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RESEARCH ARTICLE

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Use of biochar as peat substitute for growing substrates of *Euphorbia* × *lomi* potted plants

Vincenzo Dispenza[1], Claudio De Pasquale[1], Giancarlo Fascella[2], Michele M. Mammano[2] and Giuseppe Alonzo[1]

[1] Università degli Studi di Palermo, Dipartimento SAF, Viale delle Scienze Edif. 4, 90128 Palermo. Italy. [2] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Unità di Ricerca per il Recupero e la Valorizzazione delle Specie Floricole Mediterranee. S.S. 113 – Km 245.500, 90011 Bagheria (PA). Italy

Abstract

Biochar from conifers wood was used in soilless culture as growing substrate alternative to peat for ornamental crops. Potted plants of *Euphorbia* × *lomi* Rauh cv. 'Ilaria' were grown with different mixtures (v:v) of brown peat and biochar in order to evaluate main physical and chemical characteristics of this biomaterial as well as its effect on plant growth, ornamental characteristics and nutrients uptake. Biochar addition to peat increased pH, EC and K content of the growing substrates, as well as air content and bulk density. Biochar content of substrates significantly affected plant growth and biomass partitioning: higher number of shoots and leaves, leaf area and leaf dry weight were recorded in plants grown in 40% peat-60% biochar, with respect to plants grown in 100% peat and secondarily in 100% biochar. Leaf chlorophyll content was higher in plants grown in 60% and 80% biochar, while biomass water use efficiency was higher with 60% biochar. Plant uptake of K and Ca increased as biochar content of the substrates increased. Hence, a growing substrate containing 40% brown peat and 60% conifers wood biochar was identified as the more suitable mixture allowing to have a high-quality production of *Euphorbia* × *lomi* potted plants.

Additional key words: charcoal; growing media; ornamentals; peat reduction; plant growth; soilless culture.

Abbreviations used: CEC (cation exchange capacity); DMRT (Duncan's multiple range test); EC (electrical conductivity); LA (leaf area); RGR (Relative growth rate); R/S (root to shoot ratio); WUE (biomass water use efficiency).

Authors' contributions: Conceived and designed the experiments, analyzed the data and wrote the paper: GF and CDeP. Performed the experiments: GF and VD. Contributed reagents/materials/analysis tools: MMM and GA.

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Correspondence should be addressed to Giancarlo Fascella: giancarlo.fascella@crea.gov.it

Introduction

Peat is one of the main substrate components for nursery production of horticultural crops in containers because of its suitable physical properties, such as low bulk density and high total porosity, and its high nutrients exchange capacity (Bustamante *et al.*, 2008; Li *et al.*, 2009). Peat is obtained from wetlands, highly fragile ecosystems which constitute important CO_2 sinks. The increasing use of peat in horticulture has resulted in a fast depletion of wetlands determining the loss of a non-renewable resource (Maher *et al.*, 2008) and generating an increase of the price. Therefore, there is a great environmental concern in order to limit peat extraction while it is required to find a sustainable substitute.

Numerous researches on peat alternatives have been recently conducted (Chong, 2005; Li *et al.*, 2009). Different authors have suggested that some organic materials such as tree bark, sawdust, sewage sludge, municipal solid waste and agro-industrial waste, after composting could be used as partial peat substitute (Guerrero *et al.*, 2002; Benito *et al.*, 2005). Composts have physical and chemical properties superior or similar to peat because of their higher nutrients availability, not excessive water content and optimum porosity (Sanchez-Monedero *et al.*, 2004). In contrast, the percentage of compost used for potting substrates must be carefully determined to avoid negative effects (high soluble salts content, presence of heavy metals) on plant growth (Garcia-Gomez *et al.*, 2002; Ostos *et al.*, 2008).

Biochar is fine-grained and porous charcoal produced after 300-500°C biomass pyrolysis under partial exclusion of oxygen. It may be added to soils to improve soil quality because of the C fundamental role in chemical, biological and physical processes, while also reducing atmospheric CO_2 emissions (Glaser *et al.*, 2002; Lehmann, 2007; Laird, 2008). Soil application of biochar has many environmental advantages over the use of organic manures or compost, it is a porous material with a high inner surface area which helps to retain more water and increase saturated hydraulic conductivity of soils (Asai *et al.*, 2009). Biochar used as soil amendment can increase soil fertility and crop yield (Van Zwieten *et al.*, 2008), also showing high levels of micro-organic activities (Fowles, 2007). It can improve soils physical structure (Chan *et al.*, 2007) and may also modify soil hydraulic properties (Gaskin *et al.*, 2010). Because of the relatively fixed pore sizes, biochar application increases available moisture in sandy soils and decreases moisture availability in clay soils. Biochar has been found to decrease nutrients leaching thus enhancing their availability (Yamato *et al.*, 2006). Furthermore, its cation exchange capacity (CEC) is consistently higher than that of the whole soil (Lehmann *et al.*, 2003; Liang *et al.*, 2006). Some studies (Rondon *et al.*, 2007; Van Zwieten *et al.*, 2008) attributed the positive plant response to the effects of biochar on nutrients availability as well as to its capacity to increase or maintain the pH of soil, through liming. Changes in soil porosity and size aggregate distribution following biochar applications promote soil structure modifications, leading also to ameliorations of many other chemical-physical properties such as electrical conductivity (EC), CEC, pH, and water holding capacity (De Pasquale *et al.*, 2012; Ouyang *et al.*, 2013) that have a fundamental role in the standardization of substrate for greenhouse crops. Up to now several researches on biochar agricultural use have been focused on its application on soil, few studies were conducted in containers (Altland & Locke, 2013; Vaughn *et al.*, 2013; Street *et al.*, 2014; Zaccheo *et al.*, 2014), even less regarded its utilization as growing substrate for ornamental potted plants (Tian *et al.*, 2012; Zhang *et al.*, 2014).

