POINSETTIA AND EASTER LILY GROWTH AND DEVELOPMENT RESPONSES TO ROOT SUBSTRATE CONTAINING BIOCHAR

A Thesis

by

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ABSTRACT

Greenhouse production of Poinsettia (*Euphorbia pulcherrima*) and Easter lily (*Lilium longiflorum*) mainly uses peat-based root substrates. The decrease of peatland has increased the need for alternative root substrate components in the horticulture industry. Biochar, a byproduct of bio-energy production, has the potential to be an alternative root substrate component to reduce the use of peatmoss in greenhouse production. The objectives of the present studies were to determine the effects of different percentages of biochar and fertigation regimes on the growth and development of ‘Prestige Red’ poinsettia and Easter lily ‘Nellie White’ in greenhouse production.

Two experiments were conducted to evaluate different percentages of one type of biochar added to a commercial peat-based root substrate for poinsettia and Easter lily greenhouse production. In experiment one, rooted poinsettia cuttings were potted in one of the six root substrates mixes including Sunshine Mix #1 replaced by 0%, 20%, 40%, 60%, 80%, or 100% biochar (by volume) and irrigated under four fertigation regimes (100 to 200 mg•L^{-1} N, 200 to 300 mg•L^{-1} N, 300 to 400 mg•L^{-1} N, or 400 to 500 mg•L^{-1} N). Root rot and red bract necrosis were only observed in the highest fertigation regime (400-500 mg•L^{-1} N) combined with the highest biochar percentage (100%). At 100 to 400 mg•L^{-1} N fertilization rate, up to 80% of the commercial peat-based root substrate could be replaced by biochar without a significant change in poinsettia growth and quality.

In experiment two, Easter lily bulbs were potted in one of the five root substrates mixes (Sunshine Mix #1 amended with 0%, 20%, 40%, 60%, and 80% biochar) and irrigated under four fertigation regimes (constant liquid feed at 200 mg•L^{-1} N or 300 mg•L^{-1} N, and fertilization at every third watering with 200 mg•L^{-1} N or 300 mg•L^{-1} N). Neither fertigation regimes nor biochar percentages significantly affected the Easter lily growth and development. Under the four fertigation regimes used in this experiment, up to 80% peat-based root substrate could be replaced by biochar without a significant difference on the growth and development of Easter lily.

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CHAPTER I

INTRODUCTION

1.1 Introduction

Poinsettia (*Euphorbia pulcherrima*) and Easter lily (*Lilium longiflorum*) are two major potted plants in the Unites States. The quantity of potted poinsettias sold in 2012 was over thirty million (ranking No. 1) with a wholesale value of $143.7 million (ranking No. 2) in potted flowering plants in the Unites States (Floriculture Crops 2012 Summary, 2013). The quantity of potted Easter lily sold in 2012 was over five million (ranking No. 5) with a wholesale value of $ 22.2 million (ranking No. 3) in potted flowering plants in the U.S. (Floriculture Crops 2012 summary, 2013).

Root substrate is an important factor for potted plant production since a healthy functional root system is essential for plant growth and development (Bilderback, 1982). A root substrate must be able to serve four functions: holding available nutrients and water, providing gas exchange, and providing anchorage for plant growth (Nelson, 2012). Currently, most potted poinsettias and Easter lilies are produced in peat-based root substrate (Hidalgo and Harkess, 2002; Erwin, 2002).

Peatmoss is a highly valued source for root substrates in current greenhouse production for its superior properties of stability, light bulk density for transportation, and high water and nutrient holding capacities (Nelson, 2012). In recent years, there has been a rising opposition in Europe for the use and extraction of peatmoss (Carlile and Lane, 2004). A recent geological survey showed that the volume of global peatlands is decreasing at a rate of 0.05% annually due to peatmoss harvesting and land development

(Apodaca, 2014). Peatland is a fragile ecosystem and provides important functions including water regulation, biodiversity conservation, and carbon sequestration and storage (Joosten et al., 2012). However, 15% of peatland has already been drained and used for agricultural purposes, and CO_2 emissions from these drained peatlands contributed almost 6% of the total anthropogenic-emissions (Joosten et al., 2012). In the United Kingdom, environmental, scientific, and governmental agencies proposed to limit the use and extraction of peatmoss (Carlile, 2004). In the U.S., almost 90% peatmoss was sold for horticultural use (Apodaca, 2014). Thus the reduction in use of peatmoss in greenhouses, a major part of horticultural production, could reduce the use of peatmoss significantly. Currently, there are no regulations or governmental mandates to oppose peatmoss use in the U.S., but increased fuel (transportation) cost has caused increasing peatmoss price in recent years. Thus, interest in finding alternative substrates has increased among researchers (Jackson et al., 2008; Wright et al., 2009).

1.2 Alternative Substrates

Research has been conducted on using alternative root substrate components for greenhouse crop production. Though most alternative root substrates need to be amended with peatmoss (Clarke, 2008). Up to 80% (by volume) of ground noncomposted kenaf (*Hibiscus cannabinus*) woody stem core was used as an amendment to peat-based root substrate to produce potted tropical foliage and woody nursery crops, though additional irrigation and undesirable shrinkage were issues (Wang, 1994). Fain et al. (2008) reported that WholeTree, a type of root substrate made from loblolly pine (*Pinus taeda*), slash pine (*Pinus elliottii*), and longleaf pine (*Pinus* *palustris*) (hammer milled to pass through a 0.9 cm screen), could be used as an alternative root substrate to produce short-term horticultural crops, such as vinca (*Catharanthus roseus*). A ground pine tree substrate, produced from loblolly pine and ground into 2.5 cm × 2.5 cm × 1.3 cm chips, was used to grow potted chrysanthemum (*Chrysanthemum* x *grandiflora*) with additional fertilizer application (Wright et al., 2008). Boyer et al. (2008) reported up to 20% by volume clean chip residual (a byproduct from loblolly pine tree harvest, and hammer milled to pass through 1.27 cm screen) could be used as an amendment to peat-based root substrate to produce ageratum (*Ageratum houstonianum*), salvia (*Salvia* × *superba*), and impatiens (*Impatiens walleriana*) in 1-gallon containers.

Composts made from different biomass have also been studied as alternative substrates in greenhouse production. Using composted cotton (*Gossypium hirsutum*) burrs (25% to 75% by volume) as a root substrate amendment for poinsettia production resulted in lower dry weight and smaller bracts (Wang and Blessington, 1990). Without significant change in poinsettia quality, up to 50% by volume of composted poultry litter, yard trimmings, or municipal solid waste compost and 25% polymer-dewatered bio solids or crab offal composts by volume, were used as an amendment to peat-based root substrate (Ku et al., 1998). Papafotiou et al. (2004) observed reduced poinsettia growth by using olive (*Olea europaea*)-mill waste compost as a root substrate amendment, though 12.5% of olive-mill wastes compost by volume had no effect on pigmentation of the bracts and flowering. Up to 37.5% cotton gin trash compost by volume could be used as an amendment to peat-based root substrate in potted croton

(*Codiaeum variegatum*) production without affecting plant growth (Papafotiou et al., 2007). Matta et al. (2008) reported that using earthworm cast as a root substrate amendment (25%-75% by volume) increased the growth of poinsettia, marigold (*Tagetes erecta*), and chrysanthemum, and though the poinsettia performed best in 25% earthworm cast, marigold and chrysanthemum performed best in 50% earthworm cast. However, the disadvantages of these alternative substrates include additional input of fertilizer, composition variability, inconsistent availability, and potential contamination (Konduru and Evans, 1999; Gu et al., 2013).

1.3 Biochar

Biochar is the byproduct of pyrolysis (Lehmann, 2007). Pyrolysis is an industrialized thermochemical conversion process that uses high temperature and low oxygen conditions to convert biomass into biochar, bio-oil and syngas (Zhang et al., 2013). The characteristics and yield of biochar depend on pyrolysis method, temperature, and the biomass source. The temperature for pyrolysis varies from 225 °C (torrefaction of biomass; Phanphanich and Mani, 2011) to 850 °C (gasification of biomass; Salleh et al., 2010). The pH range of biochar is between 4 and 12, and as the pyrolysis temperature increases, biochar surface area and pH increase, while the biochar yield decreases (Lehmann, 2007; Zhang et.al., 2013). The cation exchange capacity of fresh biochar is low, yet it increases as biochar ages in the present of oxygen and water (Cheng et al., 2006). Considering the production cost, biochar yield, and characteristics of biochar, the optimal pyrolysis temperature of biochar for agricultural industry usage is 450-550 °C (Lehmann, 2007; Spokas et al., 2012).

Biochar has been reported as an amendment to revitalize degraded soils (Spokas et al., 2012). Amending soil with biochar was reported to increase net soil surface area (Hunt et al., 2010) and soil water and nutrient retention (Downie et al., 2009), improve water holding capacity in sandy soils (Gaskin et al., 2007; Glaser et al., 2002), increase soil pH (Chan et al., 2007), increase beneficial soil micro-organisms populations (Lehmann et al., 2011), reduce soil tensile strength (Chan et al., 2007), and reduce soil bulk density (Brady and Weil, 2004). Both increased and decreased crop yield have been reported when grown on biochar-amended soil (Gaskin et al., 2010; Haefele et al., 2011; Spokas et al., 2012). Yet a meta-analysis on 100 biochar studies showed that despite the variability of biochar applied, biochar decreased crop yield in 20% of the studies, did not change crop yield in 30% of the studies, and increased yields in 50% of the studies (Spokas et al., 2012). The increase in yield was greater for the studies conducted in weathered or degraded soils (Spokas et al., 2012).

Using biochar as a root substrate amendment in a greenhouse setting is relatively new compared to its use as a soil amendment in field studies. Previous research showed that biochar had great potential to improve root substrate physical and chemical characteristics. Compared to pre-pyrolysis material, biochar amended at 5%, 10% and 15% (by volume) increased root substrate (peat-based or bark-based substrate) cation exchange capacity, increased air-filled porosity in peat-based substrate, and increased container capacity in bark-based root substrate (Jackson et al., 2011). Fresh made biochar had similar initial leachate electrical conductivity as unfertilized peatmoss (Steiner and Harttung 2014). Amending biochar in root substrate could also increase root substrate hydraulic conductivity and water retention with desirable root substrate physical porosity (25% biochar pellets, by volume, Dumroese et al. 2011), control extreme fluctuation of macronutrients (tested up to 10%, by volume, Altland and Locke 2012), reduce nutrient run off (Beck et al., 2011), and reduce root substrate degradation (Tian et al. 2012).

Other studies also reported that amending peat-based root substrate with a suitable percentage of biochar increased plant growth and plant quality, or had no effect on plants. A greenhouse experiment done by Graber et al. (2010) suggested root substrate amended with a low rate of biochar (1% to 5% by weight) could increase tomato (L*ycopersicum esculentum*) and pepper (*Capsicum annuum*) growth. Kadota and Niimi (2004) reported that amending 10% or 30% by volume of a biochar mixture (biochar with pyroligneous acid or barnyard manure) with bedding plant medium shortened the number of days from transplantation to flowering for bedding plants, and increased the survival rate and the quality of bedding plants. Ruamrungsri et al. (2011) reported freesias (*Freesia* spp) and gloriosa lily (*Gloriosa rothschildiana*) could be grown in a 1:1:1 (by volume) of sand:rice (*Oryza sativa*) husk charcoal:coconut (*Cocos nucifera*) fiber substrate. Gu et al. (2013) reported up to 30% biochar could be used as an amendment to peat-based root substrate in ‘Fireworks’ gomphrena (*Gomphrena* spp) greenhouse production. A greenhouse study found no effects on cucumber (*Cucumis sativus*), tomato and pepper yields using biochar as a soilless root substrate compared to a coconut fiber-tuff potting root substrate (Zhang et al., 2013).

Too much biochar in greenhouse production, however, may suppress plant growth. Mini sunflower (*Helianthus annuus*) grown in root substrate with biochar (25-100% by volume) had similar plant height as plants grown in peatmoss, though lower fresh weights were observed on plants grown in 50% and 100% biochar. Calathea (*Calathea rotundifola*) grown in 50% biochar had higher total dry weight and leaf dry weight, yet those grown in 100% biochar had the lowest dry weights of three biochar percentages used in the experiment (0%, 50% or 100% biochar by volume, Tian et al. 2012)).

Biochar is an environmentally friendly and renewable product. It could be made from any biomass, such as animal manure, wood, pecan shells, peanut hulls and grass (Novak et al., 2009; Singh et al., 2010). Also, using biochar in agricultural production could stimulate biofuel production, thus reducing net CO_2 emission to the atmosphere (Turner, 1999). At the current stage, the price of biochar is a disadvantage. Compared to peatmoss, the market price of biochar is more expensive. The average retail price of biochar is around $0.087/kg (Granatstein et al., 2009), and sphagnum peatmoss is $0.062/kg (Apodaca, 2010). As the technology develops, and the biochar market expands, the price of biochar is expected to decrease in the future.

1.4 Objectives

The objectives of the present studies were to determine the effects of different percentages of biochar and fertigation regimes on the growth and development of ‘Prestige Red’ poinsettia and Easter lily ‘Nellie White’ in greenhouse production. No study has investigated the possibility of using biochar in greenhouse production of poinsettia and Easter lily. Considering the significant amount of peatmoss (38.9 million pots of peat-based substrate) used in poinsettia and Easter lily greenhouse production annually, finding an alternative root substrate suitable for poinsettia and Easter lily production could substantially reduce the use of peatmoss.

