ABSTRACT

VEAZIE, PATRICK H. Examining the Impact of Silicon Substrate Amendments and Biochar Aggregates on Plant Growth and Nutrient Uptake on Greenhouse Produced Crops (Under the direction of Brian Earl Whipker).

Greenhouse substrates traditionally utilize perlite or bark as aggregates to promote drainage within a substrate. However, alternative substrate components such as coconut coir, wood chip, wood fiber, and biochar, have been evaluated as alternative substrate components. Biochar is a charred feedstock material similar to charcoal that is the result of pyrolysis, gasification, or hydrothermal carbonization. Recently, biochar has been evaluated as an alternative soil and greenhouse substrate amendment with promising success as an alternative to traditional aggregates. Additionally, research examining calcium silicate as a substrate amendment has demonstrated it to be an effective alternative to foliar spray applications to increase silicon (Si) concentrations within a plant. However, the impact of these substrate amendments on the physical and chemical properties of soilless substrates and plant growth has not been examined together. Therefore, the objective of this work was to classify the cultural parameters with biochar as an alternative aggregate to perlite and silicon substrate amendments in greenhouse production. First, the response of Si on heavy metal micronutrient uptake and plant growth for greenhouse-cultivated cannabis at varying Si substrate amendments was evaluated. The results of this study suggest that plants that receive a Si substrate amendment can prevent toxic accumulation of micronutrients in various plant parts including the roots, most recently mature leaves, and floral material. A second objective of examining plant growth and substrate chemical properties when amending a substrate with both Si substrate amendment and biochar aggregate. Plant dry weight or plant growth metrics were not significantly impacted when both substrate amendments were incorporated for tomato (*Solanum lycopersicum*), pepper

(*Capsicum annum*), French marigold (*Tagetes patula*), or cannabis. Additionally, when examining the efficacy of paclobutrazol, a GA inhibitor plant growth regulator, was not affected by the incorporation of biochar in soilless substrates. Drench concentrations of paclobutrazol exhibited similar control for poinsettia, pansy, and begonia up to 15% or 30% biochar when compared to similar perlite aggregate substrates. This work suggests that biochar is a suitable alternative to perlite and no changes in cultural practices are needed when biochar is incorporated up to 30% for greenhouse substrates.

Examining the Impact of Silicon Substrate Amendments and Biochar Aggregates on Plant Growth and Nutrient Uptake on Greenhouse Produced Crops

by
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DEDICATION

This work is dedicated to my family who has provided me with love and support during my academic journey and continues to encourage me to expand my knowledge and try new experiences. For that I am forever grateful.

To my major professor Dr. Brian Whipker, thank you for the constant encouragement and mentorship to shape me into who I am today.

To all those who came along side in my journey to support, mentor, guide, advise, and assist. I am the person I am today because of the millions of everyday deeds and your small acts of love and kindness.

BIOGRAPHY

Patrick Veazie was born in Sherman Texas, in August 2000, to Penny and Ray Veazie. Patrick graduated from NC State University with a B.S. in Plant Breeding and Biotechnology from the Department of Horticulture Science in the spring of 2022 where he began working in the floriculture program and found his passion for research. Patrick began his graduate studies in Horticulture in the fall of 2022 and plans to pursue a Ph.D.

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CHAPTER 1

Introduction

History of Container Substrates

The use of container substrates was first depicted by Egyptian wall paintings dating back to the 21st century BC (Raviv and Leith, 2008). Prior to 1946, British scientists claimed that while it was possible to grow plants in soilless substrates and a nutrient solution, plant growth would be negatively impacted when compared to plants that were similarly grown in soil (Raviv and Leith, 2008). Greenhouse crop production commonly utilized a soil-based mix as a substrate for nearly all greenhouse crops as recently as the 1970's (Raviv and Leith, 2008). The mix consisted of one-third field soil, one-third sphagnum peat moss, and one-third horticultural grade perlite (Northup, 2013). Growers began to look for alternative substrates for soil-based substrates to lower the shipping cost and clean, herbicide free, soil sources were becoming difficult to find (Northup, 2013). As an alternative to soil-based substrates, Cornell University developed Cornell A and Cornell B in the mid-1950s (Northup, 2013). Cornell A consisted of 50% sphagnum peat moss and 50% horticulture vermiculite. The Cornell B mix consisted of 50% sphagnum peat moss and 50% horticultural grade perlite (Nelson, 2012). Since the release of Cornell A and Cornell B commercial substrate blends have varied in the ratios of their components; however, most commercially available substrate blends consist of 2/3 sphagnum peat moss and 1/3 perlite or vermiculite (Northup, 2013). Additionally, substrates are typically amended with dolomitic or calcitic limestone to achieve the manufacturer's desired pH based on the intended crop (Nelson, 2012).

Container Substrates in Horticulture

Containerized plant production faces many challenges regarding root growth (Zulfiqar et al., 2019). One challenge is that pots provide a shallow rooting area that quickly becomes saturated after watering in comparison to a soil profile which can provide more drainage (Zulfiqar et al., 2019). Also, there is limited water storage capacity between irrigations due to the limited volume of the container (Bunt, 2012). Therefore, substrates must possess physical and chemical properties that favor maximum water retention between irrigation applications as well as being well drained to promote optimal plant growth (Caron and Nkongolo, 1999).

Substrates are a mixture of materials that can be tailored for specific crops and/or a grower's specific need and are comprised of growing medium constituents measured on a percentage volume basis (Schmilewski, 2008). Substrates are composed of a variety of materials such as peat, bark, aggregates, lime, wetting agents, and pre-plant charge fertilizers (Owen, 2013). Growers must utilize high-quality, consistent component substrates that function well under their growing conditions to optimize yield. As prices of raw materials change, substrate suppliers seek to evaluate new blends that can result in similar plant growth and water use habits to the traditional substrate blends. Additionally, many growers trying to limit their use of nonrenewable resources in their substrates and move towards more environmentally friendly alternatives. However, this results in growers using different or lower quality substrate constituents that may result in lower quality crops due to limited grower experience with the new substrate constituent (Owen, 2013).

Perlite

Perlite is a heat-expanded alumio silicate ore utilized for a wide variety of applications (Bunt, 2012). The ore is heated to 982 to 1760 ◦C (1800 to 3200 ◦F), causing it to expand four to

twenty times its original size, resulting in a lightweight, porous particle with a pH between 6.5 to 7.5 (Evans, 2011). Perlite has a closed cellular structure which prevents water from being held in the center of the aggregate and only on the surface or in the pores between the aggregates (Bunt, 2012). Thus, substrates that contain a high percentage of perlite are generally well drained. The aggregate size of perlite varies widely in the horticulture industry, ranging from 55-80% #8 mesh screen (2.36 mm) for propagation mixes to 5 to 20 % volume for substrate mixes (Owen, 2013). While perlite has been used for many years in the horticulture industry, the commercial substrate industry has begun searching for more renewable alternatives to traditional peat and perlite blends due to supply chain shortages.

Concerns with Traditional Soilless Substrate Aggregates

Currently over 50% of the world's population lives in cities (Eidenbrod and Gruda, 2015). With less airable land available for agriculture production and increased urbanization there is a large need for efficient agriculture production (Gruda 2019). However, there are many concerns with the inorganic inputs to soilless agriculture production such as rockwool (Bussel and McKennie, 2004), perlite, and vermiculite (Gruda, 2019). As a result soilless substrate companies and growers are searching for organic alternatives that result in comparable or improve plant growth to traditional soilless substrates including wood chips (Owen, 2013), rice hulls (Evans 2011), and biochar (Northup 2013). Biochar has been investigated as a potential method to transform agricultural, industrial, and municipal waste that would traditionally go to landfills into organic growing media (Gruda et al., 2019). This offers the potential for a biodegradable growing material that is available from waste products unlike many traditional soilless substrate components.

Biochar Production

Biochar is a charred feedstock material similar to charcoal that is intended for use as a soil amendment (Lehmann and Joseph, 2009). Biochar is the residual material resulting from heating a feedstock using one of three main methods: pyrolysis, gasification, and hydrothermal carbonization (Huang and Gu, 2019). Pyrolysis is the thermal decomposition of biomass by heating feedstocks in the absence of oxygen around 400 ◦C to 600 ◦C (752 to 1112 ◦F) (Gvero et al., 2016). During pyrolysis, the organic matter is transformed into the vapor phase resulting in the solid biochar residue remaining. The organic matter that volatilizes during pyrolysis remains as syngas or is condensed into bio-oils. These can then be used directly or refined to produce renewable liquid fuels (Northup, 2013). Gasification is conducted under small amounts of oxygen at higher temperatures ranging from 700 ◦C to 1200 ◦C (1292 to 2192 ◦F) (Hansen et al., 2016). This method will also result in lower carbon content in the final product when compared to pyrolysis (Hansen et al., 2016; Huang and Gu., 2019). Hydrothermal carbonization is the process of utilizing water as a catalyst and lower temperatures under high pressure to convert biomass to hydrochar, a different type of biochar than what is produced by pyrolysis or gasification (Libra et al., 2011). All three methods have a large impact on the characteristics of biochar including differences in aromatic compound content, cation exchange capacity (CEC), pH, container capacity, and electrical conductivity (EC) (Neito et al., 2016; Huang and Gu, 2019). Additionally, the wide array of feedstocks ranging from plant material such as pine waste to compost material can have a large impact on the physical properties of the final product (Huang and Gu, 2019; Judd, 2019).

Biochar Feedstocks

A wide variety of feedstocks can be utilized in biochar production, and each type contributes to differences in the chemical and physical composition of the final product. Feedstocks that are derived from waste materials have been evaluated for plant growth include green waste (Tian et al., 2012), wheat straw (Vaughn et al., 2013), sugarcane (Webber et al., 2018), rice hull (Locke et al., 2013), and crab shell (Cho et al., 2017). Additionally, feedstocks derived from non-wasted materials have been evaluated such as Eucalyptus wood chips (Housley et al., 2015), oak wood (Sáez et al., 2016; Choi et al., 2018), conifer wood (Dispenza et al., 2016), and citrus wood (Graber et al., 2010). Biochar feedstocks and manufacturing process can greatly impact a wide variety of physical and chemical properties including pH and EC (Locke et al., 2013). Thus, growers and substrate manufacturers should account for variability within batches or the same material from different source locations when incorporating biochar into a container substrate.

Use of Biochar in Field Production Systems

Biochar has been evaluated extensively in field production systems for the potential increase in soil quality and enhanced carbon sequestration (Larid, 2008). Additionally, soil amendments with biochar were being examined for benefits including enhancing soil fertility and crop productivity (Lehmann and Joseph, 2015), reducing greenhouse gas emissions (Castaldi et al., 2011; Glaser et al., 2002), increasing water holding capacity (Basso et al., 2013), and reducing nutrient leaching (Vaccari et al., 2011). In 2011, Jeffery et al. compared 177 individual experiments and concluded that the beneficial effects of biochar soil amendments outweighed both the negative (decreased plant growth or yield) and neutral (no impacts on plant growth or

yield) effects and concluded that a 10% increase in crop productivity was observed on average with amending soils with biochar.

Biochar's effects on soil properties can vary based on the soil type and properties (Judd, 2016). Some of these effects can have long-term impacts on the soil and can potentially impact the soil-forming process that regulates the accumulation of soil constituents (Richter, 2007). Additionally, research suggests that biochar soil amendments can provide equivalent amounts of nitrogen (N), phosphorus (P), and potassium (K) as traditional fertilizer sources, and can impact the microbial population and dynamics (Spokas et al., 2012; Warnock et al., 2007).

Biochar has been evaluated for its potential use for bioremediation in heavy metal-contaminated soils as well as in potted substrates to prevent heavy metal uptake for a variety of crops including maize (*Zea mays* L.) (Uzoma et al., 2011) and rice (*Oryza sativa* L.) (Haefele et al., 2011). The primary focus has been on interactions of biochar with copper (Cu), arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and chromium (Cr) (Cheng et al., 2020). Research has focused on the interaction of biochar mechanism of complexation, reduction of uptake, cation exchange, electrostatic attraction, and precipitation of heavy metals (Cheng et al., 2020).

Biochar in Container Substrates

Recently, researchers have focused on the implications of biochar on container-grown plants. Results reported biochar as a viable option for replacing many commonly used substrate components such as sphagnum peat moss, perlite, pine bark, and coconut coir (Huang and Gu 2019; Judd, 2016; Northup, 2013). However, the influence of biochar on soilless substrates has been examined less than the incorporation of biochar into field soils (Atland and Locke, 2012). The first report of a product similar to biochar being used in a soilless substrate was by Santiago and Santiago (1989), who recommended the use of charcoal due to it acting as a sponge because

of the ability to retain water, gasses, and solutions. Additionally, beneficial impacts on plant growth have been reported for a variety of species including poinsettia (*Euphorbia pulcherrima*) (Guo et al., 2018), basil (*Ocimum basilicum*) and tomato (*Solanum lycopersicum*) (Huang, 2018). Biochar can impact the physical and chemical properties of a substrate such as the bulk density, container capacity, pH, EC, and CEC.

Bulk Density

Biochar has a greater bulk density when compared to other commonly used substrate components, such as peat, perlite, or vermiculite (Huang and Gu, 2019). Thus, depending on the biochar incorporation rate, significant impacts on the substrate's bulk density can occur with an increase in biochar in a substrate (Tian et al., 2012).

Container Capacity and Air Space

Container capacity is the maximum percent volume of water a substrate can hold after gravity drainage (Foneteno et al., 1995). Substrates absorb water between or inside small pores among substrate components (Huang and Gu, 2019). Particle size distribution of the various substrate components is important in determining the physical properties of a substrate including container capacity (Noguera et al., 2003). In addition to container capacity, biochar incorporation can impact air space (Huang and Gu, 2019). Air space is the proportion of air-filled macropores after drainage. Due to the different particle sizes of biochar, the effect of biochar incorporation can have a significant impact on a substrate’s physical properties. Méndez et al. (2015) reported that the incorporation of biochar at 50% (v:v) with peat increased container capacity and air space when compared to 100% peat substrates. The increase in air space and container capacity is a result of the increased macropores as a result of the biochar incorporation (Méndez et al., 2015). Similar results were reported by Zhang et al., (2014) in which incorporating 20% or 35%

(w/w) biochar with compost from green waste increased container capacity. However, other research states that the incorporation of biochar did not impact container capacity (Tian et al., 2012; Vaughn et al., 2013). The variation in results of biochar incorporation is likely due to the variation in particle size and variation of incorporation rate across experiments.