The present study concerns the use of biochar produced by a pyrolysis process of conifers wood as component of potting substrates for *Euphorbia* × *lomi* Rauh, an ornamental succulent shrub belonging to the Spurge family and usually cultivated as flowering plant (Fascella & Zizzo, 2009). Few studies regarding the cultivation of *Euphorbia* × *lomi* in peat substitutes have been carried out (De Lucia *et al.*, 2008). The aims of this work were to evaluate the main physical and chemical characteristics of growing substrates composted with decreasing content of brown peat and increasing percentages of conifers wood biochar, and to observe the effects of these substrates on growth, ornamental features and nutrients uptake of *Euphorbia* × *lomi* potted plants.

Material and methods

Greenhouse facilities and plant material

The experiment was conducted in 2012, in an unheated (28°C day/14°C night) single-span EW oriented greenhouse (25 × 8 m) with steel structure and polyethylene cover (thickness 0.15 mm), located at the Research Unit for Mediterranean Flower Species near Palermo (38° 5’ N, -13° 30’ E, 23 m above sea level), on the North Western Sicily coastal area.

Micropropagated plants of *Euphorbia* × *lomi* cv. ‘Ilaria’ were grown in plastic pots of 13 cm diameter (vol. 1 L) filled with different mixtures (v:v) of brown peat and conifers wood biochar (100% peat [P100], 80% peat-20% biochar [BC20], 60% peat-40% biochar [BC40], 40% peat-60% biochar [BC60], 20% peat-80% biochar [BC80], 100% biochar [BC100], respectively). The six growing substrates were prepared by mixing different volumes of pelletized biochar (sieved at 5 mm-mesh) and sphagnum peat (0–3 mm sized, H3 decomposition degree, pH 4.3). The two components were thoroughly but gently blended by hand in order to limit breakage of biochar particles, then mixed with 2 L of water and air dried.

Water, macro and micronutrients were supplied to plants through a drip fertigation system (1 dripper/plant, 2 L) controlled by a computer. All plants were fed with the same nutrient solution which had the following composition (mg/L): 180 N (nitrate + ammonium), 50 P, 200 K, 120 Ca, 30 Mg, 1.2 Fe, 0.2 Cu, 0.2 Zn, 0.3 Mn, 0.2 B. The EC of the nutrient solution was maintained at 1.8 mS/cm and when exceeded this threshold, water was added to the fresh nutrient solution in order to restore the EC value to the original starting point. The pH of the nutrient solution was maintained between 5.8 and 6.2 by adding nitric acid. Irrigation scheduling was performed using electronic low-tension tensiometers (Tensioswitch, Tensio-Technik, Germany) that control irrigation based on substrate matric potential. Tensiometers were installed at the midpoint of different pots in order to supply a representative reading of the moisture tension. Tensiometers were connected to an electronic programmer that controlled the beginning (-5 kPa) and the end of irrigation (-1 kPa), which correspond to high and low substrate water potential set points. The leaching fraction of the six substrates after each irrigation varied in a range from 10% (P100) to 30% (BC100) of the supplied water; this fraction was calculated by collecting the drainage solutions.

Chemical and physical characteristics of growing substrates

The used biochar was derived from mechanically chipped trunks and large branches of silver fir (*Abies alba* M.), larch (*Larix decidua* Mill.), spruce (*Picea excelsa* L.), black pine (*Pinus nigra* A.) and Scots pine (*Pinus sylvestris* L.) pyrolysed at 450°C for 48 h. As a direct product of forestry, the feedstock was free of contaminants such as metal, plastic, rubber, stones and pollutant compounds. Main chemical and physical characteristics of the tested substrates were analyzed and are reported on Table 1 and Table 2, respectively. The pH was determined with a pH-meter (GLP 21, Crison, Italy) in the settling suspension on a 60 g sample mixed with 300 mL of deionised water, after shaking for 60 min at room temperature (22°C), while the EC was measured on the same water extract (1:5 v:v) with a conductivity-meter (HI 4321, Hanna Instruments, Italy). Total nitrogen (N) content was determined by dry combustion using an elemental analyzer (Carlo Erba Instruments, Italy); correction for the ash content was obtained by loss on ignition at 600°C in an electric muffle furnace. Total contents of P, K, Ca, Mg and Na were determined using 0.2 g of dry sample (105°C for 24 h) after acid digestion in a microwave oven (CEM Mars Xpress, USA); substrate digests were filtered, diluted and analyzed by atomic absorption spectrometry (Perkin-Elmer AAnalyst 200, USA).

For the determination of main physical properties, the sample was water saturated into a doubled ring and equilibrated on a sand box at -10 cm water pressure head. After equilibration, the physical properties were calculated from the wet and dry weights of the sample in the lower ring. Water content at two water pressure heads (1 and 10 kPA, corresponding to 10 and 100 cm height of the water column needed to give these tension values) was determined by drying the sample at 105°C for 24 h and measuring pressure from the middle of the lower ring. Particle density of the sample was calculated from organic matter and ash content, considering a density of organic matter of 1550 kg/m^3 and a density of ash of 2650 kg/m^3. Dry bulk density was determined by dividing sample mass by the volume of the lower ring. Total porosity was calculated according the formula: Total porosity = 1.1 − (Bulk density/Particle density).

