

Further investigation of applying precision drought stress for high-value crops such as *Cannabis* could help maximize space for profits and maintain quality. Investigating the severity of drought stress, method of applying stress (matric or osmotic), and test species would help increase the understanding of how differing species are affected by this system.