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The Use of Silicon Substrate Amendments to Decrease Micronutrient Concentrations at Varying Micronutrient Fertility Rates with *Cannabis sativa* 'Auto CBG'

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Abstract. **Many abiotic factors impact the yield and growth of *Cannabis sativa* (cannabis). Cannabis has been reported to be a bio-accumulator of heavy metals. For growers who are targeting floral production and other byproducts for human consumption, this is a concern. Silicon (Si) has been examined as a beneficial plant element to limit the uptake of heavy metals in a variety of crops. The objective of this study was to determine the impact of Si on heavy metal micronutrient uptake and plant growth for greenhouse-cultivated cannabis at varying Si substrate amendments. 'Auto CBG' plants were grown in a 70:30 peat:perlite substrate with one of three varying calcium silicate ($CaSiO_3$) (Si) substrate amendment rates, Si_{0X}, $Si_{0.5X}$, or Si_{1X} (of 0.0, 1.04, and 2.07 kg·m^{-3} $CaSiO_3$), and one of three micronutrient fertility treatments, M_{1X} [0.49 boron (B), 0.19 copper (Cu), 4.02 iron (Fe), 0.99 manganese (Mn), 0.01 molybdenum (Mo), and 0.20 zinc (Zn) mg·L^{-1}], M_{2X}, or M_{4X}, using a modified Hoagland's solution, creating a 3 × 3 factorial. Plants grown with a Si_{1X} substrate amendment exhibited a significantly lower iron concentration in the foliage and root tissue when compared with those grown in a substrate without Si. After 6 weeks of growth, Si_{0X} plants that received a M_{4X} fertility rate exhibited greater foliar micronutrient concentrations of B, Mn, Zn, Fe, and Cu than plants that received a Si substrate amendment when provided a M_{4X} fertility rate. Additionally, lower micronutrient concentrations in floral tissue were observed in plants that received a Si substrate amendment for M_{2X} and M_{4X} when compared with plants that did not. Silicon substrate amendments had no impact on the cannabinoid concentration or plant growth metrics after 12 weeks of growth. This research suggests that using a Si substrate amendment in a greenhouse production system can limit excessive uptake and accumulation of micronutrients in the foliage, roots, and floral material of cannabis without negative impacts on plant growth or cannabinoid concentrations.**

Hemp (*Cannabis sativa* L.) has gained global popularity because of the wide array of products that can be manufactured from hemp fibers, oils, and cannabinoids (Salentijn et al. 2019). Hemp is defined as *Cannabis sativa* that contains no more than a 0.3% total tetrahydrocannabinol (THC) concentration of dry weight in any part of the plant (US Congress 2014, 2018). Hemp contains more than 100 cannabinoids, including cannabidiol (CBD), THC, and cannabigerol (CBG), which vary in concentration, and many are considered to have medical and therapeutic effects, thus leading to an increased interest in cannabis production (Salentijn et al. 2019).

Soil contamination has increased since the industrial revolution because of human activities, which are attributed to industrial waste, municipal waste, and sludge enriched with heavy metals (Galić et al. 2019). Heavy metals such as cadmium (Cd), lead (Pb), and nickel (Ni) threaten food safety and public health at any concentration (Mao et al. 2019). Heavy metals such as zinc (Zn) and copper (Cu) are required for plant growth and are not toxic to humans at low concentrations; however, higher concentrations can lead to toxic effects. Heavy metals cannot be degraded like other pollutants, thus posing a long-term negative impact on soils (Kumpiene et al. 2008). Typically, strategies to remediate polluted areas include excavation, chemical processing to immobilize metals, and using acid solutions to desorb and leach soils (Placido and Lee 2022). Toxic effects of heavy metals on plants include changes in mineral concentrations, decreased photosynthesis, oxidative stress, and growth reduction (Luyckx et al. 2021a). However, several plant species have exhibited an increase in plant growth when cultivated under heavy metal stress, including wheat (*Triticum aestivum*), maize (*Zea mays*), rice (*Oryza sativa*), peanut (*Arachis hypogaea*), and cotton (*Gossypium hirsutum*) (Liang et al. 2007). Regarding fiber hemp, one study reported that the roots of plants grown in mineral soils accumulated the greatest heavy metal concentration, followed by the stems, leaves, and seeds, respectively (Angelova et al. 2014). Although the majority of literature surrounding heavy metal accumulation in plants is focused on field-grown crops, there is still concern regarding heavy metal contamination of plants grown in soilless media.

Silicon (Si) is the second most abundant element in the soil and surface of the earth, and it is considered beneficial for plants (Liang et al. 2007). In recent years, Si has been investigated as a soil amendment to improve plant growth in heavy metal-contaminated soils and exclude heavy metal uptake. Additionally, Si has been examined to determine its ability to increase the availability and absorption of phosphorus (P) and other essential nutrients (Tripathi et al. 2015). In plants, Si decreases the heavy metal concentration through the chelation of heavy metals in the soil, which decreases the bioavailability and may prevent the translocation of heavy metals from the roots to the shoots (Khan et al. 2021). In fiber hemp, the impact of Si soil amendments in the presence of soils exhibiting high Cd concentrations resulted in less Cd accumulation in the plant when examined in a field setting; however, no change in Cd distribution within the plant was observed (Luyckx et al. 2021b).