One-way analysis of variance (ANOVA) was used to analyze differences among the six growing substrates (treatments) for chemical and physical characteristics. When ANOVA was significant, treatment means were separated with the Duncan's multiple range test (DMRT) at the 0.05 significance level.

Plant measurements and data analysis

Ten plants per treatment were harvested every 30 days and separated into stems, leaves and roots for growth measurements. In order to extract roots contained in the substrates limiting breakage and loss, roots entangled in substrate aggregates were first soaked in water; the soaked sample was then carefully passed through a mesh sieve (0.50 mm) and substrate particles caught by the sieve were discarded. Dry weight of the biomass was determined after 72 h in a forced-air oven (at 100°C) when harvested tissues reached a constant value. Plant height was determined as the distance from the surface of the substrate to the top of the plant. Stem diameter was measured at 5 cm above the substrate using an electronic caliper. Root length was determined as the distance from the base of the stem and the end of the longest root. Root to shoot ratio (R/S) was calculated by dividing root dry weight by the sum of leaf and stem dry weights. Leaf area (LA) was measured using a digital area meter (WinDIAS 2; DELTA-T DEVICES Ltd, Cambridge, UK).

Table 1. Chemical characteristics of the growing substrates as affected by biochar content.

Substrates[1]	pH	EC [2]	N [3]	P	K	Ca	Mg	Na
P100	5.7±0.1c	12±0.2c	103.1±1.7a	29.3±0.3a	102.5±1.2b	105.0±1.3a	37.5±0.4a	19.5±0.6a
BC20	6.4±0.3bc	15±0.6c	15.9±0.5b	18.6±0.5b	115.0±1.6b	67.5±0.8b	18.0±0.3b	15.5±0.5b
BC40	6.7±0.1b	16±0.4c	24.5±0.2b	16.5±0.1b	130.6±1.1a	50.2±0.2b	18.0±0.1b	14.0±0.2b
BC60	7.3±0.4ab	24±0.5b	30.4±0.4b	14.9±0.2b	132.5±1.9a	43.0±0.2b	15.0±0.7b	11.5±0.1bc
BC80	7.9±0.3a	25±0.2b	31.2±0.2b	4.6±0.2c	135.2±1.7a	17.1±0.3c	6.5±0.4c	9.5±0.3c
BC100	8.5±0.2a	36±0.1a	36.9±0.6b	3.8±0.1c	140.0±1.3a	15.5±0.1c	5.5±0.5c	7.0±0.4c
Significance	*	**	**	*	ns	**	**	*

[1]P100: 100% peat; BC20: 80% peat-20% biochar; BC40: 60% peat-40% biochar; BC60: 40% peat-60% biochar; BC80: 20% peat-80% biochar; BC100: 100% biochar. [2]EC values expressed in mS/m. [3]Nutrient ions content expressed in mg/L. Values are means ± SE. In any column, means followed by different letters are significant at $p<0.05$ (DMRT). ns, *, ** = non-significant, significant at $p<0.05$ and 0.01 respectively.

Table 2. Physical characteristics of the growing substrates as affected by biochar content.

Substrates[1]	Water content (% v:v) at:		Air content (% v:v) at:		Total porosity (% v:v)	Particle density (g/L)	Bulk density (g/L)
	1 kPa	10 kPa	1 kPa	10 kPa			
P100	78.7±0.3a	38.7±0.4a	12.7±0.2b	52.0±0.3a	89.8±0.1a	1605±2.2d	318±0.6d
BC20	76.3±0.3a	38.9±0.1a	10.5±0.3b	47.9±0.4ab	90.0±0.3a	1630±1.9cd	350±0.9cd
BC40	68.5±0.5b	38.3±0.1a	12.3±0.1b	42.3±0.2b	90.1±0.2a	1670±2.0c	419±1.1c
BC60	57.5±0.2c	34.8±0.2ab	22.6±0.1a	45.4±0.1ab	90.6±0.1a	1780±1.7b	485±1.4bc
BC80	53.2±0.1cd	33.4±0.3ab	22.2±0.2a	42.0±0.3b	91.7±0.4a	1830±1.8ab	525±1.2b
BC100	49.1±0.4d	29.3±0.5b	26.4±0.6a	46.1±0.2ab	92.2±0.5a	1860±1.8a	642±0.8a
Significance	*	*	*	ns	ns	*	*

[1] For growing substrates: see Table 1. Values are means ± SE. In any column, means followed by different letters are significant at $p<0.05$ (DMRT). ns, * = non-significant and significant at $p<0.05$ respectively.

Relative growth rate (RGR), which is considered the most widely used way of estimating the speed of plant growth by measuring the mass increase per total dry biomass produced per day, was calculated according to the formula proposed by Hoffmann & Poorter (2002): RGR = (lnW2-lnW1)/(t2-t1) where ln = natural logarithm; W1, W2 = dry weight of plant at time 1 and 2, respectively (in grams); t1, t2 = time 1 and time 2, respectively (in days). Dry weight of plants at the beginning of the experiment was the same for all treatments (0.86 g). Biomass water use efficiency (WUE), which is the ratio of water used in plant metabolism for producing biomass to water lost by plants through transpiration, was calculated as the ratio between total dry weight of plants and plants total water supply.

