| 1  | Effects of partial substitution of commercial substrates for the production of   |
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| 2  | Camellia sasanqua  |
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| 4  | J.A. Oliveira <sup>1*</sup> , E. Afif <sup>2</sup> , F. Martínez <sup>3</sup> , F.J. Estrada <sup>4</sup> and P. Palencia <sup>1</sup> |
| 5  | <sup>1</sup> Plant Production Area. Department of Organisms and System Biology, Polytechnic School                                     |
| 6  | of Mieres, Oviedo University, Mieres, 33600 Asturias, Spain.   |
| 7  | <sup>2</sup> Agroforestry Area. Department of Organisms and System Biology, Polytechnic School of                                      |
| 8  | Mieres, Oviedo University, 33600 Mieres, Asturias, Spain.  |
| 9  | <sup>3</sup> Agroforestry Department, E.T.S.I. 'La Rabida', Huelva University, Palos de la Frontera,                                   |
| 10 | 21819 Huelva, Spain.   |
| 11 | <sup>4</sup> Plantas del EO Nursery, Finca Riufelle s/n, 32760 Castropol, Asturias, Spain.   |
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| 13 | *Corresponding author's E-mail: <u>oliveira@uniovi.es.</u>   |
| 14 |  |
| 15 | ABSTRACT   |
| 16 | Commercial substrates comprising different proportions of peat moss and fermented pine bark  |
| 17 | and used for container production of ornamental plants are becoming expensive because they   |
| 18 | are not produced locally. The objective of this study was to determine whether shredded,   |
| 19 | sieved, locally produced switchgrass (Panicum virgatum L.) could at least partly replace   |

20 commercial substrates. Five test substrates were prepared with the following proportions, by

volume, of commercial substrates/switchgrass: 100/0, 75/25, 50/50, 25/75, and 0/100. Pots

- 22 were filled with the substrates, planted with the camellia (*Camellia sasanqua* Thunb.)
- 23 'Rainbow' and placed in a polyethylene greenhouse. The plants and substrates were
- 24 monitored for four months (23 April 28 August) in 2014. Shredded, sieved (5 mm)
- 25 switchgrass substrate by itself does not provide physical properties considered ideal for

container camellia plant production. The tallest plants were those grown in the substrates 26 27 containing between 0 and 50% Switchgrass, possibly because of the good values for water holding capacity, total porosity and air-filled porosity of those blends. The density of roots 28 29 decreased as the proportion of Switchgrass in the substrate increased. Switchgrass substrate 30 can be used as a substrate component for commercial container production of camellia plants 31 over 4 months, when mixed in a proportion of no more than 50% by volume with a 32 commercial substrate comprising peat moss and fermented pine bark. Nevertheless, more 33 research is required with different plants in order to confirm the results obtained so far.

34

35 Keywords: Biomass, *Camellia sasanqua*, nursery production, ornamental plants, *Panicum* 36 *virgatum*

37

## 38 INTRODUCTION

39 Camellia sasanqua Thunb. are a group of evergreen shrubs native to Asia (Sánchez de 40 Lorenzo, 18). This group of fall blooming camellias are gaining popularity for their versatility 41 and ease to grow (Green, 6). Greenhouse production of camellias involves growing the plants in containers with a substrate comprising different proportions of peat moss, fermented pine 42 bark and sometimes small amounts of other components. However, peat moss and pine bark 43 44 materials are becoming expensive because they are not produced locally. In the search for 45 alternative materials for use as nursery container substrates, different herbaceous energy crops have been evaluated (Altland and Krause, 2; Altland, 1; Altland and Locke, 3). 46 47 Of the many species of herbaceous perennial graminaceous species that could potentially be grown as bioenergy crops (Sanderson *et al.*, 19), only miscanthus (*Miscanthus* x giganteus) 48 49 and switchgrass (Panicum virgatum L.) have been considered for making potting substrates

50 (Jackson and Wright, 7). On the other hand, rice husk and hazelnut shells were found to be

suitable alternatives to peat for the cultivation of *Camellia japonica* (Larcher and Scariot, 10;
Larcher et *al.*, 9).