CHAPTER II

POINSETTIA GROWTH AND DEVELOPMENT RESPONSE TO CONTAINER ROOT SUBSTRATE WITH BIOCHAR

2.1 Overview

A greenhouse study was conducted to evaluate the growth and development of poinsettia “Prestige Red’ (*Euphorbia pulcherrima*) grown in a commercial peat-based potting mix amended with biochar at 0%, 20%, 40%, 60%, 80%, or 100% (by volume) at four different fertigation regimes: F1:100 to 200 mg•L^{-1} nitrogen (N), F2: 200 to 300 mg•L^{-1} N, F3: 300 to 400 mg•L^{-1} N, or F4: 400 to 500 mg•L^{-1} N. The experimental design was a two factor factorial design with 10 replications. As the percentage of biochar increased, root substrate pore space and bulk density increased, and root substrate container capacity decreased. Substrates with biochar had lower leachate electrical conductivity in the first two weeks of the experiment. Root rot and red bract necrosis only occurred in the highest fertigation regime (400-500 mg•L^{-1} N) combined with the highest biochar percentage (100%). Plants grown in 20% biochar had a slightly higher plant growth index and dry weight than other treatments. Plants grown in 40% biochar had a similar growth and development to those in 0% biochar. Up to u80% biochar, plants had no significant change, except on dry weight, which decreased at higher biochar percentage (60% and 80%). SPAD readings increased as fertigation N concentration increased. In summary, at a fertigation rate of 100 to 400 mg•L^{-1} N, up to 80% biochar could be used as an amendment to peat-based root substrate without significant changes in poinsettia growth and quality.

2.2 Introduction

The quantity of potted poinsettias (*Euphorbia pulcherrima*) sold in 2012 was over thirty million (ranking no. 1) with a wholesale value of $143.7 million, ranking no. 2 in potted flowering plants in the U.S. (Floriculture Crops 2012 Summary, 2013). Root substrate is important for poinsettia production since a healthy functional root system is crucial to poinsettia growth and development (Bilderback, 1982). Currently, greenhouse poinsettia production uses peat-based root substrate (Hidalgo and Harkess, 2002).

Peat-based root substrate is a dependable medium in the greenhouse industry, and most alternative substrates are peat-based amended with other root substrate components (Clarke, 2008). In Europe, environmental, scientific, and governmental agencies proposed to limit the use and extraction of peatmoss (Carlile, 2004). Although the amount of peatmoss reserve is still significant, the need to find environmentally friendly substrates is increasing due to the annually decreasing volume of global peatland, the fragility of peatlands' natural environments, and the large demand for peatmoss in the horticultural industry (Apodaca, 2014; Robertson, 1993; Rivière et al., 2008). In the U. S., currently there are no restrictions regarding peatmoss use (Jackson et al., 2008). However the increase of fuel prices in recent years has increased the transportation cost of peatmoss, which is mined and shipped from Canada. Thus, many scientists are interested in finding less expensive, renewable and locally available peatmoss substitutes to reduce the use of peatmoss in the horticultural industry (Gu et al., 2013; Jackson et al., 2008).

Many peatmoss substitutes have been evaluated in poinsettia greenhouse production. Hidalgo and Harkess (2002) reported better quality poinsettias were produced in peat-based root substrate amended with 25% earthworm castings made from sheep (*Ovis aries*) or cattle (*box taurus*) manures. Poinsettias were successfully produced in loblolly pine (*Pinus taeda*) root substrate with small particles (2.38 mm screen) or large particles (4.76 mm screen) amended with 25% peatmoss (Jackson et al., 2008). Using root substrate blended with composted cotton burrs resulted in lower dry weight and smaller bracts in poinsettias (Wang and Blessington, 1990). Composted organic materials amended with peat-based root substrate at different rates (50% for poultry litter, yard trimmings or municipal solid waste composts; 25% for polymer-dewatered bio solids or crab offal composts; 25% for olive (*Olea europaea*)-mill wastes compost; by volume) have been used for poinsettia production without significant change in plant quality (Ku et al., 1998; Papafotiou et al., 2004). However, the disadvantages of those materials as alternative substrates are lack of uniformity and risk of root substrate shrinkage during the plant production period (Gu et al., 2013; Jackson et al., 2008).

Biochar, a byproduct of thermochemical pyrolysis for bio-energy production, has been considered as a possible root substrate amendment in greenhouse production to reduce the use of peatmoss (Gu et al., 2013). Pyrolysis is a process of thermochemical decomposition of biomass at high temperatures (from 225-850 °C) with the absence of oxygen (Bridgwater et al., 1999; Salleh et al., 2010; Phanphanich and Mani, 2011). The characteristics of biochar depend on the thermal conversion process type (pyrolysis method and temperature) and the biomass source (Spokas et al., 2012). Considering the production cost, biochar yield, and characteristics of biochar, the optimum biochar for use in agricultural production is probably produced at 450–550°C (Lehmann, 2007).

In recent year, multiple studies reported that biochar has a great potential to be used as an alternative root substrate in greenhouse production. In a study performed by Altland and Locke (2012), amending 10% biochar (by volume) in peat-based root substrate could increase root substrate macronutrient retention capacity. By mixing 25% by volume biochar pellets (mixture of biochar, wood flour, polylactic acid, and starch) with 75% peat-based substrate, Dumroese et al. (2011) observed an improvement of water retention of the substrate, which also had the desirable 40% porosity, although concern was noted about lower cation exchange capacity and higher C/N. Gu et al. (2013) reported up to 30% by volume biochar could be used as an amendment to peat-based root substrate to produce 'Fireworks' gomphrena without significant changes in plant quality. Other research showed that a low rate of biochar amended with coconut (*Cocos nucifera*) fiber-tuff potting root substrate improved tomato (L*ycopersicum esculentum*) and pepper (*Capsicum annuum*) growth (Graber et al., 2010).

There is insufficient research using biochar in soilless medium for greenhouse production. Since a significant amount of peatmoss is used annually in the U.S. for poinsettia production, finding an alternative root substrate suitable for poinsettia production could substantially reduce the use of peatmoss. Research has not been reported on using biochar in root substrate for a long-season crop, such as poinsettias. The objectives of this experiment were to determine a suitable biochar percentage and its effects on fertilization regimes needed for growth and development of ‘Prestige Red’ poinsettias in greenhouse production.

2.3 Materials and Methods

2.3.1 Root substrate treatments and plant materials

Six root substrate treatments used in this experiment were sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA) amended with biochar (provided by Mississippi State University, Department of Agricultural and Biological Engineering) at 0%, 20%, 40%, 60%, 80%, or 100% by volume. The biochar used in this experiment was the byproduct of fast pyrolysis of pine wood at 450 °C (Gu et al., 2013). Particle size distribution was determined by passing 100 g biochar through 2.0-, 1.4- and 0.59-mm soil sieves, and weight was measured to determine the percentage of each particle size (Table 1, Figure 1). The biochar had an initial pH of 5.4 and an EC of 0.15 $mS{\cdot}cm^{-1}$ (using 2:1 method; Cavins et al., 2000). Poinsettia ‘Prestige Red’ rooted cuttings (Ball Horticultural Company, West Chicago, IL) were transplanted on 23 Aug. 2013, to 15 cm plastic pots (1,250 ml) with one of the six substrates. Plants were pinched (removing apical growing point to leave 7-9 nodes) on 15 Sep. 2013 to stimulate branching. Plants were grown in a glass greenhouse located on Texas A&M University campus. The average greenhouse temperature (T), relative humidity (RH), and daily light integral (DLI) in the greenhouse, recorded by Watchdogs 450 (Spectrum Technologies Inc., Paxinos, PA), were 27.2°C day /20.4 °C night, 59.8% and 8.8 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, respectively (Figure 2).

Banrot® 40 WP (ScottsMiracle-Gro Company, Marysville, OH,) was applied monthly as a drench to prevent root rot disease in poinsettias. Avid® 0.15 EC (Syngenta,

Syngenta Crop Protection Inc., Greensboro, NC) and Kontos® (OHP Inc., Mainland, PA) were sprayed weekly in rotation to control whitefly, starting in late October. No growth regulators were applied in this experiment.

Table 1. Particle size distribution of the biochar used as an alternative substrate.

Particle size (mm)	Percent of sample
>2.0	15.7
1.4-2.0	27.3
0.59-1.4	49.1
<0.59	7.9

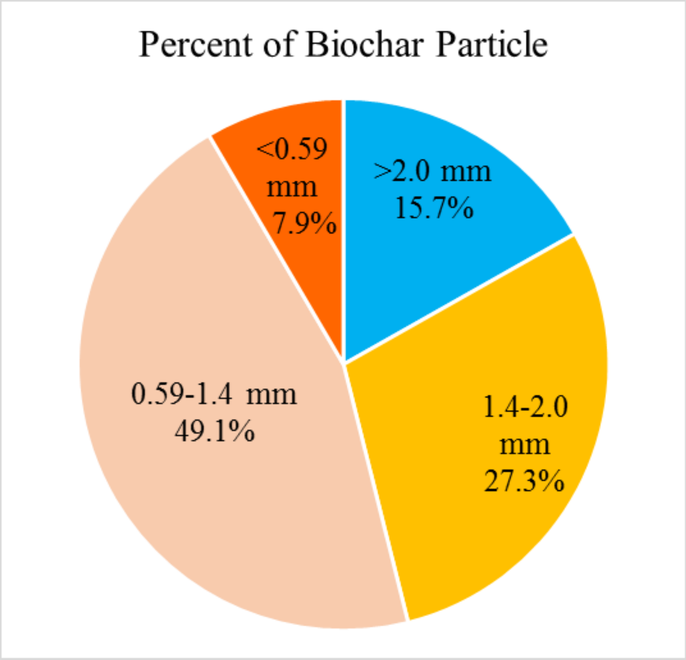


Figure 1 Biochar particle size distribution

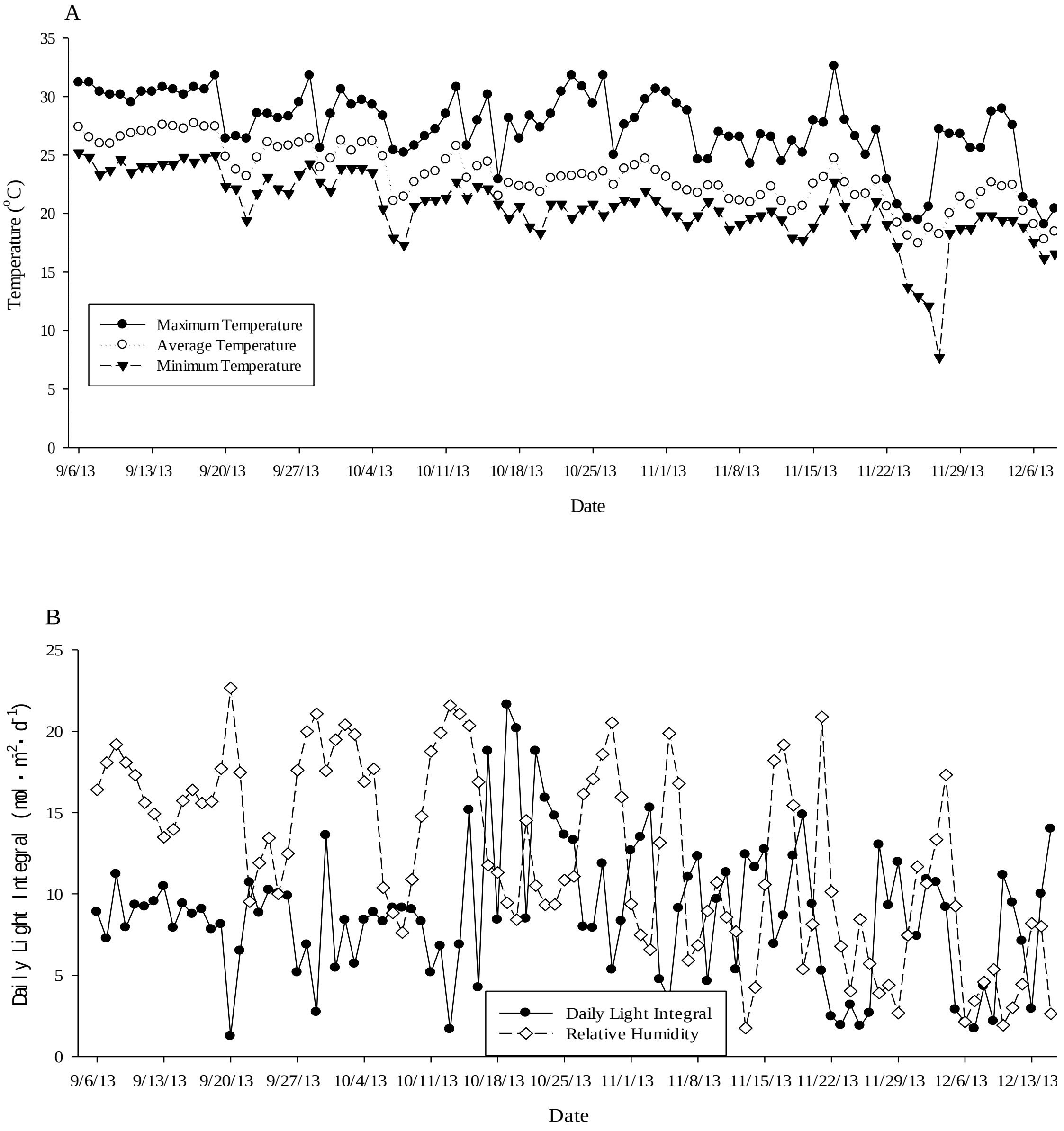


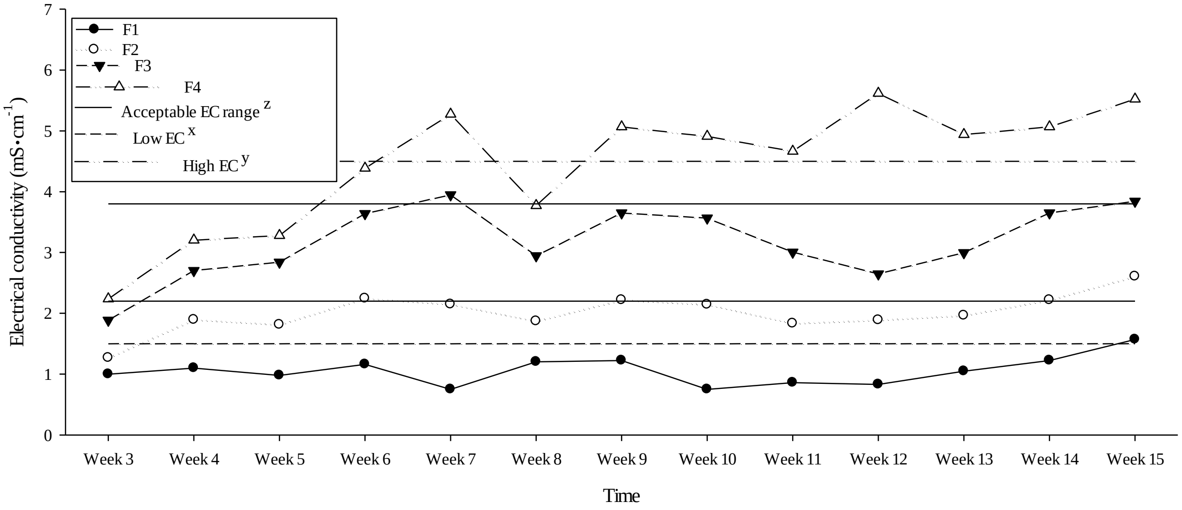
Figure 2. Daily temperatures (maximum, minimum, and average temperature), daily light integral, and relative humidity in the greenhouse from 14 days after potting (the first day of fertigation treatment) to the end of Week 16.

