

Effect of Branch-inducing Treatments on Growth of Tissue Culture and Cutting-Propagated *Hydrangea quercifolia* ‘Alice’¹

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Abstract

Cutting-propagated ‘Alice’ oakleaf hydrangea (*Hydrangea quercifolia* Bartr.) often produces a few vigorous branches with apical dominance, thus suppressing growth of other branches. As a result, the maturing canopy is sparse and develops asymmetrically, rendering plants unappealing to customers. For this reason, growers prune or apply plant growth regulators (PGRs) to encourage more branching, thereby producing a more desirable product. Propagation through tissue culture may provide another option to increase branching as an outcome of habituation. Habituation occurs when plant cultures continue to respond to a hormone that is no longer being supplied and, in turn, frequently leads to more branching. We evaluated oakleaf hydrangea growth as affected by propagation technique [tissue-cultured (TC) and cutting-propagated (CUT)] and PGR (cycilanilide and benzyladenine) application during container production. Nontreated TC plants had more branches longer than 15.2 cm (6 in) compared to nontreated CUT plants in 2008, although not in 2010. In both years, single applications of cycilanilide did not affect total branch number but two applications increased total branch number compared to nontreated plants, averaged over propagation technique. Plants treated with benzyladenine had similar or fewer total branches compared with nontreated, hand-pruned, and cycilanilide-treated plants (one or two treatments). Propagation technique did not consistently influence response to PGRs.

Index words: benzyladenine, branch architecture, cycilanilide, Configure, habituation, plant hormones, plant growth regulators, PGRs, propagation.

Species used in this study: oakleaf hydrangea (*Hydrangea quercifolia* Bartr.).

Chemicals used in this study: benzyladenine (Configure, Fine Americas); cycilanilide (Tiberon SC, Bayer Environmental Sciences); modified phthalic glycerol alkyd resin, (Latron B-1956 surfactant, J.R. Simplot Co.).

Significance to the Horticulture Industry

Hydrangea is an extremely popular landscape plant and ‘Alice’ is one of the most widely grown oakleaf hydrangea cultivars in container nursery production. However, ‘Alice’ plants are prone to asymmetrical growth, which is considered unattractive by consumers. Plants are often sparse and only consist of a few rampantly growing shoots, reducing shrub quality. Additionally, controlling growth during container production is becoming more important as growers continue to adopt fixed-height racks for transporting container crops. Identifying propagation and/or production practices that improve branching, uniformity, and overall growth control would increase the quality of plants, thus potentially making ‘Alice’ a more profitable plant to produce. This research shows that two applications of cycilanilide can increase branch number but may not consistently improve overall

plant architecture, while benzyladenine did not improve branching or plant architecture. Our results suggest that exposing ‘Alice’ to PGRs during tissue culture propagation may influence characteristics that improve the marketability and quality of a plant, however results were not consistent across the two seasons. This is the first study to evaluate the relationship between propagation technique and branching during container production of hydrangea. Therefore, more research is needed to determine if propagation techniques using branch-inducing PGRs can be a tool nursery growers can implement to improve growth characteristics, such as increased branching and plant architecture.

Introduction

Oakleaf hydrangea is a native, large ornamental shrub that has become increasingly popular among consumers and landscape designers in recent years. The inflorescences are exceptionally showy, with creamy white sepals occurring in panicles up to 30.5 cm (12 in) long in the summer. Leaves are large and coarse, turning deep shades of red, maroon, and burgundy in the fall. Shrubs are commercially propagated by tissue culture, cuttings or by seed. Tissue culture commonly utilizes auxins and cytokinins in the medium to stimulate and control root and branch development while in culture. Plant hormones used during in vitro propagation can have lasting effects on the explants known as habituation (Hartmann et al. 1997). Habituation can be inherited but is considered an epigenetic variation because it is readily reversible (Meins 1989). Nonetheless, habituation is supported anecdotally by growers who report that red maple (*Acer rubrum* L.), rhododendron (*Rhododendron* sp.) and magnolia (*Magnolia grandiflora* L. and *M. virginiana* L.) propagated by tissue culture have more branches and demonstrate better uniformity than propagated by cuttings (J. Potts, The Flower Potts, Kirksey,

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KY, personal communication and E. Kinsey, Kinsey Gardens Inc., Knoxville, TN, personal communication).

'Alice' oakleaf hydrangea easily grows in containers but often produce one or more vigorous branches that exhibit apical dominance, suppressing growth of other branches. As a result, the canopy is sparse and asymmetrical and considered poor quality (Glasgow 1997). While pruning is the nursery standard for modifying branch architecture during production, it does not always lead to an acceptable increase in branching and often times requires repeated pruning to elicit the desired branch architecture. Therefore, the use of plant growth regulators (PGRs), either exogenously applied plant hormones, inhibitors of endogenous plant hormones, or chemical pinching agents, could benefit the horticulture trade. PGRs have been successfully used to enhance branching on 'Merritt Supreme' florist hydrangea (*Hydrangea macrophylla* Thunb. ex J.A. Murr.) (Hester et al. 2013), 'Jane' hardy hydrangea (*H. paniculata* Sieb.) (Cochran and Fulcher 2013) and 'Limelight' hardy hydrangea (Cochran et al. 2013, Hester et al. 2013) and have been shown to restrict growth of florist hydrangea (Bailey 1989) and 'Alice' oakleaf hydrangea (Cole et al. 2013). However, the effect PGRs have on branch development of oakleaf hydrangea is largely unknown. Therefore, the objectives of this study were to evaluate if tissue-cultured (TC) plants are predisposed to greater branching than cutting-propagated plants (CUT) and evaluate if propagation technique influences response to application of PGRs during container production.