Currently, there is an extensive body of literature regarding the impact of Si amendments incorporated as mineral soil amendments, and there is a growing body of literature regarding supplemental Si applications for greenhouse production (Wei et al. 2020). Supplementation of Si in greenhouse crops can be achieved in multiple ways, ranging from foliar applications (Kamenidou et al. 2009; Whitted-Haag et al. 2014), incorporation of Si in hydroponic nutrient solution (Boldt and Altland 2021; Mattson and Leatherwood 2010), and Si substrate amendments (Boldt et al. 2018; Kamenidou et al. 2010). However, research investigating the impact of Si substrate amendments on greenhouse cannabis nutrient uptake and

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plant growth has not been published. Although previous studies of cannabis Si soil amendments have been conducted in mineral soils where Si is prevalent, few have examined the impact of Si amendments in soilless substrates where Si availability is limited. Therefore, the objective of this study was to determine the impact of Si on heavy metal micronutrient uptake and plant growth for greenhouse-cultivated cannabis at varying Si rates.

Materials and Methods

Seeds of a high CBG auto-flowering hemp cultivar, 'Auto CBG' (*Cannabis sativa*) (Oregon CBD, Independence, OR, USA), with a peat-based substrate were sown on 23 Sep 2021. Seeds were sown into Ellepots (3.5 cm × 4 cm; Ellepots, Kommune, Denmark) in 50-cell trays and placed under T5 full-spectrum fluorescent lights (AgroBrite T5 Full Spectrum; Hydrofarm, Petaluma, CA). Seeds were germinated in a controlled environment using an intensity of 200.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which was achieved in darkness for all supplemental light treatments using a light meter (MQ-610 ePar Meter; Apogee Instruments, Logan, UT, USA) providing 11.52 $mol \cdot m^{-2} \cdot d^{-1}$ based on a 16-hour photoperiod with an average temperature of 22.2 °C. Plants remained under these lights until 5 d after germination before being transplanted in the glasshouse with ambient lighting [35.78 °N latitude with 23.9 °C/18.3 °C (75 °F and 65 °F) day/night temperatures]. Seedlings were transplanted into 2.48-L containers containing one of three substrate treatments on 1 Oct 2021. These treatments comprised of a 70:30 (volume: volume) mix of peatmoss (Canadian sphagnum peatmoss; SunGro Horticulture Distribution Inc., Agawam, MA, USA), perlite (Horticultural Perlite; SunGro Horticulture Distribution Inc.), and wetting agent (Aqua-Gro 2000 G; Aquatrols, Cherry Hill, NJ, USA) at 600 $g \cdot m^{-3}$ with varying rates of calcium silicate ($CaSiO_3$) (SunGro Horticulture Distribution Inc.) and pH-adjusted amendment rates of dolomitic limestone (Rockydale Agricultural, Roanoke, VA, USA) to create a substrate with a pH between 5.8 and 6.2. The first substrate was amended with 0.0 $kg \cdot m^{-3}$ $CaSiO_3$ and 2.97 $kg \cdot m^{-3}$ dolomitic limestone. A second substrate was amended with 1.04 $kg \cdot m^{-3}$ $CaSiO_3$ and 2.07 $kg \cdot m^{-3}$ dolomitic limestone. A third substrate was amended with 2.07 $kg \cdot m^{-3}$ $CaSiO_3$ and 1.04 $kg \cdot m^{-3}$ dolomitic limestone. Calcium silicate rates of 0.0, 1.04, and 2.07 $kg \cdot m^{-3}$ $CaSiO_3$ are referred to as Si_{0X}, $Si_{0.5X}$, and Si_{1X}, respectively.

Fertilization treatments. All fertilizers were custom blends of the following individual technical-grade salts (Fisher Scientific, Pittsburgh, PA): calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$]; potassium nitrate (KNO_3); monopotassium phosphate (KH_2PO_4); potassium sulfate (K_2SO_4); magnesium nitrate [$Mg(NO_3)_2$]; iron chelate (Fe-DTPA); manganese chloride ($MnCl_2 \cdot 4H_2O$); zinc chloride ($ZnCl_2 \cdot 7H_2O$); copper chloride ($CuCl_2 \cdot 2H_2O$); boric acid (H_3BO_3); and sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$).

Table 1. The applied nutrient fertilizer concentration × micronutrient fertility concentration treatments.

Micronutrients ($mg \cdot L^{-1}$) Weeks 1–12						
Micronutrient rate	Fe	Mn	Cu	Zn	B	Mo
1×	4.02	0.99	0.19	0.20	0.49	0.01
2×	8.04	1.98	0.38	0.40	0.98	0.02
4×	16.08	3.96	0.76	0.80	1.96	0.04
Macronutrients ($mg \cdot L^{-1}$) Weeks 1–4 and 9–12						
All treatments	N	P	K	Ca	Mg	S
	150	20	150	128.66	54.21	53.88
Macronutrients ($mg \cdot L^{-1}$) Weeks 5–8						
All treatments	N	P	K	Ca	Mg	S
	250.3	20	250.4	200.64	54.21	53.88

Fertilization treatments began on the day of transplant. Three micronutrient fertilizer rates of 1×, 2×, and 4× (referred to M_{1X}, M_{2X}, and M_{4X}, respectively) of a modified Hoagland's solution concentration (Barnes et al. 2012) were mixed using the previously described fertilizer salts (Table 1). The macronutrient fertility rate was altered during weeks 5 through 8, during which nitrogen (N), potassium (K), and calcium (Ca) were increased in all of the examined micronutrient fertility treatments (Table 1). The fertility treatments were mixed in 100-L barrels and applied through drip irrigation as needed at every irrigation with an estimated 10% leaching fraction. The solution was delivered via pumps (model 1A; Little Giant Pump Co., Oklahoma City, OK, USA) connected to 1.9-cm-diameter irrigation tubing fitted with circular drip emitters (Dramm USA, Manitowoc, WI, USA).