2.3.2 Fertigation regimes

There were four fertigation regimes using a water soluble fertilizer (20N-4.4P-16.6K Peters 20-10-20; ScottsMiracle-Gro Company, Marysville, OH): fertigation regime 1 (F1: 100 to 200 mg•L^{-1} N) was 100 mg•L^{-1} N lower than fertigation regime 2 (F2: 200 to 300 mg•L^{-1} N), fertigation regime 3 (F3: 300 to 400 mg•L^{-1} N) was 100 mg•L^{-1} N higher than F2, and fertigation regime 4 (F4: 400 to 500 mg•L^{-1} N) was 200 mg•L^{-1} N higher than F2. Fertilizer concentration in F3 was adjusted to keep root substrate EC level around 2.2 (Ecke et al., 1990; Figure 3), and the other three fertigation regimes were adjusted accordingly every week, with all plants fertilized at 200 mg•L^{-1} N in week 1 and week 2 (Table 2). Root substrate EC was determined weekly using the pour-through method (Wright and Grueber 1990; LeBude and Bilderback 2009).

Table 2. Four fertigation regimes for poinsettia production in this experiment

Time	Fertigation 1 (mg•L^{-1} N)	Fertigation 2 (mg•L^{-1} N)	Fertigation 3 (mg•L^{-1} N)	Fertigation 4 (mg•L^{-1} N)
Week 1~2	200	200	200	200
Week 3~6	150	250	350	450
Week 7	100	200	300	400
Week 8	150	250	350	450
Week 9~11	200	300	400	500
Week 12~15	100	200	300	400



^{x} EC level considered as low for greenhouse poinsettia production using pour-through method (Ecke et al, 1990).
^{y} EC level considered as high for greenhouse poinsettia production using pour-through method (Ecke et al, 1990).
^{z} Recommended electrical conductivity rage for greenhouse poinsettia production electrical conductivity range using pour-through method (Ecke et al, 1990).
Figure 3. Weekly electrical conductivity (EC) of root substrate amended with biochar at different percentages, after four different fertigated regimes were initiated from week 3 (6 Sep. 2013) and ended in week 15 (5 Dec. 2013, termination of the experiment).

2.3.3 Measurements

The root substrate physical characteristics, including total porosity, container capacity, air space, and bulk density, were determined in a laboratory, using a porometer (North Carolina State University, NC) according to the North Carolina State University Porometer Method (Fonteno et al., 1981). Plant height and width were recorded biweekly, and growth index (GI) was calculated as: GI=plant height/2 + (plant width1+plant width2)/4. Plant height was measured from the root substrate surface to the plant growing point. Two plant widths were measured across the greatest plant width, and the perpendicular width. In week 9, gas exchange (photosynthetic rate, stomatal conductance to H_2O, and transpiration rate) was measured by putting a young fully expanded leaf in the leaf chamber (cuvette) of a CO_2 analyzer (LI-6400XT, LI-COR Inc., Lincoln, NE). The cuvette environment was maintained at 25 °C, 400 $\mu mol•m^{-2}•s^{-1}$ CO_2 concentration, and 1200 $\mu mol•m^{-2}•s^{-1}$ PPF (photosynthetic photon flux). Due to time limitation, gas exchange was only measured on five replications for each treatment combination of three biochar percentages and three fertigation regimes. Leaf greenness was quantified using a chlorophyll meter (SPAD-502 Minolta Camera Co., Osaka, Japan) in week 10, 12, and 14. Define SPAD here (SPAD) readings of three fully expanded green leaves per plant were taken from three plants per treatment. The average number of red bracts from three main shoots was recorded from week 1, when they started turning red, to week 14. Plants were harvested when there were at least two opened cyathias, which occurred week 15. The number of green leaves, red bracts, and plant dry weight (DW) were determined at harvest. Visual quality of both shoots and roots was rated on every plant based on three photos taken at different angles before harvest (Table 3; Figure 4). Shoot DW was determined after severing plant shoots at the root substrate surface and oven dried at 80 ºC to constant weight.

Table 3. Poinsettia visual rating scales.

	Poinsettia shoot visual rating
1	Less than 50% red bracts coverage on the top layer of the plant with bracts marginal necrosis, and with or without horizontal branches
2	50% to 75% red bracts coverage on the top layer of the plant with one or two horizontal
3	75% to 90% coverage of red bracts on the top layer of the plant with less than two horizontal branches
4	90% to 100% red bracts coverage on the top layer of the plant with one or two horizontal branches
5	Full coverage of red bracts on the top layer of the plant with round structure without horizontal branches or bracts marginal necrosis
	Poinsettia root visual rating
1	Less than 20% of coverage
2	20% to 40% coverage
3	40% to 60% coverage
4	60% to 80% coverage
5	Over 80% coverage

Figure 4. Photos of shoots and roots used as rating standard for visual rating. Numbers on the photo are ratings in a scale from 5 to 1.

2.3.4 Experimental design and statistical analysis

The experiment was a two-factor factorial design with 10 replications. There were six biochar percentages and four fertigation regimes. A two-way analysis of variance was used to test the effects of biochar percentage and fertigation regimes on plant growth and development (ANOVA version 9.3; SAS Institute, Cary, NC). When the main effect was significant, mean separation was conducted using Student-Newman-Keuls test. All means were separated at 5% significance level.

Quadratic regression analyses were performed to the nature of association between plant total dry weight and red bracts dry weight using SigmaPlot (Version 12.0; Systat Software Inc. San Jose, CA).

2.4 Results and Discussion

2.4.1 Root substrate characteristics

Total porosity (TP) was numerically highest (86.5%) in 20% biochar, but it was not different from values for 0% and 100% biochar (Table 4). TPs in all treatments were within the recommended range of 50% to 85%, except for 20% biochar which is slightly higher (Yeager et al., 2007). Container capacity (CC) and air space (AS) of all root substrate treatments were within the recommended range (45% to 65% and 10% to 30%, respectively; Table 4). Air space increased as biochar percentage increased, while CC decreased as biochar percentage increased (Figure 5). Root substrate without biochar had the lowest AS, while 100% biochar had the highest AS. Suitable biochar percentage (25% by weight of a pellet material made from a mixture of biochar and other ingredients) increased root substrate water holding capacity while maintaining a desirable air-filled porosity (Dumroese et al., 2011). Dole and Wilkins (2005) suggested that root substrate with approximately 20% AS and 50% CC was suitable for poinsettia growth. Bulk density increased as biochar percentage increased, however, BD at CC decreased as biochar percentage increased (Figure 6).Bulk density for all root substrate treatments was lower than the lower range of the recommendation (0.19-0.7 $g \cdot cm^{-3}$; Yeager et al., 2007). Considering that Yeager's recommended BD was for field containers, the BD at CC for greenhouse substrate, 0.64-0.96 $g \cdot cm^{-3}$, suggested by Dole and Wilkins (2005) was more suitable for comparison with the results of this experiment. For the BD at CC, 60% and 80% biochar was slightly lower than the lower suggested range, while those for 0%, 20%, 40% and 100% biochar were within the suggested range, though 80% and 100% are not significant different (Dole and Wilkins, 2005; Table 4). Jackson et al. (2011) reported peat-based root substrate replaced by 5% and 10% biochar by volume had higher AS than peat-based root substrate without biochar. However, Dumroses et al. (2011) reported decreased AS in root substrate with pelleted biochar. Particle size and type of biochar most likely influenced the physical characteristics of the substrate, and thus further tests were required for optimization (Steiner and Harttung, 2014).

Table 4. Root substrate physical properties (total porosity, TP; container capacity, CC; air space, AS; and bulk density, BD) of Sunshine Mix #1 amended with six different percentages of biochar (by volume).

Biochar percentage	TP [y] (% vol)	CC [x] (% vol)	AS [w] (% vol)	BD [v] ($g \cdot cm^{-3}$)	BD [u] at CC ($g \cdot cm^{-3}$)
0%	84.2 ab [z]	62.8 a	21.5 e	0.10 f	0.73 a
20%	86.5 a	61.5 a	24.9 d	0.11 e	0.72 a
40%	79.8 bcd	55.8 b	24.0 d	0.12 d	0.68 b
60%	75.3 d	46.3 c	29.0 c	0.14 c	0.60 d
80%	78.5 cd	47.2 c	31.3 b	0.16 b	0.63 c
100%	82.6 abc	46.9 c	35.7 a	0.18 a	0.65 c
Suitable Range [t]	50-85	45-65	10-30	0.19-0.7	0.64-0.96

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$.

[y] Total porosity is equal to container capacity + air space.

[x] Container capacity is (wet weight – dry weight)/volume of the sample.

[w] Air space is the volume of water drained from the sample/volume of the sample.

[u] Bulk density after oven drying at 80°C for one week.

[v] Bulk density just after watering at container capacity.

[t] Recommended physical properties of container root substrate by Yeager et al. (2007) and suggested acceptable range for bulk density just after watering at container capacity by Nelson (2012)

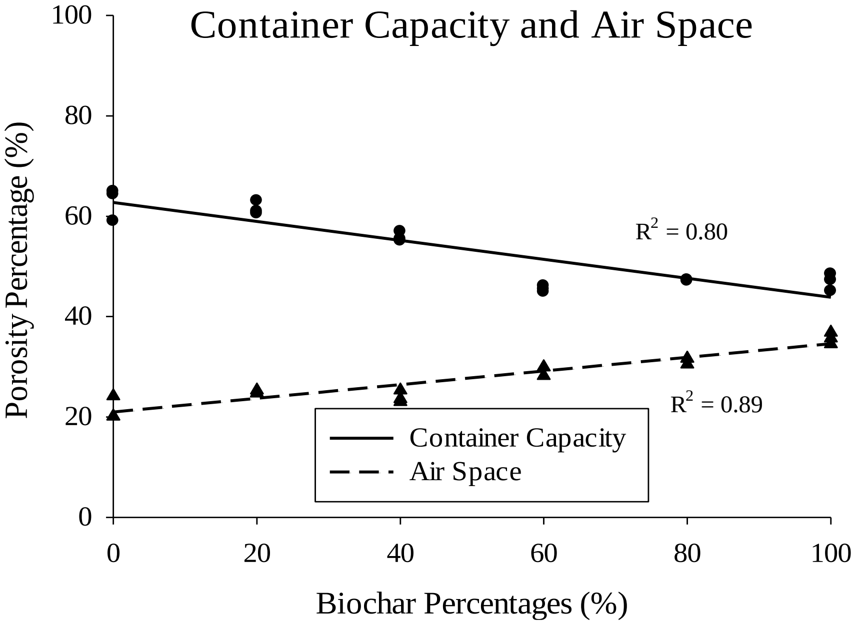


Figure 5. Container capacity and air space of six substrates.

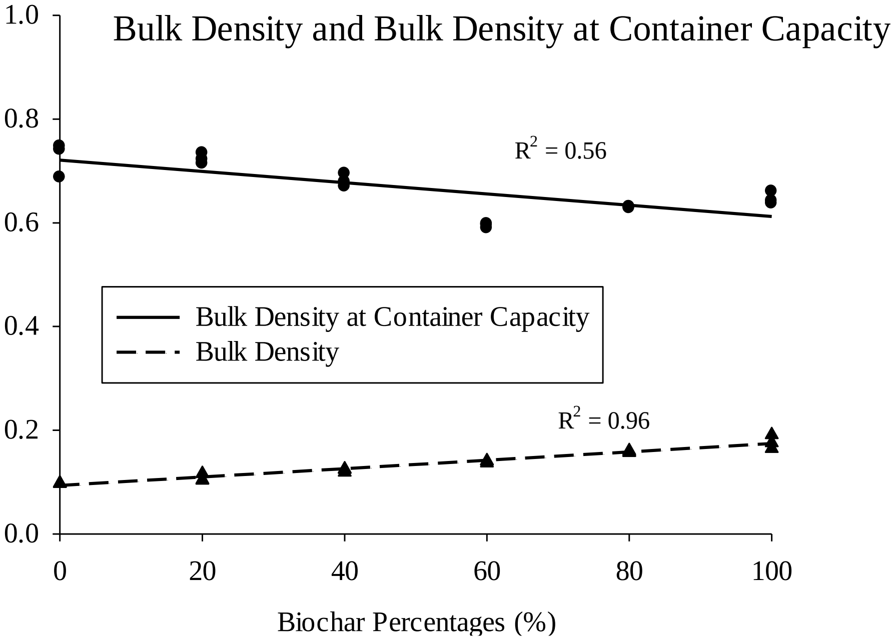


Figure 6. Bulk density and bulk density at container capacity of six substrates.