Materials and Methods

Plant source and culture. Experiments were conducted at the University of Kentucky's Horticulture Research Farm, Lexington, KY, USDA Plant Hardiness Zone 6b (U.S. Department of Agriculture 2012). Average daytime and nighttime air temperature during the experiments for the University of Kentucky's Horticulture Research Farm in 2008 were 26.7/14.4 °C [80/58 °F [34.4 maximum/−3.9 °C minimum (94/25 °F)]] with a total of 42.2 cm (16.6 in) of precipitation; in 2010 average daytime and nighttime air temperature were 28.0/15.9 °C [82.4/60.6 °F [35.6 maximum/0 °C minimum (96.1/32 °F)]] with a total of 58.4 cm (23.0 in) of precipitation [National Oceanic and Atmospheric Administration (NOAA) 2012].

TC propagules were taken from axillary cuttings and placed on Woody Plant Medium (Lloyd and McCown 1981) supplemented with N6-benzyladenine (2.5 to 10 ppm) and gibberellic acid (1.0 to 5.0 ppm). CUT plants were dipped in an indole-3-butyric acid (IBA) and naphthaleneacetic acid rooting hormone (Dip'N Grow; Clackamas, OR) solution containing 500 ppm IBA. In May 2008, 'Alice' oakleaf hydrangea TC plants [7.62 cm (3.0 in) rooted liners (A. McGill and Son Nursery, Hubbard, OR)] and CUT plants [10.16 cm (4 in) rooted liners (Spring Meadow Nursery, Grand Haven, MI)] of oakleaf hydrangea 'Alice' were potted into 3.8 liter (#1) containers with Barky Beaver Professional Grow Mix [pine bark fines, peat moss, and sand, but without the standard dolomitic lime (Barky Beaver Mulch and Soil Mix, Inc., Moss, TN)]. 'Alice' oakleaf hydrangea were fertilized with 19N-1.7P-6.6K (Harrell's 19-4-8, Lakeland, FL), 5–6 month controlled-release fertilizer at 10 g per container on May 13, 2008. On July 25–29, 2008, plants were repotted into 11.4 liter (#3) containers and top-dressed with 15N-4P-10K (Osmocote Plus 15-9-12, Marysville, OH), 3–4 month

controlled-release fertilizer at 38 g per container (75% of the medium label rate). Cyclic irrigation was provided during the growing season as needed. In 2010, TC plants were acquired from Briggs Nursery (Elma, WA), CUT plants were acquired again from Spring Meadow Nursery. Plants were potted into 11.4 liter (#3) containers between May 11th and 15th. Plants were fertilized with 19N-1.7P-6.6K (Harrell's 19-4-8, Lakeland, FL), 5–6 month controlled-release fertilizer at 10 g per container (medium label rate) on May 25, 2010.

In both years, plants were subjected to one of the following branch-inducing treatments (BIT): one application of cyclanilide (Tiberon SC, Bayer Environmental Sciences, Research Triangle Park, NC) at 100 ppm (1×), two applications of cyclanilide at 100 ppm (2×), two applications of benzyladenine [now labeled as Configure (Fine Americas, Inc., Walnut Creek, CA)] at 500 ppm, one application of water (nontreated), or a hand-pruned control (removing the most terminal three nodes on each branch). Latron B-1956 surfactant (J.R. Simplot Co., Boise, ID) was added to both cyclanilide and benzyladenine treatments at 2.3 ml per 3.8 L (0.08 fl oz per gal). Applications were made using a CO₂ backpack sprayer (R&D Sprayers, Opelousas, LA) with a flat fan nozzle tip (TP8003E, TeeJet, Springfield, IL) until the foliage was thoroughly wetted. The first application was made on July 11, 2008, or July 2, 2010, and a second application was made four weeks after the first application. Pruning of the hand-pruned control occurred concurrent with the first PGR application. TC plants had 5.1 and 5.9 branches and were 36.6 and 33 cm (14.4 and 13.0 in) in height, in 2008 and 2010, respectively. CUT plants had 5.9 and 4.5 branches and were 32.3 and 37.1 cm (12.7 and 14.6 in) in height in 2008 and 2010, respectively. The experiment was a completely randomized design with a 2 by 5 factorial arrangement (propagation technique by BIT) with 10 (2008) and 12 (2010) single pot replications.

