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Comparison of Peat–Perlite-based and Peat–Biochar-based Substrates with Varying Rates of Calcium Silicate on Growth and Cannabinoid Production of *Cannabis sativa* 'BaOx'

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Abstract. **Growers have been searching for alternative horticultural growing media components because of their desire to use sustainable resources. Biochar is a carbon-based material that has been evaluated for use as an alternative aggregate in peat-based soilless substrates. Additionally, silicon (Si) has been examined as a beneficial element to promote plant growth and plant quality in a variety of crops. However, there has been limited research regarding the interaction of biochar as an aggregate and Si in soilless substrates. This study aimed to determine the impact of Si and biochar on plant growth and nutrient uptake for greenhouse-cultivated hemp (*Cannabis sativa* L.). Hemp plants were grown in one of 12 different substrate blends: with two rates of calcium silicate ($CaSiO_3$), two aggregate types of biochar (medium or coarse) or perlite, and aggregate percentages of 85% peat + 15% aggregate and 70% peat + 30% aggregate. The cannabinoid concentration, plant height, diameter, or total plant biomass were similar across all substrate blends after 12 weeks of growth. Additionally, the use of $CaSiO_3$ as a Si substrate amendment increased Si foliar concentrations, and the addition of biochar to peat-based mixes did not limit the Si availability for plant uptake. However, Si substrate amendments did not impact plant height, diameter, or total plant biomass. This suggests that the biochar tested during this study is suitable in peat-based substrates for *C. sativa* 'BaOx' production at rates up to 30% (by volume) in peat-based substrates with $CaSiO_3$ amendments.**

Cannabis sativa L. has recently gained global popularity because of the wide array of products that contain hemp fibers, oils, and cannabinoids (Salentijn et al. 2019). Hemp is defined as *C. sativa* that contains no more than 0.3% total tetrahydrocannabinol (THC) concentration of dry weight in any part of the plant (US House of Representatives 2018). Hemp contains more than 100 cannabinoids, including cannabidiol (CBD), THC, and cannabigerol, that vary in concentration. Many cannabinoids are considered to have medical and therapeutic effects, thus leading to the recent interest in *Cannabis* production (Salentijn et al. 2019).

Currently, several products are used in the formulation of a growing media including sphagnum peat, aged or composted bark, and aggregates such as perlite and vermiculite (Nemati et al. 2015). However, recently, alternative biomasses have been evaluated for use in growing media. Evans and Gachukia (2004) reported that 10% to 40% parboiled fresh rice hulls incorporated in a peat-based substrate resulted in similar growth of tomato (*Solanum lycopersicum* L. 'Better Boy'), marigold (*Tagetes patula* L. 'Bonza Yellow'), geranium (*Pelargonium ×hortum* Bailey 'Orbit Cardinal'), vinca (*Vinca minor* L. 'Cooler Blush'), impatiens (*Impatiens walleriana* Hooker 'Dazzler Rose Star'), and pansy (*Viola ×wittrockiana* Gams 'Bingo Azure') when compared with the growth resulting from equal amounts of perlite. Additionally, the incorporation of 10% to 30% pine wood chips has been proven to be an appropriate alternative to perlite in peat-based substrates for plectranthus (*Plectranthus ciliatus* E. Mey. 'Vareigata'), sunflower (*Helianthus annuus* L. 'Pacino Gold'), French marigold (*Tagetes patula* L. 'Anemone Safari Yellow'), and zinnia (*Zinnia ×hybrida* Jacquin 'Profusion Orange') (Owen 2013).

Biochar is a black charcoal-like material produced by organic products heated to temperatures below 700 °C in an oxygen-limited environment that is intended to be used in agricultural applications (Lehmann and Joseph 2015). Recently, there has been a large initiative to use biochar in agricultural applications ranging from field amendments (Chan et al. 2007; Singh et al. 2010) to a perlite replacement in potting media (Northup 2013; Yu et al. 2019). Biochar can be created from a wide array of materials such as hardwoods, softwood, hemp fiber, or other biomasses (Glaser and Asomah 2022; Huang and Gu 2019; Yu et al. 2019). One of the largest concerns with biochar is the impact on the substrate's chemical and physical properties, such as pH, electrical conductivity (EC), and porosity (Huang and Gu 2019). In most cases, biochar has a neutral or basic pH (>7.0) and is effective at increasing the substrate pH (Dispenza et al. 2016; Northup 2013; Park et al. 2011; Zhang et al. 2014). However, the pH values of biochar materials reportedly range from 3.5 to 10.3 (Fornes et al. 2015; Khodadad et al. 2011; Nemati et al. 2015; Spokas et al. 2012) and may potentially neutralize acidity caused by peat and root growth (Bedussi et al. 2015). Incorporating biochar into substrates can increase the cation exchange capacity; however, the magnitude of increase is dependent on the biochar feedstock (Huang and Gu 2019). Because of the wide variety of feedstocks used and biochar incorporation rates in substrates, the impacts on physical and chemical properties can vary widely (Huang and Gu 2019).