Wettability

Greenhouse and nursery growers rely on the substrate's ability to capture and retain water to evenly distribute nutrients throughout the root zone (Judd et al., 2019). Biochar is a porous material with both internal and external pores impacting substrate water retention (Gray et al., 2014). Judd et al. (2016) reported that biochar created from pine wood chips or rice hulls did not reach water holding capacity after ten watering treatments suggesting hydrophilicity in biochar. However, biochar has been reported to be hydrophobic or hydrophilic depending on the feedstock (Gray et al., 2014; León et al., 2013).

Chemical Properties

pH

In most cases, biochar is neutral or has a basic pH and can effectively increase the substrate pH (Dispenza et al., 2017; Northup, 2013; Park et al., 2011; Zhang et al., 2014). However, biochar can be acidic as well. The pH of the biochar is dependent on the feedstock and the temperature used based on the production method (Huang and Gu, 2019). Different biochars can range from pH 3.5 to 10.3 (Khodadad et al., 2011; Fornes et al, 2015; Nemati et al., 2015; Spokas et al., 2012) and may have the potential to neutralize acidity caused by peat and root growth (Bedussi et al., 2015).

Electrical Conductivity

Incorporating biochar into substrates can increase the EC of a substrate due to the elevated EC of the biochar (Huang and Gu, 2019). Yet Hossain et al. (2011) reported that increasing pyrolysis temperatures resulted in a decrease in the EC of the biochar. However, Vaughn et al. (2013) reported that incorporating 5, 10, or 15% (v:v) pelletized wheat straw with hardwood biochar increased EC for container substrates containing peat moss and vermiculite. Judd (2016) reported that pine bark chip biochar EC was 2X greater when compared to pine bark chip feedstock using the same production methods. However, other researchers reported no impact of biochar incorporation on substrate EC (Northup, 2013). Thus, the differences in the results of biochar's impact on substrate EC can likely be attributed to the differences in feedstocks utilized as well as the incorporation amount.

Cation Exchange Capacity

Biochar incorporation can affect cation exchange capacity (CEC) and nutrient availability (Huang and Gu, 2019). Biochar CEC estimates are reported to have wide variability ranging from 5 to 50 cmol kg^{-1} (Munera-Echeverri et al., 2018). This wide variability of factors influences the surface properties of biochar including production temperature and the original feedstock properties (Suliman et al., 2016). Biochars that are produced using a lower temperature of less than 600 ◦C exhibit a greater CEC value (Weber and Quicker, 2018). Biochar samples with a greater oxygen:carbon ratio correlated to greater CEC values (Huff et al., 2018). This is due to the negative charge of the oxygen functional group which electrostatically attracts cations from the solution (Lee et al., 2010; Huff et al., 2018). However, with limited set of standardized protocols for determining the CEC of biochar, Munera-Echeverri et al. (2018) attribute some of

the variability to nonstandard testing procedures that may influence the results due to biochar possessing physical properties widely different than that of other substrate components.

Biochar Interacting with Other Nutrients

While there are limited studies regarding fertility and biochar-amended substrates, there are crucial factors regarding the physical properties of biochar that can impact plant growth. Most biochar exhibit a basic pH and incorporating significant amounts of biochar can raise the pH above the optimal range of nutrient availability. Fascella et al. (2017), reported that substrates incorporating 75% biochar and 25% peat exhibited significantly lower phosphorus (P) concentrations in the foliage compared to control plants of 25% biochar and 75% peat and attributed these results to the increase in pH when using a conifer wood feedstock. Additionally, due to the basic properties of many biochar feedstocks, a lower lime rate is required to increase the pH to the optimal range, which may result in the need to increase calcium (Ca) and magnesium (Mg) fertility rates that would have otherwise been provided through the substrate's lime charge.

Reported Uses of Biochar in Container Substrates

In recent years, studies have highlighted the success of biochar as a potted substrate amendment for different greenhouse species. A review developed by Huang and Gu (2019) detailed numerous studies highlighting the impacts of biochar on plant growth. Huang and Gu (2019) reported that 77.3% of the literature indicated a positive impact on plant growth when incorporating biochar to potted substrates. However, the length of the studies reported by Huang and Gu (2019) range from 3 to 28 weeks. This non-uniform experiment timeframe could impact the observance of potential impacts of biochar on plant growth depending on the species examined. Additionally, aggregate incorporation rate significantly impacts the physical

properties of potted substrates (Basso et al., 2013). These changes in physical properties can skew data if substrate treatments are watered independently or all treatments receive the same amount of water. Genetic differences of salt tolerance for species such as tomatoes (*Solanum lycopersicum* L.) when compared to marigolds (*Tagetes erecta* L.) should be considered as to why some species such as tomatoes exhibit an increase in plant dry weight while others to not (Vaughn et al., 2015). Thus, while there has been a considerable increase in research conducted on biochar incorporation rate for potted substrates, more species need to be evaluated for each feedstock and ratio to determine optimal rates for a commercial mix.

Silicon's Impact on Plant Growth

Silicon (Si) is the second most abundant element in soils and on the earth's crust (Liang et al., 2007). However, while Si is found in high concentrations in the soil, most Si compounds are insoluble and are not available for plant uptake (Richmond and Sussman, 2003). This results in soluble Si concentrations typically ranging from 0.1 to 0.6 mM L^{-1} Si (Epstein 1994). Plants only absorb soluble Si typically in the form of H_4SiO_4 which is commonly found in low concentrations in the soil (Imtaiz et al., 2016). Plants exhibit varying Si concentrations depending on the plant's ability to uptake, transport, and accumulate the nutrient and this is a result of varying transporters that impact the Si concentrations across different organs (Wu et al., 2013; Ma and Yamaji, 2015).

Silicon is not considered a plant essential nutrient, but many researchers have reported beneficial impacts on a variety of crops when exposed to abiotic or biotic stress (Epstein, 1994; Liang et al., 2007). While Si is not considered essential from a physiological perspective, it has been deemed 'agronomically essential' for rice in Japan since it is required to obtain higher yields (Ma and Takahashi, 2002). Silicon's potential benefits have been evaluated on many crops

in both field (Khan et al., 2021; Luyckx et al., 2021), soilless (Boldt et al., 2018; Kameniduo et al., 2010), and hydroponic settings (Boldt and Altland, 2021; Mattson and Leatherwood, 2010).

Silicon Foliar Sprays

Silicon is commonly applied to plants through foliar spray applications or substrate/ field amendments similar to other fertilizers. Silicon foliar sprays have been evaluated due to the assumption that foliar Si feeding could compensate for low uptake by the roots (Laane, 2018). Foliar silicate sprays can be divided into three groups based on the active Si compound: silicates, stabilized silicic acid, and nano-silica (Laane et al., 2018). Each of these components has varying effectiveness regarding different benefits associated with Si foliar applications as reviewed by Laane, 2018)

Silicon Impact on Abiotic Stress

Silicon has been highly researched for its impact on the reduction of abiotic stresses ranging from reduction of heavy metal uptake, alleviation of drought stress, and improving resistance to strong wind and rain (Guntzer et al., 2012). It was reported that Si decreases the heavy metal concentration in the plant through chelation of the 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 (*Cannabis sativa* L.), the impact of Si soil amendments in the presence of Cd stress 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 (Luycjx et al., 2021). Similarly, Si has been reported to alleviate aluminum (Al) and manganese (Mn) toxicity which can accumulate to highly toxic levels in plants (Epstein, 1999). Rizwan et al. (2015), reported Si improved plants' ability to tolerate drought stress by reducing nutrient

balance, and enhancement of photosynthetic rate to decrease the generation of reactive oxygen species below excessive levels.

Silicon's ability to regulate P uptake is one of the earliest reported Si interactions. Brenchley and Maskell (1927), reported that Si fertilization increased yields of wheat when P fertilization was limiting. Additionally, Tripathi et al. (2015) reported an increase in P uptake and other essential nutrients of Si fertilized crops when compared to plants that did not receive a Si fertility treatment.

Silicon Impact on Biotic Stress

Silicon has been examined for beneficial effects on several pathogens with a wide range of plants as reviewed by Guntzer et al. (2012). Many of the processes as to how Si assists in pathogen resistance are unclear. Epstein (1999) reported that Si boosts the defense mechanisms when the plant is under stress, including the accumulation of lignin, phenolic compounds, and phytoalexins. In some cases, such as powdery mildew, in Si fertilized plants the occurrence of powdery mildew was limited, and when it was observed the infection was kept to a minimum (Guevel et al., 2007).

Silicon in Greenhouse Crops

While Si can be readily available in field settings, in greenhouse production most crops are produced using soilless substrates that have limited Si concentrations (Kamenidou et al., 2010). In greenhouse production, Si is commonly applied through foliar sprays, substrate amendments, or the inclusion of Si into hydroponic solutions. Kamenidou et al. (2010) reported that gerbera (*Gerbera hybrid* L.) that received rice husk ash substrate incorporation, sodium silicate foliar sprays, or potassium silicate substrate drenches exhibited thicker flower peduncles, increased flower diameter, increased height, and flowered earlier when compared to plants that

did not receive supplemental Si. Plants grown using a recirculating hydroponic solution improved stem quality for roses (*Rosa* sp.) with the addition of Si (Ehret et al., 2005). However, in the production of zinnia (*Zinnia elegans*) and sunflower (*Helianthus annuus* L.), Kamenidou et al. (2008) reported that when utilizing a peat-based system that the beneficial effects of Si depended on the silicate source and the Si concentration applied.

The above literature demonstrates the potential impacts of Si and biochar substrate amendments on plant growth. However, given the deficit of information available examining the impacts of Si substrate amendments when incorporated with biochar substrates, these studies will seek to determine these effects and determine if commercial producers will need to modify production practices. The first objective will be to determine the impact of Si and biochar incorporation rates on the chemical properties of growing media independently and then jointly by examining the impact on pH, EC, plant growth, and nutrient concentrations throughout the plant. The second objective is to determine the impact of these substrate amendments on commercial growing practices such as nutrient uptake and paclobutrazol efficacy.

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Chapter 2

The Use of Silicon Substrate Amendments to Decrease Micronutrient Concentration 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 and 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 reduce the uptake of heavy metals in a variety of crops. The objective of this study is 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 one of three varying calcium silicate ($CaSiO_3$) (Si) substrate amendment rates of Si_{0X}, $Si_{0.5X}$, or Si_{1X} (of 0.0, 1.04, and 2.07 $kg \cdot m^{-3}$ $CaSiO_3$) and one of three micronutrient fertility treatments of M_{1X} (0.49 B, 0.19 Cu, 4.02 Fe, 0.99 Mn, 0.01 Mo, and 0.20 Zn $mg \cdot L^{-1}$), M_{2X}, or M_{4X} using a modified Hoagland's solution creating a 3 X 3 factorial. Plants grown with a Si_{1X} substrate amendment exhibited a significantly lower iron concentration in the foliage and the root tissue when compared to those grown in a substrate without Si. After six 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 to plants that did not. Silicon substrate amendments had no impact on cannabinoid concentration or plant growth metrics after twelve weeks of growth. This research suggests that utilizing 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 resulting in negative impacts on plant growth or cannabinoid concentrations.

Introduction

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 0.3% total tetrahydrocannabinol (THC) concentration of dry weight in any part of the plant (US Congress 2014, 2018). Hemp contains over 100 cannabinoids including cannabidiol (CBD), THC, and cannabigerol (CBG), varying in concentration, many of which are considered to have medical and therapeutic effects leading to an increased interest in cannabis production (Salentijn et al., 2019).

Soil contamination has increased since the industrial revolution due to human activities, which is 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, but higher concentrations can lead to toxic effects. Heavy metals cannot be degraded like other pollutants, which poses 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 the change in mineral concentration, a decrease in photosynthesis, oxidative stress, and growth reduction (Luyckx et al., 2021a). However, a number of 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). In fiber hemp, one study reported that 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). While the majority of literature surrounding heavy metal accumulation in plants is focused on field grown crops there is still a concern of heavy metal contamination for plants grown in soilless media.

Silicon (Si) is the second most abundant element in the soil and surface of the earth and 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 to exclude heavy metal uptake. Silicon has also been examined for its ability to increase the availability and absorption of phosphorus (P) and other essential nutrients (Tripathi et al., 2015). Silicon decreases the heavy metal concentration in plants through chelation of the 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., 2021a).

Currently, there is an extensive body of literature regarding the impact of Si amendments incorporated in as mineral soil amendments and a growing body of literature regarding supplemental Si applications for greenhouse production (Wei et al., 2020). Silicon supplementation in greenhouse crops can be achieved in multiple ways ranging from foliar applications (Kameniduo 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; Kameniduo et al., 2010). However, there is no published research investigating the impacts of Si substrate amendments on greenhouse cannabis nutrient

uptake and plant growth. While previous studies on 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 is 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), were sown on 23 Sept. 2021. Seeds were sown into Ellepots [Ellepots (3.5 cm x 4 cm), 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 utilizing 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) 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 five days after germination before being transplanted in the glasshouse with ambient lighting [35.78 °N latitude with 23.9°C/18.3°C (75 and 65°F) day/night temperatures]. Seedlings were transplanted on 1 Oct 2021, into 2.48L containers containing one of three substrate treatments. These treatments were all comprised of a 70:30 (v:v) mix of peat moss (Canadian sphagnum peat moss, SunGro Horticulture Distribution Inc., Agawam, MA), perlite (Horticultural Perlite, SunGro Horticulture Distribution Inc), and wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ) 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) 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·m^{-3} dolomitic limestone, a second substrate was amended with a 1.04 kg·m^{-3} $CaSiO_3$ and 2.07 kg·m^{-3} dolomitic limestone, and a third substrate was amended with a 2.07 kg·m^{-3} $CaSiO_3$ and 1.04 kg·m^{-3} dolomitic limestone. Calcium silicate rates of 0.0, 1.04, and 2.07 kg·m^{-3} $CaSiO_3$ will be respectively referred to as Si_{0X}, $Si_{0.5X}$, and Si_{1X}.

Fertilization Treatments. All fertilizers were custom blends of the following individual technical grade salts (Fisher Scientific, Pittsburgh, PA): calcium nitrate [$Ca(NO_3)_2·4H_2O$], 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 ($MnCl_2·4H_2O$), zinc chloride ($ZnCl_2·7H_2O$), copper chloride ($CuCl_2·2H_2O$), boric acid (H_3BO_3), and sodium molybdate ($Na_2MoO_4·2H_2O$).

Fertilization treatments began on the day of transplant. Three micronutrient fertilizer rates of 1X, 2X, and 4X (referred to M_{1X}, M_{2X}, or M_{4X}, respectively) of a modified Hoagland's solution concentration (Barnes et al., 2012) were mixed using the previously described fertilizer salts (Table 2.1). The macronutrient fertility rate was altered during weeks five through eight in which nitrogen (N), potassium (K), and calcium (Ca) were 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) connected to 1.9-cm-diameter irrigation tubing fitted with circular drip emitters (Dramm USA, Manitowoc, WI).