Leaf chlorophyll content (expressed as SPAD index) of three randomly selected fully expanded leaves of all plants in each treatment was measured with a chlorophyll meter (SPAD 502, Minolta Sensing Inc., Osaka, Japan). Leaf color was determined with a shot in the middle of the blade on three leaves of all plants of each treatment with a colorimeter (Minolta CR10, Konica Minolta Inc., Osaka, Japan) that calculated the color coordinates (CIELAB): lightness (L), a (redness) and b (yellowness); L ranges from 0 (completely opaque or black) to 100 (completely transparent or white); a varies from positive (redness) to negative (greenness) values, as well as b (positive is yellowness, negative is blueness).

The experiment was concluded six months after planting (from April 1st to October 31th 2012), when potted plants grew to commercial size. Each of the six growing substrates (treatments) was replicated three times and each replication consisted of 20 potted plants, reaching a total of 360 potted plants (6 treatments × 3 replications × 20 plants) arranged in a completely randomized design on the benches in the greenhouse. One-way analysis of variance (ANOVA) was used to determine how substrates affected plant growth and quality; when ANOVA was significant, means of treatments were compared using the *post-hoc* DMRT at 5% of probability by using the package Statistica (Statsoft Inc., Tulsa, OK, USA).

Nutrients uptake

The metal ions residues in each plant organ (leaf, stem and root) were determined through atomic absorption spectroscopy using a Shimadzu AA-6300 (Milan, Italy) with flame atomization. A CEM Microwave Accelerated Reaction System (Bergamo, Italy) was used for the digestion, following the procedure described in Tranchina *et al.* (2008). Trace metal grade nitric and perchloric acids were used with 30% hydrogen peroxide for the digestion of the vegetable samples. Nutrients uptake was determined only for the treatments containing at least 40% biochar (40% [BC40], 60% [BC60], 80% [BC80] and 100% biochar [BC100]) because of the lower values for plant growth and ornamental features recorded with the remaining two treatments (0% [P100] and 20% biochar [BC20]). Collected data on nutrients uptake in the six substrates were subjected to one-way ANOVA and, when significant, treatment means were separated with DMRT at $p\leq0.05$.

Results

Chemical and physical characteristics of growing substrates

Chemical characteristics of the growing substrates was affected by the addition of conifers wood biochar as pH increased (from 5.7 to 8.5) with the increase of biochar content (Table 1), as well as EC (from 12 to 36 mS/m for 100% peat and 100% biochar, respectively). Higher values of N, Ca and Mg were recorded in the substrate with 100% peat. The N content, since biochar usually contains very low amounts of N, did not significantly vary among biochar-amended substrates (from BC20 to BC100). P content decreased (from 29.3 to 3.8 mg/L) with higher levels of biochar, whereas K content increased (from 102.5 to 140.0 mg/L) (Table 1). Na was higher (19.5 mg/L) in 100% peat and lower (7.0 mg/L) in the 100% biochar.

Physical characteristics of the growing substrates was affected by biochar addition as a decrease of the water content at 1 kPa (from 78.7 to 49.1% v:v) was recorded together with the increase of biochar content (Table 2), while at 10 kPa the differences were less evident (from 38.9 to 29.3% v:v). An increase of air content at 1 kPa was observed by increasing biochar content of the substrates, with lower values (10.5% v/v) measured with 20% biochar, whereas limited differences were recorded at 10 kPa averaging 45.9% v:v across the six treatments (Table 2). No significant differences among treatments were recorded as regards total porosity (average 90.7%) (Table 2). Particle density and bulk density increased with the increase of biochar content in the growing substrates, ranging respectively from 1605 to 1860 g/L and from 318 to 642 g/L.

Plant growth and biomass yield

Plant height was not significantly influenced by growing substrates as an average value of 12.8 cm was recorded irrespective of the treatment (Table 3). Stem diameter increased with higher content of biochar as thicker stems (1.8 and 2.0 cm) were obtained in plants grown with 60% and 80% biochar, respectively, while lower value (1.2 cm) was recorded with 100% peat (Table 3). Biochar content of the growing substrates significantly affected also leaves production and leaf area as higher number of leaves (61.1/plant) and higher areas (897.3 cm^2) were measured in plants grown with 60% biochar; lower values of both parameters were recorded with 100% peat (29.3 leaves/plant and 426.2 cm^2, respectively) (Table 3). With regard to shoots production, higher number of shoots (5.9/plant) was obtained in plants grown in the substrate containing 60% biochar, whereas lower production (2.2 shoots/plant) was achieved in plants with 100% peat (Table 3). Root length was also affected by growing substrates as longer roots (average 16.4 cm) were recorded in plants grown with 60% biochar or more. Root to shoot ratio was significantly influenced by growing substrates as higher ratios were measured in plants grown with 80% and 100% biochar (0.54 and 0.58, respectively), whereas lower R/S (0.22) was recorded with 100% peat (Table 3). As regards water use efficiency, higher value was measured in plants grown in the substrate with 60% biochar (1.1 g/L) and lower WUE was obtained with 100% peat (0.29 g/L) (Table 3).