Number of branches less than 15.2 cm (6 in) and greater than 15.2 cm, total number of branches [any branch greater than 1.27 cm (0.5 in) in length], and growth index [GI = (height + widest width + perpendicular width) ÷ 3] were all measured at the end of each experiment (September 5, 2008; September 20, 2010). In addition, to quantify plant architecture, a branch ratio (BR = total branches ÷ GI), a height to width ratio (H:W = height ÷ average width) and a visual quality rating [1–5 scale (1 = sparse, asymmetrical branching, surface of substrate not covered by the plant; 5 = full, symmetrical branching, 100% of the surface of the container covered by the plant)] were assessed at the end of each experiment. Plants were rated for phytotoxicity symptoms on a 0–10 scale (0 = no phytotoxicity; 10 = plant death) 6 weeks after BIT treatments were applied. Data were analyzed using linear models with the GLIMMIX procedure of SAS (version 9.3; SAS Institute, Cary, NC). Pairwise treatment differences were determined using the LSMEANS statement according to the Holm-Simulation method at $\alpha = 0.05$. When there was a significant interaction (propagation technique by BIT), the SLICEDIFF option was used to examine the pairwise comparisons, using adjusted *P*-values for multiple comparisons with the ADJUST=SIMULATE and STEPDOWN options.

Results and Discussion

In 2008, there was no propagation technique effect on short branch development or total branch number; however, there

Table 1. Branch development of container-grown *Hydrangea quercifolia* 'Alice' as affected by propagation technique averaged over growth regulator treatment and plant growth regulators averaged over propagation technique in two experiments^z.

Propagation technique ^y	2008			2010		
	Small branches ^x (no.)	Long branches ^w (no.)	Total branches ^v (no.)	Small branches (no.)	Long branches (no.)	Total branches (no.)
Tissue-cultured	6.6	7.8	14.5	1.3	9.2a	10.4a
Cutting propagated	8.0	7.0	15.0	1.6	7.8b	9.5b
Branch-inducing treatment ^u						
Nontreated	5.3bc ^t	7.9	13.3b	1.4ab	7.4b	8.8bc
Hand-pruned	4.0c	7.6	11.6b	2.4a	9.3a	11.7a
Cyclanilide 1×	8.7ab	6.9	15.6b	1.4ab	9.2a	10.5ab
Cyclanilide 2×	11.7a	8.1	19.8a	1.8a	9.3a	11.2a
Benzyladenine	6.9bc	6.8	13.6b	0.3b	7.2b	7.5c
Effects						
Propagation technique	0.1082 ^s	0.0640	0.5405	0.1981	0.0010	0.0404
Branch-inducing treatment	0.0001	0.1768	0.0001	0.0002	0.0002	0.0001
PT×BT ^r	0.4701	0.0008	0.9211	0.6216	0.9204	0.9225

^zData collected 8 weeks after treatment (2008), 11 weeks after treatment (2010).

^yPropagation technique: plants propagated through tissue culture were received from A. McGill and Son Nursery (Hubbard, OR) in 2008 and Briggs Nursery (Elma, WA) in 2010 and cutting propagated plants were received from Spring Meadow Nursery (Grand Haven, MI).

^xSmall branches: branches less than 15.24 cm (6 in) counted.

^wLong branches: branches greater than 15.24 cm (6 in) counted at close of experiment.

^vTotal branches = long branches + short branches.

^uBranch-inducing treatment: Nontreated (water applied), hand-pruned (removing the most terminal three nodes on each branch), cyclanilide 1× (one application of cyclanilide at 100 ppm), cyclanilide 2× (two applications of cyclanilide at 100 ppm), and benzyladenine (two applications of benzyladenine at 500 ppm).

^sMeans within columns followed by the same letters are not significantly different according to the Holm-Simulation method for mean comparison, alpha = 0.05.

^tPvalue.

^rPT: propagation technique; BT: branch-inducing treatment.

was a difference among BITs (Table 1). Plants treated with cyclanilide (2×) had 121, 193 and 70% more short branches respectively, compared with the nontreated, hand-pruned, and benzyladenine-treated plants in 2008. Similarly, plants treated with cyclanilide (2×) had more total branches compared with all other BITs. In 2008, there was no propagation by BIT effect for short or total branches; however, there was a propagation by BIT effect for long branches, indicating that nontreated TC plants had 93% more long branches compared with nontreated CUT plants (Fig. 1).

In 2010, short branch development did not differ regardless of propagation technique; however, hand-pruned and cyclanilide- (2×) treated plants had 700 and 500% more short branches compared with benzyladenine-treated plants (Table 1). In 2010, plants treated with PGRs had 84 to 95% fewer short branches than in 2008, yet, pruned plants only had 40% fewer short branches. It is possible that due to the excessively overcast and rainy 2010 season, light levels were very low within the plant canopy except for plants that received the pruned treatment, which would explain some of the reduction in short branches across the two seasons. Propagation technique did affect long and total branch number, with TC plants having 19 and 10% more long and total branches respectively, compared with CUT plants. While we cannot rule out that differences in post-propagation practices at the propagation nurseries influenced branch development during our studies, practices that we were able to ascertain were relatively similar and representative of conventional nursery production. Both nurseries incorporated controlled release fertilizer at 1.8 to 3.0 kg·m⁻³ (3 to 5 lb·yd⁻³) of either 15-9-12 or 18-5-9