A commercial greenhouse substrate additive that is growing in popularity is silicon (Si). Silicon is considered a beneficial element for plants and is the second most abundant element in the soil and surface of the earth (Liang et al. 2007). To date, few studies have investigated Si substrate amendments during greenhouse cultivation because most greenhouse crops are low accumulators of Si (Bolt and Altland 2021). Silicon supplementation in greenhouse crops can be achieved in multiple ways, including foliar applications (Kamenidou et al. 2009; Whitted-Haag et al. 2014), incorporation of Si in hydroponic nutrient solution (Bolt and Altland 2021; Mattson and Leatherwood 2010), or Si substrate amendments (Boldt et al. 2018; Kamenidou et al. 2010). Although the published effects of Si greenhouse amendments are limited, the effects of Si amendments on mineral soils have been studied extensively. Silicon has been examined as a soil amendment to improve plant growth in soils contaminated with heavy metals and exclude the uptake of heavy metals (Luyckx et al. 2021; Khan et al. 2021; Pavlovic et al. 2021). Silicon has the ability to increase the availability and absorption of phosphorus and other essential nutrients (Tripathi et al. 2015). In fiber hemp, the impact of Si soil amendments in the presence of Cd stress resulted in less Cd accumulation in the plant; however, no change in Cd distribution within the plant was observed (Luyckx et al. 2021). Silicon chelates heavy metals in the soil, thus decreasing their bioavailability and ultimately leading to lower heavy metal concentrations in the plant (Khan

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Table 1. Summary of substrate treatments and evaluation of biochar as a perlite replacement for *Cannabis sativa* ‘BaOx’.

		Substrate properties					
Substrate	Aggregate type	Substrate composition (by volume)	Peat %	Biochar %	Perlite %	Silicon ($kg\cdot m^{-3}$)	Limestone ($kg\cdot m^{-3}$)
1	Perlite	85:15	85	0	15	0.0	3.56
2			85	0	15	0.50	2.37
3		(70:30)	70	0	30	0.0	3.56
4			70	0	30	0.50	2.37
5	Medium biochar[i]	(85:15)	85	15	0	0.0	3.56
6			85	15	0	0.50	2.37
7		(70:30)	70	30	0	0.0	3.56
8			70	30	0	0.50	2.37
9	Coarse biochar[ii]	(85:15)	85	15	0	0.0	2.97
10			85	15	0	0.50	1.78
11		(70:30)	70	30	0	0.0	2.97
12			70	30	0	0.50	1.78

[i] Medium biochar aggregates are smaller than 6 mm.

[ii] Coarse biochar aggregates are larger than 6 mm.

et al. 2021). Furthermore, Si can enhance cell wall binding sites and alleviate certain nutrients accumulating to toxic levels, such as copper (Pavlovic et al. 2021).

Although the beneficial effects of biochar and Si are known independently, there is limited research regarding their interaction when combined for potted plants. One study reported that when both biochar and Si were used in combination, significant increases in the growth of maize (*Zea mays* L. ‘ICI-8914’) roots, shoots, and seedlings occurred when grown under drought conditions (Sattar et al. 2020). However, there is limited published research regarding the impacts of Si amendments when applied to a growing substrate amended with biochar or oil production of hemp. This study aimed to determine the impact of Si and biochar on plant growth and nutrient uptake for greenhouse-cultivated hemp.

Materials and Methods

Plant material. We obtained unrooted cuttings of the high CBD hemp cultivar BaOx from 12-week-old mother stock plants. Terminal vegetative exterior canopy cuttings were taken on 1 Feb 2022. Cuttings were inserted into 50-cell trays (3.5 cm × 4 cm individual cell size; VidaWool cubes, Owens Corning, Toledo, OH, USA) and humidity domes were placed over the unrooted plant cuttings. Cuttings were placed under T5 full-spectrum fluorescent lamps (AgroBrite T5 Full Spectrum;

Table 2. Growth metrics of *Cannabis sativa* ‘BaOx’ grown in soilless substrate amended with three different aggregates (perlite, medium biochar, or coarse biochar) at two different incorporation rates (15% or 30% by volume) and with or without silicon (Si) amendments (Si_{0X} or Si_{1X}) 6 weeks after transplantation.