Data Collection. Twenty single plant replicates were transplanted for each treatment (Si rate x micronutrient fertility rate). At weeks 1, 3, 6, 9, and 12, substrate pH and electrical conductivity (EC) were evaluated using the pour-through method on 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 minutes before each data collection and 75 mL of deionized water (DI) was poured over the pots to displace 50 mL of leachate. The leachate was analyzed for pH and EC using a Hanna portable pH/EC meter (HI9813-6, Hanna Instruments; Smithfield, RI).

At weeks six and twelve, six plants were sampled for plant height was measured from the substrate level to the apical meristem, as well as diameter [(widest diameter + perpendicular axis) ÷2]. After six weeks of growth, 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 washed in a solution of 0.5 M HCl for 1 min, and again rinsed with deionized water (DI) water (Henry et al., 2018). The remaining shoot tissue was harvested separately and dried to calculate total plant biomass. At week twelve, plants were destructively harvested in which floral material was collected for cannabinoids, heavy metal, and micronutrient analysis while the remaining above-ground portion of the plant was collected to determine biomass production.

Upon sampling, the plant tissues and the remaining above-ground plant biomass were dried at 70 °C for 96 hours, and the dry mass was weighed and recorded. After drying, leaf tissue was ground in a Foss Tecator Cyclotec™ 1093 sample mill (Analytical Instruments, LLC; Golden Valley, MN; ≤0.5 mm sieve). The ground tissue was then placed in vials containing ~3 g of tissue and analyzed at the Waters Lab (Warsaw, NC). Plant material (0.5 g) was first rinsed in nitric acid (10 mL of HNO_3 at 15.6N) and digested in a microwave digestion system for 30 minutes (MARS 6 Microwaves; CEM Corp., Matthews, NC). After microwave digestion, the solution was diluted with 50 mL of DI and then vacuum filtered through acid-washed paper (Laboratory Filtration Group; Houston, TX). After dilution, plant mineral tissue concentration was determined using an

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) machine (Spectro Arcos EOP; Mahwah, NJ).

Floral chemical analysis. During the flowering harvest (eight 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 utilized for heavy metal analysis by Waters Lab. The metals were analyzed using EPA methods 3050B for digestion and 6010S for analyses by ICAP-OES. Plant material (0.5g) was rinsed using 70:30 (v:v) nitric acid: hydrogen peroxide and digested using an Environmental Express HotBlock metals digestion system (Environmental Express, Charleston, SC). After digestion, plant samples were analyzed using a Spectro ARCOS ICP-OES (Spectro Arcos EOP).

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

Cannabinoid analysis included both the active (decarboxylated) and acid forms of CBG, THC, CBD, and cannabichromene (CBC). Additional cannabinoids and forms exist but are not reported here [e.g., cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV)], given their concentrations were either too low to detect, were not tested for, or were present in similar

concentrations regardless of treatment. Total CBD and THC were calculated by the following equations:

Δ^9 THC + (0.877 x tetrahydrocannabinol acid (THCA) = Total THC [1]

CBD + (0.877 x cannabidiol acid (CBDA)) = Total CBD [2]

Statistical Analysis. Statistical analysis was conducted using SAS (version 9.4; SAS Inst., Cary, NC). Plant growth metrics and leaf and flower nutrient values were analyzed for differences within each data collection (n=6) as a 3X3 factorial of micronutrient fertility rate X 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 on a percentage basis from the controls (M_{1X} X Si_{0X}).

Results and Discussion

Substrate pH and Electrical Conductivity

Regarding the interaction of micronutrient fertility X Si substrate amendment at sampling intervals of six and twelve weeks, differences were observed among the substrate pH of the various treatments in which a general trend of plants that received a Si_{1X} or $Si_{0.5X}$ amendment exhibited a lower substrate pH than those that received a Si_{0X} amendment with a respective P-value of 0.043 and <0.001 (Tables 2 &3). When examining the simple effects of Si rate plants that did not receive an Si amendment exhibited significantly greater substrate pH than those that received an Si amendment of $Si_{0.5X}$ or Si_{1X} ($P < 0.001$) at both sampling intervals (Tables 2.2 & 2.3). 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 2.1). However, the

difference was less than 0.4 units at both sampling dates and likely had no impact on plant growth (Table 2.2 & 2.3).

After six weeks of growth, there were no observed differences in substrate EC for the interaction of micronutrient fertility X Si substrate amendment or any of the examined simple effects (Table 2.2). However, at week 12, while there were no significant differences for the interaction, but when examining the simple effect of 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 2.3). In previous studies, researchers 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 increased EC values can likely be related to the uptake patterns of the crop and that excess nutrients were not available in the container.

Plant Growth Metrics

After six weeks of growth, plant height, diameter, and shoot dry weight were not significantly different when comparing the interaction of micronutrient fertility X Si substrate amendment (Table 2.2). However, plant dry weight was significant when examining the simple effect of micronutrient fertility rate, with plants that received a M_{1X} fertility rate had significantly less biomass when compared to those that received a fertility rate of M_{2X} or M_{4X} with an observed P-value of <0.001(Table 2.2). Additionally, no phytotoxicity was observed after six weeks of growth on any of the treatments and similar growth was observed (Fig. 2.1).

After twelve 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. 2.2). 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 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 different when comparing the interaction or simple effects of micronutrient fertility rate X Si substrate amendment after twelve weeks of growth (Table 2.3).

Currently, there is limited published research examining the use of calcium silicate as a substrate amendment, however, researchers have examined other forms of Si supplements in soilless substrates, one of which is rice hulls. The results of this study vary from those reported by Boldt et al., (2018), which reported that a decrease sunflower and petunia total above ground dry weight when amending peat: perlite substrates with a 20% rice-hull incorporation. However, this could likely be due to the change in physical properties of the substrate and not as a result of Si.

Root Tissue Analysis

After six weeks of growth, when examining the interaction of micronutrient fertility rate X Si rate significant differences were observed for B, Zn, Mn, Fe, and Cu with a general trend of plants that received a silicon substrate amendment of Si_{1X} and M_{4X} fertility rate exhibited the lowest elemental concentrations (Table 2.4). Plants receiving a M_{1X} fertility rate and a $Si_{0.5X}$ or Si_{1X} rate exhibited 48% and 52% lower, respectively, Fe concentrations when compared to plants grown without Si (Table 2.4). When the highest micronutrient rate was applied (M_{4X}) and a Si_{1X} rate, the plant's root tissue exhibited lower B, Zn, Fe, Mn, and Cu concentrations of 64.5%, 67.3%, 74%, 67.2%, and 77.4%, respectfully, when compared to plants that received a $Si_{0.5X}$ or

Si_{0X} rate (Table 2.4). These trends show that under excessive micronutrient conditions, the addition of Si helps limit micronutrient accumulation into the roots.

Foliar Tissue Analysis

Boron leaf tissue concentrations exhibited significant difference for the interaction of micronutrient fertility rate and Si rate ($P < 0.001$) (Table 2.5). When examining the simple effects of micronutrient fertility rate for B, Zn, Fe, and Cu, significant differences were observed (Table 2.5). Silicon concentration in the foliar tissue was significant when examining the interaction of micronutrient fertility rate and Si rate (P-value 0.039). However, when examining the simple effect of Si amendment rate, plants that received a Si concentration of 1X exhibited a significantly greater Si concentration compared to those that did not receive Si (Si_{0X}) ($P <0.001$) (Table 2.5). Silicon is reported to alleviate Mn toxicity in cucumber (*Cucumbis sativus L.*) (Rogalla and Römheld, 2002) however, in this study we did not observe a decrease in foliar Mn concentrations with increasing Si rate. Manganese toxicity has been reported in Cannabis to occur in plants as low as 47.88 $mg \cdot kg^{-1}$ (Cockson et al., 2019), however, even in plants that did not receive Si (Si_{0X}) and a micronutrient fertility rate of 1X exhibited a Mn foliar concentration of 193.80 $mg \cdot kg^{-1}$without exhibiting visual Mn foliar toxicity symptoms.

The interaction of Si soil amendments and Fe nutrition has been widely examined with many different species, the results suggest that Si amendments strongly regulates the Fe availability in the soil, the root apoplast, and the transport of Fe, thus lowering the Fe concentration and distribution within the plant (Gonzalo et al., 2013; Becker et al., 2020). Additionally, the interaction of Si and Cu-toxicity has been examined for wheat (Nowakowski and Nowakowska. 1997) and *Arabidopsis (*Khandekar and Leisner. 2011). Silicon alleviation of Cu-toxicity was the result of increased binding sites in the cell wall, and although there was not a

decrease in 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 six weeks of growth for plants that were grown using a Si substrate amendment.

Floral Tissue Analysis

After twelve weeks of growth, B, Zn, Mn, Fe, Cu, and Si floral tissue concentration were significantly different when examining the interaction of micronutrient fertility rate X Si rate (Table 2.6). As a general trend plants that received a greater micronutrient fertility rate exhibited a greater micronutrient concentration in the floral tissue excluding Fe (Table 2.6). Inversely, plants that received a Si amendment generally exhibited a greater Si concentration compared to those that did not receive and Si amendment (Table 2.6). In most cases, Si concentrations in floral material yielded a lower Si concentration when compared to the foliar material, this trend is similar to that reported by Boldt et al., (2018) in which sunflower leaves exhibited the greatest concentration followed by roots, stems, and flowers. Silicon has been reported to reduce the infection of gray mold (*Botrytis cinerea*) in lettuce, tomato, and pepper when supplying Si in a hydroponic nutrient solution (Pozo et al., 2015). Gray mold is one of the most important diseases in Cannabis production that results in the greatest losses in yield (McPartland et al., 2000). Thus, it suggests that with 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 twelve 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 2.7).

Conclusion

Growing *Cannabis sativa* 'Auto CBG' under increasing 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 a rate of 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 resulted in no phytotoxicity being observed after twelve weeks of growth on Si_{1X} plants that received a M_{4X} fertility rate while all other Si rates did 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 into 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 avoidance of heavy metal contamination in *Cannabis sativa*.

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Figure 2.1. Impact of micronutrient fertility treatment and calcium silicate substrate amendment on *Cannabis sativa* 'Auto CBG' six weeks after transplant.

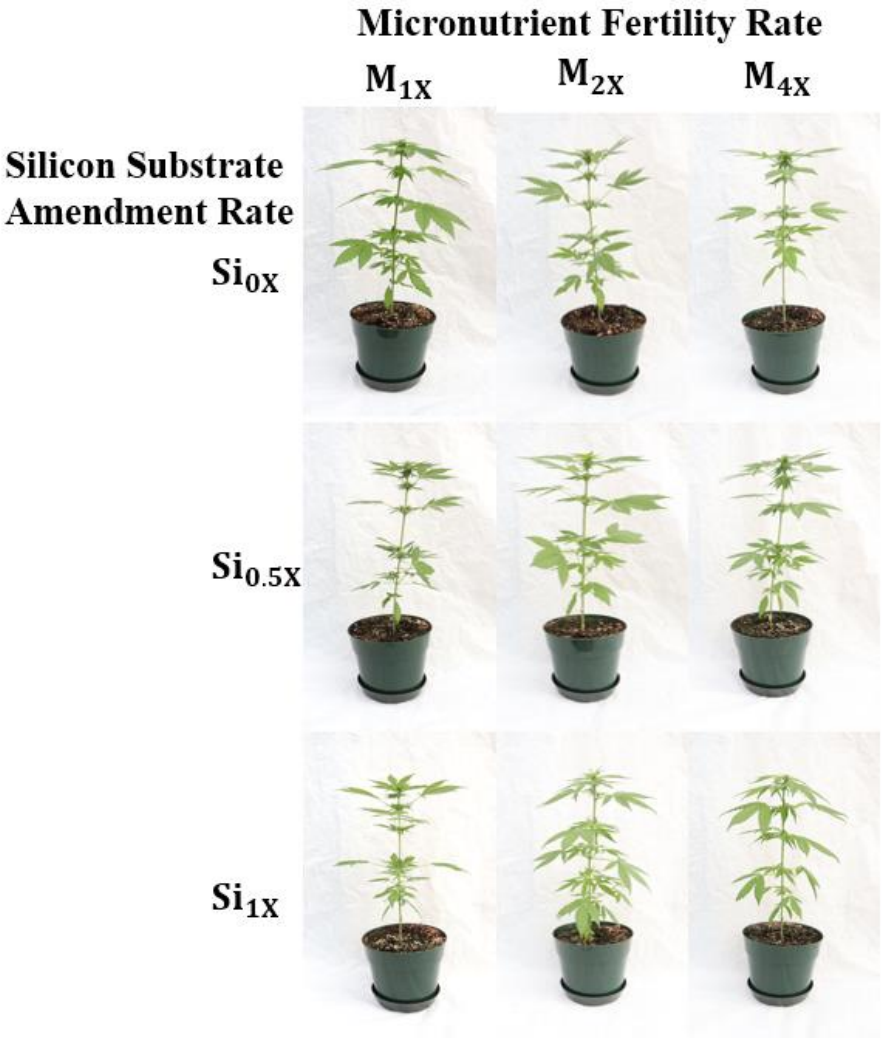

Figure 2.2: Lower leaf phytotoxicity observed on *Cannabis sativa* ‘Auto CBG’ plants grown without a calcium silicate substrate amendment and 4X micronutrient fertility treatment.

Table 2.1: The applied nutrient fertilizer concentrations by micronutrient fertility concentrations treatments.

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

Table 2.2. Growth metrics of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for six weeks from transplant.

Impact of Si Substrate Amendment					
Calcium Silicate	pH	Electrical conductivity (mS/cm)	Height[2] (cm)	Diameter[2] (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[3]	*	NS	NS	NS	NS
Impact Of Micronutrient Fertility Rate					
Micronutrient Fertility Rate	pH	Electrical conductivity	Height[2]	Diameter[2]	Shoot Dry Weight
1X	5.31 AB	1.86	33.48	26.75	2.23 B
2X	5.25 B	1.97	32.77	27.79	2.79 A
4X	5.36 A	1.93	34.28	28.59	2.80 A
Significance[3]	*	NS	NS	NS	**
Interaction					
Micros X Si Rate	pH	Electrical conductivity	Height[2]	Diameter[2]	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[3]	*	NS	NS	NS	NS

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

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

[3]*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Table 2.3. Growth metrics of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for twelve weeks from transplant.

