Liner Propagation Method Influences Growth of Container Grown Bottlebrush

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Keywords. Callistemon viminalis, cuttings, growth index, nursery production, tissue culture

Abstract. Bottlebrush (Callistemon viminalis) is a widely propagated and cultivated ornamental large shrub with large red bottlebrush-like flowers. Traditional clonal propagation using stem cuttings may be replaced with tissue cultured liners. In this study, we established a container-grown field experiment of bottlebrush ‘Little John’ using liners propagated from both rooted stem cuttings and tissue culture. Growth index was recorded by propagation method periodically through the 34-week period, and both fresh and dry weights of roots and shoots recorded at experiment’s end. Final growth index of plants grown from tissue cultured liners were significantly greater than growth index of plants started from rooted stem cuttings. Both fresh and dry root weight means were significantly greater in plants propagated by tissue culture. Further testing of containerized bottlebrush production, through the flowering stage, will better determine whether tissue-cultured liners accelerate production time vs. liners from stem cuttings.

Tissue culture allows establishment of plant organs and tissues in aseptic culture, thereby, growing new plants (Davies et al. 2017). This form of propagation is an accelerated form of clonal propagation. It is the propagation method of choice to propagate plants that are slow-to-root or cannot be clonally propagated some other way. Tissue culture is a highly efficient method of vegetative propagation used in the breeding of many tree and shrub species (Hazubska-Przybyl and Bojarczuk 2008; Silva de Oliveira et al. 2015). However, propagation technique effectiveness can vary depending on species. Therefore, it would be wise to investigate a variety of techniques on each species to optimize the process (Hamill 2016).

Economically, tissue culture tends to be a more expensive form of propagation compared with traditional propagation methods (Espinosa-Leal et al. 2018). This is due to intensive labor, maintenance of laboratory conditions, and a greater technical expertise requirement of managers (Shokri et al. 2012). Evidence also exists that tissue-cultured (TC) propagules have greater vigor than do propagules of the same species derived from cuttings (Grout et al. 1986). Greater vigor can lead to a faster cropping time in the field compared with traditionally propagated plants over a 30- to 40-week time frame. A potential trade-off to the higher cost of a TC plant is less cropping time in the field. Bottlebrush (Callistemon viminalis) and related species have shown positive performance in vitro (Raj et al. 2010), meaning that growers may be able to reap the aforementioned benefits by electing to pay a higher price for TC plantlets in lieu of traditional cuttings. For this to occur, research must be conducted on the performance of both of these options in a nursery production setting.

Bottlebrush is native to the Australian continent and typically used as a medium to large shrub. In addition to being known for its showy red, bottlebrush-like flowers in spring, it serves important ecological functions; bottlebrush has demonstrated both saline soil and drought tolerance (University of Florida 2021). Pollinators also use the flowers as a source of nectary (Latif et al. 2016). In landscape design, bottlebrush can be considered a more sustainable plant for landscape management, requiring little care after establishment. A study by Curtis and Cowee (2007) had Nevada homeowners rank plant attributes in order of importance, and drought resistance out-ranked other characteristics. Drought resistance, along with the other aforementioned sustainable qualities, make bottlebrush a value-added product, shown to yield a higher willingness to pay from consumers.

Traditionally, nursery growers propagate bottlebrush as stem cuttings taken in summer (Abrameit B, personal communication; University of Florida 2013). TC bottlebrush may be an option to speed up the overall cropping cycle. For this plant with its growing popularity, the economic benefit of tissue culture and the faster cropping time remains unknown. Potential outcomes for an expedited cropping time could be saved production and labor inputs for the grower, as well as opening up nursery space for the next crop (Hamill 2016).

Our research focus is on characterizing effects of propagation method on production metrics such as cropping time, plant size, and plant health of bottlebrush ‘Little John’. The objective of this study was to compare growth parameters of potted bottlebrush from both TC and conventionally propagated liners.