Leaf chlorophyll content was also influenced by biochar content of the growing substrates as higher SPAD indexes were recorded in plants grown with 60% and 80% biochar (54.7 and 53.1, respectively) and lower values (42.6) with 100% peat (Table 4). Leaf color, expressed through the three CIELAB coordinates, was affected by the substrates composition as lightness (L) increased with the increase of biochar percentage in the substrates (from 35.6 to 44.3), yellowness (b) was higher with 80% biochar (24.6) while no significant differences for redness (a) were observed among treatments (average -14.4) (Table 4). These variations among substrates in the SPAD values and color coordinates of leaves correspond to more intense green leaves in plants grown with 60% biochar perceived by the consumers, thus to a higher ornamental and commercial value.

Dry biomass yield was significantly affected by biochar content of growing substrates as higher total biomass were recorded in plants grown with 60% and 80% biochar (60.8 and 51.1 g, respectively), whereas lower values were observed with 0% and 20% biochar (20.8 and 22.4 g, respectively) (Fig. 1). With regard to the biomass partitioning, higher dry weight of leaves was measured in plants grown with 60% biochar (35.0 g), while lower weight was achieved with 100% peat

Table 3. Effect of biochar content in the growing substrates on plant height, stem diameter, leaves and shoots production, leaf area, root length, root to shoot ratio and biomass water use efficiency (WUE) of *Euphorbia* × *lomi* potted plants.

Substrates[1]	Plant height (cm)	Stem diameter (cm)	Leaves (no.)	Leaf area (cm^2)	Shoots (no.)	Root length (cm)	Root/shoot	WUE
P100	10.8±0.3b	1.2±0.04b	29.3±0.5c	426.2±1.2d	2.2±0.03d	11.7±0.4b	0.22±0.01c	0.29±0.02c
BC20	11.2±0.4b	1.4±0.02b	39.8±0.3bc	465.3±1.5cd	3.1±0.05c	12.8±0.1b	0.34±0.03bc	0.33±0.01c
BC40	13.9±0.2a	1.8±0.02a	44.8±0.4b	568.5±1.4c	4.6±0.04b	13.0±0.3b	0.39±0.02bc	0.64±0.05b
BC60	14.7±0.3a	2.0±0.05a	61.1±0.6a	897.3±2.0a	5.9±0.10a	16.9±0.2a	0.46±0.04b	1.1±0.07a
BC80	14.0±0.5a	1.7±0.01ab	46.1±0.2b	736.8±1.7b	4.1±0.04b	16.4±0.3a	0.54±0.03a	0.85±0.03ab
BC100	12.1±0.1b	1.6±0.03ab	41.9±0.2bc	519.9±1.3cd	2.9±0.06c	15.8±0.5a	0.58±0.02a	0.40±0.03bc
Significance	ns	*	*	*	*	*	*	**

[1] For growing substrates: see Table 1. Values are means ± SE. In any column, means followed by different letters are significant at $p<0.05$ (DMRT). ns, *, ** = non-significant, significant at $p<0.05$ and 0.01 respectively.

Table 4. Effect of biochar content in the growing substrates on leaf chlorophyll content (SPAD index) and leaf color coordinates (lightness, redness, yellowness) of *Euphorbia* × *lomi* potted plants.

Substrates[1]	SPAD	Lightness (L)	Redness (a)	Yellowness (b)
P100	42.6±0.4b	35.6±0.2b	-13.1±0.2a	17.4±0.1b
BC20	44.3±0.2b	36.2±0.2b	-13.3±0.1a	18.5±0.3b
BC40	47.7±0.3b	37.4±0.1b	-14.8±0.3a	19.7±0.2ab
BC60	54.7±0.5a	36.0±0.3b	-16.5±0.2a	20.5±0.4ab
BC80	53.1±0.6a	40.1±0.2ab	-15.6±0.1a	24.6±0.5a
BC100	46.4±0.2 b	44.3±0.4a	-13.0±0.1a	17.8±0.2b
Significance	*	*	ns	*

[1]For growing substrates: see Table 1. Values are means ± SE. In any column, means followed by different letters are significant at $p<0.05$ (DMRT). ns, * = non-significant and significant at $p<0.05$ respectively.

(7.1 g) (Fig. 1); no significant differences were recorded among treatments as regards stem dry weight (average 11.6 g) and root dry weight (average 7.5 g).

Biochar content of the growing substrates significantly influenced RGR of potted *Euphorbia* × *lomi* as higher RGR values (from 1.6 to 4.0 g/g day) were observed in plants grown with 60% biochar throughout the experiment, lower rates (from 0.4 to 1.8 g/g day) were recorded with 100% peat (Fig. 2).

Nutrients uptake

An increase of K content was measured in leaves (from 17.7 to 21.6 mg/g), stem (from 12.6 to 17.0 mg/g) and root (from 11.3 to 13.8 mg/g) together with the increase of biochar content in the growing substrates (Table 5). Ca content increased in leaves (from 8.6 to 10.9 mg/g) and stem (from 15.2 to 19.3 mg/g) only, together with the increase of biochar percentage in the substrates. A decrease of Mg (from a maximum of 4.8 mg/g in stem to a minimum of 2.4 mg/g in root), Fe (from 716.9 µg/g in root to 80.1 µg/g in stem) and Mn (from 46.2 µg/g in leaves to 24.4 µg/g in stem) content in plant tissues were progressively recorded from the 40% to the 100% biochar (Table 5). In contrast, Zn (from a minimum of 1.8 mg/g in root to a maximum of 6.8 mg/g in leaves), Na and Cu content evidenced an increase in leaves, stem and root as biochar percentage in the growing substrates increased.