Impact of Si Substrate Amendment					
Calcium Silicate	pH	Electrical conductivity (mS/cm)	Height[2] (cm)	Diameter[2] (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[3]	***	NS	NS	NS	NS
Impact Of Micronutrient Fertility Rate					
Micronutrient Fertility Rate	pH	Electrical conductivity	Height[2]	Diameter[2]	Shoot Dry Weight
1X	5.33 B	2.59 A	38.41	25.93	11.44
2X	5.46 A	2.08 B	39.42	26.25	12.23
4X	5.46 A	1.90 B	39.41	26.83	11.92
Significance[3]	***	*	NS	NS	NS
Interaction					
Micros X Si Rate	pH	Electrical conductivity	Height[2]	Diameter[2]	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[3]	***	NS	NS	NS	NS

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

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

[3]*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Table 2.4. Root tissue nutrient concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for six weeks from transplant.

Impact of Si Substrate Amendment						
Calcium Silicate		B $mg \cdot kg^{-1}$	Zn $mg \cdot kg^{-1}$	Mn $mg \cdot kg^{-1}$	Fe $mg \cdot kg^{-1}$	Cu $mg \cdot kg^{-1}$
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[2]		***	NS	***	***	*
Impact Of Micronutrient Fertility Rate						
Micronutrient Fertility Rate[1]		B	Zn	Mn	Fe	Cu
1X		17.22 B	192.13	174.56 A	316.31 A	22.04 A
2X		19.61 A	194.98	177.72 A	351.12 A	15.19 B
4X		19.89 A	174.61	116.31 B	198.31 B	15.33 AB
Significance[2]		*	NS	***	***	*
Interaction						
Micros X 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	158.67ABC	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[2]		***	***	**	***	***

[1]Micronutrient fertility rates based on X times the standard concentration.

2*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Table 2.5. Leaf tissue micronutrient concentration of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for six weeks from transplant.

Impact of Si Substrate Amendment						
Calcium Silicate	B	Zn	Mn	Fe	Cu	Si
	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	%
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[2]	*	NS	*	**	NS	***
Impact Of Micronutrient Fertility Rate						
Micronutrient Fertility Rate	B	Zn	Mn	Fe	Cu	Si
1X	69.10 B	62.56 B	241.07	152.60 B	5.27 C	2.15 A
2X	72.90 B	65.56 B	230.08	162.32 A	6.49 B	2.11 A
4X	141.78 A	78.11 A	226.76	149.50 B	8.04 A	2.25 A
Significance[2]	***	**	NS	**	**	NS
Interaction						
Micros X 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[2]		***	NS	NS	NS	NS	NS

[1]Micronutrient fertility rates based on X times the standard concentration.

[2]*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Table 2.6. Floral tissue nutrient concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for twelve 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[3]		***	**	**	NS	*	***
Impact Of Micronutrient Fertility Rate							
Micronutrient Fertility Rate		B	Zn	Mn	Fe	Cu	Si
1X		49.69 C	121.38	393.25 B	478.56	12.75 B	1.38 A
2X		57.18 B	131.24	450.41 AB	457.40	17.94 AB	1.03 B
4X		85.06 A	123.00	513.17 A	521.74	22.94 A	0.97 B
Significance[3]		***	NS	**	NS	**	***
Interaction							
Micros X 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[3]		***	**	**	**	*	*

[1]Micronutrient fertility rates based on X times the standard concentration.

[2] All micronutrient concentrations are listed as ppm or mg·kg$^{-1,}$ Si concentration is listed as percentage of dry weight.

3*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Table 2.7. Cannabinoid concentrations of *Cannabis sativa* 'Auto CBG' grown in soilless substrate amended with (Si_{0X}, $Si_{0.5X}$, or Si_{1X}) and suppled with micronutrient concentrations (M_{1X}, M_{2X}, or M_{4X}) for twelve weeks from transplant.

Impact of Si Substrate Amendment					
Calcium Silicate		CBG[2]	CBGA[2]	Total CBG[2]	THCA[2]	Total THC[2]
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[3]		NS	NS	NS	NS	NS
Impact Of Micronutrient Fertility Rate						
Micronutrient Fertility Rate[1]		CBG[2]	CBGA[2]	Total CBG[2]	THCA[2]	Total THC[2]
1X		0.05	6.59	5.87	0.10	0.08
2X		0.06	6.15	5.41	0.12	0.11
4X		0.05	6.97	6.21	0.09	0.08
Significance[3]		NS	NS	NS	NS	NS
Interaction						
Micros X Si Rate		CBG[2]	CBGA[2]	Total CBG[2]	THCA[2]	Total THC[2]
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[3]		NS	NS	NS	NS	NS

[1]Micronutrient fertility rates based on X times the standard concentration.

[2]Abbreviations are as follows: Tetrahydrocannabinol (THC) and Cannabigerol (CBG). Any variance of the above 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 non-acid forms through decarboxylation. Total CBD and THC are calculated on a concentration basis of $mg \cdot g^{-1}$ of a composite sample which 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 freezedried composite weight.

[3]*, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

[v] Statistically significant based on *F* test at $P \leq 0.05$

Chapter 3
Comparison of Peat-Perlite 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 with a 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, limited research exists on 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. Cannabinoid concentration, plant height, diameter, or total plant biomass were similar across all substrate blends after twelve weeks of growth. Additionally, the use of $CaSiO_3$ as a Si substrate amendment increased Si foliar concentrations, and biochar addition 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 in this study is suitable in peat-based substrates for *C. sativa* 'BaOx' production at rates up to 30% (by vol.) in peat-based substrates with $CaSiO_3$ amendments.

Introduction

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 over 100 cannabinoids including cannabidiol (CBD), THC, and cannabigerol (CBG), that vary in concentration. Many cannabinoids are considered to have medical and therapeutic effects leading to a 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, in recent years, alternative biomasses have been evaluated for use in growing media. Evans and Gachukia (2004), reported that parboiled fresh rice hulls incorporated between 10 to 40% in a peat-based substrate resulted in similar growth of tomato (*Solanum lycopersicum* L. 'Better Boy'), marigold (*Tagetes patula* L. 'Bonza Yellow'), geranium (*Pelargonium x hortum* Bailey 'Orbit Cardinal'), vinca (*Vinca minor* L. 'Cooler Blush'), impatiens (*Impatiens walleriana* Hooker 'Dazzler Rose Star'), and pansy (*Viola x wittrockiana* Gams 'Bingo Azure') compared to those of equal amounts of perlite. Additionally, pine wood chips have proven to be an appropriate alternative to perlite as well at 10 to 30% incorporation into 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 x hybrida* Jacquin 'Profusion Orange') (Owen, 2013).

Biochar is a black charcoal-like material produced from organic products heated to temperatures below 700 ºC 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 utilize biochar in agricultural applications ranging from field amendments (Chan et al., 2007; Singh et al., 2010) or as 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; Yu et al., 2019; Huang and Gu, 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 substrate pH (Zhang et al., 2014; Dispenza et al., 2017; Park et al., 2011; Northup, 2013). However, pH of biochar materials were reported to 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 the increase is dependent on the biochar feedstock (Huang and Gu, 2019). Due to the wide variety of feedstocks utilized and biochar incorporation rates into 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 Si accumulators (Bolt and Altland, 2021). Silicon supplementation in greenhouse crops can be achieved in multiple ways ranging from foliar applications (Kameniduo et al., 2009; Whitted-Haag et al., 2014), incorporation of Si in hydroponic nutrient solution (Boldt and Altland, 2021; Mattson and Leatherwood, 2010), or as Si substrate amendments (Boldt et al., 2018; Kameniduo

et al., 2010). While 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 heavy metal-contaminated soils and to exclude heavy metal uptake (Khan et al., 2021; Luyckx et al., 2021; Pavlovic et al., 2021). Silicon is noted for its 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 cadmium (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 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).

While the beneficial effects of biochar and Si are known independently, limited research reports the 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 investigating the impacts of Si amendments when applied to a growing substrate amended with biochar or oil production of hemp. This study aims 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) and humidity domes were placed over the unrooted plant cuttings. Cuttings were placed under T5 full spectrum fluorescent lamps (AgroBrite T5 Full Spectrum; Hydrofarm, Petaluma, CA) delivering delivering200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at cutting height as measured with a quantum meter (MQ-610 ePAR Meter; Apogee Instruments, Logan, UT) 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 20L H_2O). On 21 Feb., after 21d of propagation, rooted cuttings were transplanted into 7.8-L plastic pots filled with a respective substrate and grown in a greenhouse (35.78 °N lat.) with 23.9°C/18.3°Cday/night temperatures. Plants received ambient solar radiation and night interruption (NI) lighting was deployed from 2200 to 02000 HR during the first four weeks to prevent floral initiation. After four weeks, NI ceased, and long-days were initiated to induce reproductive floral development for the subsequent eight weeks. Plants were fertilized at each delivery with Ultrasol 13N-2P-13K, (SQM, Atlanta, Ga) 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 were comprised of an 85:15 or 70:30 (v:v) mix of Canadian sphagnum peat moss fluffed from a compressed bales (Sun Gro Horticulture Company, Agawam, MA) and coarse perlite, (horticultural coarse perlite; Sun Gro Horticulture Company) or one of two wood biochar aggregates including medium (<6 mm) or coarse (>6mm; Sun Gro Horticulture Company), each with a pH ~9.0. Substrates were also amended with wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ) at 600 $g \cdot m^{-3}$, a micronutrient starter charge (M.O.S.T.; J.R.

Peters, Inc., Allentown, PA) at 1,186.6 $g \cdot m^{-3}$, 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 3.1).

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-hr 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 for pH and EC using a Hanna portable pH/EC meter (HI 9813-6; Hanna Instruments, Smithfield, RI).

At weeks six and 12, six plants per treatment were destructively harvested. Plant height was measured from the substrate surface to the apical meristem, and diameter [(widest diameter + perpendicular axis) ÷2]. At week six, 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 washed in a solution of 0.5 N hydrochloric acid (HCl) for 1 min, and rinsed again with DI water (Henry et al., 2018), dried in an oven at 70 °C for 96 hr, and weighed to determine sampled leaf biomass. The remaining plant shoot was harvested, bagged individually, and dried in an oven at 70 °C for 96 hr, and weighted to determine plant biomass. 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). The ground tissue was then placed in vials containing ~3 g of tissue and submitted for nutrient analysis of N,

P, K, Ca, Mg, S, B, Cu, Zn, Fe, and Mn concentration (Waters Lab; Warsaw, NC). Dried 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 minutes (MARS 6 Microwaves; CEM Corp., Matthews, NC). After microwave digestion, the plant material was diluted with 50 mL of DI water and then vacuum-filtered through acid-washed paper (Laboratory Filtration Group; Houston, TX). After dilution, plant mineral tissue concentration was determined using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) machine (Spectro Arcos EOP; Mahwah, NJ).

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. The composite sample was then freeze-dried (Harvest Right; North Salt Lake, UT) for 30 hr. 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). Upon arrival, the material was lyophilized, ground, and a 2 g (1.98 – 2.02 g) sub-sample from the composite sample was obtained. Analysis for cannabinoids was accomplished through high-pressure liquid chromatography (Shimadzu 8050 & 8040 Triple Quadrupole UHPLC/MS/MS analysis; Austin, TX). Exact details regarding cannabinoid analysis cannot be provided as Delta 9 Analytics utilizes a proprietary protocol.

Cannabinoid analysis included both the active (decarboxylated) and acid forms of ~~(~~CBG~~)~~, THC, CBD, and cannabichromene. Additional cannabinoids and forms exist but 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):

Δ^9 THC + ([0.877 × tetrahydrocannabinol acid)] = Total THC [1]

CBD + [0.877 × cannabidiol acid] = Total CBD [2]

Statistical analysis. Statistical analysis was conducted using SAS (version 9.4; SAS Inst., Cary, NC). Plant growth metrics, leaf nutrient values, and cannabinoids were analyzed for differences within each data collection (n=6) as an aggregate (3 levels) x aggregate percentage (2 levels) x Si amendment (2 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 (HSD) at $P \leq 0.05$). Deviations in plant metrics, total plant dry weights, and leaf tissue values were calculated on a percentage basis from the control substrate (15% perlite without Si).

Results and Discussion

Substrate pH, EC, and growth metrics. After six weeks of growth, the three-way interaction of aggregate type × aggregate percentage × Si incorporation rate did not significantly impact the substrate pH (Table 3.2). However, when examining the three simple effects of aggregate type, aggregate rate, and Si amendment independently, significant differences were observed (Table 3.2) Substrates that received a Si substrate amendment or utilized biochar as the aggregate compared to perlite exhibited a higher substrate pH after six weeks of growth (Table 3.2).

After 12 weeks of growth, the three-way interaction significantly impacted substrate pH and similar trends were observed for the simple effects (Table 3.3). However, the greatest difference among substrates was only 0.5 pH units and likely did not impact plant growth. Whipker et al. (2019) stated hemp is tolerant of substrate pH between 5.5 and 6.5, while they recommend growers target 5.8 to 6.2. During this study, most of the substrate pH means were reported within

the tolerant range of 5.5 to 6.5 reported by Whipker et al. (2019). The difference observed in the substrate pHs was a result of the varying lime charges that were utilized in this experiment to offset the higher alkaline pH characteristics of biochar and $CaSiO_3$ (Table 1).

At weeks six and 12, there were no observed differences in plant height, diameter, total plant biomass, or EC for any of the examined interactions or simple effects of aggregate type, aggregate percentage, or calcium silicate rate (Tables 3.2 and 3.3). Additionally, there were no visual impacts on plant morphology or growth at weeks six or 12 (Fig. 3.1 and 3.2).

These results are concurrent with Northup (2013), in which replacing perlite with biochar 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 peat moss with biochar, we did not observe an increase in EC which is contrasted with observations published by Northup (2013). However, due to utilizing biochar from different feedstocks and varying biochar physical properties in 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 transplant, the three-way interaction significantly impacted calcium (Ca) and magnesium (Mg) (Table 3.4). The difference observed in the Ca and Mg foliar concentrations is most likely a result of the varying limestone charges that were utilized in this experiment and the alkaline characteristics associated with biochar (Table 3.1) 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 3.4). While differences in foliar tissue concentration were observed, all reported foliar tissue concentrations were above the deficient concentrations reported by

Cockson et al., (2019) and were within the survey ranges reported by Kalinowski et al., (2019). Additionally, Mg foliar concentrations were within the recommended reported by Veazie et al., (2021) for plants fertilized with 75 to 100 $mg \cdot L^{-1}$ Mg.