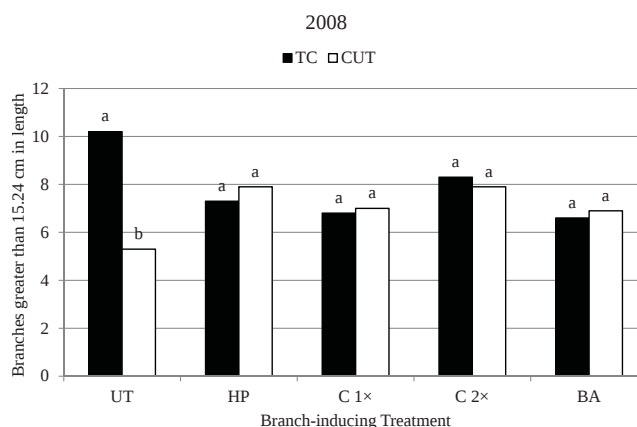


Fig. 1. Long branch [branches greater than 15.24 cm (6 in) in length] development of container-grown *Hydrangea quercifolia* 'Alice' affected by propagation technique (TC: tissue cultured; CUT: cutting propagated) and branch-inducing treatment. Branch-inducing treatments were nontreated [UT (application of water)], hand-pruned [HP (removing the three most terminal nodes)], a single application of cyclanilide at 100 ppm (C 1×), two applications of cyclanilide at 100 ppm (C 2 ×), and two applications of benzyladenine at 500 ppm (BA). Means with the same letters are not significantly different according to the SLICEDIFF option to examine the pairwise comparisons and using adjusted P values for multiple comparisons with the ADJUST=SIMULATE and STEPDOWN options, $\alpha = 0.05$.

ratio and both nurseries fertigated newly-rooted liners (rate and analysis not available). For both nurseries, irrigation was based on environmental conditions (either based on daily light integral or based on light level, temperature, and humidity) and automatically controlled. Latitude is 46° 59' 25.944" in Elma, WA, and 42° 57' 56.096" in Grand Rapids, MI, therefore natural daylength is relatively similar at the two locations. However, the Elma, WA, location maintains a 16 h photoperiod while supplemental lighting is used on an as-needed basis in the Grand Rapids, MI, location.

Hand-pruned and plants treated with cyclanilide (2×) had more long and total branches than nontreated and benzyladenine-treated plants in 2010 (Table 1). There was no propagation by BIT effect for short branches, long branches or total branch number in 2010.

In 2008, TC plants were 15% taller and 11% narrower than CUT plants but that accounted for only a 5% difference in GI (Table 2). Cyclanilide (1× and 2×) and benzyladenine were successful in controlling plant height with 21, 18, and 24% shorter plants than hand-pruned plants. There were no differences in average plant width or GI with the exception of cyclanilide (1×) treatment having a smaller GI than hand-pruned plants. There was no propagation by BIT effect for height, width, or GI in 2008. In 2010, TC plants were only 5% narrower than CUT plants and there were no differences in height or GI. In 2010, all plants were similar in height with the exception of plants treated with cyclanilide (1×), which were 11% shorter than benzyladenine-treated plants. GI was similar among all branch-inducing treatments with the exception of hand-pruned plants having a smaller GI compared with benzyladenine-treated plants in 2010. In 2010, there was no propagation technique by BIT effect for growth (height, width, or GI).

In 2008, branch ratio was unaffected by propagation technique but was affected by BIT (Table 3). For example, branch ratio was 32 and 45% greater in cyclanilide- (1× and 2×) treated plants, respectively, compared with hand-pruned plants. Conversely, there was a 27% difference in height/width (H:W) ratio between TC (0.88) and CUT (0.67) plants but there were no differences among BITs in 2008. There was no propagation by BIT effect for branch ratio or H:W ratio in 2008; however, there was an effect on plant quality. In 2008, nontreated TC plants had a 46% greater quality rating compared to nontreated CUT plants (Fig. 2). In 2010, TC plants had an 87, 9, and 29% greater branch ratio, H:W ratio, and quality rating, respectively, compared with CUT plants (Table 3). Hand-pruned plants and plants treated with cyclanilide (1× and 2×) had more branches per unit of growth compared with nontreated and benzyladenine treated plants. There were no differences in H:W ratio among PGR treatments but hand-pruned plants had a H:W ratio closer to 1 (0.86) compared to nontreated, cyclanilide 1×, cyclanilide 2×, 0.78, 0.76, 0.73, respectively, but not benzyladenine (0.79). Plant quality rating was greater for hand-pruned plants compared with nontreated and benzyladenine treated plants. There was no propagation technique by BIT effect for branch ratio, H:W ratio, or quality rating in 2010. Phytotoxicity was observed following cyclanilide application (1× and 2×) but was not apparent by the end of either season, when the plants would have been marketed (data not shown).

Hydrangeas are known to have both flower-bearing and non-flower-bearing shoots (Zhou and Hara 1988). Non-flower-bearing shoots have the potential to branch during the next growing season more than they contribute to aesthetics during the current season of growth. This was notably evident in the current study with the TC plants. In 2008,

Table 2. Height, width and final growth of container-grown *Hydrangea quercifolia* 'Alice' as affected by propagation technique averaged over growth regulator treatment and plant growth regulators averaged over propagation technique in two experiments².