			pH	EC (mS/cm)	Ht[ii] (cm)	Diameter[ii] (cm)	Total plant biomass (g)
			Aggregate type				
Perlite			5.90 C	2.31	61.22	66.95	23.26
Medium biochar			6.30 A	2.14	57.66	64.48	20.01
Coarse biochar			6.11 B	2.19	58.15	64.51	19.42
Significance[iii]			***	NS	NS	NS	NS
			Aggregate percentage				
15			5.94 B	2.22	59.60	66.60	20.86
30			6.27 A	2.21	58.42	64.03	20.94
Significance			***	NS	NS	NS	NS
			Si rate[i]				
0.0			6.04 B	2.12	59.61	66.04	21.84
0.50			6.16 A	2.32	58.41	64.59	19.95
Significance			**	NS	NS	NS	NS
			Second-order interactions				
Aggregate type × aggregate percentage			**	NS	NS	NS	NS
Aggregate type × Si rate			NS	NS	NS	NS	NS
Aggregate percentage × Si rate			*	NS	NS	NS	NS
			Aggregate type × aggregate percentage × Si rate				
Aggregate type	Aggregate	Silicon[i]					
Perlite	15	0.0	5.67	2.53	59.73	66.93	24.17
Perlite	15	0.50	5.67	2.06	56.92	63.76	18.70
Perlite	30	0.0	5.97	2.10	67.13	70.98	29.38
Perlite	30	0.50	6.30	2.55	61.10	66.15	20.78
Medium biochar	15	0.0	6.02	2.15	60.73	67.38	20.75
Medium biochar	15	0.50	6.18	2.28	60.60	69.88	21.00
Medium biochar	30	0.0	6.40	2.01	53.38	61.48	19.73
Medium biochar	30	0.50	6.60	2.13	55.93	59.18	18.57
Coarse biochar	15	0.0	6.10	1.96	61.45	68.71	21.00
Coarse biochar	15	0.50	6.00	2.37	58.17	62.92	19.53
Coarse biochar	30	0.0	6.12	1.94	55.25	60.73	16.02
Coarse biochar	30	0.50	6.23	2.51	57.73	65.68	21.13
Significance			NS	NS	NS	NS	NS

[i] Silicon (calcium silicate) substrate amendments are reported as $kg\cdot m^{-3}$.

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

[iii] *, **, and *** Statistically significant differences between sample means based on *F* test at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, 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 Tukey-Kramer adjustment ($P > 0.05$) was used to compare differences among means.

EC = electrical conductivity; NS = not significant.

Table 3. Growth metrics of *Cannabis sativa* 'BaOx' grown in soilless substrate amended with three different aggregates (perlite, medium biochar, or coarse biochar) at two different incorporation rates (15% or 30% by volume) and with or without silicon (Si) amendments (Si_{0X} or Si_{1X}) 12 weeks after transplantation.

			pH	EC (mS/cm)	Ht[ii] (cm)	Diameter[ii] (cm)	Total plant biomass (g)
Aggregate type							
Perlite			6.11 C	1.39	63.26	63.12	62.66
Medium biochar			6.61 A	1.57	65.78	60.92	70.78
Coarse biochar			6.28 B	1.23	62.60	59.40	72.50
Significance[iii]			***	NS	NS	NS	NS
Aggregate percentage							
15			6.23 A	1.45	64.19	60.67	68.84
30			6.44 B	1.34	63.57	61.63	68.45
Significance			***	NS	NS	NS	NS
Si rate[i]							
0.0			6.31	1.33	63.91	60.50	72.87
0.50			6.35	1.45	63.85	61.79	64.42
Significance			NS	NS	NS	NS	NS
Second-order interactions							
Aggregate type × aggregate percentage			***	NS	NS	NS	NS
Aggregate type × Si rate			*	NS	NS	NS	NS
Aggregate percentage × Si rate			**	NS	NS	NS	NS
Aggregate type × aggregate percentage × Si rate							
Aggregate type	Aggregate	Silicon					
Perlite	15	0.0	5.85 DE	1.01	60.68	57.13	50.47
Perlite	15	0.50	5.80 E	1.62	63.00	64.36	66.73
Perlite	30	0.0	6.18 CD	1.56	66.65	68.04	76.57
Perlite	30	0.50	6.63 AB	1.35	62.70	62.96	56.87
Medium biochar	15	0.0	6.60 AB	1.90	71.63	63.95	83.67
Medium biochar	15	0.50	6.55 AB	1.75	63.45	61.41	65.37
Medium biochar	30	0.0	6.73 A	1.15	60.28	58.10	63.70
Medium biochar	30	0.50	6.58 AB	1.47	67.78	60.20	70.38
Coarse biochar	15	0.0	6.38 BC	1.21	60.45	53.96	84.65
Coarse biochar	15	0.50	6.20 C	1.21	65.93	63.20	62.17
Coarse biochar	30	0.0	6.15 CD	1.16	63.78	61.84	78.17
Coarse biochar	30	0.50	6.38 BC	1.34	60.25	58.61	65.00
Significance			**	NS	NS	NS	NS

[i] Silicon (calcium silicate) substrate amendments are reported as $kg \cdot m^{-3}$.