Si foliar and floral concentration. After six weeks of growth, neither the three-way interaction nor any of the two-way interactions exhibited significant differences in Si foliar concentrations (Table 3.4). After 12 weeks of growth, similar trends were observed regarding Si foliar concentrations of which none of the examined interactions exhibited significant differences in Si floral concentrations (Table 3.5). However, for the simple effects, plants that received a Si amendment exhibited a 61.8% increase in floral Si concentrations when compared to plants that did not receive Si (Table 3.5). This suggests that the use of $CaSiO_3$ can effectively increase Si concentrations in the foliar and floral tissue of *C. sativa* 'BaOx'. When Si was added to a hydroponic nutrient solution a decreased infection rate of gray mold (*Botrytis cinerea*) in lettuce, tomato, and pepper was observed (Pozo et al., 2015). Gray mold is one of the most important diseases in *Cannabis* production that results in the greatest losses in yield (McPartland et al., 2000). Thus, it suggests that with floral material accumulating Si without disease pressure 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 examining the three-way interaction or any of the two-way interactions (Table 3.5). However, total THC concentration exhibited significant differences when examining the simple effect of aggregate type (Table 3.5). Plants that utilized coarse biochar exhibited significantly greater total THC when compared to plants that were grown utilizing medium biochar; however, the difference is only 0.07% and is likely not biologically significant (Table 3.5).

This would suggest that after twelve 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 biochar addition to peat-based mixes did not limit the Si availability for plant uptake.

Conclusion

The twelve different substrates evaluated in this study are all suitable and acceptable to grow *C. sativa* 'BaOx' without any negative impact on plant growth. Plants that received a Si substrate amendment (0.50 $kg \cdot m^{-3}$ Si) exhibited increased Si foliar concentrations with substrates composed of 15% aggregate compared to similar substrates that did not receive Si (0 $kg \cdot m^{-3}$ 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 performing equally to a peat:perlite mix for plant production.

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Figure 3.1. Impact of different substrate aggregates; perlite, medium (M) biochar, or coarse (C) biochar at 15% (by vol.) and 30% (by vol.) with (+) and without (-) calcium silicate (silicon; Si) on *Cannabis sativa* 'BaOX' plants at six weeks after transplant.

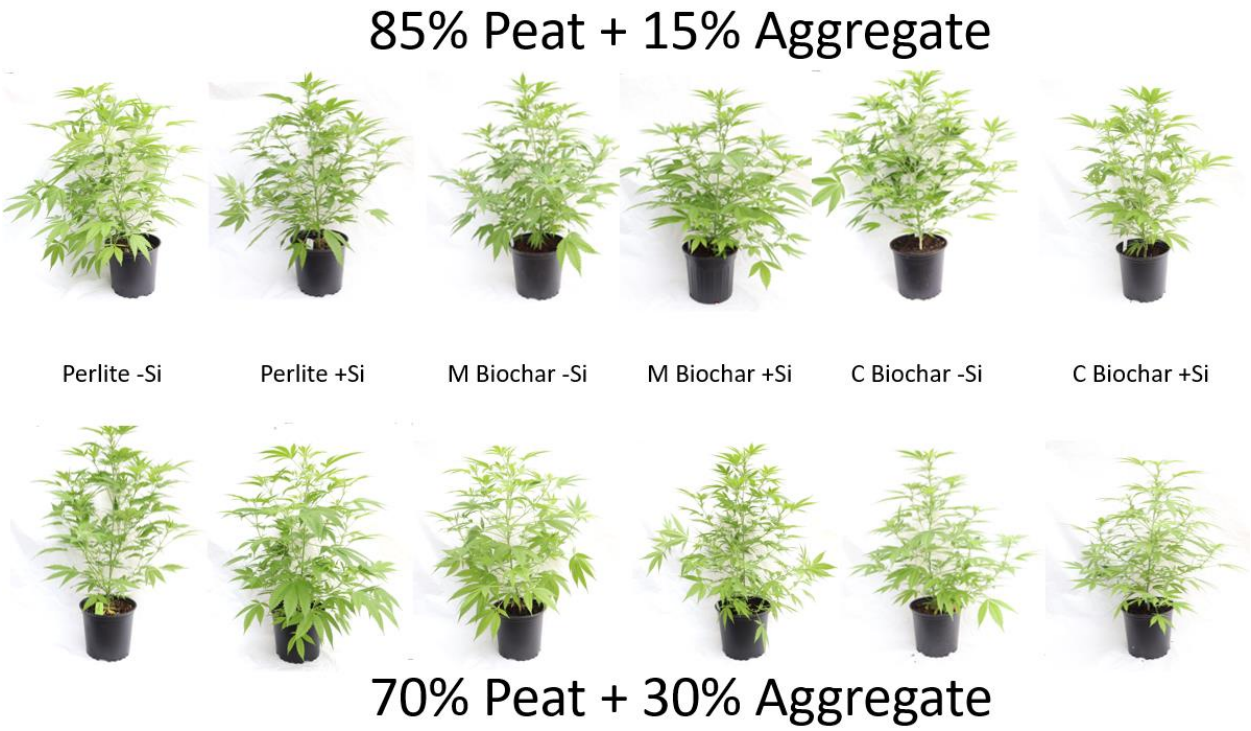

Figure 3.2. Impact of different substrate aggregates; perlite, medium (M) biochar, or coarse (C) biochar at 15% (by vol.) and 30% (by vol.) with (+) and without (-) calcium silicate (silicon; Si) on *Cannabis sativa* 'BaOX' plants at 12 weeks after transplant.

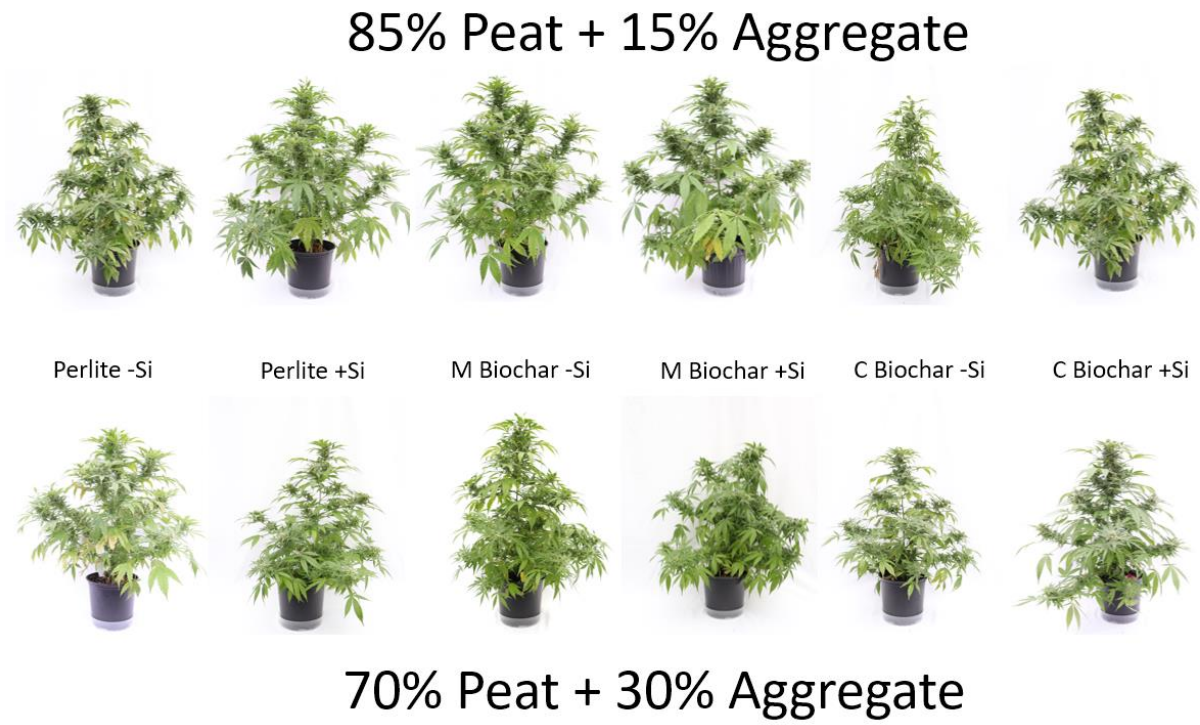

Table 3.1: Summary of substrate treatments evaluating biochar as a perlite replacement for *Cannabis sativa*.

		Substrate Properties					
Substrate	Aggregate type	V: V	Peat%	Biochar%	Perlite %	Silicon[1]	Lime Rate[1]
1	Perlite	(85:15)	85	0	15	0	3.56
2	Perlite	(85:15)	85	0	15	2.08	2.37
3	Perlite	(70:30)	70	0	30	0	3.56
4	Perlite	(70:30)	70	0	30	2.08	2.37
5	Medium Biochar[2]	(85:15)	85	15	0	0	3.56
6	Medium Biochar	(85:15)	85	15	0	2.08	2.37
7	Medium Biochar	(70:30)	70	30	0	0	3.56
8	Medium Biochar	(70:30)	70	30	0	2.08	2.37
9	Coarse Biochar[3]	(85:15)	85	15	0	0	2.97
10	Coarse Biochar	(85:15)	85	15	0	2.08	1.78
11	Coarse Biochar	(70:30)	70	30	0	0	2.97
12	Coarse Biochar	(70:30)	70	30	0	2.08	1.78

[1] Dolomitic Lime and silicon (calcium silicate) substrate amendments are reported as $kg \cdot m^{-3}$

[2] Medium biochar aggregates are 2-6mm in size

[3] Course biochar aggregates are greater than 6mm in size

Table 3.2: Growth metrics of *Cannabis sativa* 'BaOx' grown in soilless substrate amended with three different aggregates (perlite, medium or coarse biochar), at two different incorporation rates (15% or 30%) and with or without silicon amendments (Si_{0X} or Si_{1X}) six weeks from transplant.

			pH	Electrical Conductivity (mS/cm)	Height[2] (cm)	Diameter[2] (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[3]			***	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
Silicon Rate							
0.0			6.04 B	2.12	59.61	66.04	21.84
2.08			6.16 A	2.32	58.41	64.59	19.95
Significance			**	NS	NS	NS	NS
Second Order Interactions							
Aggregate Type X Aggregate Percentage			**	NS	NS	NS	NS
Aggregate Type X Si Rate			NS	NS	NS	NS	NS
Aggregate Percentage X Si Rate			*	NS	NS	NS	NS
Aggregate Type X Aggregate Percentage X Si Rate							
Aggregate Type	Aggregate	Silicon[1]					
Perlite	15	0.0	5.67	2.53	59.73	66.93	24.17
Perlite	15	2.08	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	2.08	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	2.08	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	2.08	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	2.08	6.00	2.37	58.17	62.92	19.53

Table 3.2 Continued

Coarse Biochar	30	0.0	6.12	1.94	55.25	60.73	16.02
Coarse Biochar	30	2.08	6.23	2.51	57.73	65.68	21.13
Significance			NS	NS	NS	NS	NS

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

[2] All height and diameter measurements are based on cm. The diameter was calculated by taking the widest two points on a plant taken 90º 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.

[3] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P > 0.05$) was used to compare differences among means.

Table 3.3: Growth metrics of *Cannabis sativa* 'BaOx' grown in soilless substrate amended with three different aggregates (perlite, medium or coarse biochar), at two different incorporation rates (15% or 30%) and with or without silicon amendments (Si_{0X} or Si_{1X}) 12 weeks from transplant.

			pH	Electrical Conductivity (mS/cm)	Height[2] (cm)	Diameter[2] (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[3]			***	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
Silicon Rate							
0.0			6.31	1.33	63.91	60.50	72.87
2.08			6.35	1.45	63.85	61.79	64.42
Significance			NS	NS	NS	NS	NS
Second Order Interactions							
Aggregate Type X Aggregate Percentage			***	NS	NS	NS	NS
Aggregate Type X Si Rate			*	NS	NS	NS	NS
Aggregate Percentage X Si Rate			**	NS	NS	NS	NS
Aggregate Type X Aggregate Percentage X Si Rate							
Aggregate Type	Aggregate	Silicon[1]					
Perlite	15	0.0	5.85 DE	1.01	60.68	57.13	50.47
Perlite	15	2.08	5.80 E	1.62	63.00	64.36	66.73

Table 3.3 Continued

Perlite	30	0.0	6.18 CD	1.56	66.65	68.04	76.57
Perlite	30	2.08	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	2.08	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	2.08	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	2.08	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	2.08	6.38 BC	1.34	60.25	58.61	65.00
Significance			**	NS	NS	NS	NS

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

[2] All height and diameter measurements are based on cm. The diameter was calculated by taking the widest two points on a plant taken 90º 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.

[3] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P > 0.05$) was used to compare differences among means.

Table 3.4: Foliar macronutrient and Si concentrations of *Cannabis sativa* 'BaOx' grown in soilless substrate amended with three different aggregates (perlite, medium or coarse biochar), at two different incorporation rates (15% or 30%) and with or without silicon amendments (Si_{0X} or Si_{1X}) six weeks from transplant.

			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[2]			**	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
Silicon Rate									
0.0			4.98	0.63	3.42	1.46	3.67	0.36	0.74
2.08			5.14	0.68	3.44	1.08	3.95	0.38	1.62
Significance			*	NS	NS	***	*	**	***
Second Order Interactions									
Aggregate Type X Aggregate Percentage			NS	NS	NS	NS	NS	*	NS
Aggregate Type X Si Rate			NS	NS	NS	NS	NS	NS	NS
Aggregate Percentage X Si Rate			*	NS	NS	NS	NS	*	NS
Aggregate Type X Aggregate Percentage X Si Rate									
Aggregate Type	Aggregate	Silicon[1]							
Perlite	15	0.0	5.22	0.57	3.34	1.39	3.33	0.36	0.63
Perlite	15	2.08	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	2.08	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	2.08	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	2.08	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	2.08	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	2.08	4.86	0.66	3.44	1.10	3.95	0.40	1.56
Significance			NS	NS	NS	*	*	NS	NS

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

[24] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Table 3.5: Cannabinoid and Si concentrations of *Cannabis sativa* 'BaOx' grown in soilless substrate amended with three different aggregates (perlite, medium or coarse biochar), at two different incorporation rates (15% or 30%) and with or without silicon amendments (Si_{0X} or

			Total CBD	Total CBG	Total THC	Total Cannabinoids	Si %
Aggregate Type							
Perlite			10.09	0.53	0.36 AB	12.63	1.40 B
Medium Biochar			8.81	0.68	0.30B	11.24	1.79 A
Coarse Biochar			11.16	0.49	0.37 A	13.82	0.81 C
Significance[3]			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	*
Silicon Rate							
0.0			9.80	0.57	0.34	12.31	1.02 B
2.08			10.24	0.57	0.35	12.81	1.65 A
Significance			NS	NS	NS	NS	***
Second Order Interactions							
Aggregate Type X Aggregate Percentage			NS	NS	NS	NS	NS
Aggregate Type X Si Rate			NS	NS	NS	NS	NS
Aggregate Percentage X Si Rate			NS	NS	NS	NS	NS
Aggregate Type X Aggregate Percentage X Si Rate							
Aggregate Type	Aggregate	Silicon[1]					
Perlite	15	0.0	11.36	0.38	0.42	14.00	0.94
Perlite	15	2.08	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	2.08	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	2.08	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	2.08	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	2.08	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	2.08	10.01	0.59	0.33	12.56	0.62
Significance			NS	NS	NS	NS	NS

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

[2] Abbreviations are as follows: Tetrahydrocannabinol (THC), Cannabigerol (CBG), Cannabidiol (CBD), Total CBD and THC are calculated on a concentration basis 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 concentration ($mg \cdot g^{-1}$) of 2 g freeze-dried composite weight.