Propagation technique ^a	2008			2010		
	Height (cm)	Width (cm)	GI (cm) ^c	Height (cm)	Width (cm)	GI (cm)
Tissue-cultured	57.7a	67.7b	64.4b	63.0	77.9b	72.9
Cutting propagated	50.4b	76.0a	67.5a	60.3	81.7a	74.6
Branch-inducing treatment ^w						
Nontreated	56.3ab ^v	70.6	65.8ab	62.6ab	80.9ab	74.8ab
Hand-pruned	61.4a	73.0	69.1a	62.1ab	73.6b	69.8b
Cyclanilide 1×	50.8b	68.9	62.9b	58.8b	79.2ab	72.4ab
Cyclanilide 2×	51.9b	70.8	64.5ab	59.2ab	82.0a	74.4ab
Benzyladenine	49.7b	76.0	67.2ab	65.5a	83.3a	77.4a
Effects						
Propagation technique	0.001 ^u	0.0001	0.0112	0.0639	0.0399	0.2708
Branch-inducing treatment	0.0053	0.0971	0.0161	0.0318	0.0119	0.0281
PT×BT ^t	0.4425	0.2926	0.1278	0.6441	0.8936	0.9454

²Height and width data collected 8 weeks after treatment (2008), 11 weeks after treatment (2010).

^aPropagation technique: plants propagated through tissue culture were received from A. McGill and Son Nursery (Hubbard, OR) in 2008 and Briggs Nursery (Elma, WA) in 2010 and cutting propagated plants were received from Spring Meadow Nursery (Grand Haven, MI).

^cGI = (height + widest width + perpendicular width) ÷ 3).

^wbranch-inducing treatment: nontreated (water applied), hand-pruned (removing the most terminal three nodes on each branch), cyclanilide 1× (one application at 100 ppm), cyclanilide 2× (two applications at 100 ppm), and benzyladenine (two applications at 500 ppm).

^umeans within columns followed by the same letters are not significantly different according to the Holm-Simulation method for mean comparison, alpha = 0.05.

^vPvalue.

^tPT: propagation technique; BT: branch-inducing treatment.

Table 3. Branch ratio, height to width ratio, and quality rating of container-grown *Hydrangea quercifolia* ‘Alice’ as affected by propagation technique averaged over growth regulator treatment and plant growth regulators averaged over propagation technique in two experiments^a.

Propagation technique ^y	2008			2010		
	Branch ratio ^x	H:W ratio ^w	Quality rating ^v (1 – 5 scale)	Branch ratio	H:W ratio	Quality rating (1 – 5 scale)
Tissue-cultured	0.23	0.88a ^u	3.3	0.14a	0.82a	3.6a
Cutting propagated	0.22	0.67b	3.1	0.13b	0.75b	2.8b
Branch-inducing treatment ^t						
Nontreated	0.20bc	0.84	2.9b	0.12b	0.78b	3.0b
Hand-pruned	0.17c	0.86	4.1a	0.17a	0.86a	3.7a
Cyclanilide 1×	0.25ab	0.76	3.0b	0.15a	0.76b	3.3ab
Cyclanilide 2×	0.31a	0.76	3.2b	0.15a	0.73b	3.2ab
Benzyladenine	0.21bc	0.66	2.8b	0.10b	0.79ab	2.8b
Effects						
Propagation technique	0.7347 [*]	0.0001	0.0579	0.0039	0.0006	0.0010
Branch-inducing treatment	0.0001	0.0531	0.0010	0.0001	0.0056	0.0042
PT×BT ^r	0.6150	0.5265	0.0120	0.7341	0.3142	0.4536

^aCalculated from data collected 8 weeks after treatment (2008) and 11 weeks after treatment (2010).

^yPropagation technique: plants propagated through tissue culture were received from A. McGill and Son Nursery (Hubbard, OR) in 2008 and Briggs Nursery (Elma, WA) in 2010 and cutting propagated plants were received from Spring Meadow Nursery (Grand Haven, MI).

^xBranch ratio = final branch count ÷ GL.

^wH:W ratio: height:width = (height ÷ average width).

^vQuality rating: 1–5 scale (1 = sparse, asymmetrical branching, surface of substrate not covered by the plant; 5 = full, symmetrical branching, 100% of the surface of the container covered by the plant).

^uMeans within columns followed by the same letters are not significantly different according to the Holm-Simulation method for mean comparison, alpha = 0.05.

^tBranch-inducing treatment: nontreated (water applied), hand-pruned (removing the most terminal three nodes on each branch), cyclanilide 1× (one application at 100 ppm), cyclanilide 2× (two applications at 100 ppm), and benzyladenine (two applications at 500 ppm).

^{*}Pvalue.