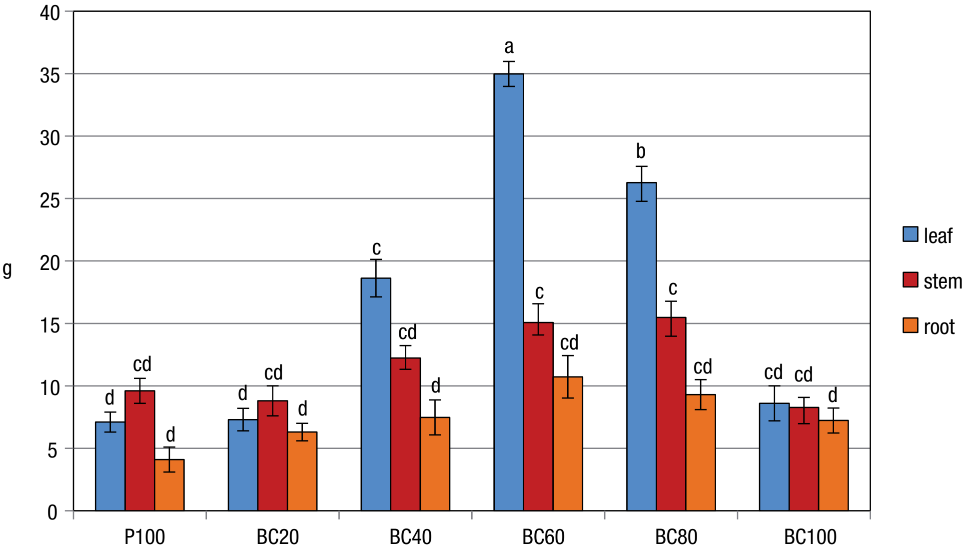

Figure 1. Effect of biochar content in the growing substrates on dry biomass partitioning of *Euphorbia* × *lomi* potted plants measured for each plant organ (leaf, stem and root). P100: 100% peat; BC20: 80% peat-20% biochar; BC40: 60% peat-40% biochar; BC60: 40% peat-60% biochar; BC80: 20% peat-80% biochar; BC100: 100% biochar. Vertical bars are means ± SE. Different letters are significant at $p<0.05$ (DMRT).

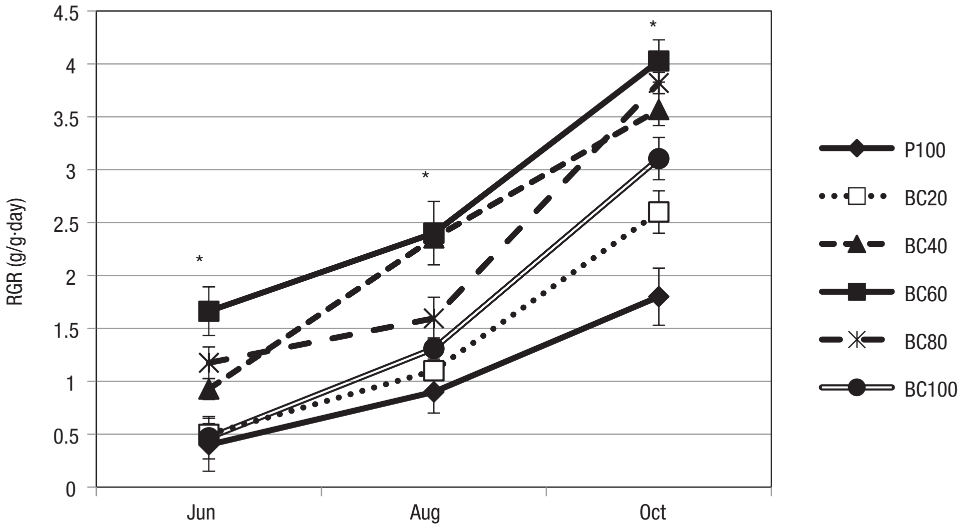

Figure 2. Effect of biochar content in the growing substrates on Relative Growth Rate (RGR) of *Euphorbia × lomi* potted plants throughout the experiment [April (time 0)-October 2012]. P100: 100% peat; BC20: 80% peat-20% biochar; BC40: 60% peat-40% biochar; BC60: 40% peat-60% biochar; BC80: 20% peat-80% biochar; BC100: 100% biochar. Values are means ± SE. * = significant at $p<0.05$ (DMRT).

Discussion

Chemical and physical characteristics of growing substrates

Results from our experiment showed an increase of pH, EC and K as well as a decrease of P of the growing substrates with the increase of conifers wood biochar content (Table 1). Our outcomes on chemical characterization of the substrates are in line with those from Tian *et al.* (2012) who reported lower pH values in a peat-based substrate than in green waste biochar and in their mixture. Karami *et al.* (2011) referred that available P was lower in a soil amended with biochar than in green waste compost-amended soil. Altland & Locke (2013), in a study on the impact of biochar amendment of sphagnum peat:perlite on nutrients retention and leaching, reported that increasing levels of biochar will add a substantial quantity of K to the substrate and should be accounted for in fertility programs though representing a modest source of P for ornamental plant production. Zaccheo *et al.* (2014)

Table 5. Effect of biochar content in the growing substrates on macro and micronutrients content in leaf, stem and root of *Euphorbia × lomi* potted plants.