Data collection. Twenty single plant replicates were transplanted for each treatment (Si rate × micronutrient fertility rate). At weeks 1, 3, 6, 9, and 12, substrate pH and electrical conductivity (EC) were evaluated using the pour-through method for the same six replicates for each treatment (Cavins et al. 2004). Plants were initially irrigated with 250 mL of the fertilizer solution to reach container capacity 30 min before each data collection, and 75 mL of deionized water (DI) was poured over the pots to displace 50 mL of

Table 2. Growth metrics of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with Si_{0X}, $Si_{0.5X}$, or Si_{1X} and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 6 weeks from transplant.

Impact of Si substrate amendment					
Calcium silicate	pH	EC (mS/cm)	Height[ii] (cm)	Diameter[ii] (cm)	Shoot dry weight (g)
Si_{0X}	5.54 A	1.97	33.21	27.75	2.52
$Si_{0.5X}$	5.19 B	1.93	35.38	28.44	2.69
$Si_{1.0X}$	5.18 B	1.85	31.95	26.95	2.60
Significance[iii]	*	NS	NS	NS	NS
Impact of micronutrient fertility rate					
Micronutrient Fertility Rate[i]	pH	EC	Height[ii]	Diameter[ii]	Shoot dry weight
1×	5.31 AB	1.86	33.48	26.75	2.23 B
2×	5.25 B	1.97	32.77	27.79	2.79 A
4×	5.36 A	1.93	34.28	28.59	2.80 A
Significance[iii]	*	NS	NS	NS	**
Interaction					
Micros × Si rate	pH	EC	Height[ii]	Diameter[ii]	Shoot dry weight
Si_{0X} M_{1X}	5.60 A	1.95	34.78	27.01	2.34
Si_{0X} M_{2X}	5.50 AB	2.00	31.33	27.65	2.83
Si_{0X} M_{4X}	5.52 AB	1.97	33.50	28.58	2.40
$Si_{0.5X}$ M_{1X}	5.13 C	2.03	33.90	27.49	2.23
$Si_{0.5X}$ M_{2X}	5.12 C	1.77	36.28	28.39	2.75
$Si_{0.5X}$ M_{4X}	5.33 BC	2.00	35.95	29.43	3.08
$Si_{1.0X}$ M_{1X}	5.18 C	1.61	31.77	25.76	2.11
$Si_{1.0X}$ M_{2X}	5.13 C	2.13	30.68	27.33	2.78
$Si_{1.0X}$ M_{4X}	5.22 C	1.82	33.40	27.75	2.91
Significance[iii]	*	NS	NS	NS	NS

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] The diameter was calculated by taking the widest two points on a plant 90° from each other. These numbers were summed and divided by 2 to get the diameter measurement. All dry weights were based on oven-dried material.

[iii] *, **, or *** indicates statistically significant differences between sample means based on the *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the *F* test difference between sample means was $P > 0.05$. When the *F* test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

leachate. The leachate was analyzed to determine the pH and EC using a Hanna portable pH/EC meter (HI9813–6; Hanna Instruments, Smithfield, RI, USA).

At weeks 6 and 12, six plants were sampled to determine plant height and measured from the substrate level to the apical meristem [(widest diameter + perpendicular axis) ÷ 2]; the diameter was also measured. After 6 weeks of growth, six plants were destructively harvested, and the most recently matured leaves and a sample of the roots were collected to evaluate the micronutrient and macronutrient tissue concentrations of each treatment. The root samples were washed to remove the substrate before drying. The most recently matured leaves and root samples were initially rinsed with DI; then, they were washed in a solution of 0.5 M HCl for 1 min and rinsed again with DI water (Henry et al. 2018). The remaining shoot tissue was harvested separately and dried to calculate the total plant biomass. At week 12, six plants were destructively harvested, during which floral material was collected for the cannabinoids, heavy metal, and micronutrient analysis; the remaining aboveground portion of the plant was collected to determine biomass production.

Upon sampling, the plant tissues and remaining aboveground plant biomass were dried at 70 °C for 96 h, and the dry mass was weighed and recorded. After drying, leaf tissue was ground in a Foss Tecator Cyclotec™ 1093 sample mill (≤0.5-mm sieve; Analytical Instruments, LLC, Golden Valley, MN, USA). Then, the ground tissue was placed in vials containing ∼3 g of tissue and analyzed at the Waters Laboratory (Warsaw, NC, USA). Plant material (0.5 g) was first rinsed in nitric acid (10 mL of HNO_3 at 15.6 N) and digested in a microwave digestion system for 30 min (MARS 6 Microwaves; CEM Corp., Matthews, NC, USA). After microwave digestion, the solution was diluted with 50 mL of DI; then, it was vacuum-filtered through acid-washed paper (Laboratory Filtration Group, Houston, TX, USA). After dilution, the plant mineral tissue concentration was determined using an inductively coupled plasma-optical emission spectrometry (ICP-OES) machine (Spectro Arcos EOP; Mahwah, NJ, USA).