2.4.2 Root substrate electrical conductivity

Biochar had a significant effect on electrical conductivity (EC) of the root substrate leachate in the first two weeks after transplanting (Table 5). Root substrate leachate EC was reduced as biochar percentage increased in week 1 (Figure 7). In week 2, 20% to 100% biochar had similar EC values, though they were lower than that with 0% biochar. From week 3, root substrate EC was mainly affected by fertigation regime and data were pooled from different biochar percentages (Figure 3). Starting from week 3, EC of fertigation regime 1 (F1) was lower than the low EC level (1.5 $mS \cdot cm^{-1}$) of poinsettia production (Ecke et al, 1990). The EC of root substrate fertilized under fertigation regime 2 (F2) was at the lower portion of the acceptable range (2.2 to 3.8 $mS \cdot cm^{-1}$), that of root substrate fertilized under fertigation regime 3 (F3) was at the higher portion of the acceptable range (Ecke et al, 1990), and the EC of root substrate fertilized under fertigation regime 4 (F4) was near the high EC level of poinsettia production (Figure 6 B). Steiner and Harttung (2014) reported that the initial leachate EC of fresh biochar was similar to that of unfertilized peatmoss. The lower leachate EC with higher biochar percentages in week 1 may have been caused by the starter nutrients charge added in the Sunshine Mix #1. The lower leachate EC of root substrate with biochar regardless of percentage at the first two weeks of the experiment could also be caused by biochar’s moderating effect on extreme fluctuation of macronutrients (Altland and Locke, 2012).

Table 5. Analysis of variance (ANOVA) table showing root substrate electrical conductivity (EC) of first two weeks after transplanting, total dry weight (DW; total DW= leaf DW+ stem DW+ red bract DW), leaf DW, stem DW, red bract DW, the total number of red bracts, the number of green leaves, the total number of leaves, and final shoot rating of 'Prestige Red' poinsettia grown in Sunshine Mix #1 amended with six different percentages of biochar and fertigated at four regimes. All data (except the EC data) were collected 15 weeks after transplanting.

Treatment	EC (mS·cm-1)		Dry Weight (g)				Number of Bracts	Number of Green Leaves	Total Number of Leaves	Final Shoot Rating
	Week 1	Week 2	Total DW	Leaf DW	Stem DW	Bract DW				
Biochar	***	***	***	***	***	***	***	*	***	***
Fertigation	NS	NS	***	***	NS	***	NS	NS	NS	***
Biochar x Fertigation	-	-	NS	NS	NS	***	*	NS	*	**

NS (nonsignificant) or significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***).

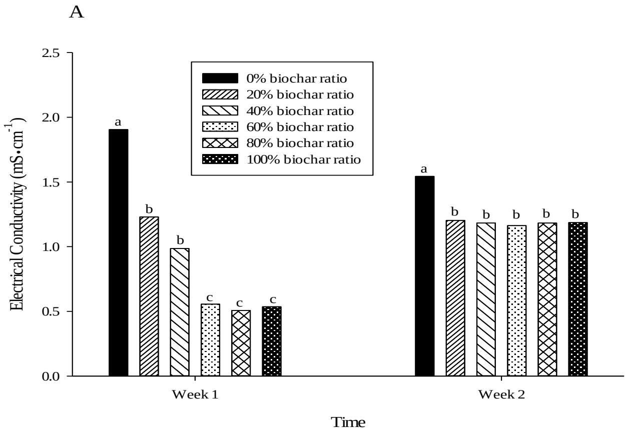


Figure 7. Week1 and week 2 electrical conductivity (EC) of root substrate amended with biochar at different percentages. All plants were fertigated with 200 mg•L^{-1} N. Columns followed by the same letter within week are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05 (week 1, six days after potting, and week 2, 13 days after potting).

2.4.3 Plant growth

There was no significant interaction between biochar percentage and fertigation regime on poinsettia growth index (GI; Table 6). There was no difference in GI between plants grown in 0% or 40% biochar. Plant GI was the highest in plants grown in 20% biochar in Week 11 and Week 13. In week 13 and week 15, the GI of plants grown in 0%, 40%, 60% or 80% biochar were not significantly different, and plants grown in 100% biochar had the lowest GI. Fertigation regimes had no significant effect on plant GI in week 1, 3, 5, or 9. There were no significant differences in plant GI among plants fertigated at F2, F3, or F4 in week 11, 13 and 15. In week 13 and week 15, plants fertigated at F2, F3, or F4 had higher GI than plants fertigated at F1.

Total dry weight (total DW= leaf DW + red bracts DW + stem DW) was correlated with GI at the final week (week 15). There was no significant interaction between biochar percentage and fertigation regime for total DW, green leaf DW and stem DW (Table 5). Plant DW increased as biochar percentage increased at 20% biochar, then decreased as biochar percentage increased over 40%. (Figure 8). There were no difference for green leaf DW and stem DW between 0% and 40% biochar (Table 7). Plants grown in 100% biochar and 80% biochar had significant lower total leave DW and stem DW. Fertigation regime had no significant effect on stem DW. Plants fertigated at F2, F3, and F4 had higher total DW and green leaf DW than plants fertigated at F1. The results indicated that 20% biochar increased plant growth, as reflected in higher plant total DW.

Growth index and DW of poinsettia was previously found to be affected by root substrate compositions (Jackson et al., 2008; Wang and Blessington, 1990). In our experiment, the low plant DW and the small plant GI in plants grown in 100% biochar may be caused by lower CC of 100% biochar (Table 4). Jackson et al. (2008) reported a similar reduction in poinsettia DW for plants grown in lower CC substrate. The results of this experiment indicated that 20% biochar increased plant growth, as reflected in higher plant total DW. Supportive of this result, Graber et al. (2010) reported a small amount of biochar (1-5% by weight) could increase tomato and pepper growth in soilless medium (Graber et al., 2010). Tian et al. (2012) reported Calathea (*Calathea rotundifola cv. Fasciata*) plants grown in 50% biochar had higher total dry weight, yet those grown in 100% biochar had the lowest dry weights of three biochar percentages (0%, 50% or

100% biochar by volume). These results suggest that amending biochar with peat-based root substrate could provide better root substrate physical properties and higher nutrient retention for plant growth than commercial peat-based substrate, though the suitable percentage of biochar may depend on species and biochar type. However, Steiner and Harttung (2014) reported no increase in fresh weight or plant height for mini sunflower grown in root substrate with biochar, and lower fresh weights were observed for those grown in 50% and 100% biochar compared to plants grown in 0% biochar. The different plant growth response could be caused by the different type and particle size of biochar used in the experiment.

Table 6. Growth index of ‘Prestige Red’ poinsettia grown in Sunshine Mix #1 amended with six different percentages of biochar and fertigated at four regimes from Week 1 to Week 15.

	Growth Index (cm)							
Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 11	Week 13	Week 15
				Biochar				
0%	12.3 a	20.8 a	25.4 a	29.0 a	32.7 ab	37.7 b	40.8 b	44.1 ab
20%	12.5 a	20.1 ab	24.7 a	30.0 a	33.7 a	39.5 a	42.6 a	45.2 a
40%	12.3 a	20.3 a	24.9 a	29.1 a	33.0 ab	37.5 b	40.8 b	44.2 ab
60%	12.5 a	19.5 b	24.5 a	28.6 a	32.2 b	36.9 b	40.6 b	43.7 ab
80%	12.0 a	18.7 c	24.2 a	28.4 a	30.7 c	35.7 c	39.5 b	42.7 b
100%	11.8 a	17.0 d	22.3 b	24.6 b	26.4 d	32.5 d	35.1 c	37.5 c
				Fertigation				
F1	12.1 a	19.4 a	24.8 a	29.1 a	31.6 a	35.6 b	37.6 b	40.9 b
F2	12.3 a	19.2 a	23.6 a	28.6 ab	31.7 a	36.8 ab	40.4 a	43.1 a
F3	12.2 a	19.7 a	24.7 a	27.9 bc	31.4 a	37.2 a	40.8 a	44.0 a
F4	12.4 a	19.1 a	24.2 a	27.1 c	31.0 a	36.9 ab	40.8 a	43.7 a
Significant								
Biochar	NS	***	***	***	***	***	***	***
Fertigation	NS	NS	NS	***	NS	***	***	***
Biochar x Fertigation	NS	NS	NS	NS	NS	NS	NS	NS

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$.

[y] NS (nonsignificant) or significant at $P \leq 0.001$ (***).

Table 7. Total dry weight (Total DW = green leaf DW+ red bract DW+ stem DW), green leaf and stem DW of ‘Prestige red’ poinsettia grown in Sunshine Mix #1 amended with six different percentages of biochar and fertigated at four different regimes. All data were collected at 15 weeks after transplanting.

	Dry Weight (g)		
Treatment	Total DW	Green Leaf DW	Stem DW
		Biochar	
0%	39.8 b[z]	15.1 ab	10.6 a
20%	43.1 a	16.1 a	10.9 a
40%	38.1 bc	14.7 b	10.7 a
60%	35.9 c	14.2 b	9.7 a
80%	32.0 d	12.4 c	8.7 b
100%	24.1 e	9.3 d	6.7 c
		Fertigation	
Fertigation 1	32.5 b	12.2 b	9.5 a
Fertigation 2	36.3 a	13.9 a	10.0 a
Fertigation 3	36.6 a	14.1 a	9.4 a
Fertigation 4	38.0 a	14.9 a	9.5 a

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

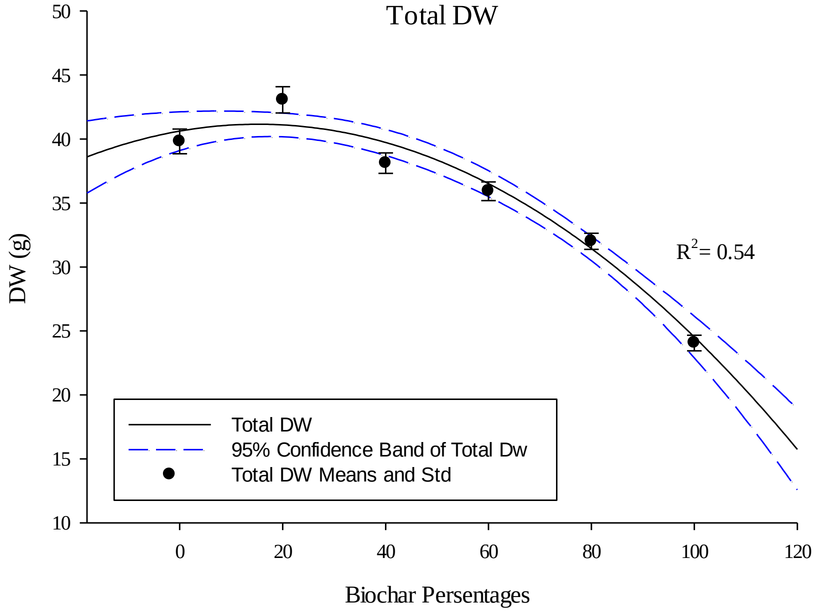


Figure 8. Plant total dry weight at 15 weeks after transplanting regression.

2.4.4 Gas exchange

There were no interactions between biochar percentage and fertigation regime for all leaf gas exchange parameters (Table 8). Plants grown in root substrate without biochar had the highest photosynthetic rate. There were no differences in photosynthetic rate among plants grown in 40% and 100% biochar. No differences were found in stomatal conductance and transpiration rate among the three biochar percentages. The photosynthetic rate, stomatal conductance, and transpiration rate increased as fertigation rate increased, which might explain the lower plant DW and GI with F1. Previous research showed that higher EC and fertilizer concentration could be the reason for increased plant photosynthetic rate and growth (Ku and Hershey 1991; Yelanich and Biernbaum 1993).

Table 8. Leaf gas exchange (photosynthetic rate, stomatal conductance to H2O, transpiration rate) of ‘Prestige Red’ poinsettia nine weeks after transplanting in root substrate amended with 0%, 40% and 100% biochar and fertigated with fertigation regimes 1, 2 and 3.

Treatment	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mol H_2O m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})
		Biochar	
0%	10.83 a[z]	0.41 a	4.47 a
40%	8.13 b	0.41 a	4.55 a
100%	8.11 b	0.42 a	4.58 a
		Fertigation	
Fertigation 1	7.09 b	0.36 b	4.23 b
Fertigation 2	9.47 a	0.42 ab	4.63 ab
Fertigation 3	10.51 a	0.45 a	4.74 a
Significance			
Biochar	** [y]	NS	NS
Fertigation	***	*	*
Biochar x Fertigation	NS	NS	NS

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

[y] NS (nonsignificant) or significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***).

2.4.5 Plant quality

Red bract DW and the number of red bracts were determined because red bract is an important parameter of poinsettia’s quality and visual appeal (Jackson et al., 2008). The number of leaves are also important for plant quality. Lower total number of leaves could reduce plant visual appeal.