Switchgrass is a graminaceous species native to the North American continent, ranging from 53 54 northern Mexico to Canada. It is adapted to subtropical and cold temperate climates and is grown as a herbaceous summer crop. It is drought tolerant and displays a high potential for 55 biomass production under diverse soil and climate conditions (Parrish and Fike, 15). 56 57 In a two-year-long trial of six switchgrass cultivars carried out in Carreño (Asturias, NW Spain), an average yield of 13.4 t DM/ha and year was obtained (Oliveira et al., 14). 58 The objective of this study was to determine whether shredded, sieved switchgrass could 59 60 supplement the commercial substrate used for container production of ornamental camellia plants. 61

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## 63 MATERIAL AND METHODS

The switchgrass cultivar 'Kanlow' (SG) was obtained from a field trial carried out in Candás 64 (43° 35' 03.95'' N, 5° 46' 56.32'' W elevation, 77 m, Spain) and established on an Inceptisol 65 Typic Dystrudept soil (Principado de Asturias, 16) under a temperate oceanic climate (Rivas-66 Martinez, 17). The experimental trial was carried out in the "Plantas del EO" nursery (a 67 producer of forest and ornamental plants) in Castropol, Asturias (43° 31' 25.28'' N, 7° 00' 68 69 45.87" W, elevation, 16 m, Spain). The SG was harvested at the end of February 2014 and processed in an electric shredder 70 (Viking GE355: Power 2.500 W) before being passed through a sieve of 5 mm mesh size to 71 72 obtain the material used to make the substrates.

The commercial substrate (CS) routinely used in the nursery is composed of 70% peat moss
(fibrous, particle size 20-40 mm, pH 5.5), 30% fermented pine bark, and clay (bulk density 40

75 kg/m<sup>3</sup>).

76 The CS and SG were mixed in five different proportions, by volume, to make the test

77 substrates (CS/SG): 100/0, 75/25, 50/50, 25/75 and 0/100.

Controlled release fertilizer (14N-5.9P-10.8K, Osmocote<sup>®</sup>, KB, Scotts France SAS, Bourth,
France), effective for 6 months, was incorporated into the substrate at a rate of 4 kg/m<sup>3</sup>.
Rooted cuttings of 'Rainbow' camellia (obtained in the nursery from cuttings taken in autumn
2013) were planted in black polyethylene containers (2.5 l) filled with 2.5 l of the different
substrates.

83 The containers were placed in a polyethylene greenhouse without temperature or light control,

in March 2014. The containers were watered throughout the trial by micro-spray irrigation,

for 20 minutes daily in summer and twice weekly in spring, with the aim of maintaining the

86 pots at field capacity.

87 Ten individual plants (one cutting per container) were considered as replicates for each

treatment (substrate mixture) and the containers were placed randomly in the greenhouse.

89 The physical and chemical characterization of the substrate mixtures was carried out

90 following the methods proposed respectively by Ansorena (4). The following parameters were

91 determined in triplicate: bulk density and real density, air-filled porosity and total porosity,

92 water holding capacity, EC (ds/m) and pH (measured with a SevenMulti<sup>™</sup>

93 pH/Conductivity/Ion meter Mettler Toledo<sup>®</sup>). All measurements were made in the

94 Agroforestry Engineering Laboratory, University of Oviedo.

95 The first plant reached commercial size and quality 4 months after planting the cuttings. At

96 this point, the response variables of the camellia plants in the different substrate mixtures

97 were measured and recorded: final height of the plants, fresh and dry weights of the aerial

98 plant parts and density of roots in the root ball.

99 The final height of all of the plants was measured from the surface of the substrate to the apex

100 of the plant. The fresh and dry weights of the aerial plant parts were determined in 3 plants

101 per treatment, after respectively cutting and drying the material in a forced air oven at 70°C to 102 dry weight. The density of roots in the root ball was determined in all plants, on a scale of 0-103 10, where 0 = no roots observed on the exterior surface of the root ball and 10 = root ball 104 totally covered by roots.

105 At the end of the study, foliar analysis of 3 plants per treatment was carried out after first

106 washing the samples with deionized water and drying them at 70°C to constant weight. The

107 dried samples were ground in an ultracentrifuge mill ZM 100 Retsch<sup>®</sup> (Retsch GmbH & Co.

108 KG, Haan, Germany) and sieved (1 mm). Foliar analysis was carried out after wet extraction

109 with perchloric acid and nitric acid (JONES et al. 1991), followed by dilution of samples with

110 1N HCl. The extract was used to determine Ca, Mg and K by atomic adsorption spectroscopy

111 (PerkinElmer<sup>®</sup> AAnalyst<sup>TM</sup> 200, Shelton, CT, USA). The concentrations of P were

112 determined by colorimetric analysis (PerkinElmer<sup>®</sup> Lambda<sup>TM</sup> 35 UV/VIS

113 Spectrophotometer, Shelton, CT, USA) after combustion for 4 hours in a muffle furnace at

114 450°C, and dissolution of the ashes with 6 N HCl.

115 The N was determined in a PerkinElmer<sup>®</sup> spectrophotometer Lambda 35 UV/Vis (Shelton,

116 CT, USA) after mineralization of the samples in a Foss Tecator<sup>TM</sup> 2020 digester (Hillerød,

117 Denmark) with concentrated sulphuric acid at 350°C for 2 h, by the Kjeldahl method.

118 The data were analysed by analysis of variance (ANOVA), with the factor type of substrate (x

119 5 types) in a completely randomized design. When the ANOVA indicated the substrate factor

120 as significant, the LSD test for comparison of means was applied. Differences between means

121 were considered significant at  $P \le 0.05$ . All statistical analyses were carried out with SPSS v.