Floral chemical analysis. During the flowering harvest (8 weeks into floral development), the main apical meristem and four terminal axillary buds were harvested, creating a composite floral sample; half of this sample (∼4 g) was used for the heavy metal analysis by Waters Laboratory. The metals were analyzed using Environmental Protection Agency methods 3050B for digestion and 6010S for analyses by the ICAP-OES. Plant material (0.5 g) was rinsed using 70:30 (volume:volume) nitric acid:hydrogen peroxide and digested using an Environmental Express HotBlock metals digestion system (Environmental Express, Charleston, SC, USA). After digestion, plant samples were analyzed using a Spectro Arcos ICP-OES (Spectro Arcos EOP).

Table 3. Growth metrics of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with Si_{0X}, $Si_{0.5X}$, or Si_{1X} and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 12 weeks from transplant.

Impact of Si substrate amendment						
Calcium silicate		pH	EC (mS/cm)	Height[ii] (cm)	Diameter[ii] (cm)	Shoot dry weight (g)
Si_{0X}		5.59 A	2.10	38.16	25.55	11.10 B
$Si_{0.5X}$		5.38 B	2.20	41.38	27.24	12.96 A
$Si_{1.0X}$		5.27 C	2.29	37.70	26.22	11.52 AB
Significance[iii]		***	NS	NS	NS	NS
Impact of micronutrient fertility rate						
Micronutrient fertility rate[i]		pH	EC	Height[ii]	Diameter[ii]	Shoot dry weight
1×		5.33 B	2.59 A	38.41	25.93	11.44
2×		5.46 A	2.08 B	39.42	26.25	12.23
4×		5.46 A	1.90 B	39.41	26.83	11.92
Significance[iii]		***	*	NS	NS	NS
Interaction						
Micros × Si rate		pH	EC	Height[ii]	Diameter[ii]	Shoot dry weight
Si_{0X}	M_{1X}	5.43 B	2.46	39.68	24.73	11.62
Si_{0X}	M_{2X}	5.65 A	2.24	37.58	25.35	10.75
Si_{0X}	M_{4X}	5.70 A	1.59	37.22	26.58	10.93
$Si_{0.5X}$	M_{1X}	5.43 B	2.54	38.27	26.72	11.57
$Si_{0.5X}$	M_{2X}	5.40 B	1.79	43.97	26.46	13.93
$Si_{0.5X}$	M_{4X}	5.30 B	2.26	41.90	28.54	13.38
$Si_{1.0X}$	M_{1X}	5.13 C	2.78	37.28	26.35	11.13
$Si_{1.0X}$	M_{2X}	5.32 B	2.22	36.72	26.94	12.00
$Si_{1.0X}$	M_{4X}	5.37 B	1.86	39.10	25.37	11.43
Significance[iii]		***	NS	NS	NS	NS

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] The diameter was calculated by taking the widest two points on a plant 90° from each other. These numbers were added together and divided by 2 to obtain the diameter measurement. All dry weights were obtained based on oven-dried material.

[iii] *, **, or *** indicates statistically significant differences between sample means based on the *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the *F* test difference between sample means was $P > 0.05$. When the *F* test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

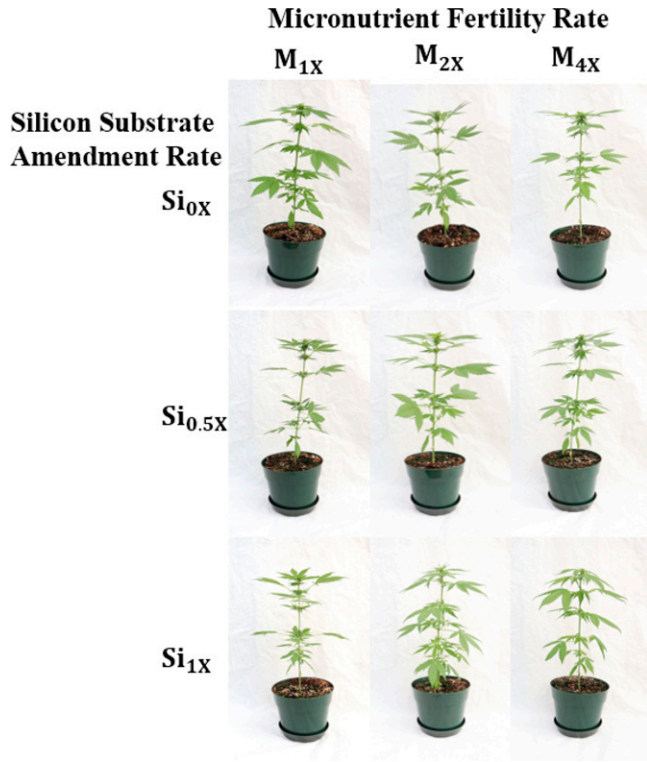

Fig. 1. Impact of the micronutrient fertility treatment and calcium silicate substrate amendment on *Cannabis sativa* 'Auto CBG' 6 weeks after transplant.

The remainder of the composite floral sample was used for the cannabinoid analysis. The composite sample was freeze-dried (Harvest Right, North Salt Lake, UT, USA) for 30 h. Dry mass was weighed, recorded, and submitted for the cannabinoid and terpene analysis (Delta 9 Analytics, Raleigh, NC, USA). On arrival, samples were lyophilized and ground; a 2-g (1.98–2.02 g) sub-sample from the composite buds was obtained. The analysis of cannabinoids was accomplished through high-pressure liquid chromatography (SHIMADZU 8050 and 8040 Triple Quadrupole UHPLC/MS/MS analysis; Austin, TX, USA). Exact details regarding the cannabinoid analysis cannot be provided because Delta 9 Analytics uses a proprietary protocol.