[3] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, HSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Si_{1X}) twelve weeks from transplant.

Chapter 4
Evaluation of Biochar as a Sustainable Aggregate in Horticultural Growing Media for Greenhouse Production

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Title: Evaluation of Biochar as a Sustainable Aggregate in Horticultural Growing Media for Greenhouse Production

Abstract

Several organic/inorganic materials have been used in the formulation of growing media. Traditionally, *Sphagnum* peat, coir, and aggregates, such as bark, perlite and vermiculite, are used. However, in recent years, alternative biomasses have been evaluated for use in growing media including biochar. Biochar is a carbon-based material. Regionally produced biochar materials have been evaluated previously by other researchers as horticultural substrate additives to promote microbiological and chemical characteristics for the rhizosphere environment improvement for plant roots. In North America, perlite is a major aggregate component to engineer horticultural substrates. Therefore, the biochar aggregate was evaluated by comparing the performance of peat-perlite mixes. Three species, French marigold, pepper, and tomato were grown in one of four different substrates with either perlite or wood biochar, and aggregate ratios of 15% or 30% by volume. Plant height and diameter were similar across all examined substrates for all species. This suggests that biochar with its lower environmental impact is a comparable substrate aggregate to perlite without any cultural practice adjustments such as irrigation and fertilization regime.

Keywords Biochar, Alternative aggregate, Sphagnum Peat, Perlite, Growing Media

Introduction

The traditional composition of growing media has a variety of components that include peat, bark, and aggregates, such as perlite and vermiculite (Nemati et al., 2015). However, in recent years, alternative aggregates have been evaluated for use in growing media. Evans and Gachukia (2004) reported that parboiled fresh rice hulls incorporated between 10-40% in a peat-

based substrate exhibited similar growth compared to those of equal amounts of perlite. Additionally, pine wood chips have proven to be an appropriate alternative to perlite as well at 10-30% incorporation into peat-based substrates (Owen, 2013).

Biochar is a black charcoal-like material that is produced from 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, biochar has been evaluated as 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; Yu et al., 2019; Huang and Gu, 2019). However, with the wide array of feedstocks used the biochar chemical and physical properties can vary widely based on the feedstock utilized in the production of biochar. 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. In most cases, biochar has a neutral or basic pH (> 7.0 pH) and is effective at increasing substrate pH. However, biochar pH's have been reported ranging from 3.5 to 10.3 (Fornes et al, 2015; Khodadad et al., 2011; Nemati et al., 2015; Spokas et al., 2012) and may have the potential to neutralize acidity caused by peat and root growth (Bedussi et al., 2015). Incorporating biochar into substrates can increase the cation exchange capacity (CEC), however, the magnitude of the increase is dependent on many factors of the initial biochar feedstock (Huang and Gu, 2019). The cost and availability of horticultural grade perlite has been challenging during the last few years. A possible alternative could be to use biochar to replace perlite in a growing mix, but there is uncertainty of how well plants will perform. Therefore this study sought to compare the type of aggregate used for growing plants in a peat and perlite-based mix and a peat and biochar-based substrate.

Materials and Methods

Seeds of tomato (*Solanum lycopersicon L.*) 'Tasmanian Chocolate OG', pepper (*Capsicum annuum*) 'Cupid', and French marigold (*Tagetes patula*) 'Queen Sophia' were sown on 11 Aug. Seeds were sown in 105 cell flats and placed under full spectrum halogen lights (AgroBrite T5 Full Spectrum grow lights, Hydrofarm, Petaluma, CA) for three weeks. On 1 September seedlings were transplanted into 14-cm diameter azalea plastic pots (1.3L) (ITML Horticulture Products, Middlefield, OH) filled with one of four substrates. The plants were grown in a greenhouse in Raleigh, NC, and grown with 23 ˚C Day/ 17 ˚C night air temperature settings. Plants were irrigated as needed using 13-2-13 at 10.7 mmol/L N, 0.325 mmol/L P, and 3.18 mmol/L K (Ultrasol, SQM, Atlanta, Ga) beginning the day after transplant.

Seedlings were transplanted into one of four substrate treatments, these treatments were comprised of an 85:15 or 70:30 (v:v) mix of Canadian Sphagnum peat moss (Sun Gro Horticulture Company, Agawam, MA), perlite (horticultural coarse perlite Sun Gro Horticulture Company, Agawam, MA) or pine wood biochar (2-6mm, pH~9.0)(Sun Gro Horticulture Company, Agawam, MA), wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ) at 600 $g \cdot m^{-3}$, micronutrient starter charge (J.R. Peters, Allentown, PA) at 1186.6 $g \cdot m^{-3}$ and, varying rates of dolomitic limestone (Sun Gro Horticulture Company, Agawam, MA) (Table 4.1).

The experiment was a completely randomized design with five single plant replicates (n=5) of four substrates. Peppers and tomatoes were terminated three weeks after transplant, and French marigolds were terminated six weeks after transplant. This was to determine if biochar can be utilized as an alternative aggregate for plants being produced for garden center sales. Plant height was measured from the substrate line to the apical tip, diameter (measured at the greatest width,

turned 90°, and averaged), and total above-ground dry biomass. At termination substrate pH and electrical conductivity (EC) were evaluated using the pour-through method (Cavins et al., 2004). Plants were well irrigated two hours before each data collection and 75 mL of deionized water (DI) was poured over the pots to displace 50 mL of leachate. The leachate was analyzed for pH and EC using a Hanna portable pH/EC meter (HI9813-6, Hanna Instruments; Smithfield, RI).

Statistical analysis was conducted using SAS (version 9.4; SAS Inst., Cary, NC). Plant growth metrics were analyzed for differences within each data collection regarding the substrate as the explanatory variables using PROC GLM. Where the *F*-test was significant, LSD with a Tukey Kramer adjustment ($P < 0.05$) was used to compare differences among means. Deviations in plant growth metrics were calculated on a percentage basis from the controls.

RESULTS AND DISCUSSION

Substrate pH

For tomato and pepper, the final substrate pH was significantly higher, 6.18 and 6.56 with 30% biochar compared to 30% perlite substrate (Tables 4.2 & 4.3). Additionally, pepper plants grown utilizing 85% peat:15% biochar exhibited higher substrate pH compared to 85% peat:15% perlite substrate (Table 4.3). The difference in substrate pH between perlite and biochar aggregate substrates was likely caused by the basic properties associated with biochar. Biochar pH is greatly influenced by the feedstock that is utilized (Fornes et al, 2015; Khodadad et al., 2011; Nemati et al., 2015; Spokas et al., 2012). This is in comparison to perlite which exhibits a neutral pH (Owen, 2013). These results are also supported by the varying rates of dolomitic lime that were utilized to adjust for this difference in aggregate pH (Table 4.1).

Electrical Conductivity

Electrical conductivity (EC) was greater in tomato plants that were grown in biochar-containing substrates when compared to substrates that contained perlite at the same incorporation rate (Table 4.2). Additionally, pepper plants that were grown utilizing an 85% peat and 15% perlite substrate exhibited greater EC concentration when compared to plants grown in 85% peat and 15% biochar substrates (Table 4.3).

Plant Growth Metrics

After three weeks of growth, height, diameter, or plant dry weight of tomato plants were not significantly different for any of the four examined substrates (Table 4.2). However, pepper plants grown in 70% peat: 30% perlite substrate exhibited greater plant height compared to those that were grown in 70% peat: 30% biochar (Table 4.3). Additionally, when comparing all four substrates, significant differences were observed in pepper total plant biomass, however, no clear trends were observed (Table 4.3). After six weeks of growth, no differences in plant height, diameter, or dry weight were observed for French marigolds across any of the examined substrates (Table 4.4). This limited difference in plant growth metrics across the examined aggregates and aggregate incorporation rates suggests that biochar is a suitable alternative aggregate for peat-based substrates.

CONCLUSION

The four different substrates tested in this study are all suitable and acceptable to grow tomato, pepper, or French marigolds without a negative impact on plant growth or quality. When comparing aggregate percentages and aggregate types, limited differences in plant growth metrics were observed across all three species other than an slightly elevated pH when biochar was used as aggregates compared to perlite at 30%. However, substrate pH can be adjusted by modify the

limestone incorporation rate. Thus, commercial growing media producers should monitor substrate pH and can likely reduce lime rates depending on the biochar's physical and chemical properties. This research demonstrates that wood biochar is a suitable soilless substrate aggregate that can be utilized for greenhouse crops without needing to modify cultural practices.

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Table 4.1: Summary of substrate formulation to evaluate the suitability of biochar compared to the performance of perlite in peat-based substrates.

Substrate	Aggregate type	V: V	Peat%	Biochar%	Perlite %	Lime Rate[1]
1	Perlite	(85:15)	85	0	15	3.56
2	Perlite	(70:30)	70	0	30	2.96
3	Biochar[2]	(85:15)	85	15	0	2.96
4	Biochar	(70:30)	70	30	0	2.96

[1] Dolomitic limestone rates are reported as $kg \cdot m^{-3}$

[2] Biochar aggregates are 2-6mm in size

Table 4.2: Comparison between biochar and perlite substrate aggregate incorperation on *Solanum lycopersicon* L. 'Tasmanian Chocolate OG' pH, EC, and total plant biomass after three weeks of plant growth.

***Solanum lycopersicon* L. 'Tasmanian Chocolate OG'**						
15% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(85:15)	5.92	0.69 B	26.36	55.47	8.82
Biochar	(85:15)	5.84	0.83 A	25.90	53.18	8.88
Significance[2]		NS	*	NS	NS	NS
30% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(70:30)	5.94 B	0.73 B	25.96	54.78	8.35
Biochar	(70:30)	6.18 A	0.87 A	26.13	54.06	8.65
Significance[1]		***	**	NS	NS	NS
Comparison of all substrates		**	**	NS	NS	NS

[1] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, LSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Table 4.3 Comparison between biochar and perlite substrate aggregate incorperation on *Capsicum annuum* 'Cupid' pH, EC, and total plant biomass after three weeks of plant growth.

***Capsicum annuum* 'Cupid'**						
15% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(85:15)	6.04 B	0.75 A	28.1	45.45	5.52
Biochar	(85:15)	6.26 A	0.63 B	29.14	45.59	5.98
Significance[2]		**	*	NS	NS	NS
30% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(70:30)	6.18 B	0.71	29.38 A	45.95	5.58
Biochar	(70:30)	6.56 A	0.69	26.44 B	43.73	5.02
Significance[1]		***	NS	*	NS	NS
Comparison of all substrates		***	*	NS	NS	*

[1] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, LSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Table 4.4: Comparison between biochar and perlite substrate aggregate incorporation on *Tagetes patula* 'Queen Sophia' pH, EC, and total plant biomass after three weeks of plant growth.

***Tagetes patula* 'Queen Sophia'**						
15% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(85:15)	6.96	1.87	23.62	31.95	28.26
Biochar	(85:15)	7.04	1.78	23.08	34.56	24.66
Significance[2]		NS	NS	NS	NS	NS
30% Aggregate Substrates						
Aggregate Type	V: V	pH	Electrical Conductivity (ms/cm)	Height (cm)	Diameter (cm)	Total Plant Biomass (g)
		Mean	Mean	Mean	Mean	Mean
Perlite	(70:30)	7.10	1.57	24.70	32.42	25.14
Biochar	(70:30)	7.10	1.73	24.02	32.76	25.42
Significance[1]		NS	NS	NS	NS	NS
Comparison of all substrates		*	NS	NS	NS	NS

[1] *, **, or *** indicates 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 $P > 0.05$. Where the *F*-test was significant, LSD with a Tukey-Kramer adjustment ($P < 0.05$) was used to compare differences among means.

Chapter 5
Evaluation of Biochar as a Sustainable Aggregate in Horticultural Growing Media for Greenhouse Production

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Title: Peat Substrates Amended with Wood-based Biochar Do Not Influence the Efficacy of Paclobutrazol Drenches

Additional index words: Plant growth regulators, soilless substrate, pansy, poinsettia, begonia

Abstract.

Various soilless substrate components have been evaluated for many years to identify sustainable resources that do not negatively impact plant growth. Biochar is a carbon-based material that has been evaluated for use as an alternative aggregate in peat-based soilless substrates. Additionally, the use of carbon adsorption for compound removal is widely used in groundwater remediation, municipal water filtration, and volatile organic compounds. Experiment one aimed to determine the impact of coarse biochar (<6mm) on paclobutrazol efficacy when incorporated at 15 or 30% by volume in a peat-based substrate when compared to a perlite amended substrate at the same incorporation volumes. In experiment one, a single paclobutrazol drench application of 0, 0.5, 1.0, 2.0, and 4.0 $mg \cdot L^{-1}$ was applied to 'Princettia Red' and 'Princettia White' poinsettias (*Euphorbia pulcherrima*×*Euphorbia cornastra*). In experiment two, two different biochar particle sizes of coarse (<6mm) and extra coarse (>6mm) were examined at the same incorporation volumes as experiment one and compared to a perlite amended substrate at the same incorporation volumes. However, during experiment two continual drench applications at times of irrigation of 0.0, 6.25, 12.5, 25.0, 50, and 100 $\mu g \cdot L^{-1}$ (ppb) paclobutrazol were applied to pansy (*Viola*×*wittrockiana*) 'Matrix Blue Blotch' and begonia (*Begonia*×*hybrida*) 'Big Red Bronze Leaf'. The efficacy of paclobutrazol drenches for controlling growth in all species was not affected by the substrate composition regarding aggregate type or aggregate incorporation rate. Thus, even though biochar is often used for bioremediation and wastewater treatment, it did not negatively impact the efficacy of paclobutrazol drenches at the concentrations used. This

research suggests that when biochar is used as an amendment to peat moss it will not influence paclobutrazol drench efficacy when incorporated up to 30% by volume for the examined species.

Introduction

Horticultural soilless substrates used in floriculture crop production are often composed of a wide variety of materials ranging from peat moss, bark, and aggregates, such as perlite and vermiculite (Nemati et al., 2015). A wide range of alternative aggregates including wood chips, rice hulls, and biochar have been evaluated as alternative aggregates for horticultural substrates (Evans and Gachukia, 2004; Guo et al., 2018; Jackson et al., 2008; Owen, 2013; Woldetsadik et al., 2018). In most cases, a 10-30% incorporation rate of alternative aggregates such as biochar, wood chips, or rice hulls, into a peat-based substrate resulted in similar growth when compared to perlite (Owen, 2013; Northup, 2013).