^rPT: propagation technique; BT: branch-inducing treatment.

nontreated TC plants had more long branches compared with nontreated CUT plants. In addition, all TC plants had more long branches than CUT plants in 2010. These long branches [≥ 15.2 cm (6 in)] appeared to be responsible for desirable metrics of plant aesthetics and overall size. The short branches [1.27 to 15.2 cm (0.5 to 6 in)] lacked flowers and for the shortest branches, 1.3 cm (0.5 to 1.5 in) appeared to be composed of latent buds. Similar research with blueberries (*Vaccinium* sp.) indicated that micropropagated plants were more uniform, grew more vigorously, and produced more and longer shoots than cutting-derived plants (Litwińczuk et al. 2005). Our results were also similar to Ghrist et al. (1991) who reported greater branching in weigela (*Weigela florida* Bunge.) propagated through tissue culture compared with cutting propagated plants.

Propagating plants through tissue culture exposes explants to medium enriched with substances that promote survival, shoot initiation, and root initiation (Murashige 1974). This exposure can result in habituation, an epigenetic variation that can have long-lasting changes to the genome (Smulders and Klerk 2011). In TC plants, it is common for auxin or cytokinin habituation to occur since they are used as growth hormones to promote continuous proliferation during *in vitro* propagation (Meins 1989). Increased shoots is a known response of plants grown *in vitro* treated with synthetic cytokinins (Baraldi et al. 1988, Paek and Yeung 1991). In the current study, it is likely that cytokinin habituation occurred since there were more long branches, a higher plant quality rating, and a H:W ratio closer to 1 (indicating more uniform

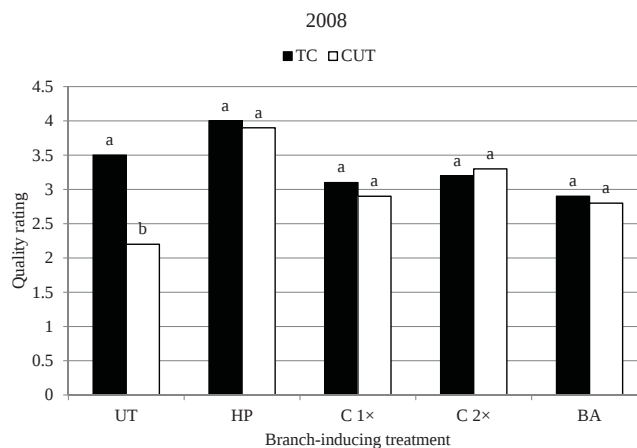


Fig. 2. Quality rating of container-grown *Hydrangea quercifolia* ‘Alice’ affected by propagation technique (TC: tissue cultured; CUT: cutting propagated) and branch-inducing treatment. Branch-inducing treatments were nontreated [UT (application of water)], hand-pruned [HP (removing the three most terminal nodes)], a single application of cyclanilide at 100 ppm (C 1×), two applications of cyclanilide (C 2×), and two applications of benzyladenine (BA). Quality rating was assessed visually on a 1 to 5 scale rating; 1 = sparse, asymmetrical branching, surface of substrate not covered by the plant; 5 = full, symmetrical branching, 100% of the surface of the container covered by the plant. Means with the same letters are not significantly different according to the SLIC-EDIFF option to examine the pairwise comparisons and using adjusted P values for multiple comparisons with the ADJUST=SIMULATE and STEPDOWN options, $\alpha = 0.05$.

mity) in TC plants treated with benzyladenine (a synthetic cytokinin) *in vitro*, compared with CUT plants.

Uniformity in growth (H:W ratio) is a highly desirable characteristic, especially by container nursery growers. Excessive growth of one or two shoots in containers can lead to top-heavy plants and/or rapid depletion of nutrients, ultimately affecting overall plant growth. Top-heavy plants are prone to tip over with even the slightest wind or worker contact, leading to fertilizer loss and stem breakage. In addition to the benefits of controlling and/or maintaining plant growth during production, producing plants with uniform growth is vital because consumers prefer compact plants with symmetrical growth (Glasgow et al. 1998; Jeffers et al. 2009; Townsley-Brascamp and Marr 1995).

In this study we evaluated plant architecture by determining a branch ratio as an indirect indication of branch density (number of branches per unit of GI), an H:W ratio to quantify plant symmetry, and a visual quality rating to assess plant symmetry. Studies evaluating human perception on geometric beauty have indicated a preference for 1:1 altitude-to-base ratios (Austin and Sleight 1951, Friedenberg 2012). Friedenberg (2012) indicated individuals preferred triangles with smaller altitude-to-base ratios, which they related to symmetry. Thus, a symmetrical plant can be interpreted as the following: for every unit in height there is an equal unit in width with a perfectly symmetrical plant having a value of 1. In our study, TC- propagated 'Alice' hydrangea had an H:W ratio closer to 1 than CUT plants, in both years. Furthermore, the H:W ratio data indicated that TC plants were only slightly wider with only a 12% (2008) and 18% (2010) difference from 1 compared with CUT plants that were considerably wider than tall with a 33% (2008) and 25% (2010) difference from 1. This growth response was similar to Remphrey and Pearn (2006) who reported micropropagated Saskatoon serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.] that were only slightly wider than tall when compared with seed propagated plants.