There were interactions between biochar percentage and fertigation regime for the total number of leaves, the number of red bracts and red bract DW (Table 5). The total number of leaves and the total number of red bracts were only significantly affected by biochar percentage, and red bracts DW was affected by both the biochar percentage and fertigation regimes. At F1, the total number of leaves, the total number of red bracts, and red bract DW decreased as biochar percentage increased (Table 9). At F2, F3 and F4, plants grown in 100% biochar had the lowest total number of leaves, total number of red bracts, and red bract DW. For the total number of leaves and the total number of red bracts, there were no difference among 0%, 20%, 40%, 60%, and 80% biochar at F2, F3 or F4. At F2, F3, or F4. In general, at F2, F3 and F4, bracts DW decreased in response to increase of biochar percentage when the biochar percentage was over 80% (Figure 9). Plants grown in 60%, 80% and 100% biochar had lower red bract DW than those grown in root substrate without biochar. For plants grown in root substrate without biochar, fertigation regimes had no effect on red bract DW. For plants grown in root substrate with biochar, bract DW was lower at F1.

There was no interaction between biochar percentage and fertigation regime for the number of green leaves, which was only significantly affected by biochar percentage

(Table 5). There was no difference in the number of green leaves among plants grown in 0% to 80% biochar, only 100% biochar reduced the number of green leaves significantly (data not shown). Supportive of this result, Tian et al. (2012) reported that Calathea grown in 100% biochar had lower leaf biomass, lower leaf number, and smaller leaf surface area than those grown in 0% biochar.

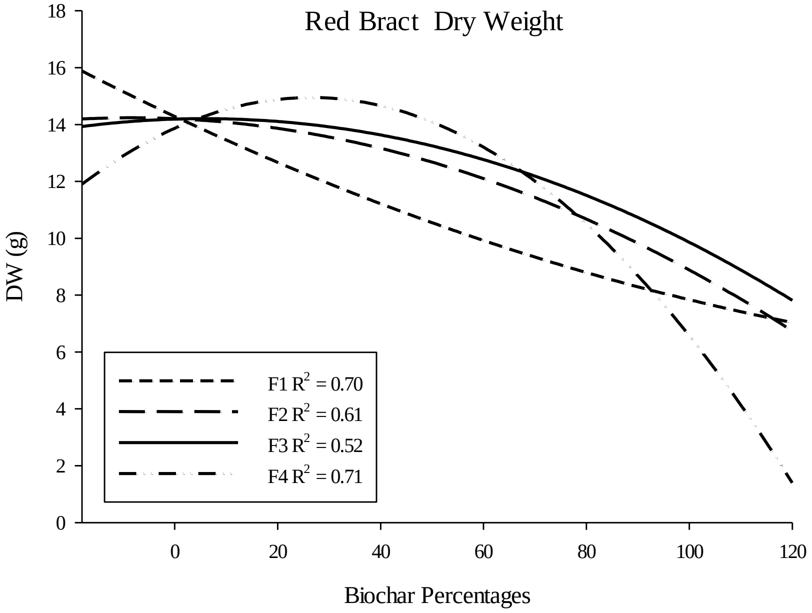


Figure 9. Plant red bract dry weight at 15 weeks after transplanting regression.

Table 9. The total number of leaves (green leaf + red bract), the total number of red bracts, and red bract dry weight of 'Prestige Red' poinsettia 15 weeks after transplanted in Sunshine Mix #1 amended with six different percentages of biochar and fertigated at four different regimes. All data were collected at 15 weeks after transplanting.

Biochar	Fertigation 1	Fertigation 2	Fertigation 3	Fertigation 4
		Total Number of Leaves		
0%	235.7 a[z]	219.2 a	225.3 a	211.5 a
20%	214.6 ab	217.8 a	232.8 a	221.8 a
40%	222.5 ab	210.4 a	202.7 ab	231.0 a
60%	188.4 b	213.3 a	224.9 a	224.0 a
80%	194.2 b	190.0 ab	209.3 ab	188.3 a
100%	155.0 c	161.4 b	187.0 b	118.8 b
		Total Number of Red Bracts		
0%	143.9 a	128.3 a	137.2 ab	136.1 a
20%	127.7 ab	130.8 a	143.9 a	137.5 a
40%	128.7 ab	122.1 a	121.9 ab	142.1 a
60%	106.9 bc	123.0 a	134.0 ab	136.4 a
80%	113.8 bc	107.0 ab	120.9 ab	115.4 a
100%	96.8 c	96.9 b	115.7 b	66.5 b
		Red Bract Dry Weight (g)		
0%	13.8 a[z] A[y]	13.7 ab A	13.8 ab A	13.9 ab A
20%	12.2 a B	15.0 a A	15.1 a A	15.4 a A
40%	10.3 b C	12.4 bc B	12.7 bc B	14.4 ab A
60%	9.9 b B	12.1 c A	12.7 bc A	12.7 b A
80%	9.6 b B	11.0 c A	11.9 c A	11.0 c A
100%	7.1 c BC	8.4 d AB	9.7 d A	5.9 d C

[z] Means within a column under each parameter followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

[y] Means within a row under each parameter followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

There was no significant interaction between biochar percentage and fertigation regime for SPAD reading, the average number of red bracts (from three main shoots), and final root rating (Table 10). Biochar treatments had no significant effect on plant SPAD reading. Plants fertigated at F2, F3, or F4 had higher SPAD reading than plants fertigated at F1. SPAD readings are highly correlated with leaf nitrogen concentration and could have been affected by increasing N fertilizer rate (Gaborcik 2003; Li et al., 1998; Neilsen et al., 1995; Sibley et al., 1996). Higher SPAD readings on plants fertigated at F3 and F4 could be caused by higher leaf nitrogen level, which is normally associated with higher fertilizer concentration. Plants grown in 20% biochar had a higher average number of red bracts than other biochar percentages in week 12 (Table 10). Compare to plants grown in 0% biochar, the average number of red bracts were significantly affected by biochar percentages in week 12 and week 14. Plants fertigated with F3 or F4 had a higher average number of red bracts than plants fertigated with F1, and no difference between F1 and F2. Final root rating showed no differences among 0%, 40% and 60% biochar, and no difference between among 20% and 80% biochar (Table 10). Plants grown in 100% biochar had the lowest root rating. Plants fertigated with F1, F2, or F3 had higher root rating than plants fertigated with F4.

Tian et al. (2012) did not report any change in plant root biomass. Hidalgo and Harkess (2002) reported that higher AS (12.8%) and lower CC (58.7%) had the greatest root development, and that root development was unrelated with the shoot performance. In this experiment, 60% biochar had relatively higher AS and lower CC, and the highest root rating. However, AS was high and CC was low in 80% and 100% biochar, but root rating was decreased. Altland and Locke (2012) also showed that low biochar percentage (1% to 10%, by volume) could moderate extreme nutrient fluctuation in container substrates over time, which is desirable for plant growth and root development.

There were significant interactions between biochar percentage and fertigation regime for the final shoot rating, but only poinsettias grown in 100% biochar and fertigated with F4 had a significantly reduced visual quality (Table 5, Table 11), with bracts necrosis on eight out of ten replications, in which five of them were already dead due to pythium (*Pythium* spp.) root rot before week 15.

Bract necrosis could be caused by any condition leading to reduced calcium uptake, like root rot, and low EC level and low percentage of ammonium (Dole and Wilkins, 2005). On the other hand, high to medium EC could increase plants' susceptibility to root disease (Dole and Wilkins, 2005). The high bract necrosis and pythium root rot rate in plants grown in 100% biochar at F4 could be caused by the high fertilizer concentration of F4, biochar nutrient retention ability, and poor root development, as reflected in lower root rating.

Plants grown in root substrate with biochar had no differences compared to those in root substrate without biochar for plant quality including: SPAD reading, final shoot rating (except plants grown under the 100% biochar combined with F4), and average number of red bracts, indicating that low concentration of fertilizer at 100 mg•L^{-1} to 200 mg•L^{-1} N was enough for poinsettia greenhouse production.

Table 10. SPAD reading at week 10, 12, and 14, the average number of red bracts from week 11 to 14, and final root visual rating of ‘Prestige Red’ poinsettia 15 weeks after transplanting in root substrate amended with six different percentages of biochar and fertilized with four fertigation regimes.

Treatment	SPAD			Average Number of Red Bracts				Final Root Rating
	Week 10	Week 12	Week 14	Week 11	Week 12	Week 13	Week 14	
				Biochar				
0%	41.7 a[z]	45.1 a	53.1 a	1.5 ab	5.2 b	8.9 a	10.1 ab	3.7 ab
20%	41.5 a	45.6 a	53.7 a	1.8 a	5.8 a	9.1 a	10.5 a	3.2 c
40%	40.1 a	44.1 a	54.1 a	1.3 ab	4.9 b	8.4 a	9.8 ab	3.7 ab
60%	40.3 a	45.4 a	54.4 a	1.2 ab	4.8 b	8.4 a	10.0 ab	4.0 a
80%	40.1 a	46.2 a	54.2 a	1.2 ab	4.8 b	8.3 a	9.7 b	3.3 bc
100%	39.9 a	45.4 a	54.9 a	1.1 b	4.8 b	8.4 a	9.5 b	2.6 d
				Fertigation				
Fertigation 1	39.0 b	43.5 b	53.0 b	1.0 b	4.6 c	8.1 b	9.3 b	3.67 a
Fertigation 2	40.4 ab	45.1 a	54.1 ab	1.2 ab	4.8 bc	8.4 ab	9.7 b	3.53 a
Fertigation 3	41.5 a	46.4 a	55.0 a	1.5 a	5.2 ab	8.9 a	10.2 a	3.48 a
Fertigation 4	41.5 a	46.2 a	54.1 ab	1.6 a	5.5 a	8.9 a	10.3 a	2.98 b
Significance								
Biochar	NS[y]	NS	NS	**	***	**	**	***
Fertigation	**	***	**	**	***	***	***	***
Biochar x Fertigation	NS	NS	NS	NS	NS	NS	NS	NS

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$.

[y] NS (nonsignificant) or significant at $P \leq 0.01$ (**), or 0.001 (***).

Table 11. Shoot final visual rating of 'Prestige Red' poinsettia 15 weeks after transplanting in root substrate amended with six different percentages of biochar and fertilized with four fertigation regimes.

	Final Shoot Rating			
Biochar	Fertigation 1	Fertigation 2	Fertigation 3	Fertigation 4
0%	4.4 a[z] A[y]	4.1 a AB	4.5 a A	3.9 a B
20%	4.6 a A	4.0 a A	4.2 a A	4.2 a A
40%	4.9 a A	4.1 a B	4.1 a B	4.4 a B
60%	4.4 a A	4.2 a A	4.2 a A	3.6 a A
80%	4.5 a A	4.3 a A	4.1 a A	3.3 a B
100%	4.5 a A	3.3 a A	4.3 a A	1.8 b B

[z] Means within a column under each parameter followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

[y] Means within a row under each parameter followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

2.5 Conclusion

The results of this experiment indicated that peat-based commercial root substrate (Sunshine Mix #1) amended with 80% biochar could be used in poinsettia greenhouse production. Plants grown in 20% biochar had greater growth than those in 0% biochar, as reflected in higher plant total dry weight. On the other hand, 60% and 80% biochar treatments reduced dry weight, yet this reduction had no effect on plant final visual rating and plant grown index. Plants grown in 40% biochar were similar to those grown in Sunshine Mix #1. Treatment with 100% biochar suppressed plant growth as reflected in plant growth index, plant dry weight, the total number of leaves, the total number of red bracts, and final root visual rating. Higher fertigation regime (fertigation regime 4) combined with 100% biochar increased the susceptibility of plants to root rot and bracts necrosis. Fertigation regime 1 slightly decreased plant SPAD reading and the average number of red bracts, but the effects were minor. Root substrate with biochar had lower leachate EC during the first two weeks of the experiment, which did not affect plant growth and development. Thus, low fertilization regime (Fertigation regime 1, 100 mg•L^{-1} to 200 mg•L^{-1} N) could be used for poinsettia production without affecting the quality of plants.

Biochar is a byproduct of pyrolysis, where high temperatures of the production process makes it a weed-, pathogen-, and insect-free root substrate amendment. Physical and chemical properties of biochar may vary due to differences in the production process and biomass source. Biochar used in this experiment had acceptable bulk density, container capacity, air space, and total porosity. These physical characteristics showed a potential for amending certain percentage biochar with peat-based root substrate in greenhouse production. Further experiments may be conducted to determine the suitable biochar percentage for biochar made from other sources using different pyrolysis methods with different particle size, as well as for other popular greenhouse crops, such as orchid (*Orchis* spp), rose (*Rosa* spp), chrysanthemums, and Easter lilies.

CHAPTER III

EASTER LILY GROWTH AND DEVELOPMENT RESPONSE TO CONTAINER ROOT SUBSTRATE WITH BIOCHAR

3.1 Overview

Biochar, a byproduct of bio-energy production, may have a great potential to be used as a greenhouse root substrate amendment to reduce the use of peatmoss. A greenhouse study was conducted to evaluate the growth and flowering of Easter lily (*Lilium longiflorum*) ‘Nellie White’ grown in a commercial potting mix (Sunshine Mix #1) amended with biochar at 0%, 20%, 40%, 60%, or 80% (by volume) and fertigated at four different regimes (constant feeding at 200 $mg•L^{-1}$ or 300 $mg•L^{-1}$ N, and fertigation at every third waterings with 200 $mg•L^{-1}$ or 300 $mg•L^{-1}$ N). The experimental design was a split-plot design with fertigation regimes as the main plot and biochar percentage as the subplot. There was no interaction between fertigation regimes and biochar percentage on any parameter measured in this experiment. Neither fertigation regime nor biochar percentage significantly affected number of days before full flower, number of flowers, total shoot dry weight, number of leaves, and leaf gas exchange rate. Root substrate with 80% biochar had lower leachate electrical conductivity than the other biochar treatments during the experiment. Plants grown in 80% biochar had shorter stems than plants grown in 20% and 40% biochar, yet the stem length were not significantly different compared to plants grown in root substrate without biochar. The ratio of stem length with green leaves to total stem length (LSG/TSL) increased as biochar percentage increased, and plants grown in root substrate with 80% biochar had the highest LSG/TSL. Plants in the two constant feeding groups had higher SPAD readings than those fertigated at every third watering in week 17. In summary, up to 80% biochar could be used as an amendment to peat-based root substrate without significant changes in Easter lily growth and development.