Materials and methods

Establishment of experiment. Bottlebrush ‘Little John’ rooted liners were obtained from a nearby nursery grower in Nov 2019 and held in a greenhouse until the experiment began. The rooted liners consisted of one 50-cell tray grown from conventionally (CO) propagated stem cuttings, and TC-rooted liners in an established 72-cell tray were obtained from the same nursery. Liners of both propagation types were well rooted, with roots to the sides and bottom of the individual cell in the tray. This is a typical standard for determining the maturity and readiness for transplant. Protocols for propagating both types of liners were considered proprietary information by the nursery donating the liners. A 0.078% solution of alkyl

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dimethyl benzyl ammonium chloride (Green-Shield®, BASF, Research Triangle Park, NC, USA) was used to soak #3 nursery (2.7 gallon; Nursery Supplies, Inc., Kissimmee, FL, USA) containers for 30 min before filling with soilless substrate. Substrate was a composted bark-based substrate (Scotts Miracle-Gro Potting Soil®, The Scotts Company, Marysville, OH, USA). After filling nursery pots with substrate, all liners were installed on Nov 18 with one plant per pot and watered. All rooted liners (a total of 50 CO pots and 72 TC pots) were started, and a 32-week experiment time was predetermined. A substrate sample was obtained at experiment’s initiation and submitted to the Texas A&M AgriLife Extension Service Soil Testing Laboratory (College Station, TX, USA) for analysis. Analytes included pH, electrical conductivity (EC), macronutrients, iron, zinc, copper, manganese, and boron. Substrate pH and EC was determined using a hydrogen selective electrode (PH800 Laboratory PH Meter; Apera Instruments, Columbus, OH, USA). Substrate EC was determined using a conductivity probe (EC950 Conductivity Meter, Apera Instruments, Columbus, OH, USA).

**STUDY SITE.** This study took place at Sam Houston State University’s Agriculture Center. The location is lat. 30°70’34.7” N, long. 95°55’82.0” W, elevation 450 ft in Huntsville, TX, USA. The experiment was established and completed outdoors from 18 Nov 2019 to 29 Jun 2020. This location is US Department of Agriculture 2012 Plant Hardiness Zone 8b.

**MAINTENANCE OF EXPERIMENT.** Experimental units were watered by hand when the substrate was dry to the touch at 1” deep using City of Huntsville tap water (pH of ≈7.9 and alkalinity of ≈102 ppm). Leaves, stems, and root systems were inspected weekly to identify any potential pest. Plants were not pinched or trimmed during the experiment and were grown in full sun. An 18N-2.6P-10K (Osmocote®; ICL Specialty Fertilizers, Charleston, SC, USA) controlled-release fertilizer was top-dressed in each pot at a rate of 1 tablespoon per pot on 15 Jan 2020. On 27 Feb 2020, substrate samples were obtained from both CO and TC pots and submitted to the Texas A&M AgriLife Extension Service Soil Testing Laboratory for analysis. Analytes included pH, conductivity, macronutrients, iron, zinc, copper, manganese, and boron. Substrate for analysis was removed by hand from a range of 2 to 6 inches deep from the surface from randomly selected pots. A 0.39% solution of acephate (Orthene®, Ortho Co., Marysville, OH, USA) was sprayed onto stems and leaves of all plants on 29 Apr 2020 for a minor mealybug infestation.

**VARIABLES EVALUATED.** A growth index (GI) was calculated from the growth parameters of height, length, and width [GI = plant height/2 + (plant width1 + plant width2)/4 (Niu et al. 2007)] among all pots in both CO and TC. Height was measured adjacent to the stem and from top of the substrate to the tallest point on the plant. Length and width were measured perpendicular to each other at a point on the stem determined to be 50% of height. These growth parameters were measured at the end of weeks 4, 8, 12, 16, 24, and 32. During week 32, a destructive harvest took place to obtain fresh weights of the shoots/leaves and of the roots. Eight pots were randomly selected from both CO and TC pots for a destructive harvest. Each pot’s shoot/leaf system was removed at the crown and weight recorded for fresh weight. Each group of tissues was placed into paper bags, labeled appropriately, and then placed into a drying oven at 65°C (149 °F) for 7 d. Bags were then removed from the oven, and tissue systems were reweighed to obtain dry weights.

Week 32 substrate pH and EC were measured from the randomly selected pots before root elutriation. Substrate was obtained from a 2- to 6-inch depth in pots, removed and air-dried for 72 h in a 72 °F climate-controlled room before a 1:2 substrate-to-water (v:v) slurry was prepared to measure pH and EC.

**EXPERIMENTAL DESIGN AND DATA ANALYSIS.** Data analysis was performed using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA). The experiment was a randomized complete design with two treatments of rooted liners (CO and TC) having 48 CO replicates and 70 TC replicates. Mean separations for GI, pH, EC, and fresh and dry weights of shoots and roots were conducted using PROC TTEST with effects considered significant at $P \leq 0.05$. The $P$ value of the Welch’s $t$ test was used to determine significant differences.