Traditionally, hand-pruning or mechanical pruning are the techniques preferred by growers to control growth; however, with limitations in labor availability, these options will not always be the most economical choices (Banko and Stefani 1996, Holland et al. 2007). An alternative to hand-pruning and/or mechanical pruning methods is using PGRs. PGRs have been used since the 1960s (Cathey et al. 1966) but results are not always consistent between cultivars (Hilgers et al. 2005, Starman et al. 2004), or species (Currey and Erwin 2012, Norcini et al. 1994). At the time of this experiment, cyclanilide (registered for use in cotton as a harvest aid - defoliant) was being evaluated as a woody plant branching agent; however, after inconsistent experimental results on woody ornamental plants, it is currently not marketed for use in nursery production.

Previous research indicated that single applications of cyclanilide increased branching in fruit trees (Elfvig and Visser 2006), woody ornamentals (Holland et al. 2007), and herbaceous perennials (Latimer et al. 2011); whereas, other research reported two applications of cyclanilide were required for enhanced branching (Gibson 2006, Fulcher 2008, Keever 2006). Results from the current experiments were similar in that single applications of cyclanilide had no effect on total branch number. Two applications, though, significantly increased total branch number compared to water controls. In both years, plants treated with benzylad-

enine had fewer short and total branches compared with cyclanilide-treated plants (2×), and similar or fewer branches than nontreated and hand-pruned plants.

Generally in nursery production, the desired responses of PGRs are to increase branching and control growth to promote dense and uniform plants. In our 2008 study, two applications of cyclanilide enhanced branching compared with all other BITs; however, two treatments of cyclanilide were not successful in reducing overall growth (GI). Furthermore, there were more branches per unit of growth (BR) for plants treated with two applications of cyclanilide compared to nontreated and hand-pruned plants but there was no difference in H:W ratio. In 2010, application of cyclanilide (1× and 2×) did not increase branching, control growth, promote more branches per unit of growth (bushiness), or uniformity compared with hand-pruned plants and inconsistently improved branching and plant growth compared with nontreated plants. For example, plants treated with two applications of cyclanilide may have had more total branches and more branches per unit of growth (BR) compared with nontreated plants, but GI and H:W ratio were similar. Thus, our results do not support the use of cyclanilide on 'Alice' oakleaf hydrangea since only increased branching was achieved. In addition, application of benzyladenine did not improve branching or control growth compared with nontreated or hand-pruned plants. Therefore, our data do not support using this product on 'Alice' oakleaf hydrangea at the stage of growth at which they were treated during this study, but more research is needed to investigate application to other stages of growth, in particular younger plants.

In terms of propagation technique, results were not consistent enough to recommend TC plants over CUT plants. While all efforts were made to control for confounding variables, temperature and rainfall differed between the two years. In 2010, newly-potted plants received more than the average amount of rainfall for Lexington, KY, during the months of May, June, and July, 16.3 cm (6.4 in) and more rainfall than in 2008, which may have affected shoot growth due to suppressed root growth as may have lower light levels associated with frequent precipitation. Additionally, the maximum air temperature was 1.2 C (2.1 F) greater in 2010 than in 2008. PGRs are known to be sensitive to environmental conditions, thus response can vary with locations and seasons (Cochran and Fulcher 2013, Hester et al. 2013) as can magnitude of response (Cochran et al. 2013). For example, Latimer and Whipker (2012) report that producing plants under wetter conditions will increase PGR use. Additionally, Dasoju et al. (1998) documented reduced efficacy of paclobutrazol applied to sunflower (*Helianthus annuus* L. 'Pacino') when temperatures were higher. Currey and Erwin (2012) postulate that PGR efficacy is reduced under low light conditions, i.e., low daily light integral, that promote stem elongation. Further research evaluating TC plants with CUT plants grown in controlled environments is warranted.

Literature Cited

- Austin, T.R. and R.B. Sleight. 1951. Aesthetic preference for isosceles triangles. *J. Appl. Psychol.* 35:430–431.
- Bailey, D.A. 1989. Uniconazole effects on forcing of florists' hydrangeas. *HortScience* 24:518.
- Banko, T.J. and M.A. Stefani. 1996. Growth response of large, established shrubs to Cutless, Atrimmec, and Trim-cut. *J. Environ. Hort.* 14:177–181.