[ii] All height and diameter measurements are based on cm. The diameter was calculated by taking the widest two points on a plant taken 90° from each other. These numbers were then added together and divided by 2 to get the diameter measurement. All dry weights were in grams and taken based on oven-dried material.

[iii] *, **, and *** Statistically significant differences between sample means based on 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.

EC = electrical conductivity; NS = not significant.

Hydrofarm, Petaluma, CA, USA) delivering 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at cutting height as measured with a quantum meter (MQ-610 ePAR Meter; Apogee Instruments, Logan, UT, USA) for 16 h to maintain a daily light integral of 11.52 $mol \cdot m^{-2} \cdot d^{-1}$. After root emergence, young plants were irrigated with a nurse solution [33.4 g KNO_3, 33.4 g $Ca(NO_3)_2 \cdot 4H_2O$, 6.6 g KH_2PO_4, and 13.2 g $MgSO_4 \cdot 7H_2O$ in 20 L H_2O]. On 21 Feb, after 21 d of propagation, rooted cuttings were transplanted into 7.8-L plastic pots filled with a respective substrate and grown in a greenhouse (lat. 35.78°N) with 23.9 °C/18.3 °C day/night temperatures. Plants received ambient solar radiation, and night interruption lighting was deployed from 2200 to 0200 HR during the first 4 weeks to prevent floral initiation. After 4 weeks, night interruption ceased, and long days were initiated to induce reproductive floral development for the subsequent 8 weeks. Plants were fertilized at each delivery with Ultrasol 13N–2P–13K, (SQM, Atlanta, GA, USA) to provide the following ($mg \cdot L^{-1}$): 150 N, 10.1 P, 125 K, 69.2 Ca, 34.6 Mg, 0 S, 0.196 B, 0.231 Cu, 1.15 Fe, 1.15 Mn, 0.0115 Mo, and 0.346 Zn.

Substrate treatments. Rooted cuttings were transplanted into one of 12 substrate treatments. These treatments comprised an 85:15 or 70:30 (volume:volume) mix of Canadian sphagnum peatmoss fluffed from compressed bales (Sun Gro Horticulture Company, Agawam, MA, USA) and coarse perlite (horticultural coarse perlite; Sun Gro Horticulture Company) or one of two wood biochar aggregates including medium (<6 mm) or coarse (>6 mm; Sun Gro Horticulture Company), each with a pH of ∼9.0. Substrates were also amended with wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ, USA) at 600 $g \cdot m^{-3}$, a micronutrient starter charge (M.O.S.T.; J.R. Peters, Inc., Allentown, PA, USA) at 1186.6 $g \cdot m^{-3}$, and varying rates of dolomitic limestone (Sun Gro Horticulture Company) at 1.78, 1.97, 2.37, or 3.56 $kg \cdot m^{-3}$ to achieve a target pH of 6.0 based on a 21-d incubation study and 0 or 0.50 $kg \cdot m^{-3}$ Si provided from calcium silicate ($CaSiO_3$; Sun Gro Horticulture Company) (Table 1).

Substrate and plant analysis. Eighteen single-plant replicates were transplanted into each substrate treatment. At weeks 1, 3, 6, 9, and 12, substrate pH and EC were evaluated using the pour-through method (Cavins et al. 2004). Plants were irrigated to container capacity 11-h before each data collection, and 75 mL of deionized water (DI) was poured over the substrate surface to collect ∼50 mL of leachate. The leachate was analyzed to determine pH and EC using a Hanna portable pH/EC meter (HI 9813-6; Hanna Instruments, Smithfield, RI, USA).