122 22.0.

123

## 124 **RESULTS AND DISCUSSION**

*Physical properties.* The physical properties of the five substrates were significantly different 125 126 for all parameters (Table 1). Bulk density and water holding capacity decreased and the air filled porosity increased as the percentage of SG increased. The total porosity and its 127 128 components, air-filled porosity and water holding capacity, are very important for container production of plants: if these physical properties are inadequate it would be difficult to modify them 129 130 once the crop has been established and therefore their prior characterization is necessary (Cabrera, 5). 131 Adequate values reported by some authors (Ansorena, 4; Yeager *et al.*, 21) are 60-80% (by 132 volume) for total porosity, 10-30% for air-filled porosity, and 40-60% for water holding capacity. On the basis of these values, only those substrates comprising 50% or less SG can 133 134 be considered adequate for water holding capacity and total porosity, whereas for air-filled porosity, only substrates with 25% or less SG are adequate. The bulk density of substrates 135 containing 50% or less SG was in the optimal range of 0.15 to 0.60 g/ml (Nappi, 13). Bulk 136 137 density values in the substrates with 75% or more SG were low and not suitable for potting substrates. 138

139 Chemical properties. The chemical parameters evaluated mainly affect the plants at 140 establishment, particularly pH and EC (Table 2). The pH has an important influence on the assimilation of nutrients, as it facilitates or hinders dissolution of these elements (Ansorena, 141 4). The pH of the different substrates did not differ significantly: the mean value was pH 5 at 142 143 the start and 5.5 at the end of the trial, indicating that the SG substrate tended to cause an 144 increase in pH when added to CS. Although the ideal pH depends on the type of crop, the values obtained in this study can be considered adequate (desirable pH between 5.0 and 6.5) 145 146 for this type of plant (Yeager et al., 21).

147 Non-significant differences were observed in the EC of the five substrates, and the values for
148 all substrates were within the usual range (Ansorena, 4). The EC of the substrates decreased
149 as the proportion of SG biomass increased.

150 *Plant growth.* Significant differences in the height of the plants were observed in relation to

151 the substrate (Table 3) and the tallest plants were those grown in the substrates containing

between 0 and 50% SG, possibly because of the good values for water holding capacity, total

153 porosity and air-filled porosity of those blends.

154 There were no significant differences between the substrates in the dry weight of the aerial

155 plant parts measured at the end of the study.

156 There were significant differences in the density of roots in the root ball of the plants grown in

157 the different substrates. The density of roots decreased as the proportion of SG in the substrate

158 increased. However, in the experiment carried out by Treder *et al.* (20) growing media did not

159 influence the dynamics and quality of plantlet rooting.

160 As reported in other studies evaluating energy crops as substrates for container production of

161 plants (Altland and Krause, 2; Altland, 1; Altland and Locke, 3; Locke and Altland, 11),

shredded, sieved (5 mm) switchgrass biomass mixed with at least 50% (by volume) of a

163 commercial substrate could be used for container production of ornamental plants to achieve

164 the proper ratio of air-filled porosity to water holding capacity.

165 Foliar nutrient contents. Regarding the foliar mineral contents in camellia plants grown in

166 different substrates, significant differences were only observed in the Mg contents, and the

167 values were highest in substrates containing 75% or more SG (Table 4).

168 There were no significant differences between the different substrates in the N, P, Ca, and K169 contents.

170 In the *Camellia sasanqua* plants, the foliar mineral contents were within the usual range for

171 Ca (6.9-14.6 g/kg) and lower than usual for N (13.9-35.4 g/kg), P (0.8-1.1 g/kg), Mg (1.4-2.8

172 g/kg) and K (6.8-11.1 g/kg), according to reports by Mills and Jones (12).

173 Switchgrass substrate can be used as a substrate component for commercial container

174 production of camellia plants over 4 months, when mixed in a proportion of no more than

175 50% by volume with a commercial substrate comprising peat moss and fermented pine bark.

176 Nevertheless, more research is required with different plants in order to confirm the results

177 obtained so far.