Biochar is a charcoal-like material that is produced from organic feedstocks by using pyrolysis, gasification, or hydrothermal carbonization (Huang and Gu, 2019). Pyrolysis is the thermal decomposition of biomass by heating the feedstocks to 400 ˚C to 600 ˚C in the absence of oxygen (Gvero et al., 2016). The product of the pyrolysis process can have a wide array of physical properties and can have significant impacts on substrate pH, electrical conductivity (EC) and porosity, and is primarily associated with the properties of the feedstock (Huang and Gu, 2019). The pH of biochar is generally considered to be basic (pH $\geq$ 7.0), however, pH has been reported ranging from 3.5 to 10.3 (Fornes et al, 2015; Khodadad et al., 2011; Nemati et al., 2015; Spokas et al., 2012). The basic properties of biochar may be useful to neutralize the acidity caused by peat in most blends leading to a decrease in the amount of liming material required to achieve an optimal growing pH (Bedussi et al., 2015). Guo et al. (2018) examined the impact of

pinewood biochar incorporation rate into a commercially available substrate and fertility rates on poinsettia (*Euphorbia pulcherrima)* growth and reported that biochar incorporation rates of up to 80% can yield acceptable results when amended into a peat-based substrate. Additionally, Yu et al. (2023) reported when poinsettias were inoculated with root rot (*Pythium aphanidermatum*) plants grown in hardwood biochar, 20% by volume, exhibited significantly higher shoot dry weight and lower disease severity when compared to perlite aggregate substrates at the same percentage. However, while most published research focuses on the impact of biochar on plant growth, limited research has been conducted to determine how production practices would need to be modified for implementation in a commercial setting.

Plant growth regulators (PGR) allow the production of uniform compact plants that can be tightly spaced in a growing area (Smith, 2019). Additional benefits associated with the use of PGRs, such as ancymidol, daminozide, chlormequat chloride, flurprimidol, paclobutrazol, and uniconizole, include increased chlorophyll concentrations resulting in greener leaves, reduced water stress, and disease suppression (Whipker, 2023). Controlling stem elongation of poinsettias is crucial to producing desired market size and shaped plants, controlling stem elongation is commonly done utilizing PGRs to retard plant growth (Faust et al., 2001; Niu et al., 2002). Paclobutrazol is a triazole PGR that is effective on poinsettias when applied as either a spray or drench (Newman and Tant, 1995). The triazole class of PGRs are not readily transported through phloem so increased efficacy occurs when they can be transported by the xylem stream when applied as a drench (Barrett, 2001; Desta and Amare, 2021). While there are many common methods of PGR applications, drenches offer precision of application, application uniformity, and reduction of potential drift that is associated with foliar sprays (Owen et al., 2016).

While drench applications provide an effective method for plant uptake through the roots, substrate composition has been reported to impact the application rate. Quarrels and Newman (1994) reported a significant reduction of paclobutrazol drench efficacy on poinsettias when applied to pine bark substrates. However, Owen et al., (2016), reported no significant differences in paclobutrazol drench rate efficacy when amending peat-based substrates with wood chip aggregates as a perlite replacement. The use of carbon adsorption for compound removal is widely used in groundwater remediation, municipal water filtration, and volatile organic compounds (Ioannidou et al., 2010). Granular activated carbon has been shown to reliably remove pesticides and herbicides from water (Brooks et al., 2000). Additionally, a 50 $\mu g \cdot L^{-1}$ paclobutrazol solution that was in contact with granular activated carbon for 59 seconds resulted in a 36% greater begonia dry weight when compared to plants irrigated with a 50 $\mu g \cdot L^{-1}$ paclobutrazol solution that did not pass through granular activated carbon filter (Grant et al., 2018). While paclobutrazol is routinely used on many vigorous floriculture crops such as poinsettias, other crops such as vinca (*Vinca minor*), begonia (*Begonia x hybrida)*, and pansy (*Viola x wittrockiana*) are considered to be highly sensitivity to paclobutrazol (Million et al., 1999). In one study researchers reported 30% less growth when begonias were exposed to 5 $\mu g \cdot L^{-1}$ paclobutrazol by constant feed sub-irrigation (Million et al., 2002).

Past research has highlighted uses of biochar filters for remediation of low concentrations of paclobutrazol and biochar incorporation rates impact on the growth of floriculture crops (Grant et al., 2018). Currently, there is no published research examining paclobutrazol efficacy on biochar incorporated substrates. The goal of these experiments was to evaluate the impact of two different grades of biochar at two incorporation rates on the efficacy of paclobutrazol drench applications for low and high-sensitive greenhouse species.

Materials and Methods

Experiment One:

Cuttings of two rooted poinsettia hybrids ‘Princettia Red’ and ‘Princettia White’ (*Euphorbia pulcherrima x Euphorbia cornastra)* (Suntory Flowers, Tokyo, Japan) were transplanted on 25 Aug. 2022. Cuttings were rooted and shipped in ten cell liners [3 x 3 x 4.41 cm (length x width x height)] and were transplanted into 14-cm diameter azalea plastic pots (1.4L) (ITML Horticulture Products, Middlefield, OH). A 2 × 2 × 5 factorial design (aggregate type × aggregate rate × PGR rate) was created where cultivars were sampled independently of one another.

Substrate Treatments. Rooted cuttings were transplanted into one of four substrate treatments, these treatments were comprised of an 85:15 or 70:30 (v:v) mix of Canadian sphagnum peat moss (Sun Gro Horticulture Company, Agawam, MA) fluffed from compressed bales and either horticultural coarse perlite (Sun Gro Horticulture Company) or coarse wood biochar (<6 mm) (Sun Gro Horticulture Company) with fine particles removed (< 2 mm) with an initial pH of ~9.0. A wetting agent (AquaGro 2000 G; Aquatrols, Cherry Hill, NJ) at 600 $g \cdot m^{-3}$, starter charge 15N-2.2P- 12.6K (J.R. Peters, Allentown, PA) at 1186.6 $g \cdot m^{-3}$, dolomitic limestone (Sun Gro Horticulture Company) at 3.56 $kg \cdot m^{-3}$ to achieve a target pH of 6.0 were incorporated (Table 5.1).

The plants were grown in a glasshouse in Raleigh, NC with 23 ˚C day/ 17 ˚C night air temperature settings. Plants were irrigated as necessary with a water-soluble fertilizer (Ultrasol 20N-4.4P-16.4K, SQM, Atlanta, GA) to provide the following ($mg \cdot L^{-1}$): 150 N, 32.7 P, 125 K, 0 Ca, 3.75 Mg, 11.5 S, 0.128 B, 0.15 Cu, 0.75 Fe, 0.75 Mn, 0.0075 Mo, and 0.225 Zn for two weeks and then irrigated with Ultrasol 13N-0.87P-10.79K (SQM, Atlanta, GA) 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 for the remainder of the trial. Plants were pinched after the first two weeks of vegetative growth on 9 Sept. Supplemental lighting was utilized between 20:00 to 2:00 nightly for the first four weeks of growth and was turned off the day of PGR treatment. On 27 Sept., 87 ml of the solution containing 0.0, 0.5, 1.0, 2.0, or 4.0 $mg \cdot L^{-1}$ paclobutrazol (Piccolo 10 XC; Fine Americas, Walnut Creek, CA) was applied per 1.4L container.

Data Collection. The experiment was a completely randomized design with ten single plant replicates (n=10). On 20 Nov., six plants were sampled for plant height, diameter (measured at the greatest width, turned 90°, and averaged), and bract diameter (measured at the greatest width, turned 90°, and averaged).

Experiment Two:

Pansy (*Viola x wittrockiana*) 'Matrix Blue Blotch' seeds (Syngenta Flower, Gilroy, CA) were sown 21 Dec. 2022 into 288 cell trays [2.05 x 2.05 x 2.87 cm individual cells (length x width x height)] containing Sunshine #4 mix (Sun Gro Horticulture Company). Seeds were germinated under full spectrum fluorescent lights (AgroBrite T5 Full Spectrum, Hydrofarm, Petaluma, CA) with an intensity of 200.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with no additional light sources using a was established using a light meter (MQ-610 ePar Meter, Apogee Instruments, Logan, UT) providing 11.52 $mol \cdot m^{-2} \cdot d^{-1}$ based on a 16-hour photoperiod (out of 24 hours). On 17 Jan. 2023 plugs were transplanted into 0.67L containers filled with each substrate (Table 2) and watered in with tap water. On 17 Feb., begonia 'Big Red Bronze Leaf' (*Begonia x hybrida*) (Ball Horticultural Company, West Chicago, IL) seedlings were received in 162 cell trays [2.54 x 2.54 x 4.06 cm individual cells (length x width x height)] and were transplanted the same day. Plants

were grown in a glass greenhouse in Raleigh, NC, with a 23 ˚C day/ 17 ˚C night air temperature setting. All containers had emitters on a ring drip system and were watered as needed depending on weather conditions. Plants were fertilized at each irrigation with Ultrasol 13N-0.87P-10.79K (SQM, Atlanta, GA) mixed with respective PGR treatment from 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) connected to 1.9-cm-diameter irrigation tubing fitted with circular drip emitters (Dramm USA, Manitowoc, WI). A 3 × 2 × 6 factorial design (aggregate type × aggregate rate × PGR rate) was created where species were sampled independently of one another.

Substrate Treatments. Plugs were transplanted into one of six substrate treatments. Treatments were comprised of an 85:15 or 70:30 (v:v) mix of Canadian sphagnum peat moss with either, horticultural coarse perlite, coarse (<6 mm), or extra coarse (>6 mm) wood biochar with fines removed (< 2 mm) (SunGro Horticulture Company). Wetting agent (AquaGro 2000 G; Aquatrols) at 600 $g \cdot m^{-3}$, starter charge 15N-2.2P- 12.6K (J.R. Peters) at 1186.6 $g \cdot m^{-3}$, varying rates of dolomitic limestone (Sun Gro Horticulture Company) to achieve a substrate pH of 6.0 (Table 5.2) were also incorporated.

Plant Growth Regulator Treatments. Plants received one of six paclobutrazol treatments of 0, 6.25, 12.5, 25, 50, or 100 $\mu g \cdot L^{-1}$ at each irrigation in the fertilizer solution. The experiment was a completely randomized design with ten single plant replicates.

Data Collection. On 10 Mar. 2023, six single plant replicates (n=6) were sampled for plant height (measured from the substrate line to the highest leaf), and plant diameter (measured width at widest point, turned 90˚, and averaged) were recorded for each plant. Shoots were cut at the substrate surface, dried at 70$^{\circ C}$ for 96 hours, and weighed.

Statistical Analysis. Statistical analysis for both experiments was conducted using the GLIMMIX procedure in SAS (version 9.4; SAS Inst., Cary, NC). Substrate treatment (discrete), PGR rate (continuous), and their interactions were treated as fixed effects. Higher order polynomial regression models were fit and models with the highest order significant polynomial were selected. For all analyses, a $P \leq 0.05$ was used to determine significant effects.

Results and Discussion

Experiment One:

Plant Height. Plant height of 'Princettia Red' and 'Princettia White' were not significantly different when examining the interaction between the three-way interaction of aggregate type × aggregate incorporation rate × paclobutrazol concentration. However, for 'Princettia Red' the interaction of aggregate type × aggregate rate was significant ($P = 0.028$), but the greatest range difference was 1.3 cm and would not be considered commercially significant (data not shown). Additionally, 'Princettia White' exhibited a significant difference when examining the simple effect of aggregate type ($P = 0.008$), however, the difference between aggregate types was 0.8 cm and was not commercially significant (data not shown). The efficacy of paclobutrazol drench on plant height was not significantly different among substrates incorporated with biochar and perlite at two aggregate incorporation rates of 15 and 30%. Thus, the plant height data for each cultivar were pooled from all substrates before determining the impact of paclobutrazol concentration on plant height. There was a significant quadratic relationship between paclobutrazol concentration and 'Princettia Red' plant height (Fig. 5.1A). Plant height was 23.4%, 30.3%, 39.9%, and 46.1% shorter than the untreated control for 0.5, 1.0, 2.0, and 4.0 $mg \cdot L^{-1}$ concentrations, respectively. Also, a significant quadratic relationship between plant height and paclobutrazol concentrations for 'Princettia White' (Fig. 5.2A) occurred. Plant height

was 20.0%, 29.5%, 36.9%, and 43.0% shorter respectively, than the untreated control for 0.5, 1.0, 2.0, and 4.0 mg·L^{-1}.

Plant Diameter. Plant diameter followed a similar trend as plant height in which 'Princettia Red' and 'Princettia White' were not significantly different when examining the three-way interaction of aggregate type × aggregate incorporation rate × paclobutrazol concentration. However, for 'Princettia Red' the simple effect of aggregate rate was significant ($P = 0.011$), but the greatest range difference was 0.8 cm and would not be considered commercially significant (data not shown). Additionally, 'Princettia White' exhibited significant differences when examining the simple effects of aggregate type and aggregate rate ($P < 0.001$ and $P = 0.033$, respectively), however, the values ranged 1.1 cm and 0.8 cm, respectively, and were not commercially significant (data not shown). Thus the data was pooled to examine the effect of paclobutrazol concentration on plant diameter. There was a significant quadratic relationship between paclobutrazol concentration and 'Princettia Red' plant diameter (Fig. 5.1B). Plant diameter was 16.3%, 24.8%, 32.2%, and 39.2% smaller than the untreated control for 0.5, 1.0, 2.0, and 4.0 mg·L^{-1}, respectively. Also, a significant quadratic relationship between plant diameter and paclobutrazol concentration for 'Princettia White' (Fig. 5.2B) was observed. Plant diameter was 16.8%, 23.2%, 30.2%, and 37.5% smaller than the untreated control for 0.5, 1.0, 2.0, and 4.0 mg·L^{-1}, respectively.

Bract Diameter. Significant differences in bract diameter were not observed when examining the three-way interaction of aggregate type × aggregate rate × paclobutrazol concentrations for either cultivar. However, 'Princettia Red' exhibited significant differences when examining the simple effects of aggregate type and aggregate rate ($P < 0.001$ and $P = 0.001$, respectively)

however the values ranged 0.9 cm and 0.5 cm, respectively, and were not commercially significant (data not shown).