3.2 Introduction

The quantity of potted Easter lilies (*Lilium longiflorum*) sold in 2012 was over five million (ranking no. 5) with a wholesale value of $ 22.2 million (ranking no. 3) in potted flowering plants in the U.S. (Floriculture Crops 2012 summary, 2013). Most growers use peat-based substrates for potted Easter lily production (Erwin, 2002). A well-drained and aerated medium is required to grow high-quality plants with good root systems (Dole and Wilkins, 2005).

Peatmoss is a highly valued source for potted plant culture root substrate by the current horticulture industry (Clarke, 2008). However, peatmoss harvest and land development reduce the volume of global peatland at a rate of 0.05% annually (Apodaca, 2014). Peatlands are fragile and unique ecosystems, and the decrease of peatlands results in a decline in biodiversity, increasing greenhouse gas emissions and a shrinking carbon sink (Henson, 2007). In the U.K., multiple environmental, scientific, and governmental agencies have proposed to limit the use and extraction of peatmoss (Carlile, 2004). There are no restrictions regarding peatmoss use in the U.S. (Jackson et al., 2008). Yet the cost of peat-based root substrate has risen in recent years due to transportation costs and growing environmental concerns over peatland in Canada and Europe (Wright et al., 2009). Therefore, interest in finding alternative root substrate components to replace or reduce the use of peatmoss has increased among researchers.

Alternative substrates have previously been investigated to reduce the use of peatmoss in nursery and greenhouse crop production. Reports showed ground loblolly pine (*Pinus taeda*) has potential to replace peatmoss as a greenhouse root substrate with an increase of fertigation concentration (Fain et al., 2008; Wright et al., 2008; Jackson et al., 2009). Up to 20% by volume clean chip residual (a byproduct from loblolly pine tree harvest, and hammer milled to pass through 1.27 cm screen was used as an amendment to peat-based root substrate to produce ageratum (*Ageratum houstonianum*), salvia (*Salvia* × *superba*), and impatiens (*Impatiens walleriana*) (Boyer et al., 2008). Peat-based root substrate amended with 25% earthworm cast by volume (from sheep (*Ovis aries*) or cattle (*Box taurus*) manure) increased the growth of poinsettia (*Euphorbia pulcherrima*), and 50% earthworm cast by volume increased the plant quality of marigold (*Tagetes erecta*), and chrysanthemum (*Dendranthema ×grandiflora*) (Matta et al., 2008). Composted organic materials amended with peat-based root substrate at different rates (from 20% to 50% for different composts by volume) was used for greenhouse production without significant changes in plant quality (Ku et al., 1998; Papafotiou et al., 2004). Yet there are limits for those alternative substrates, such as additional input of fertilizers, composition variability, inconsistent availability, and contamination such as glass, metal fragment, lead, mercury (Konduru and Evans, 1999; Gu et al., 2013).

To reduce the use of peatmoss by the greenhouse industry, a fine-grained porous byproduct from pyrolysis, biochar, has been investigated as an alternative container root substrate (Gu et al., 2013). Pyrolysis is an industrialized thermochemical conversion process which converts biomass to biochar, bio-oil and syngas at high temperatures with low or no oxygen conditions (Zhang et al., 2013). The characteristics and yield of biochar depend on method, temperature, and the biomass used for pyrolysis (Spokas et al. 2012). The pyrolysis temperature varies from 225 °C (torrefaction of biomass; Phanphanich and Mani, 2011) to 850 °C (gasification of biomass; Salleh et al., 2010). As pyrolysis temperature increases, the surface area and pH of biochar increase, while the yield decreases (Lehmann, 2007; Zhang et.al., 2013). Considering the production cost, biochar yield, and characteristics of biochar, the optimal temperature of biochar for agricultural usage should be 450-550 °C (Lehmann, 2007).

Studies have revealed the potential of biochar to be used as an alternative root substrate to reduce peatmoss use in greenhouse production. Steiner and Harttung (2014) reported that fresh biochar had similar initial leachate electrical conductivity as unfertilized peatmoss. Tian et al. (2012) reported using biochar as a root substrate amendment could reduce root substrate particle size degradation during the production period. In a study performed by Dumroese et al. (2011), amending 25% by volume biochar pellets (made from a mixture of biochar, wood flour, polylactic acid and starch) with peat-based root substrate increased root substrate hydraulic conductivity and water retention while maintaining a desirable root substrate physical porosity. By testing multiple root substrate leachates macronutrient composition after one fertilizer event, Altland and Locke (2012) reported that root substrate with biochar had higher macronutrient retention capacity.

Other studies also reported that amending peat-based root substrate with a suitable percentage of biochar increased plant growth and plant quality, or had no effect on plants. A greenhouse experiment by Graber et al. (2010) suggested that root substrate with low rates of biochar (1 to 5% by weight) could increase tomato (*Lycopersicum esculentum*) and pepper (*Capsicum annuum*) growth. Gu et al. (2013) reported up to 30% biochar could be used as an amendment to peat-based root substrate in 'Fireworks' gomphrena (*Gomphrena* spp.) greenhouse production. A greenhouse study found no effects on crop yields of cucumber, tomato and pepper using biochar as a soilless root substrate compared to a coconut (*Cocos nucifera*) fiber-tuff potting root substrate (Zhang et al., 2013).

However, amending too much biochar in root substrate may suppress plant growth. Mini sunflower grown in root substrate with biochar (25-100% by volume) had similar height as plants grown in peatmoss, and lower fresh weights were only observed on plants grown in 50% and 100% biochar (Steiner and Harttung 2014). Altland and Locke (2012) reported Calathea (*Calathea rotundifola*) grown in 50% biochar had higher total dry weight and leaf dry weight, yet those grown in 100% biochar had the lowest dry weights in three biochar percentages used in the experiment (0%, 50% or 100% biochar by volume).

No study has investigated the possibility of using biochar in greenhouse production of Easter lily. Considering the significant amount of the peatmoss (5.1 million pots of peat-based substrate) used annually in Easter lily greenhouse production, an alternative root substrate suitable for Easter lily production could substantially reduce the use of peatmoss. The objectives of this experiment were to determine of effects of five different percentages of biochar and four fertigation regimes on growth and development of Easter lily in greenhouse production.

3.3 Materials and Methods

3.3.1 Plant materials and root substrate treatments

Substrates were formulated by mixing Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA) with biochar (provided by Mississippi State University) at 0%, 20%, 40%, 60%, or 80% by volume. The biochar used in this experiment was the byproduct of fast pyrolysis of pine wood at 450 °C (Gu et al., 2013). Particle size distribution was determined by passing 100 g biochar through 2.0-, 1.4- and 0.59-mm soil sieves, and weight was measured to determine the percentage of each particle size (Table 12). The biochar had an initial pH of 5.4 and an EC of 0.15 $mS \cdot cm^{-1}$ (using 2:1 method; Cavins et al., 2000). The pre-chilled 'Nellie White' Easter lily bulbs obtained from Gloeckner (Fred C. Gloeckner & Company Inc., Harrison, NY) were potted on 17 Dec. 2013 (week 1 of the experiment) in 15 cm plastic pots (1,680 ml) with one of five different substrates, and placed in a glass greenhouse located on the Texas A&M University campus. The greenhouse environment, recorded by Watchdogs 450 (Spectrum Technologies Inc., Paxinos, PA), was maintained at temperature of 21.5°C day /12.8 °C night, relative humidity of 57.2%, and daily light integral of 13.2 $mol \bullet m^{-2} \bullet d^{-1}$ (Figure 10).

Banrot® 40 WP (Scotts Miracle-Gro Company, Marysville, OH; applied from Dec. 2013 to Feb. 2014), a mixture of Truban® 30 WP (Scotts Miracle-Gro Company, Marysville, OH) and Cleary's 3336® F (Cleary Chemicals LLC, Dayton, NJ; applied from Mar. 2014 to Apr. 2014) were applied monthly at the labeled rates to prevent root rot disease. Soapy water (5 ml olive oil:15 ml liquid hand soap for one gallon water) were sprayed weekly to control aphids beginning March 10, 2014. No growth regulators were applied in this experiment.

3.3.2 Fertigation

A water soluble fertilizer 15 N-2.2 P-12.2 K (Peters 15-5-15; Scotts Miracle-Gro Company, Marysville, OH) was used in this experiment. A 400 mg•L^{-1} N fertilizer solution was added to all plants after potting on 17 Dec. 2013. Four fertigation regimes were initiated on 6 Jan. 2014 (week 4): constant feeding at 200 mg•L^{-1} N or 300 mg•L^{-1} N, or 200 mg•L^{-1} N or 300 mg•L^{-1} N at every third watering.

The recommended feeding frequency of Easter lily was constant feeding at 200 mg•L^{-1} N or 150 mg•L^{-1} N, or 300-400 mg•L^{-1} N at every second watering (Erwin 2014). Since root substrate with biochar has be reported to have higher macronutrient retention capacity (Altland and Locke 2012), we reduced the fertigation frequency to every third watering in this experiment to test whether amending biochar in root substrate could reduce the use of fertilizer in Easter lily production or not.

Table 12. Particle size distribution of the biochar used as an alternative substrate.

Particle Size (mm)	Percent of Sample
>2.0	15.7
1.4-2.0	27.3
0.59-1.4	49.1
<0.59	7.9

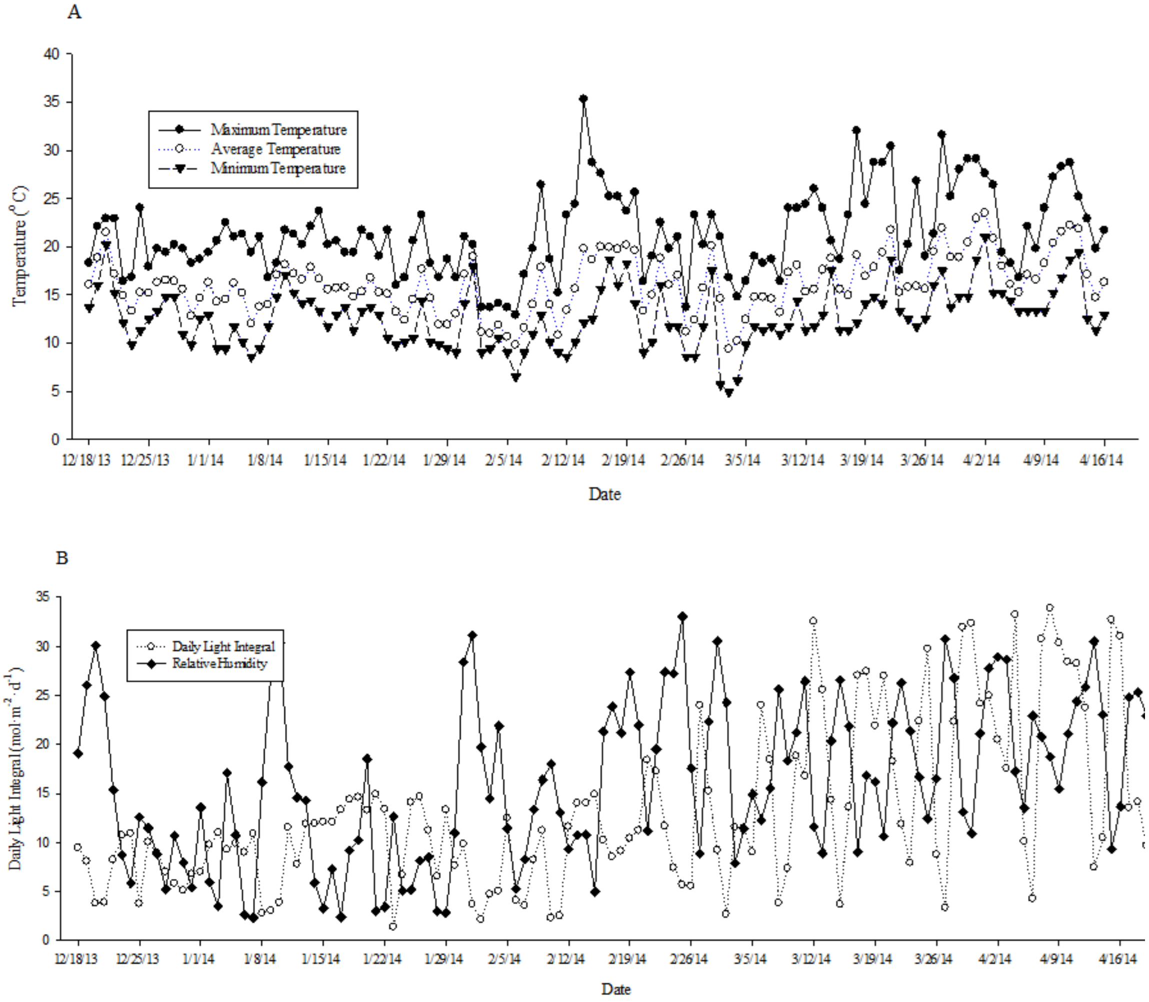


Figure 10. Average daily temperatures (maximum, minimum, and average temperature), daily light integral, and relative humidity in the greenhouse during the experimental period.