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Table 1. Comparison of physical properties of substrates composed of different proportions of
commercial substrate (CS) and shredded, sieved switchgrass (SG), measured at the start of the
study. Standard deviation in parentheses.

| Substrate   | Bulk density Water holding capacity |            | Air-filled   | Total porosity |  |
|-------------|-------------------------------------|------------|--------------|----------------|--|
|             | (g/ml)                              | (%)        | porosity (%) | (%)            |  |
| 100%CS+0%SG | 0.22(0.01)a <sup>z</sup>            | 58.3(1,4)a | 12.4(0.1)e   | 70.6(1.2)e     |  |
| 75%CS+25%SG | 0.21(0.01)a                         | 49.6(0.7)b | 25.2(0.8)d   | 74.8(0.1)d     |  |
| 50%CS+50%SG | 0.16(0.01)b                         | 41.7(0.3)c | 36.5(0.1)c   | 78.2(0.2)c     |  |
| 25%CS+75%SG | 0.14(0.01)c                         | 33.7(0.4)d | 48.5(0.4)b   | 82.2(0.1)b     |  |
| 0%CS+100%SG | 0.09(0.01)d                         | 25.1(1.6)e | 60.5(2.3)a   | 85.7(0.7)a     |  |

<sup>250</sup> <sup>z</sup>Mean values in the same column indicated by the same letter are not statistically different

251 (LSD,  $P \le 0.05$ )

| 264 | Table 2. Comparison of pH and EC (ds/m) of the substrate composed of different proportions    |
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| 265 | of commercial substrate (CS) and shredded, sieved switchgrass (SG), measured at the start and |
| 266 | at the end of the study. Standard deviation in parentheses.                                   |

| Substrate   | pHinitial | pHfinal  | ECinitial  | ECfinal    |
|-------------|-----------|----------|------------|------------|
| 100%CS+0%SG | 4.4(1.8)  | 5.1(0.5) | 0.08(0.01) | 0.22(0.08) |
| 75%CS+25%SG | 4.8(0.4)  | 5.2(0.1) | 0.07(0.02) | 0.15(0.08) |
| 50%CS+50%SG | 5.1(0.6)  | 5.7(0.4) | 0.06(0.03) | 0.09(0.04) |
| 25%CS+75%SG | 5.2(0.2)  | 5.8(0.2) | 0.03(0.02) | 0.07(0.01) |
| 0%CS+100%SG | 5.3(0.3)  | 5.8(0.4) | 0.01(0.01) | 0.05(0.02) |
|             |           |          |            |            |
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Table 3. Comparison of mean response variables in camellia plants grown in substrates composed of different proportions of commercial substrate (CS) and shredded, sieved switchgrass (SG), measured at the end of the study. Standard deviation in parentheses.

| Substrate Height of pla |                          | Dry weight of  | Density of roots |
|-------------------------|--------------------------|----------------|------------------|
|                         | (cm)                     | aerial portion | in root ball     |
|                         |                          | (g)            | (scale 0-10)     |
| 100%CS+0%SG             | 36.3(6.1)ab <sup>x</sup> | 6.6(2.7)       | 8.7(0.5)a        |
| 75%CS+25%SG             | 38.0(4.3)a               | 6.8(1.4)       | 7.6(0.5)b        |
| 50%CS+50%SG             | 36.6(2.5)ab              | 5.5(2.3)       | 6.5(0.5)c        |
| 25%CS+75%SG             | 33.6(3.1)b               | 3.0(1.2)       | 4.7(0.7)d        |
| 0%CS+100%SG             | 29,0(2.2)c               | 4.2(0.8)       | 2.5(0.5)e        |

<sup>x</sup>Mean values in the same column indicated by the same letter are not statistically different

285 (LSD P  $\leq$  0.05)

Table 4. Comparison of foliar nutrient levels in camellia plants grown in substrate composed
of different proportions of commercial substrate (CS) and shredded, sieved switchgrass (SG),
measured at the end of the study. Standard deviation in parentheses.

| Substrate   | Ν          | Р          | Ca          | Mg                 | K          |
|-------------|------------|------------|-------------|--------------------|------------|
|             | (g/kg)     | (g/kg)     | (g/kg)      | (g/kg)             | (g/kg)     |
| 100%CS+0%SG | 4.09(0.26) | 0.24(0.01) | 6.91(5.02)  | $1.14(0.02)bc^{y}$ | 4.36(0.12) |
| 75%CS+25%SG | 3.57(0.97) | 0.21(0.06) | 10.31(1.05) | 1.09(0.05)c        | 4.63(0.22) |
| 50%CS+50%SG | 3.08(0.24) | 0.18(0.01) | 9.67(0.92)  | 1.13(0.01)bc       | 4.40(0.08) |
| 25%CS+75%SG | 5.13(2.02) | 0.30(0.12) | 9.35(0.59)  | 1.22(0.02)a        | 4.32(0.13) |
| 0%CS+100%SG | 6.20(2.78) | 0.36(0.16) | 9.02(0.88)  | 1.19(0.04)ab       | 4.25(0.17) |

300 <sup>y</sup>Mean values in the same column indicated by the same letter are not statistically different 301 (LSD P  $\leq$  0.05)