At weeks 6 and 12, six plants per treatment were destructively harvested. Plant height was measured from the substrate surface to the apical meristem, and diameter was measured as [(widest diameter + perpendicular axis) ÷ 2]. At week 6, the most recently matured leaves were collected to evaluate the critical micronutrient and macronutrient tissue concentrations for each substrate. The collected leaves were initially rinsed with DI; then, they were washed in a solution of 0.5 N hydrochloric acid (HCl) for 1 min, rinsed again with DI water (Henry

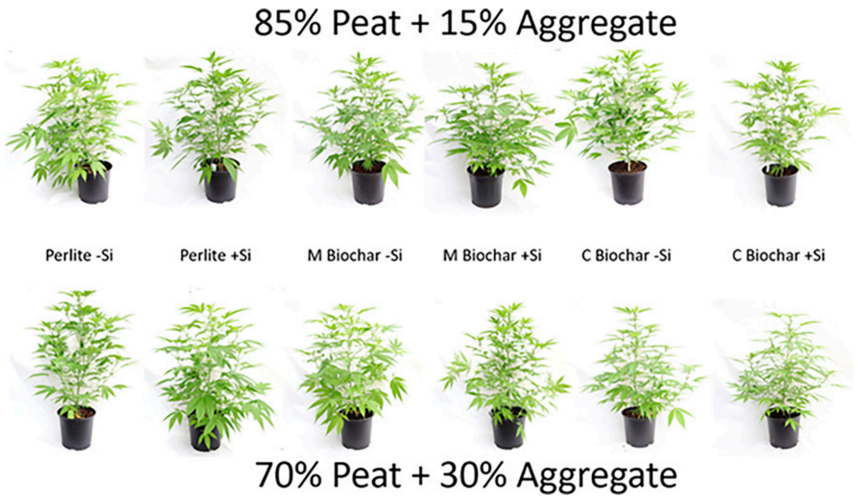

Fig. 1. Impact of perlite, medium (M) biochar, and coarse (C) biochar at 15% (by volume) and 30% (by volume) with (+) and without (−) calcium silicate [silicon (Si)] on *Cannabis sativa* 'BaOX' plants at 6 weeks after transplantation.

et al. 2018), dried in an oven at 70 °C for 96 h, and weighed to determine the sampled leaf biomass. The remaining plant shoot was harvested, bagged individually, dried in an oven at 70 °C for 96 h, and weighed to determine plant biomass. The total plant biomass (leaf biomass + plant biomass) was calculated for each plant.

After determining leaf biomass, dried tissue was ground to ≤0.5 mm (Foss Tecator Cyclotec™ 1093 sample mill; Analytical Instruments, LLC, Golden Valley, MN, USA). The ground tissue was placed in vials containing ∼3 g of tissue and submitted for a nutrient analysis of N, P, K, Ca, Mg, S, B, Cu, Zn, Fe, and Mn concentrations (Waters Laboratory, Warsaw, NC, USA). Dried plant material (0.5 g) was 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 plant material was diluted with 50 mL of DI water and 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 machine (Spectro Arcos EOP; Mahwah, NJ, USA).

Cannabinoid analysis. After 8 weeks of floral development, during the flowering harvest, the main apical meristem and four-terminal axillary flowers were excised, creating a composite floral sample. Then, the composite sample was freeze-dried (Harvest Right; North Salt Lake, UT, USA) for 30 h. The floral composite sample dry mass was weighed and recorded. After drying, dried tissue (∼8 g) samples were placed into vials and submitted for cannabinoid analysis (Delta 9 Analytics, Raleigh, NC, USA). Upon arrival, the material was lyophilized, ground, and a 2-g (1.98–2.02 g) sub-sample from the composite sample was obtained. An analysis to determine cannabinoids was performed using high-pressure liquid chromatography (8050 & 8040 Triple Quadrupole UHPLC/MS/MS analysis; Shimadzu, Austin, TX, USA). Exact details regarding cannabinoid analysis could not be provided because Delta 9 Analytics uses a proprietary protocol.

The cannabinoid analysis included both the active (decarboxylated) and acid forms of cannabigerol, THC, CBD, and cannabichromene. Additional cannabinoids and forms exist, but they are not reported here. Cannabidivarin and tetrahydrocannabivarin, given their concentrations, were either too low to detect, were not tested for, or were present in the same concentrations regardless of treatment. Total CBD and THC were calculated by the following equations reported by Citti et al. (2018):