3.3.3 Measurements

The electrical conductivity (EC) of root substrate leachates of each treatment was measured at every third watering according to the pour-through method (Wright et al., 1990; LeBude and Bilderback, 2009). Plant height was measured biweekly starting at week 6 (after stems emerged above the root substrate surface) from the root substrate surface to the top of the plant. Net photosynthetic rate (A), stomatal conductance to H_2O (g_s) and transpiration rate (E) were measured at week 15 and week 17 by placing a young fully expanded leaf in the leaf chamber (cuvette) of a portable infrared gas exchange analyzer (LI-6400XT, LI-COR Inc., Lincoln, NE). The cuvette environment was set at 25 °C, 400 µmol/s CO_2 flow rate, and 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux. There were five replications for each treatment combination tested for plant gas exchange. Leaf greenness was quantified as SPAD readings using a chlorophyll meter (SPAD-502 Minolta Camera Co., Osaka, Japan) at weeks 15, 16, and 17. SPAD readings of three fully expanded green leaves per plant were taken from three plants per treatment. The number of days to first open flower were recorded as days from planting until the first petal parted exposing reproductive organs on the first developed flower per plant . The number of flowers, the length of stem with brown, yellow and green leaves, and the total stem length were recorded on 18 April 2014 (week 18). All plants were separated into flowers, leaves and stems, and the dry weight was recorded after being oven-dried at 80 ºC until constant weight.

3.3.4 Experimental design and statistical analysis

This experiment utilized a split-plot design with fertigation regimes as the main plot and biochar amendment percentages as the subplot with eight replications per treatment. Easter lily responses to different biochar percentages and fertigation regimes were analyzed by a two-way analysis of variance (ANOVA version 9.4; SAS Institute, Cary, NC). When the main effect was significant, mean separation was conducted using Student-Newman-Keuls test at 5% significance level.

Quadratic regression analyses were performed to the nature of association between plant total dry weight and red bracts dry weight using SigmaPlot (Version 12.0; Systat Software Inc. San Jose, CA).

3.4 Results and Discussion

3.4.1 Root substrate electrical conductivity

There were no significant interactions between fertigation regime and biochar percentage for any parameters measured (Tables 13, 14, 15 and 18). However, both biochar percentage and fertigation regime had significant effects on the root substrate leachate EC at each watering cycle (Table 13). Root substrate leachate EC increased as fertigation concentration and fertigation frequency increased. Substrates under constant fertigation at 300 mg•L^{-1} N resulted in the highest leachate EC, followed by constant fertigation at 200 mg•L^{-1} N. Leachate EC of the two root substrate groups under fertigation with 200 mg•L^{-1} N or 300 mg•L^{-1} N at every third watering were similar, except in week 11 and week 16, and lower compared to constant feeding treatments. Root substrate leachate EC was the lowest with 80% biochar except week 8, followed by

60% biochar. No differences were found in leachate EC among 0%, 20% and 40% biochar. Our results were similar to Steiner and Harttung's report (2014) on leachate EC of substrates with high percentages of biochar (50%, 75% and 100% by volume) six weeks after application of a slow release fertilizer. The lower root substrate leachate EC with high percentage of biochar may be caused by biochar's moderating effect on extreme fluctuations of macronutrients (Altland and Locke, 2012).

Table 13. Electrical conductivity of root substrate leachate at Weeks 5, 8, 11, 14 and 16 of Easter lily grown in Sunshine Mix #1 amended with five different percentages of biochar and fertilized with four fertigation regimes.

	Electrical Conductivity (mS·cm^{-1})				
Treatment	Week 5	Week 8	Week 11	Week 14	Week 16
			Fertigation		
200 mg•L^{-1}/3ed watering	1.0 c[z]	0.4 c	0.4 d	0.3 c	0.6 d
300 mg•L^{-1}/3ed watering	1.0 c	0.4 c	0.6 c	0.4 c	0.8 c
200 mg•L^{-1}	1.6 b	1.4 b	1.6 b	1.6 b	2.1 b
300 mg•L^{-1}	2.5 a	2.0 a	2.6 a	2.5 a	3.3 a
			Biochar		
0%	1.8 a	1.0 a	1.4 a	1.3 a	1.8 ab
20%	1.6 a	1.1 a	1.4 a	1.3 a	1.9 a
40%	1.6 a	1.1 a	1.3 a	1.2 a	1.8 ab
60%	1.3 b	1.0 a	1.2 a	1.1 b	1.6 b
80%	1.1 c	0.7 a	0.7 b	0.6 c	0.9 c
Significance					
Fertigation	***[y]	***	***	***	***
Biochar	**	NS	***	**	**
Biochar x Fertigation	NS	NS	NS	NS	NS

[z] Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$.

[y] NS (nonsignificant) or significant at $P \leq 0.01$ (**), or 0.001 (***).

3.4.2 Plant growth

Biochar percentage had significant effects on plant heights from week 6 to week 14, while fertigation did not affect plant height (Table 14). Root substrate with 80% biochar resulted in the shortest plants until week 12. At week 16 and week 18, after flower buds emerged, the differences in plant height were insignificant.

The percentages of biochar and different fertigation regimes did not affect growth and development parameters of Easter lily, number of days until full bloom, number of flowers, flower dry weight, stem dry weight, total shoot dry weight (a sum of flower dry weight, leaf dry weight, and stem dry weight), number of leaves, length of stem with green leaf (LSG; Table 15), or leaf gas exchange parameters (photosynthetic rate, stomatal conductance, and transpiration rate) at week 15 and week 17 (data not shown).

Leaf dry weight was significantly affected by fertigation regime (Table 15). Leaf dry weight increased as the frequency of fertigation increased (Table 16). Plants with constant feeding (200 mg/L N or 300 mg•L^{-1} N at every watering) had higher leaf dry weight than those fertigated at every third watering.

Table 14. Plant height (from the root substrate surface to the top of plants) of Easter lily grown in Sunshine Mix #1 amended with five different percentages of biochar and fertigated at four regimes from week 6 to week 16. Flower buds emerged after week 14.

Treatment	Height (cm)						
	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week18
				Biochar			
0%	8.2 b[z]	14.4 b	18.0 a	22.6 a	27.8 ab	35.3 a	41.0 a
20%	8.9 a	15.8 a	19.5 a	23.7 a	29.5 a	36.2 a	42.6 a
40%	8.5 ab	15.0 ab	19.2 a	23.7 a	29.4 a	36.3 a	43.0 a
60%	7.9 bc	14.1 b	18.4 a	22.8 a	27.9 ab	35.4 a	41.5 a
80%	7.4 c	12.9 c	16.6 b	20.4 b	26.0 b	33.9 a	39.8 a
Significance							
Fertigation	NS	NS	NS	NS	NS	NS	NS
Biochar	***	***	***	***	***	NS	NS
Biochar x Fertigation	NS	NS	NS	NS	NS	NS	NS

[z] Means within a column followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$.

[y] NS (nonsignificant) or significant at $P \leq 0.001$ (***).

Table 15. Analysis of variance (ANOVA) table showing number of days before full bloom (NFB), the number of flowers (NF), flower dry weight (FDW), leaf dry weight (LDW), stem dry weight (SDW), total shoot dry weight (TSDW = FDW+LDW+SDW), number of leaves (NL), total stem length (TSL), length of stem with brown leaf (LSB), length of stem with yellow leaf (LSY), length of stem with green leaf (LSG), the sum of LSB and LSY, and the ratio of LSG/TSL of Easter lily grown in root substrate amended with five different percentages of biochar and fertigated at four different regimes.

	NFB	NF	FDW	LDW	SDW	TSDW	NL	TSL	LSB	LSY	LSG	LSB+LSY	LSG/TSL
Fertigation	NS[z]	NS	NS	***	NS	NS	NS	NS	***	***	NS	NS	NS
Biochar	NS	NS	NS	NS	NS	NS	NS	**	**	**	NS	***	**
Biochar x Fertigation	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] NS (nonsignificant) or significant at $P \leq 0.01$ (**), or 0.001 (***).

Table 16. The leaf dry weight (LDW) of Easter lily grown in Sunshine Mix #1 amended with five different percentages of biochar and fertigated at four regimes. All data were collected at 18 weeks after bulbs were potted.

Fertigation	LDW (g)
200 mg•L^{-1}/3rd watering	3.7 b^{z}
300 mg•L^{-1}/3rd watering	3.8 b
200 mg•L^{-1}	4.2 a
300 mg•L^{-1}	4.3 a

^{z} Means within a column followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at $P = 0.05$

3.4.3 Plant quality

Fertigation regime significantly affected length of stems with brown leaves (LSB) and length of stems with yellow leaves (LSY) but did not affect total stem length (TSL), sum of LSB and LSY, or the ratio of length of stem with green leaf and total stem length (LSG/TSL) (Table 15). Easter lily plants with constant feeding (200 mg•L^{-1} N or 300 mg•L^{-1} N at every watering) had lower LSB, but higher LSY (Table 17). Biochar percentage significantly affected TSL, LSB, LSY, the sum of LSB and LSY, the ratio of LSG/ TSL (Table 15). There was no significant difference for TSL among 0%, 20%, 40%, and 60% biochar, or among 0%, 60%, and 80% biochar (Table 17). The increase of biochar percentage tended to improve Easter lily visual quality by reducing the percentage of leaf chlorosis, as reflected in a significant linear correlation of LSG/TLS and biochar percentages ($r^2=0.1027$, $P<0.0001$).

Higher biochar percentage in root substrate increased root substrate air space (21.5% for peat-based substrate, and 35.7% for biochar), which could contribute to higher LSG/TLS and better root growth of Easter lily since the production of Easter lily requires well-drained and aerated root substrate (Dole and Wilkins, 2005). The higher LSG/TLS in root substrate with high biochar percentage may also be due to higher nutrient holding capacity of biochar (Downie et al., 2009; Altland and Locke, 2012) since lower leaf chlorosis would be caused by nutrient deficiencies (Nelson, 2012). Some of the other causes of leaf chlorosis and death of lower leaves are: root injury, root loss (due to high soluble salt levels, insufficient medium aeration, overwatering, and root rot), insufficient light at the base of the plant and high EC level (3.5 $dS{\cdot}m^{-1}$ for saturated paste extract or 2.0 $dS{\cdot}m^{-1}$ for 2:1 method) (Dole and Wilkins, 2005). Erwin (2014) suggested that leaf chlorosis may be caused by perlite in the peat-based root substrate that contains fluoride. The leaf yellowing in Easter lily during greenhouse production could be prevented by applying growth regulator solutions containing gibberellins 4 and 7 (GA_{4+7}), or benzyladenine (BA) combining with GA_{4+7} (Han, 2000). Application of the commercial growth regulator Fascination (Valent, Sumitomo Chemical Co., Ltd, Canada), which contains 1.8% BA and 1.8% GA_{4+7} by weight, to the lower leaves immediately prior to and after the visible bud date could prevent lower leaf yellowing and leaf abscission (Erwin, 2014).

SPAD readings were significantly affected by fertigation regime (Table 18). Plants with constant fertigation had higher SPAD readings than those with fertigation at every third watering. Plants irrigated with 300 $mg{\bullet}L^{-1}$ N at every third watering had a slight higher SPAD reading than plants irrigated with 200 $mg{\bullet}L^{-1}$ N at every third watering.

Leaf nitrogen concentration, which was affected by fertilizer concentrations, had a strong correlation with SPAD readings (Gaborcik 2003; Li et al., 1998; Neilsen et al., 1995; Sibley et al., 1996). Biochar percentage had no effect on Easter lily SPAD reading, number of days before full bloom, number of flowers, flower dry weight, stem dry weight, leaf dry weight, total shoot dry weight (a sum of flower dry weight, leaf dry weight, and stem dry weight), number of leaves, length of stem with green leaf (Table 15), or leaf gas exchange parameters, indicating that lower leachate EC at higher biochar percentage (80%) had no effect on Easter lily plant quality.

Unlike the previous study of poinsettia growth and development responses to root substrate with biochar, Easter lily growth and flowering was not affected by the percentage of biochar used in substrate. This may be caused by the different characteristics of plants since the biochar used in these two experiments was the same. Easter lily might be less sensitive to the lower container capacity than poinsettia, and thus were less affected by biochar percentage. Steiner and Harttung (2014) reported the average height of mini sunflower was not affected by different percentages of biochar (25-100% by volume), though lower fresh weights were observed for plants grown in 50% and 100% biochar. Zhang et al. (2013) reported no change in yield when using biochar as an alternative root substrate in greenhouse production of cucumber, tomato and pepper. Tian et al. (2014) reported that compared to 0% biochar, Calathea grown in 100% biochar had smaller total dry weight, and those grown in 50% had greater total dry weight. The difference between these experiments may be caused by the difference of the species, characteristics of the tested plants, and the different root substrate characteristics due to the type and particle size of biochar. Therefore, further experimentation is required to determine the optimum biochar type and particle size for other horticultural crops.

Table 17. Total stem length (TSL), length of stem with brown leaf (LSB), length of stem with yellow leaf (LSY), the sum of LSB and LSY, and the ratio of LSG/TSL of Easter lily grown in Sunshine Mix #1 amended with five different percentages of biochar and fertilized at four fertigation regimes. All data were collected at 18 weeks after bulb were potted.

Treatment	TSL	LSB	LSY	LSB+LSY	LSG/TSL (%)
			Fertigation		
200 mg•L^{-1}/3rd watering	26.1 a^{z}	6.0 a	4.7 b	10.7 a	58.9 a
300 mg•L^{-1}/3rd watering	25.8 a	5.7 a	4.5 b	10.1 a	60.3 a
200 mg•L^{-1}	27.4 a	4.8 b	6.7 a	11.5 a	58.0 a
300 mg•L^{-1}	27.7 a	4.7 b	6.8 a	11.5 a	58.6 a
			Biochar		
0%	26.7 ab	5.6 ab	5.3 ab	10.9 ab	58.6 abc
20%	27.7 a	5.9 a	6.4 a	12.3 a	54.8 c
40%	27.5 a	5.5 ab	6.6 a	12.1 a	59.1 bc
60%	26.5 ab	4.5 c	5.5 ab	10.0 bc	62.3 ab
80%	24.6 b	4.9 bc	3.8 b	8.7 c	64.4 a

^{z} Means within a column under each main factor followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

Table 18. SPAD reading at Week 15, 16, and 17 of Easter lily grown in Sunshine Mix #1 amended with five different percentages of biochar and fertilized at four fertigation regimes.