The cannabinoid analysis included both the active (decarboxylated) and acid forms of CBG, THC, CBD, and cannabichromene. Additional cannabinoids and forms exist, but they are not reported here (e.g., cannabidivarin and tetrahydrocannabivarin) because their concentrations were too low to detect, were not evaluated, or were present in similar concentrations regardless of treatment. The total CBD and THC were calculated by the following equations:

$$\Delta^9\text{THC} + [0.877 \times \text{tetrahydrocannabinol acid (THCA)}] = \text{Total THC} \quad [1]$$

$$\text{CBD} + [0.877 \times \text{cannabidiol acid (CBDA)}] = \text{Total CBD} \quad [2]$$

Statistical analysis. The statistical analysis was conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA). Plant growth metrics and leaf and flower nutrient values were analyzed for differences within each data collection (n = 6) as a 3 × 3 factorial of micronutrient fertility rate × Si amendment rate with substrate PROC GLM. Means were separated with Tukey's honest significant difference ($P < 0.05$). Deviations in plant metrics, total plant dry weights, and leaf tissue nutrient values were calculated based on the percentage of controls ($M_{1X} \times Si_{0X}$).

Results and Discussion

Substrate pH and EC. Regarding the interaction of micronutrient fertility × Si substrate amendment at sampling intervals of 6 and 12 weeks, differences were observed among the substrate pH of the various treatments in which a general trend of plants that received the Si_{1X} or $Si_{0.5X}$ amendment exhibited a lower substrate pH than those that received the Si_{0X} amendment ($P = 0.043$ and $P < 0.001$, respectively) (Tables 2 and 3). When examining the simple effects of the Si rate, plants that did not receive a Si amendment exhibited significantly greater substrate pH than those that received a Si amendment of $Si_{0.5X}$ or Si_{1X} ($P < 0.001$) at both sampling intervals (Tables 2 and 3). These differences can be attributed to the lower amount of dolomitic limestone added to the $Si_{0.5X}$ or Si_{1X} treatments to account for the basic properties of Si (Table 1). However, the difference was less than 0.4 units at both sampling dates and likely had no impact on plant growth (Tables 2 and 3).

After 6 weeks of growth, there were no observed differences in substrate EC for the interaction of micronutrient fertility × Si substrate amendment or any of the examined simple effects (Table 2). However, at week 12, although there were no significant differences in the interaction, when examining the simple effect of the micronutrient fertility rate, plants that received a fertility rate of M_{1X} exhibited significantly greater EC than all other micronutrient fertility treatments ($P = 0.002$) (Table 3). Previous studies reported an increase in substrate EC for sunflowers grown using substrates that received Si from a 20% rice hull amendment; however, petunias grown under the same conditions did not exhibit significant differences in EC (Boldt et al. 2018). This variability in differences regarding Si resulting in increased EC values can likely be related to the uptake patterns of the crop and the fact that excess nutrients were not available in the container.

Plant growth metrics. After 6 weeks of growth, plant height, diameter, and shoot dry weight were not significantly different when comparing the interaction of micronutrient fertility × Si substrate amendment (Table 2). However, plant dry weight was significant when examining the simple effect of the micronutrient fertility rate; plants that received a M_{1X} fertility rate had significantly less biomass when compared with those that received a fertility rate of M_{2X} or M_{4X} ($P < 0.001$) (Table 2). Additionally, no phytotoxicity was observed after 6 weeks of growth on any of the treatments, and similar growth occurred (Fig. 1).

After 12 weeks of growth, plants were screened for phytotoxicity caused by micronutrient accumulation. Plants that received a Si_{0X} rate and a M_{4X} fertility treatment exhibited the most severe phytotoxicity (Fig. 2). Additionally, less severe phytotoxicity was observed on plants that received $Si_{0.5X}$ and a M_{4X} fertility rate. Phytotoxicity was not observed on plants that were grown with a M_{4X} micronutrient fertility rate and received a Si_{1X} substrate amendment or any other treatment. Additionally, plant height, diameter, and shoot dry weight were not significantly

Fig. 2. Lower leaf phytotoxicity observed on *Cannabis sativa* 'Auto CBG' plants grown without a calcium silicate substrate amendment and 4× micronutrient fertility treatment.

different when comparing the interaction or simple effects of micronutrient fertility rate × Si substrate amendment after 12 weeks of growth (Table 3).

Currently, limited published research has examined the use of calcium silicate as a substrate amendment; however, researchers have examined other forms of Si supplements in soilless substrates, with rice hulls being one of them. The results of this study vary compared with those reported by Boldt et al. (2018), who reported decreased sunflower and petunia total aboveground dry weight when amending peat:perlite substrates with 20% rice hull incorporation. However, this could likely be due to the change in the physical properties of the substrate and not as a result of Si.