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

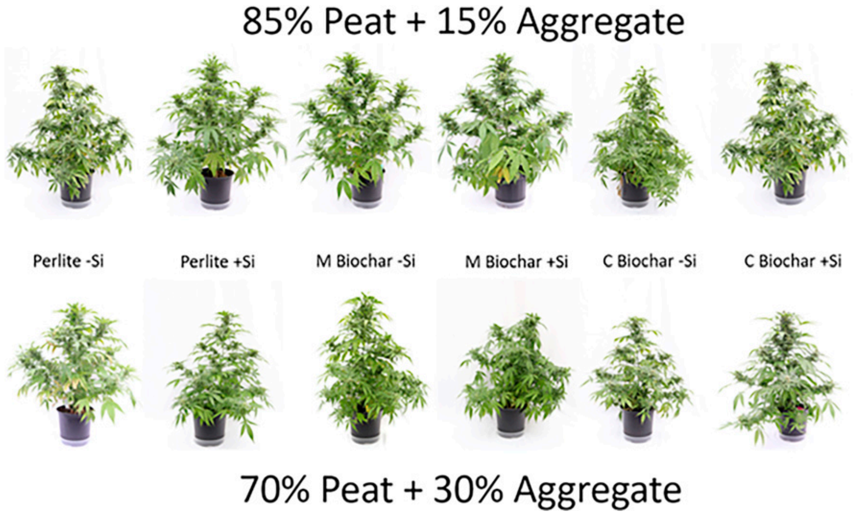

Fig. 2. Impact of perlite, medium (M) biochar, and coarse (C) biochar at 15% (by volume) and 30% (by volume) with (+) and without (−) calcium silicate [silicon (Si)] on *Cannabis sativa* 'BaOX' plants at 12 weeks after transplantation.

Table 4. Foliar macronutrient and Si concentrations of *Cannabis sativa* ‘BaOx’ grown in soilless substrate amended with three different aggregates (perlite, medium biochar, or coarse biochar) at two different incorporation rates (15% or 30% by volume) and with or without silicon (Si) amendments (Si_{0X} or Si_{1X}) 6 weeks from transplantation.

			N %	P %	K %	Ca %	Mg %	S %	Si %
Aggregate type									
Perlite			5.20	0.63	3.42	1.29	3.76	0.38	1.06
Medium biochar			5.11	0.68	3.47	1.26	3.92	0.37	1.23
Coarse biochar			4.87	0.66	3.39	1.26	3.75	0.36	1.25
Significance[ii]			**	NS	NS	NS	NS	NS	NS
Aggregate percentage									
15			5.16	0.65	3.41	1.25	3.75	0.37	1.15
30			4.97	0.66	3.45	1.30	3.87	0.37	1.21
Significance			*	NS	NS	NS	NS	NS	NS
Si rate[i]									
0.0			4.98	0.63	3.42	1.46	3.67	0.36	0.74
0.50			5.14	0.68	3.44	1.08	3.95	0.38	1.62
Significance			*	NS	NS	***	*	**	***
Second-order interactions									
Aggregate type × aggregate percentage			NS	NS	NS	NS	NS	*	NS
Aggregate type × Si rate			NS	NS	NS	NS	NS	NS	NS
Aggregate percentage × Si rate			*	NS	NS	NS	NS	*	NS
Aggregate type × aggregate percentage × Si rate									
Aggregate type	Aggregate	Silicon							
Perlite	15	0.0	5.22	0.57	3.34	1.39	3.33	0.36	0.63
Perlite	15	0.50	5.30	0.62	3.56	1.19	4.17	0.38	1.62
Perlite	30	0.0	5.08	0.60	3.28	1.55	3.74	0.36	0.56
Perlite	30	0.50	5.23	0.72	3.51	1.05	3.80	0.40	1.43
Medium biochar	15	0.0	5.16	0.72	3.38	1.32	3.48	0.38	0.64
Medium biochar	15	0.50	5.27	0.65	3.46	1.06	4.10	0.38	1.64
Medium biochar	30	0.0	4.77	0.67	3.56	1.55	3.94	0.34	0.77
Medium biochar	30	0.50	5.26	0.69	3.49	1.10	4.15	0.36	1.87
Coarse biochar	15	0.0	5.08	0.63	3.54	1.53	3.90	0.35	0.74
Coarse biochar	15	0.50	4.95	0.73	3.18	0.99	3.52	0.36	1.61
Coarse biochar	30	0.0	4.61	0.63	3.41	1.44	3.64	0.35	1.09
Coarse biochar	30	0.50	4.86	0.66	3.44	1.10	3.95	0.40	1.56
Significance			NS	NS	NS	*	*	NS	NS

[i] Silicon (calcium silicate) substrate amendments are reported as $kg \cdot m^{-3}$.

[ii] *, **, and *** 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.