	SPAD		
Treatment	Week 15	Week 16	Week 17
	Fertigation		
200 mg•L^{-1}/3rd watering	48.7 b[z]	54.0 c	52.1 c
300 mg•L^{-1}/3rd watering	50.7 ab	55.2 bc	55.8 b
200 mg•L^{-1}	52.5 a	56.7 ab	58.7 a
300 mg•L^{-1}	52.4 a	58.1 a	59.7 a
Significance			
Fertigation	***[y]	***	***
Biochar	NS	NS	NS
Biochar x Fertigation	NS	NS	NS

[z] Means within a column followed by the same letter are not significantly different according to Student-Newman-Keuls multiple comparison at *P* = 0.05.

[y] NS (nonsignificant) or significant at $P \leq 0.01$ (**), or 0.001 (***).

3.5 Conclusion

The results of this experiment indicated that peat-based root substrate amended with 80% biochar had no noticeable effects on Easter lily plant quality. Root substrate with 80% biochar had lower leachate electrical conductivity, and shorter plant stems compared to 20% and 40% biochar, yet these differences did not affect Easter lily quality. In addition, high biochar percentage (80%) could reduce plant leaf chlorosis more than the other root substrate treatments.

Biochar is a renewable byproduct from pyrolysis, a method for bio-energy production. Replacing peatmoss with biochar can protect the peatland environment, add value as a byproduct of bio-energy production, and thus make greenhouse production more environmentally friendly. In addition, biochar is a suitable alternative root substrate since it is weed-, pathogen-, and insect-free due to high temperatures used during the pyrolysis process. Since the characteristics of biochar are largely dependent on its source and pyrolysis methods, further experiments are required to investigate other horticultural crops with various types and particle size of biochar.

CHAPTER IV

SUMMARY OF FINDINGS

- The physical properties, total porosity, container capacity, air space, and bulk density of the root substrate amended with biochar at various percentages were generally within the recommended range for greenhouse production. The total porosity of root substrate with 20% biochar was slightly higher than the recommended range.
- Substrates with biochar had lower leachate electrical conductivity (EC) during the first two weeks of the poinsettia experiment. Higher percentage of biochar (80%) caused a lower leachate EC value compared to other biochar treatments (0% to 60%) from the beginning to the end of the Easter lily experiment. However, the lower leachate EC phenomenon in these two experiments did not affect plant growth and development.
- EC, dry weight, and SPAD readings increased as fertilizer concentration or fertigation frequency increased in both experiments.
- Poinsettia grown in 20% biochar had higher shoot dry weight. Plants grown in 60% or 80% biochar had smaller dry weight than those grown in 0% biochar, yet this reduction had no effect on poinsettia final visual rating and plant growth index. The 100% biochar treatment suppressed poinsettia growth in terms of plant growth index, dry weight, the total number of leaves and total red bracts, and root final visual rating.

- High fertigation concentration (400 mg•L^{-1} - 500 mg•L^{-1} N) combined with high percentage of biochar (100%) increased the susceptibility of plants to root rot and bracts necrosis, which significantly reduced the market value of poinsettias.
- Root substrate with 80% biochar resulted in shorter total stem length and shorter plant height of Easter lily until week 12, yet the differences in plant height were not significant after flower buds emerged.
- High biochar percentage (80%) reduced plant leaf chlorosis compared to other root substrate treatments. Neither biochar or fertigation regime had significant effects on number of days before full bloom, number of flowers, flower dry weight, stem dry weight, total shoot dry weight, number of leaves, length of stem with green leaf, or leaf gas exchange parameters (photosynthetic rate, stomatal conductance, and transpiration rate) of Easter lily at week 15 and week 17.
- In summary, peat-based root substrate (Sunshine Mix #1) amended with 80% biochar could be used in poinsettias and Easter lily greenhouse production. In addition, the 100-200 mg•L^{-1} N was suitable for poinsettia plants production.

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APPENDIX

Poinsettia weekly pH of root substrate amended with biochar at different percentages from week 1 to week 15.

Root substrate leachate pH				
		Week 1		
Biochar	Fertigation 1	Fertigation 2	Fertigation 3	Fertigation 4
0%	6.5 ± 0.2	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.2
20%	6.3 ± 0.2	6.3 ± 0.1	6.2 ± 0.0	6.2 ± 0.1
40%	6.5 ± 0.2	6.3 ± 0.2	6.5 ± 0.2	6.4 ± 0.2
60%	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	6.7 ± 0.2
80%	7.0 ± 0.2	7.0 ± 0.3	7.0 ± 0.2	7.0 ± 0.2
100%	7.0 ± 0.5	7.5 ± 0.1	7.5 ± 0.2	7.4 ± 0.1
		Week 2		
0%	6.5 ± 0.0	6.5 ± 0.1	6.3 ± 0.1	6.6 ± 0.0
20%	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.2	6.4 ± 0.1
40%	6.5 ± 0.1	6.5 ± 0.2	6.5 ± 0.1	6.5 ± 0.1
60%	6.6 ± 0.0	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.0
80%	6.6 ± 0.0	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
100%	6.8 ± 0.1	6.7 ± 0.7	6.7 ± 0.1	6.6 ± 0.1
		Week 3		
0%	6.6 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
20%	6.6 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
40%	6.7 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
60%	6.6 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.1 ± 0.1
80%	6.6 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
100%	6.5 ± 0.2	6.4 ± 0.1	6.1 ± 0.1	5.9 ± 0.0
		Week 4		
0%	6.3 ± 0.1	6.2 ± 0.1	5.9 ± 0.1	6.0 ± 0.1
20%	6.4 ± 0.1	6.2 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
40%	6.5 ± 0.1	6.2 ± 0.2	5.9 ± 0.1	5.8 ± 0.0
60%	6.5 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	5.8 ± 0.1
80%	6.5 ± 0.2	6.1 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
100%	6.4 ± 0.0	6.1 ± 0.1	5.9 ± 0.0	5.7 ± 0.2
		Week 5		
0%	6.5 ± 0.0	6.2 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
20%	6.5 ± 0.1	6.2 ± 0.2	5.9 ± 0.1	5.8 ± 0.1
40%	6.6 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	5.8 ± 0.1

60%	6.5 ± 0.0	6.2 ± 0.1	5.8 ± 0.2	5.8 ± 0.2
80%	6.5 ± 0.0	6.2 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
100%	6.3 ± 0.1	6.0 ± 0.0	5.7 ± 0.1	5.6 ± 0.1
		Week 6		
0%	6.4 ± 0.0	6.0 ± 0.1	5.7 ± 0.1	5.7 ± 0.2
20%	6.5 ± 0.0	6.0 ± 0.2	5.7 ± 0.0	5.7 ± 0.1
40%	6.4 ± 0.1	5.9 ± 0.1	5.8 ± 0.2	5.5 ± 0.2
60%	6.5 ± 0.1	6.0 ± 0.2	5.6 ± 0.2	5.7 ± 0.1
80%	6.3 ± 0.1	6.0 ± 0.0	5.7 ± 0.1	5.7 ± 0.1
100%	6.1 ± 0.1	5.9 ± 0.0	5.6 ± 0.2	5.3 ± 0.2
		Week 7		
0%	6.5 ± 0.2	5.8 ± 0.2	5.6 ± 0.1	5.4 ± 0.2
20%	6.5 ± 0.2	5.9 ± 0.2	5.5 ± 0.2	5.4 ± 0.2
40%	6.4 ± 0.2	5.8 ± 0.2	5.5 ± 0.2	5.2 ± 0.1
60%	6.4 ± 0.2	5.7 ± 0.1	5.4 ± 0.2	5.3 ± 0.2
80%	6.2 ± 0.5	5.8 ± 0.2	5.4 ± 0.1	5.4 ± 0.1
100%	6.1 ± 0.2	5.7 ± 0.1	5.4 ± 0.0	5.2 ± 0.2
		Week 8		
0%	6.1 ± 0.0	5.9 ± 0.1	5.6 ± 0.1	5.5 ± 0.0
20%	6.1 ± 0.1	5.8 ± 0.0	5.6 ± 0.1	5.5 ± 0.2
40%	6.1 ± 0.1	5.7 ± 0.0	5.6 ± 0.0	5.4 ± 0.1
60%	6.0 ± 0.1	5.7 ± 0.0	5.5 ± 0.2	5.4 ± 0.0
80%	5.9 ± 0.1	5.8 ± 0.2	5.3 ± 0.1	5.3 ± 0.1
100%	5.6 ± 0.1	5.3 ± 0.1	5.2 ± 0.2	4.9 ± 0.2
		Week 9		
0%	6.1 ± 0.0	5.7 ± 0.0	5.4 ± 0.2	5.2 ± 0.1
20%	6.0 ± 0.0	5.8 ± 0.0	5.4 ± 0.3	5.2 ± 0.1
40%	5.9 ± 0.2	5.5 ± 0.1	5.6 ± 0.2	5.3 ± 0.2
60%	5.9 ± 0.1	5.6 ± 0.2	5.2 ± 0.1	5.1 ± 0.0
80%	5.8 ± 0.1	5.4 ± 0.2	5.2 ± 0.2	5.1 ± 0.2
100%	5.5 ± 0.1	5.5 ± 0.0	5.0 ± 0.2	4.9 ± 0.4
		Week 10		
0%	6.3 ± 0.1	5.8 ± 0.2	5.3 ± 0.1	5.3 ± 0.1
20%	6.2 ± 0.1	5.8 ± 0.2	5.3 ± 0.0	5.0 ± 0.1
40%	6.2 ± 0.1	5.6 ± 0.1	5.2 ± 0.1	5.0 ± 0.2
60%	6.0 ± 0.1	5.7 ± 0.2	5.1 ± 0.0	5.1 ± 0.0
80%	5.9 ± 0.1	5.5 ± 0.2	5.1 ± 0.1	5.0 ± 0.1
100%	5.7 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	4.9 ± 0.1
		Week 11		

0%	6.2 ± 0.2	5.7 ± 0.1	5.3 ± 0.2	5.3 ± 0.0
20%	6.1 ± 0.2	5.7 ± 0.1	5.2 ± 0.2	5.1 ± 0.1
40%	6.1 ± 0.2	5.6 ± 0.2	5.5 ± 0.2	5.3 ± 0.2
60%	6.0 ± 0.1	5.6 ± 0.2	5.2 ± 0.1	5.1 ± 0.1
80%	5.9 ± 0.1	5.5 ± 0.2	5.1 ± 0.2	5.1 ± 0.1
100%	5.6 ± 0.1	5.3 ± 0.2	5.0 ± 0.1	5.0 ± 0.3
Week 12				
0%	6.4 ± 0.1	5.9 ± 0.1	5.4 ± 0.2	5.3 ± 0.3
20%	6.4 ± 0.2	5.8 ± 0.2	5.3 ± 0.1	5.0 ± 0.2
40%	6.3 ± 0.1	5.9 ± 0.1	5.4 ± 0.1	5.1 ± 0.2
60%	6.1 ± 0.1	5.6 ± 0.1	5.2 ± 0.1	5.1 ± 0.0
80%	6.0 ± 0.2	5.5 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
100%	5.8 ± 0.2	5.7 ± 0.3	5.2 ± 0.1	5.0 ± 0.1
Week 13				
0%	6.5 ± 0.1	6.4 ± 0.3	5.7 ± 0.2	5.5 ± 0.3
20%	6.4 ± 0.1	6.0 ± 0.0	5.5 ± 0.0	5.0 ± 0.2
40%	6.4 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	5.1 ± 0.2
60%	6.2 ± 0.1	5.9 ± 0.1	5.5 ± 0.2	5.0 ± 0.1
80%	5.9 ± 0.1	5.6 ± 0.3	5.4 ± 0.2	5.1 ± 0.1
100%	6.0 ± 0.2	5.6 ± 0.1	5.4 ± 0.2	5.1 ± 0.3
Week 14				
0%	6.8 ± 0.2	6.7 ± 0.2	6.2 ± 0.1	6.3 ± 0.3
20%	6.8 ± 0.1	6.6 ± 0.3	6.1 ± 0.1	6.0 ± 0.4
40%	6.7 ± 0.1	6.5 ± 0.2	6.3 ± 0.2	5.9 ± 0.2
60%	6.5 ± 0.1	6.5 ± 0.4	6.2 ± 0.3	6.1 ± 0.1
80%	6.4 ± 0.1	6.5 ± 0.3	5.9 ± 0.0	6.2 ± 0.1
100%	6.5 ± 0.1	6.4 ± 0.2	6.3 ± 0.2	6.6 ± 0.1
Week 15				
0%	7.7 ± 0.1	7.7 ± 0.1	6.9 ± 0.1	6.6 ± 0.3
20%	7.6 ± 0.1	7.4 ± 0.2	6.7 ± 0.3	6.3 ± 0.1
40%	7.7 ± 0.1	7.3 ± 0.1	6.9 ± 0.3	6.3 ± 0.1
60%	7.8 ± 0.2	7.3 ± 0.2	7.0 ± 0.5	6.4 ± 0.1
80%	7.7 ± 0.2	7.5 ± 0.2	6.7 ± 0.2	6.7 ± 0.2
100%	7.7 ± 0.1	7.5 ± 0.1	7.2 ± 0.3	6.8 ± 02