Root tissue analysis. After 6 weeks of growth, when examining the interaction of the micronutrient fertility rate × Si rate, significant differences were observed for B, Zn, Mn, Fe, and Cu, and there was a general trend of plants that received a silicon substrate amendment of Si_{1X} and M_{4X} fertility rate exhibiting the lowest elemental concentrations (Table 4). Plants that received a M_{1X} fertility rate and $Si_{0.5X}$ or Si_{1X} rate exhibited 48% and 52% lower Fe concentrations, respectively, when compared with plants grown without Si (Table 4). When the highest micronutrient rate was applied (M_{4X}) with the Si_{1X} rate, the root tissue of the plant exhibited B, Zn, Fe, Mn, and Cu concentrations that were lower by 64.5%, 67.3%, 74%, 67.2%, and 77.4%, respectively, when compared with plants that received the $Si_{0.5X}$ or Si_{0X} rate (Table 4). These trends show that under excessive micronutrient conditions, the addition of Si helps limit micronutrient accumulation in the roots.

Foliar tissue analysis. The B leaf tissue concentrations exhibited significant differences in the interaction of the micronutrient fertility rate × Si rate ($P < 0.001$) (Table 5). When examining the simple effects of the micronutrient fertility rates for B, Zn, Fe, and Cu, significant differences were observed (Table 5). The Si concentration in the foliar tissue was significant when examining the interaction of the micronutrient fertility rate × Si rate ($P = 0.039$). However, when examining the simple effect of the Si amendment rate, plants that received a Si concentration of 1× exhibited a significantly greater Si concentration compared with those that did not receive Si (Si_{0X}) ($P < 0.001$) (Table 5). It has been reported that Si alleviates Mn toxicity in cucumber (*Cucumbis sativus* L.) (Rogalla and Römheld 2002); however, during this study, we did not observe a decrease in foliar Mn concentrations with the increasing Si rate. It has been reported that Mn toxicity can occur with concentrations as low as 47.88 mg·kg^{-1} in cannabis plants (Cockson et al. 2019); however, even plants that did not receive Si (Si_{0X}) and a micronutrient fertility rate of 1× exhibited an Mn foliar concentration of 193.80 mg·kg^{-1} without visual Mn foliar toxicity symptoms.

Table 4. Root tissue nutrient concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with Si_{0X}, $Si_{0.5X}$, or Si_{1X} and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 6 weeks from transplant.

Impact of Si substrate amendment						
Calcium silicate		B mg·kg^{-1}	Zn mg·kg^{-1}	Mn mg·kg^{-1}	Fe mg·kg^{-1}	Cu mg·kg^{-1}
Si_{0X}		18.33 B	175.58	149.22 B	411.43 A	17.59 AB
$Si_{0.5X}$		22.06 A	204.53	185.26 A	259.28 B	20.98 A
$Si_{1.0X}$		16.33 B	181.61	134.11 B	195.03 C	14.00 B
Significance[ii]		***	NS	***	***	*
Impact of micronutrient fertility rate						
Micronutrient fertility rate[i]		B	Zn	Mn	Fe	Cu
1×		17.22 B	192.13	174.56 A	316.31 A	22.04 A
2×		19.61 A	194.98	177.72 A	351.12 A	15.19 B
4×		19.89 A	174.61	116.31 B	198.31 B	15.33 AB
Significance[ii]		*	NS	***	***	*
Interaction						
Micros × Si rate		B	Zn	Mn	Fe	Cu
Si_{0X}	M_{1X}	16.00 C	157.40 BC	147.00 BC	467.25 A	15.60 ABC
Si_{0X}	M_{2X}	17.50 BC	143.17 DC	161.50 ABC	490.20 A	12.17 BC
Si_{0X}	M_{4X}	21.50 B	226.17 AB	139.17 C	276.83 B	25.00 AB
$Si_{0.5X}$	M_{1X}	17.17 BC	169.50 BC	203.17 AB	248.83 B	31.20 A
$Si_{0.5X}$	M_{2X}	20.83 BC	220.60 AB	213.00 A	283.17 B	16.40 ABC
$Si_{0.5X}$	M_{4X}	28.17 A	223.50 BC	139.60 C	245.83 B	15.33 BC
$Si_{1.0X}$	M_{1X}	18.50 BC	249.50 A	173.50 ABC	232.83 B	19.33 ABC
$Si_{1.0X}$	M_{2X}	20.50 BC	221.17 AB	158.67 ABC	280.00 B	17.00 ABC
$Si_{1.0X}$	M_{4X}	10.00 D	74.17 D	70.17 D	72.25 C	5.67 C
Significance[ii]		***	***	**	***	***

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] *, **, or *** indicates statistically significant differences between sample means based on the *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the *F* test difference between sample means was $P > 0.05$. When the *F* test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Table 5. Leaf tissue micronutrient concentration of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 6 weeks from transplant.