**Results and discussion**

The GI of TC was significantly greater than that of CO throughout the production cycle ($P < 0.001$, Fig. 1) increasing from 9.2 to 26.9 for TC, whereas CO increased to only 21.2. The GI increased slowly for both TC and CO treatments until week 16; between weeks 16 and 24, the GI increased substantially, corresponding with the beginning of spring.

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**Fig. 1.** Effect of propagation method of bottlebrush (Callistemon vinimalis) ‘Little John’, conventional cuttings (CO) and tissue culture (TC), on weekly growth index [GI = plant height/2 + (plant width 1 + plant width 2)/4] from production weeks 4 through 32. Error bars represent standard error. *** Significant at $P \leq 0.001$. 1 cm = 0.3937 inch.
Table 1. Final soilless substrate pH and electrical conductivity (EC) and including growth parameters growth index (GI), fresh weight (FW), and dry weight (DW) for bottlebrush (Callistemon vinimalis) ‘Little John’ propagated by tissue culture (TC) and conventionally rooted stem cuttings (CO) after 32 weeks of production time in Huntsville, TX, USA on 29 Jun 2020.

<table>
<thead>
<tr>
<th>Propagation method</th>
<th>pH</th>
<th>EC (mmho/cm)</th>
<th>Final GI (cm)</th>
<th>FW (g)</th>
<th>DW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stems</td>
<td>Roots</td>
</tr>
<tr>
<td>CO</td>
<td>5.34 a</td>
<td>2.45 a</td>
<td>21.2 b</td>
<td>82.3 a</td>
<td>99.3 b</td>
</tr>
<tr>
<td>TC</td>
<td>5.29 a</td>
<td>2.73 a</td>
<td>26.9 a</td>
<td>87.3 a</td>
<td>352.3 a</td>
</tr>
</tbody>
</table>

1 g = 0.0353 oz.  
1 mmho/cm = 1 mS cm⁻¹.  
GI = plant height/2 + (plant width1 + plant width2)/4.  
1 cm = 0.3937 inch.  
* Different letters in columns indicate significant differences in mean separation by Welch’s t test at P ≤ 0.05.

This result is similar to a study by Goyali et al. (2015) where lowbush blueberry (Vaccinium angustifolium) plants started from tissue culture were more vigorous vegetatively (new shoots and rhizomes) than those plants started from softwood cuttings. Similarly, Grout et al. (1986) found that the growth rate of highbush blueberry (Vaccinium corymbosum) tissue culture propagated plant was 3 times that of cutting propagated plants.

Despite a significant difference in GI between the two treatments, stem fresh and dry weight means of TC and CO were not significantly different (Table 1). The TC mean stem fresh weight was 87.3 g compared with 82.3 g for CO. The stem dry weight means were 38.2 and 33.7 g for TC and CO, respectively; neither weight difference was significant at the P = 0.05 level.

At week 32, plants started from TC had a mean root fresh weight of 352.3 g compared with CO mean of 99.3 g. The root dry weight mean for TC was 88.8 g and for CO was 22.0 g (Table 1). TC plants had root fresh and dry weight means 254.7% greater and 303.6% greater than CO root fresh and dry weight means, respectively. Each of the root weight differences was statistically significant. Larger root system size of container-grown plants can be a positive influencing factor in plant establishment success from herbaceous annuals to woody plants (Mathes et al. 2007; NeSmith and Duval 1998).

At the end of the experiment, media chemical properties of pH and EC revealed no significant differences between the two treatments. The pH means were similar at 5.34 and 5.29 for CO and TC, respectively. Media EC means of the two treatments were similar at 2.45 and 2.73 mmhos/cm for CO and TC, respectively.

**Conclusions**

Propagation method influenced plant canopy growth index and root system weight in ‘Little John’ bottlebrush. The tissue culture environment stage of root initiation (stage 3) is high in auxins, and this remnant factor may have influenced the subsequent root growth vigor of the TC treatment. The enhanced plant size of TC bottlebrush plants may positively affect the production finishing time and may also reduce the time needed for plant establishment in the amenity landscape. Growers who choose to plant TC liners vs. cutting propagated liners of bottlebrush can potentially shorten production in the field because TC-induced vigor has also been reported in azalea (Rhododendron hybrids; Ettinger and Preece 1985; Smith 1981), blueberry (Vaccinium sp.; Grout et al. 1986), and several field grown species. A shortened field production time would mean less cost per unit and potentially a greater profit on the crop.


**References cited**