Substrates[1]	K [2]	Ca	Mg	Zn	Na	Fe [3]	Cu	Mn
				Leaf				
BC40	17.7±0.2b	8.6±0.4b	4.0±0.03a	2.0±0.02c	1.5±0.03c	202.1±1.4a	6.3±0.1c	46.2±0.3a
BC60	18.4±0.1b	9.9±0.1b	3.8±0.04a	4.0±0.04b	1.8±0.02c	97.2±0.9b	10.5±0.4b	36.8±0.2b
BC80	20.1±0.3ab	10.6±0.2a	3.7±0.01a	4.7±0.03b	2.9±0.05b	99.3±1.0b	13.4±0.3a	27.1±0.1c
BC100	21.6±0.2a	10.9±0.1a	3.4±0.02b	6.8±0.06a	5.3±0.08a	80.3±1.2b	15.3±0.3a	25.0±0.1c
				Stem				
BC40	12.6±0.3b	15.2±0.2c	4.8±0.04a	0.1 a	0.8±0.02c	169.6±1.1a	6.9±0.2b	45.1±0.5a
BC60	13.3±0.1b	17.3±0.3b	4.0±0.06a	0.1 a	2.7±0.04b	142.8±0.8ab	7.1±0.1b	37.6±0.2b
BC80	16.2±0.5a	17.9±0.1b	3.5±0.02b	0.1 a	3.7±0.02b	123.5±0.9b	8.5±0.1ab	33.0±0.3b
BC100	17.0±0.6a	19.3±0.4a	3.2±0.05b	0.1 a	5.5±0.06a	80.1±0.8c	10.4±0.3a	24.4±0.1c
				Root				
BC40	11.5±0.1b	6.8±0.3a	2.9±0.02a	1.8±0.03b	5.6±0.02c	716.9±2.3a	20.7±0.6b	44.4±0.4a
BC60	11.3±0.2b	5.7±0.2b	3.2±0.01a	2.0±0.01b	5.9±0.01c	670.0±1.8a	21.8±0.3b	37.3±0.2b
BC80	11.6±0.2b	4.3±0.1c	2.6±0.03b	2.0±0.02b	7.0±0.03b	625.6±1.9b	21.9±0.2b	35.7±0.2b
BC100	13.8±0.4a	4.6±0.2c	2.4±0.02b	2.7±0.05a	8.7±0.07a	443.6±1.5c	33.0±0.8a	27.4±0.1c

[1] For growing substrates: see Table 1. [2] K, Ca, Mg, Zn and Na content expressed in mg/g. [3] Fe, Cu and Mn content expressed in µg/g. Values are means ± SE. In any column of each plant organ, means followed by different letters are significant at $p<0.05$ (DMRT).

reported an effectiveness of an alkaline pine wood biochar in correcting the acidity of a white peat substrate. Bedussi *et al.* (2015) observed that spruce wood biochar amendment to peat allowed the maintenance of stable and high levels of K in the pore water, both in the root free substrate and in the rhizosphere.

The present experiment evidenced that increasing biochar percentage of the growing substrates corresponds, as expected by adding a highly porous material, to a decrease of water content and an increase of air content and bulk density (Table 2). The reduction in water content of a substrate generally corresponds (according to its physical properties) to a diminution in the available water, that is the amount of water that can be stored in the substrate and be easily absorbed by plant roots with consequent effects on plant growth and quality. Our results on physical characteristics of the substrates agree with those from Tian *et al.* (2012) who referred that the addition of green waste biochar to a peat-based substrate tended to increase the bulk density of the mixture as well as the percentage of 0.25–2.00 mm particle-size fraction optimal for plant growth as retaining sufficient water and also providing adequate gas exchanges. Zaccheo *et al.* (2014) showed that additional benefits of pine wood biochar include improving substrates physical properties such as an increase in air content, a reduction in water availability and a lower shrinkage, suggesting that biochar can be used in place of lime in peat-based growing media. Vaughn *et al.* (2013) referred that, in a study for replacement of peat moss in soilless substrates used for containerized crops, wheat straw and wood biochars had significantly higher pHs, ECs and bulk densities than peat. Mendez *et al.* (2015) observed that addition of biochar improved the chemical and hydrophysical properties of peat-based growing media by increasing air space, water holding capacity and total porosity. Nieto *et al.* (2016) reported an increase of air space volume of peat based growing substrates after addition with commercial charcoal and pruning waste biochar but only the latter lead to adequate water holding capacity and porosity values. Conte *et al.* (2013) studying water-saturated poplar biochar suggested that water molecules are bound to the solid carbonaceous material through nonconventional hydrogen bonds: the comprehension of water-biochar interactions is fundamental to understand the molecular mechanisms through which water can be drained into biochar-amended substrates affecting their physical-chemical properties.

Plant growth and biomass yield

Our outcomes showed that higher plant growth and biomass production were recorded with high (60-80%) biochar content in the substrates, followed by a decline with 100% biochar (Table 3, Figs. 1-2). The plant growth and biomass increases observed in the mixture with 60% biochar are probably related to more equilibrate water content and available water for plants, to the improve of air condition and structure with respect to peat as well as to the neutral pH, higher K content and lower EC and Na values. Lehmann & Rondon (2006) reported an improvement of soil structure and water capacities after biochar addition. Lehmann *et al.* (2003) referred that biochar can retain high amounts of exchange cations because of its high porosity and surface/volume ratio and can improve plant nutrients uptake and availability (Yamato *et al.*, 2006; Chan *et al.*, 2007). The formation of surface functional groups and adsorption sites on biochar may affect its CEC (Liang *et al.*, 2006); therefore, the increased *Euphorbia* × *lomi* growth might be due to a higher nutrients availability of the substrates containing adequate amounts (60%) of biochar as the carbonization process creates a fine-grained, highly porous charcoal that helps soil retain nutrients and water (Laird, 2008).