Impact of Si substrate amendment							
Calcium silicate		B mg·kg^{-1}	Zn mg·kg^{-1}	Mn mg·kg^{-1}	Fe mg·kg^{-1}	Cu mg·kg^{-1}	Si %
Si_{0X}		88.20 B	66.11	193.07 C	159.48 A	6.38	1.61 B
$Si_{0.5X}$		104.10 A	72.17	264.57 A	157.44 A	6.17	2.22 AB
$Si_{1.0X}$		91.48 AB	67.94	240.27 B	147.50 B	7.26	2.69 A
Significance[ii]		*	NS	*	**	NS	***
Impact of micronutrient fertility rate							
Micronutrient fertility rate[i]		B	Zn	Mn	Fe	Cu	Si
1×		69.10 B	62.56 B	241.07	152.60 B	5.27 C	2.15 A
2×		72.90 B	65.56 B	230.08	162.32 A	6.49 B	2.11 A
4×		141.78 A	78.11 A	226.76	149.50 B	8.04 A	2.25 A
Significance[ii]		***	**	NS	**	**	NS
Interaction							
Micros × Si rate		B	Zn	Mn	Fe	Cu	Si
Si_{0X}	M_{1X}	58.83 B	62.00	193.80	159.80	4.80	1.38
Si_{0X}	M_{2X}	66.60 B	58.50	182.33	157.80	5.50	1.90
Si_{0X}	M_{4X}	139.17 A	77.83	203.08	160.83	8.83	1.55
$Si_{0.5X}$	M_{1X}	73.67 B	62.67	267.00	152.00	4.83	2.19
$Si_{0.5X}$	M_{2X}	85.80 B	74.67	275.50	170.50	6.17	2.30
$Si_{0.5X}$	M_{4X}	152.83 A	79.17	251.20	149.83	7.50	2.16
$Si_{1.0X}$	M_{1X}	74.80 B	63.00	262.40	146.00	6.17	2.88
$Si_{1.0X}$	M_{2X}	66.31 B	63.50	232.40	158.67	7.80	2.14
$Si_{1.0X}$	M_{4X}	133.33 A	77.33	226.00	137.83	7.80	3.05
Significance[ii]		***	NS	NS	NS	NS	NS

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] *, **, or *** indicates statistically significant differences between sample means based on the *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the *F* test difference between sample means was $P > 0.05$. When the *F* test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Table 6. Floral tissue nutrient concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with Si_{0X}, $Si_{0.5X}$, or Si_{1X} and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 12 weeks from transplant.

Impact of Si substrate amendment							
Calcium silicate		B mg·kg^{-1}	Zn mg·kg^{-1}	Mn mg·kg^{-1}	Fe mg·kg^{-1}	Cu mg·kg^{-1}	Si %
Si_{0X}		73.41 A	138.12 A	529.88 A	505.59	21.29 A	0.94 B
$Si_{0.5X}$		59.69 B	114.81 B	425.44 B	480.26	16.63 AB	1.05 B
$Si_{1.0X}$		60.83 B	122.33 B	409.50 B	474.72	16.33 B	1.39 A
Significance[ii]		***	**	**	NS	*	***
Impact of micronutrient fertility rate							
Micronutrient fertility rate[i]		B	Zn	Mn	Fe	Cu	Si
1×		49.69 C	121.38	393.25 B	478.56	12.75 B	1.38 A
2×		57.18 B	131.24	450.41 AB	457.40	17.94 AB	1.03 B
4×		85.06 A	123.00	513.17 A	521.74	22.94 A	0.97 B
Significance[ii]		***	NS	**	NS	**	***
Interaction							
Micros × Si rate		B	Zn	Mn	Fe	Cu	Si
Si_{0X}	M_{1X}	49.50 C	120.50 B	380.00 C	387.83 B	10.67 C	1.42 AB
Si_{0X}	M_{2X}	75.00 B	170.60 A	636.40 A	510.80 AB	24.40 AB	0.80 CD
Si_{0X}	M_{4X}	96.00 A	128.67 B	591.00 AB	619.00 A	29.33 A	0.61 D
$Si_{0.5X}$	M_{1X}	49.50 C	118.00 B	405.50 BC	536.25 AB	15.50 BC	1.11 ABCD
$Si_{0.5X}$	M_{2X}	48.83 C	110.67 B	368.33 C	440.80 AB	14.17 BC	1.00 BCD
$Si_{0.5X}$	M_{4X}	77.33 B	116.83 B	495.83 ABC	482.40 AB	19.83 ABC	1.03 BCD
$Si_{1.0X}$	M_{1X}	50.00 C	124.50 B	398.33 C	530.83 AB	13.00 BC	1.60 A
$Si_{1.0X}$	M_{2X}	50.67 C	119.00 B	377.50 C	429.50 B	16.33 BC	1.29 AB
$Si_{1.0X}$	M_{4X}	81.83 AB	123.50 B	452.67 BC	463.83 AB	19.67 ABC	1.26 ABC
Significance[ii]		***	**	**	**	*	*

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] *, **, or *** indicates statistically significant differences between sample means based on the *F* test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the *F* test difference between sample means was $P > 0.05$. When the *F* test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

The interaction of Si soil amendments × Fe nutrition has been widely examined among many different species. The results suggested that Si amendments strongly regulate the transport of Fe and Fe availability in the soil and root apoplast, thus lowering the Fe concentration and distribution within the plant (Becker et al. 2020; Gonzalo et al. 2013). Additionally, the interaction of Si × Cu toxicity has been examined in wheat (Nowakowski and Nowakowska. 1997) and *Arabidopsis (*Khandekar and Leisner 2011). The alleviation of Cu toxicity by Si resulted from the increased binding sites in the cell wall; although there was no decrease in the Cu concentration in the shoot, the Si deposits formed Cu-binding sites that prevented the high Cu concentrations from negatively impacting the plant (Pavlovic et al. 2021). This could potentially explain why, in our study, there were no differences in Cu concentrations of the foliage after 6 weeks of growth for plants that were grown using a Si substrate amendment.