Ca = calcium; EC = electrical conductivity; K = potassium; Mg = magnesium; N = nitrogen; NS = not significant; P = phosphorus; S = sulfur.

$$\text{CBD} + [0.877 \times \text{cannabidiol acid}] = \text{Total CBD} \qquad [2]$$

Statistical analysis. Statistical analysis was conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA). Plant growth metrics, leaf nutrient values, and cannabinoids were analyzed for differences within each data collection ($n = 6$) as an aggregate (three levels) × aggregate percentage (two levels) × Si amendment (two levels) factorial regarding the substrate aggregates and Si incorporation rate as the explanatory variables using the general linear model procedure (PROC GLM). Means were separated with Tukey’s honest significant difference at $P \leq 0.05$. Deviations in plant metrics, total plant dry weights, and leaf tissue values were calculated based on the percentage from the control substrate (15% perlite without Si).

Results and Discussion

Substrate pH, EC, and growth metrics. After 6 weeks of growth, the three-way interaction of aggregate type × aggregate percentage × Si incorporation rate did not significantly impact the substrate pH (Table 2). However, when examining the three simple effects of aggregate type, aggregate rate, and Si amendment independently, significant differences were observed (Table 2) Substrates that received the Si substrate amendment or used biochar as the aggregate compared with perlite exhibited a higher substrate pH after 6 weeks of growth (Table 2).

After 12 weeks of growth, the three-way interaction significantly impacted substrate pH, and similar trends were observed for the simple effects (Table 3). However, the greatest difference among substrates was only 0.5 pH units, and likely did not impact plant growth. Whipker et al. (2019) stated that hemp is tolerant of substrate pH between 5.5 and 6.5, and they recommended that growers should target 5.8 to 6.2. During this study, most of the mean substrate pH values were within the tolerant range of 5.5 to 6.5 reported by Whipker et al. (2019). The difference observed in the substrate pH values resulted from the varying limestone charges that were used during this experiment to offset the higher alkaline pH characteristics of biochar and $CaSiO_3$ (Table 1).

At weeks 6 and 12, no differences in plant height, diameter, total plant biomass, or EC were observed for any of the examined interactions or simple effects of aggregate type, aggregate percentage, or $CaSiO_3$ rate (Tables 2 and 3). Additionally, there were no visual impacts on plant morphology or growth at weeks 6 or 12 (Figs. 1 and 2).

These results are concurrent with those of Northup (2013), who replaced perlite with biochar and observed that it did not negatively impact plant growth and that the addition of biochar can reduce the amount of limestone needed to achieve the targeted substrate pH range for potted plants in a peat-based substrate. Additionally, when amending sphagnum peatmoss with biochar, we did not observe an increase in EC, which is in contrast to the observations of Northup (2013). However, because biochar from different feedstocks and varying biochar physical properties were used during the experiment conducted by Northup (2013) and this experiment, limited comparisons can be made without knowing the feedstock and physical and chemical properties of each biochar material.

Foliar nutrient concentrations. Six weeks after transplantation, the three-way interaction significantly impacted Ca and Mg (Table 4). The differences observed in the Ca and Mg

Table 5. Cannabinoid and silicon (Si) concentrations of *Cannabis sativa* ‘BaOx’ grown in soilless substrate amended with three different aggregates (perlite, medium biochar, or coarse biochar) at two different incorporation rates (15% or 30% by volume) and with or without silicon amendments (Si_{0X} or Si_{1X}) 12 weeks from transplantaton.

			Total CBD[i]	Total CBG[ii]	Total THC[i]	Total cannabinoids	Si %
Aggregate type							
Perlite			10.09	0.53	0.36 AB	12.63	1.40 B
Medium biochar			8.81	0.68	0.30 B	11.24	1.79 A
Coarse biochar			11.16	0.49	0.37 A	13.82	0.81 C
Significance[iii]			NS	NS	*	NS	**
Aggregate percentage							
15			10.15	0.54	0.36	12.68	1.56 A
30			9.89	0.60	0.33	12.44	1.11 B
Significance			NS	NS	NS	NS	*
Si rate[i]							
0.0			9.80	0.57	0.34	12.31	1.02 B
0.50			10.24	0.57	0.35	12.81	1.65 A
Significance			NS	NS	NS	NS	***
Second-order interactions							
Aggregate type × aggregate percentage			NS	NS	NS	NS	NS
Aggregate type × Si rate			NS	NS	NS	NS	NS
Aggregate percentage − Si rate			NS	NS	NS	NS	NS
Aggregate type × aggregate percentage × Si rate							
Aggregate type	Aggregate	Silicon					
Perlite	15	0.0	11.36	0.38	0.42	14.00	0.94
Perlite	15	0.50	10.15	0.60	0.36	12.76	2.21
Perlite	30	0.0	7.24	0.68	0.26	9.41	1.16
Perlite	30	0.50	11.63	0.48	0.39	14.35	1.28
Medium biochar	15	0.0	8.43	0.62	0.30	10.75	1.33
Medium biochar	15	0.50	8.09	0.64	0.27	10.34	2.52
Medium biochar	30	0.0	8.87	0.78	0.30	11.41	1.38
Medium biochar	30	0.50	9.86	0.69	0.32	12.47	1.92
Coarse biochar	15	0.0	11.15	0.55	0.37	13.86	1.00
Coarse biochar	15	0.50	11.71	0.41	0.41	14.41	1.34
Coarse biochar	30	0.0	11.76	0.40	0.38	14.44	0.29
Coarse biochar	30	0.50	10.01	0.59	0.33	12.56	0.62
Significance			NS	NS	NS	NS	NS