Additionally, ‘Princettia White exhibited significant differences when examining the simple effects of aggregate type × aggregate rate ($P < 0.001$ and $P = 0.0014$, respectively) however the values ranged 0.9 cm and 0.5 cm, respectively, and were not commercially significant (data not shown). Thus, the bract diameter data for each cultivar were pooled from all substrates before determining the impact of paclobutrazol concentration efficacy on bract diameter. There was a significant quadratic relationship between paclobutrazol concentration and ‘Princettia Red’ bract diameter (Fig. 5.1C). Bract diameter was 5.6%, 13.4%, 19.4%, and 26.9% smaller than the untreated control for 0.5, 1.0, 2.0, and 4.0 $mg \cdot L^{-1}$, respectively. Also, a significant quadratic relationship between bract diameter and paclobutrazol concentration for ‘Princettia White’ (Fig. 5.2C) occurred. Bract diameter was 5.4%, 9.0%, 16.4%, and 23.9% smaller than the untreated control for 0.5, 1.0, 2.0, and 4.0 $mg \cdot L^{-1}$, respectively.

This research suggests that wood biochar is suitable as a substrate component up to 30% by volume for peat-based substrates for poinsettia production while still achieving similar performance compared to traditional peat-perlite based substrates. Additionally, when wood biochar was incorporated with sphagnum peat up to 30% by volume, paclobutrazol efficacy was not negatively affected when compared to a 15% perlite amended substrate. These results were similar to those observed by Owen (2013), in which wood based alternative aggregates generally did not decrease the efficacy of paclobutrazol for species that are not labeled as highly sensitive. However, additional research is needed to determine if paclobutrazol efficacy is impacted when biochar incorporation rates increase > 30% or if the feedstock is not wood-based.

Experiment Two:

Plant Height. Significant differences in plant height were not observed when examining the three-way interaction of aggregate type × aggregate rate × paclobutrazol concentrations for either species. Begonias did exhibit a significant difference regarding the simple effect of aggregate (P = 0.012), however, the range of values were 0.8 cm and was not considered commercially significant (data not shown). Therefore, based on these results, the incorporation of biochar, either at coarse or extra-coarse grade and when utilizing either a 15 or 30% incorporation rate did not impact the efficacy of low dose paclobutrazol drench applications. Thus, the plant height data for each species were pooled from all substrates before determining the impact of paclobutrazol concentration on plant height. There was a significant quadratic relationship between paclobutrazol concentration and begonia plant height (Fig. 5.3A). Plant height was 22.8%, 31.0%, 46.3%, 58.5%, and 63.29% shorter than the untreated control for 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively. Also, a significant quadratic relationship between plant height and paclobutrazol rate occurred with pansies (Fig. 5.4A). Plant height was 38.1%, 53.0%, 61.4%, 70.1%, and 75.8% shorter than the untreated control for 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively.

Plant Diameter. Plant diameter followed a similar trend as plant height in which neither begonia nor pansies were significantly different when examining the three-way interaction of aggregate type × aggregate incorporation rate × paclobutrazol concentration. However, the simple effects of aggregate and aggregate rate for begonias were significant (P = 0.0057 and P = 0.015, respectively), however the values 0.7 cm and 0.5 cm, respectively, and were not commercially significant (data not shown). Thus, the data were pooled to examine the effect of paclobutrazol concentration on plant diameter. There was a significant quadratic relationship between paclobutrazol concentration and begonia plant diameter (Fig. 5.3B). Plant diameter was 12.6%,

18.5%, 24.7%, 40.6%, and 47.4% smaller than the untreated control for 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively. Also, a significant quadratic relationship between plant diameter and paclobutrazol concentration occurred for pansy (Fig. 5.4B). Plant diameter was 38.3%, 54.5%, 63.4%, 70.1%, and 72.2% smaller than the untreated control for 0.5, 1.0, 2.0, 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively.

Shoot Dry Weight. Significant differences in shoot dry weight were not observed when examining the three-way interaction of aggregate type × aggregate rate × paclobutrazol concentrations for either species. However, for begonia the interaction of aggregate × aggregate rate was significant ($P = 0.0058$), however, the difference was 0.46 g and would not be considered biologically different (data not shown). Thus, the shoot dry weight data for each cultivar were pooled from all substrates before determining the impact of paclobutrazol rate on shoot dry weight. There was a significant quadratic relationship between paclobutrazol concentration and begonia shoot dry weight (Fig. 5.3C). Shoot dry weight was 14.8%, 32.0%, 43.3%, 62.7%, and 66.8% less than the untreated control for 0.5, 1.0, 2.0, 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively. Also, a significant quadratic relationship between shoot dry weight and paclobutrazol concentration for pansy occurred (Fig. 5.4C). Shoot dry weight was 54.4%, 70.8%, 79.8%, 86.1%, and 89.6% less than the untreated control for 0.5, 1.0, 2.0, 6.25, 12.5, 25, 50, and 100 $\mu g \cdot L^{-1}$, respectively.

This research suggests that wood biochar is a suitable aggregate material to incorporate it with sphagnum peat moss up to 30% by volume in horticultural substrates for greenhouse begonia and pansy production while still achieving similar growth compared to traditional peat-perlite substrates. The comparison of an alternative aggregate up to 30% by volume is considered to be the upper end of commercial aggregate usage for most peat-based substrates in greenhouse

crops (Owen, 2016). In previous research, paclobutrazol was successfully filtered out when exposed to a small scale granular activated carbon system for 59 seconds resulting in begonias yielding similar dry weight when exposed to a 50 $\mu g \cdot L^{-1}$ paclobutrazol filtered solution to those that were exposed to 0 $\mu g \cdot L^{-1}$ paclobutrazol solution (Grant et al., 2018). Additionally, Million et al (1998), reported that increased percentages of pine bark, up to 60% pine bark, would decrease the efficacy of paclobutrazol drenches. While the incorporation of two different particle size biochar's up to 30% did not result in result in reduced efficacy of paclobutrazol, this is likely due to not a high enough percentage to yield enough contact with the biochar for paclobutrazol to bind to the biochar particles or preventing binding to the peat. Another potential reason that paclobutrazol efficacy was not negatively impacted by biochar incorporation up to 30% in a growing media is that the particle sizes examined may not have resulted in enough particle surface area to contact the paclobutrazol. Additional research examining various biochar particle sizes and incorporation rates is needed to determine the effect of surface area of paclobutrazol binding to biochar.

Conclusion

The biochar amended substrates evaluated in this study were all adequate for growing low and high-sensitive species, poinsettia, pansy, and begonia without any negative impact on plant growth when compared to plant grown in peat-perlite substrates. In both experiments, substrate composition did not decrease the paclobutrazol efficacy when comparing the response curves to perlite amended substrates. This suggests that biochar can be used as an alternative aggregate preforming comparable to a peat-perlite substrate for horticultural crop production.

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Table 5.1. Summary of substrate treatments used during experiment one for evaluating biochar as an alternative aggregate for high concentration of paclobutrazol applications to poinsettias.

Aggregate type	Peat%	Biochar %	Perlite %	Lime Rate ($kg \cdot m^{-3}$)
Perlite	85	0	15	3.56
Perlite	70	0	30	3.56
Biochar	85	15	0	3.56
Biochar	70	30	0	3.56

Table 5.2. Summary of substrate treatments used during experiment two for evaluating two grades of biochar as alternative aggregates for low concentration of paclobutrazol applications to begonias and pansies.

Aggregate type	Peat%	Biochar%	Perlite %	Lime Rate ($kg \cdot m^{-3}$)
Perlite	85	0	15	3.56
Perlite	70	0	30	3.56
Coarse Biochar[i]	85	15	0	3.56
Coarse Biochar	70	30	0	3.56
Extra Coarse Biochar[i]	85	15	0	2.97
Extra Coarse Biochar	70	30	0	2.97

[i] Coarse and extra coarse biochar aggregates are smaller than 6mm and larger than 6 mm in size, respectively.

Figure 5.1. Impact of paclobutrazol drench concentrations on ‘Princettia Red’ plant height, diameter, and bract diameter.

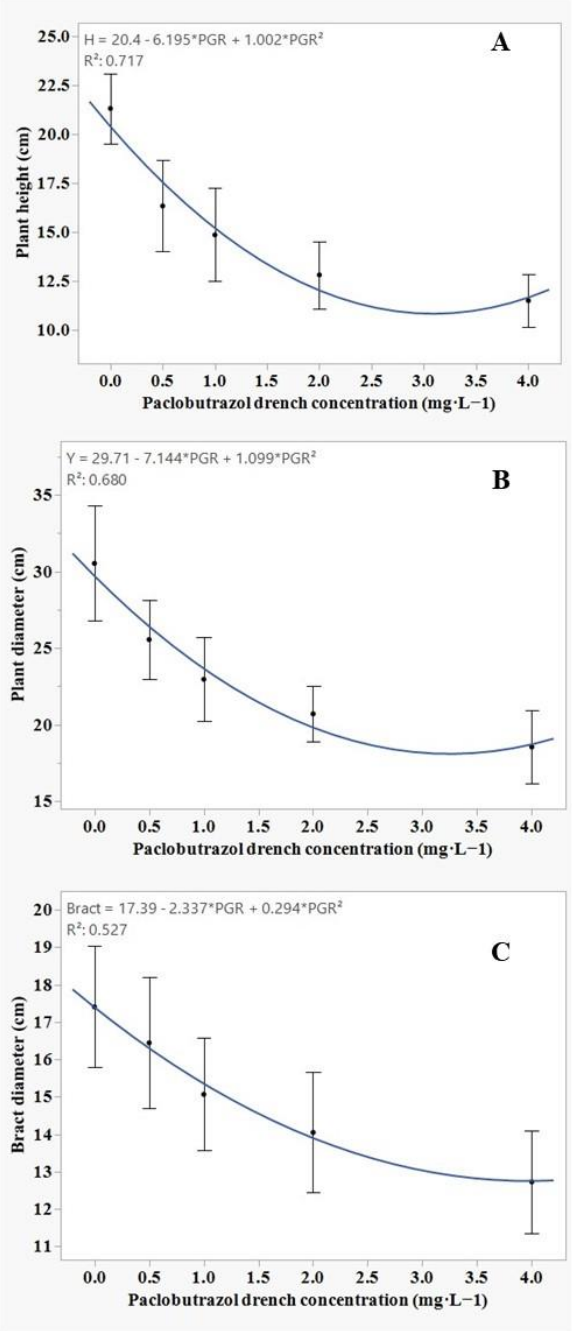

Figure 5.2. Impact of paclobutrazol drench concentration on ‘Princettia White’ plant height, diameter, and bract diameter.

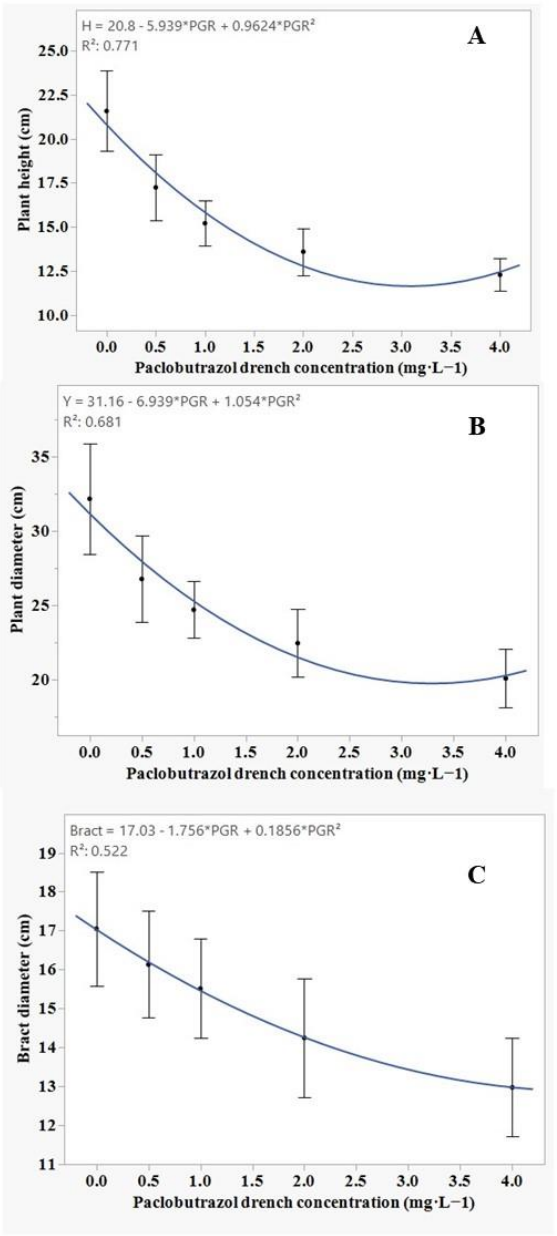

Figure 5.3. Impact of paclobutrazol drench concentrations on *Begonia x hybrida* ‘Big Red Bronze Leaf’ plant height, diameter, and shoot dry weight.

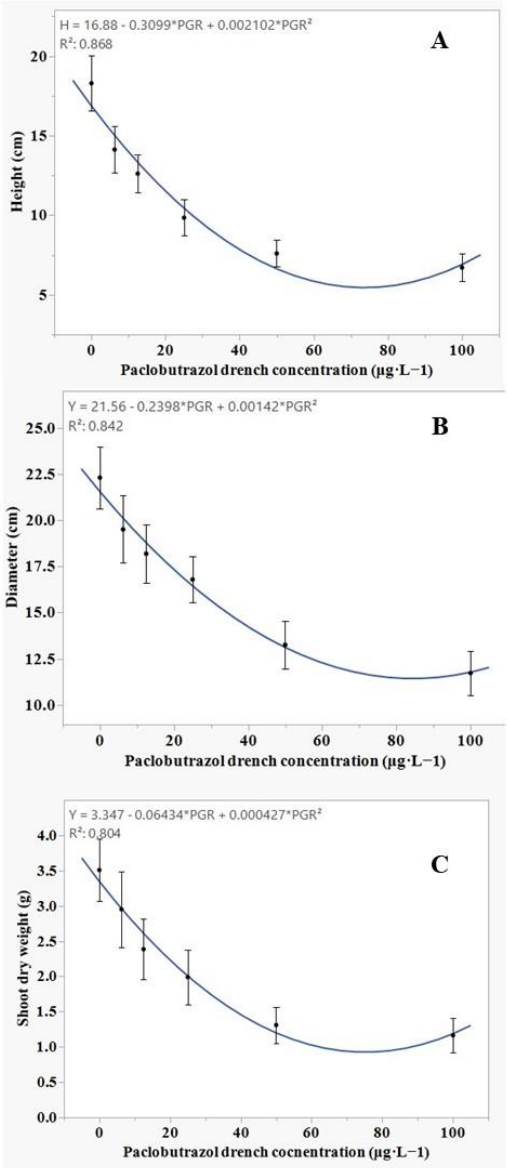

Figure 5.4. Impact of paclobutrazol drench concentrations on *Viola x wittrockiana* ‘Pansy Matrix Blue Blotch’ height, diameter, and dry weight.