Best growing conditions and performances of plants potted with 60% biochar is supported by higher water use efficiency, expressed as the ratio of biomass production to water use. Highest WUE of BC60 is mainly related to the fact that the major part of plant biomass was allocated in the leaves of this treatment; this higher allocation is associated to the greater leaves production and leaf area recorded with this substrate (Table 3, Figs. 1-2).

Positive influence of biochar amendment on plant growth and quality is also confirmed by higher SPAD index and leaf color of Euphorbias grown with 60-80% biochar (Table 4), two parameters which are strictly correlated to the plant nutritional status. The increases in leaf SPAD and color are most likely linked to a better availability in the substrates of macro (K) and micronutrients (Fe, Mn, Zn) that play a fundamental role in the biosynthesis of chlorophyll and other pigments involved in the photosynthetic activity (Netto *et al.,* 2005).

Beneficial effects of biochar addition on increasing growth, biomass production and pigments formation have been previously described by other authors. Tian *et al.* (2012) referred that mixing green waste biochar with peat provided a better physical environment (and an increased release of nutrients) for pot cultivation of *Calathea rotundifola* than biochar alone and peat alone, so plant and leaf biomass as well leaf area were significantly higher in the mixture. Vaughn *et al.* (2013) reported that addition of wheat straw and wood biochars (at rates of 5%, 10% and 15%) on peat-based substrate for potted marigolds significantly increased plant heights in all treatments but the 5% wood biochar.

Zhang *et al.* (2014) observed that, using different percentages of biochar and green-waste compost on potted *Calathea insignis*, highest plant growth and photosynthetic pigments were achieved when the compost was amended with 20% biochar whereas the lowest values were obtained with nonamended compost.

Results from Baronti *et al.* (2010) showed that a 1.7% coppiced woodland-biochar application to soil caused the maximum dry matter production in potted perennial ryegrass, due to an overall amelioration of growth condition, but above this threshold a general reduction of biomass was observed, probably because of changes in some soil properties. Lower performances of *Euphorbia* × *lomi* grown in the substrate containing 100% biochar are probably due to the deterioration of chemical (alkaline pH, higher EC and Na) and physical conditions (lower water content and its availability for plants, excessive air content); this response is in line with that from Rondon *et al.* (2007) who observed that the addition of high rate of Eucalyptus wood biochar to a poor soil in a pot experiment resulted in a decrease of crop yield probably due to a micronutrient deficiency induced by increasing soil pH.

Nutrients uptake

Our results evidenced an increase of some cations (K, Ca, Zn, Na and Cu) and a decrease of other ions (Mg, Fe and Mn) in plant tissues as the biochar content in the growing substrates increased (Table 5). Greater growth and quality response of Euphorbias grown with 60% biochar could be better explained by the more equilibrate concentration of metal ion residues in each plant organ of this treatment with respect to peat and biochar alone. Higher amount of K and some micronutrients usually involved in the photosynthetic process on leaves of 60% biochar-plants seem to confirm higher leaf chlorophyll content (SPAD) and biomass production recorded in this treatment.

Biochar has been previously shown to result in high concentrations of available nutrients that support increases in nutrients content of plant tissues (Lehmann & Rondon, 2006). Lehman *et al.* (2003) explained higher nutrients availability by biochar addition with its greater nutrients retention and changes in soil microbial dynamics. Altland & Locke (2013) reported that wood biochar amendment resulted in more leached K than the control substrate (sphagnum peat:perlite), but relatively little compared with rice hull biochar and sawdust biochar amendments. Street *et al.* (2014), studying the effects of green waste biochar amendment on apple rootstock in pot trials, observed that the addition of biochar was associated with significant increases in leaf concentrations of Ca for plants grown in sand. Finally, Zhang *et al.* (2014) referred that nutrients content in *Calathea insignis* leaves significantly increased when plants were grown in media containing composted green waste and 20% coir biochar. However, outcomes available in literature suggest a wide variability on plants uptake of macro and micronutrients depending on the feedstocks used in the pyrolysis process for producing biochar.

In conclusion, the results presented show that the growing substrate containing 60% of conifers wood biochar had a positive effect, in terms of number of shoots and leaves, leaf area, root length, leaf chlorophyll content and color as well as total dry biomass and leaf dry weight, on the growth and ornamental value of *Euphorbia* × *lomi* potted plants. The specific combination of peat and biochar had a synergistic effect with a greater efficacy for enhancing quality of this hybrid. Positive plant response may be due to the favorable physical-chemical characteristics of this particular mixture, showing a pH close to the neutrality, a moderate EC, an equilibrate nutrients content as well as adequate water and air contents. In contrast, lower performances recorded in plants grown with 100% peat and secondarily with 100% biochar may be linked to deep modifications of main properties, so creating suboptimal conditions, of the substrate by the lower/higher rate of biochar applied. Therefore, its application rate depends on substrate types and crops. In fact, though our results seem to confirm beneficial effects of biochar addition to the growing medium, application rates have to be carefully assessed in order to avoid negative effects. This study is an attempt to evaluate the potentiality of conifers wood biochar as substrate component for sustainable floriculture, even if other researches are necessary to observe the effects of this by-product coming from different feedstocks when combined with other growing media and ornamental species.

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