[i] Silicon (calcium silicate) substrate amendments are reported as $kg \cdot m^{-3}$.

[ii] Total CBD and THC are calculated based on a concentration of $kg \cdot g^{-1}$ 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 concentration ($mg \cdot g^{-1}$) of 2 g of freeze-dried composite weight.

[iii] *, **, and *** 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 the 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.

CBD = cannabidiol; CBG = cannabigerol; NS = not significant; THC = tetrahydrocannabinol.

foliar concentrations are most likely attributable to the varying limestone charges that were used during this experiment and the alkaline characteristics associated with biochar (Table 1) to offset the higher pH associated with biochar and $CaSiO_3$. When examining the simple effects, the Si amendment rate exhibited significant differences in N, Ca, Mg, and S foliar concentration (Table 4). Although differences in the foliar tissue concentration were observed, all reported foliar tissue concentrations were above the deficient concentrations reported by Cockson et al. (2019) and within the survey ranges reported by Kalinowski et al. (2020). Additionally, Mg foliar concentrations were within the recommended range reported by Veazie et al. (2021) for plants fertilized with 75 to 100 $mg \cdot L^{-1}$ Mg.

Si foliar and floral concentration. After 6 weeks of growth, neither the three-way interaction nor any of the two-way interactions exhibited significant differences in Si foliar concentrations (Table 4). After 12 weeks of growth, similar trends in Si foliar concentrations were observed; of those, none of the examined interactions exhibited significant differences in Si floral concentrations (Table 5). However, regarding the simple effects, plants that received an Si amendment exhibited a 61.8% increase in floral Si concentrations when compared with plants that did not receive Si (Table 5). This suggests that the use of $CaSiO_3$ can effectively increase Si concentrations in the foliar and floral tissues of *C. sativa* ‘BaOx’. When Si was added to a hydroponic nutrient solution, a decreased infection rate of gray mold (*Botrytis cinerea*) on lettuce, tomato, and pepper was observed (Pozo et al. 2015). Gray mold is one of the most important diseases in *Cannabis* production and results in the greatest losses in yield (McPartland et al. 2000). Thus, it is suggested that floral material accumulates Si without disease pressure; therefore, further research is needed to determine if the increased Si concentration can prevent yield losses in hemp caused by botrytis.

Cannabinoids. Cannabinoid concentrations did not vary significantly when the three-way interaction or any of the two-way interactions were examined (Table 5). However, the total THC concentration exhibited significant differences when the simple effect of the aggregate type was examined (Table 5). Plants that used coarse biochar exhibited significantly greater total THC when compared with plants that were grown using medium biochar. However, the difference was only 0.07%; therefore, it is likely not biologically significant (Table 5).

This would suggest that after 12 weeks of total growth, biochar is a suitable alternative aggregate for peat-based substrates using either of the particle sizes or aggregate percentages examined without any adverse impact on cannabinoid concentrations. Additionally, the use of $CaSiO_3$ as a Si substrate amendment increased Si foliar concentrations, and the biochar addition to peat-based mixes did not limit the Si availability for plant uptake.

Conclusion

The 12 different substrates evaluated during this study are all suitable and acceptable for growing *C. sativa* ‘BaOx’ without any negative impact on plant growth. Plants that received a Si substrate amendment (0.50 $\mathrm{kg \cdot m^{-3}}$ Si) exhibited increased Si foliar concentrations with substrates composed of 15% aggregate compared with similar substrates that did not receive Si (0 $\mathrm{kg \cdot m^{-3}}$ Si). When comparing the aggregate type, perlite or biochar of either aggregate size resulted in no significant differences in plant growth (plant height and diameter) and development (plant biomass). Additionally, the use of biochar and $CaSiO_3$ amendment did not decrease cannabinoid concentrations. This suggests that biochar can be used as an alternative aggregate with performance equal to that of a peat:perlite mix for plant production.

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