Floral tissue analysis. After 12 weeks of growth, B, Zn, Mn, Fe, Cu, and Si floral tissue concentrations were significantly different when the interaction of the micronutrient fertility rate × Si rate was examined (Table 6). As a general trend, plants that received a greater micronutrient fertility rate exhibited a greater micronutrient concentration in the floral tissue, excluding that of Fe (Table 6). Inversely, plants that received an Si amendment generally exhibited a greater Si concentration compared with those that did not receive an Si amendment (Table 6). In most cases, Si concentrations in floral material yielded a lower Si concentration when compared with the foliar material; this trend is similar to that reported by Boldt et al. (2018), who found that sunflower leaves exhibited the greatest concentration, followed by roots, stems, and flowers. It has been reported that Si reduces the infection of gray mold (*Botrytis cinerea*) in lettuce, tomato, and pepper when it is supplied in a hydroponic nutrient solution (Pozo et al. 2015). Gray mold is one of the most important diseases in cannabis production because it results in the greatest losses in yield (McPartland et al. 2000). Thus, regarding floral material accumulating Si without disease pressure, further research is needed to determine if the increased Si concentration can prevent yield losses caused by botrytis.

After 12 weeks of growth, CBG, CBGA, total CBG, total THC, and total cannabinoid concentrations were similar across all examined Si rates or micronutrient fertility treatments (Table 7). Therefore, the inclusion of Si in the substrate offers the advantage of not detrimentally impacting cannabinoid concentrations in cannabis.

Conclusion

Growing *Cannabis sativa* 'Auto CBG' under increasing heavy metal micronutrient concentrations of a modified Hoagland's solution while varying the rate of a Si substrate amendment resulted in significant differences in micronutrient concentrations in the foliage, roots, and floral tissue. Plants that received Si_{1X} exhibited less Fe foliar concentrations at M_{1X} and M_{4X} and decreased concentrations in the roots at all micronutrient fertility treatments. This decrease in Fe concentration is likely what caused no phytotoxicity after 12 weeks of growth on Si_{1X} plants that received the M_{4X} fertility rate, whereas all other Si rates exhibited phytotoxicity when grown under the same M_{4X} treatment. Additionally, the various cannabinoid concentrations were not negatively impacted by the addition of Si substrate amendments. The results obtained from this study suggest that substrates amended with Si offer the advantage of avoiding excessive micronutrient incorporation in the plant under greenhouse conditions. Furthermore, a significant reduction in micronutrient accumulation, such as B, Mn, Fe, and Cu, was observed in the floral material with the addition of the Si_{1X} substrate amendment, which is important for quality control and the avoidance of heavy metal contamination in *Cannabis sativa*.

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Table 7. Cannabinoid concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with Si_{0X}, $Si_{0.5X}$, or Si_{1X} and supplied with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for 12 weeks from transplant.

Impact of Si substrate amendment						
Calcium silicate		CBG[ii]	CBGA[ii]	Total CBG[ii]	THCA[ii]	Total THC[ii]
Si_{0X}		0.04	6.43	5.79	0.13	0.11
$Si_{0.5X}$		0.06	6.39	5.62	0.09	0.08
$Si_{1.0X}$		0.06	6.89	6.07	0.09	0.08
Significance[iii]		NS	NS	NS	NS	NS
Impact of micronutrient fertility rate						
Micronutrient fertility rate[i]		CBG[ii]	CBGA[ii]	Total CBG[ii]	THCA[ii]	Total THC[ii]
1×		0.05	6.59	5.87	0.10	0.08
2×		0.06	6.15	5.41	0.12	0.11
4×		0.05	6.97	6.21	0.09	0.08
Significance[iii]		NS	NS	NS	NS	NS
Interaction						
Micros × Si rate		CBG[ii]	CBGA[ii]	Total CBG[ii]	THCA[ii]	Total THC[ii]
Si_{0X}	M_{1X}	0.05	5.95	5.47	0.09	0.08
Si_{0X}	M_{2X}	0.04	6.39	5.61	0.20	0.18
Si_{0X}	M_{4X}	0.04	6.94	6.28	0.10	0.09
$Si_{0.5X}$	M_{1X}	0.05	6.48	5.70	0.11	0.10
$Si_{0.5X}$	M_{2X}	0.08	5.40	4.75	0.08	0.07
$Si_{0.5X}$	M_{4X}	0.05	7.29	6.42	0.09	0.08
$Si_{1.0X}$	M_{1X}	0.05	7.33	6.43	0.09	0.08
$Si_{1.0X}$	M_{2X}	0.05	6.65	5.86	0.09	0.08
$Si_{1.0X}$	M_{4X}	0.07	6.69	5.92	0.08	0.07
Significance[iii]		NS	NS	NS	NS	NS

[i] Micronutrient fertility rates based on X times the standard concentration (Table 1).

[ii] Any variance of the cannabinoids (CBDA, CBGA, THCA, CBCA, etc.) indicates the acid form of the molecule. The acidic version of the molecule is present in larger quantities in the plant and is converted to the nonacid forms through decarboxylation. The total CBD and THC are calculated based on the concentration of $mg \cdot g^{-1}$ of a composite sample that had been lyophilized (1.98–2.02 g). The "Total" column indicates the concentration of cannabinoids calculated by the equations listed in the Materials and Methods. All values are expressed in terms of the concentration ($mg \cdot g^{-1}$) of a 2-g freeze-dried composite weight.

[iii] *, **, or *** indicates statistically significant differences between sample means based on the F test at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively. NS (not significant) indicates the F test difference between sample means was $P > 0.05$. When the F test was significant, the honest significant difference with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

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