- Baraldi, R., F. Rossi, and B. Lercari. 1988. In vitro shoot development of *Prunus* GF 655-2: Interaction between light and benzyladenine. *Physiologia Plantarum* 74:440–443.
- Cathey, H.M., G.L. Steffens, N.W. Stuart, and R.H. Zimmerman. 1966. Chemical pruning of plants. *Science* 153:1382–1383.
- Cochran, D.R. and A. Fulcher. 2013. Type and rate of plant growth regulator influence vegetative, floral growth, and quality of Little Lime™ hydrangea. *HortTechnology* 23:306–311.
- Cochran, D.R., A. Fulcher, and G. Bi. 2013. Efficacy of dikegulac sodium applied to pruned and unpruned ‘Limelight’ hydrangea grown at two locations in the southeastern United States. *HortTechnology* 23:836–842.
- Cole, J.C., R.O. Brown, and M.E. Payton. 2013. Two cultivars of oakleaf hydrangea respond to ancymidol, uniconazole, or pinching. *HortTechnology* 23:339–346.
- Currey, C.J. and J.E. Erwin. 2012. Foliar applications of plant growth regulators affect stem elongation and branching of 11 kalanchoe species. *HortTechnology* 22:338–344.
- Dasoju, S., M.R. Evans, and B.E. Whipker. 1998. Paclobutrazol drenches control growth of potted sunflowers. *HortTechnology* 8:235–237.
- Elfving, D.C. and D.B. Visser. 2006. Cyclanilide induces lateral branching in sweet cherry trees. *HortScience* 41:149–153.
- Friedenberg, J. 2012. Aesthetic judgment of triangular shape: compactness and not the golden ratio determines perceived attractiveness. *i-Perception* 3:163–175.
- Fulcher, A. 2008. IR-4 woody ornamental branching program. <http://ir4.rutgers.edu/Ornamental/OrnData/20090316b.pdf>. Accessed February 2, 2014.
- Ghrist, A.C., L.C. Stephens, and J.L. Weigle. 1991. Growth habit of *Weigela florida* as affected by stock plant propagation history. *J. Environ. Hort.* 9:123–127.
- Gibson, J. 2006. IR-4 woody ornamental branching program. <http://ir4.rutgers.edu/Ornamental/OrnData/20080116p.pdf>. Accessed February 2, 2014.
- Glasgow, T., T. Bilderback, T. Johnson, and C. Safley. 1997. Consumer perceptions of plant quality. *Proc. South. Nur. Assn. Res. Conf.* 42:378–380.
- Glasgow, T.E., T.E. Bilderback, T. Johnson, K.B. Perry, and C.D. Safley. 1998. Evaluating consumer perceptions of plant quality. *Proc. South. Nur. Assn. Res. Conf.* 43:497–500.
- Hartmann, H., D. Kester, F. Davies, and R. Geneve. 1997. *Plant Propagation: Principles and Practices*. 6th ed. Prentice Hall, Upper Saddle River, NJ. p. 578–580.
- Hester, K.A., G. Bi, M.A. Czarnota, A. Fulcher, G.J. Keever, J.H. Lieth, J.D. Orsi, B.E. Whipker, K. Sullivan, and C.L. Palmer. 2013. Impact of Augeo, Configure and Florel on Hydrangea branching. *J. Environ. Hort.* 31:27–29.
- Hilgers, K.R., C. Haynes, and W.R. Graves. 2005. Chemical height control of containerized seashore mallow. *HortTechnology* 15:330–332.
- Holland, A.S., G.J. Keever, J.R. Kessler, and F. Dane. 2007. Single cyclanilide applications promote branching of woody ornamentals. *J. Environ. Hort.* 25:139–144.
- Jeffers, A.H., M.A. Palma, W.E. Klingeman, C.R. Hall, D.S. Buckley, and D.A. Kopsell. 2009. Assessments of bare-root liner quality and purchasing decisions made by green industry professionals. *HortScience* 44:717–724.
- Keever, G. 2006. IR-4 woody ornamental branching program. <http://ir4.rutgers.edu/Ornamental/OrnData/20060912a.pdf>. Accessed February 2, 2014.
- Latimer, J.G., J. Freeborn, and V. Groover. 2011. Cyclanilide increases branching of herbaceous perennials. *Acta Hort.* 886:159–162.
- Latimer, J. and B. Whipker. 2012. Selecting and using plant growth regulators on floriculture crops. VA Coop. Ext. Pub 430-102. <http://pubs.ext.vt.edu/HORT/HORT-43P/HORT-43P-pdf.pdf>. Accessed September 21, 2014.
- Litwińczuk, W. G. Szczerba, and D. Wrona. 2005. Field performance of highbush blueberries (*Vaccinium ×corymbosum* L.) cv. ‘Herbert’ propagated by cuttings and tissue culture. *Scientia Hort.* 106:162–169.
- Lloyd, G. and B. McCown. 1981. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *Comb. Proc. Int. Plan Prop. Soc.* 30:421–427.
- Meins, F. 1989. Habituation: Heritable variation in the requirement of cultured plant cells for hormones. *Annu. Rev. Genet.* 23:395–408.
- Murashige, T. 1974. Plant propagation through tissue culture. *Annu. Rev. Plant Physiol.* 25:135–166.
- Norcini, J.G., J.H. Aldrich, and J.M. McDowell. 1994. Flowering response of *Bougainvillea* cultivars to dikegulac. *HortScience* 29:282–284.
- Paek, K.Y. and E.C. Yeung. 1991. The effects of 1-naphthaleneacetic acid and N⁶-benzyladenine on the growth of *Cymbidium forrestii* rhizomes in vitro. *Plant Cell Tissue and Organ Culture* 24:65–71.
- Remphrey, W.R. and L.P. Pearn. 2006. A comparison of seed-propagated and micropropagated *Amelanchier alnifolia* (Saskatoon): Yield and yield components in relation to crown architecture characteristics. *Can. J. Plant Sci.* 86:499–510.
- Smulders, M.J.M. and G.J. de Klerk. 2011. Epigenetics in plant tissue culture. *Plant Growth Regulation* 63:137–146.
- Starman, T.W., M.C. Robinson, and K.L. Eixmann. 2004. Efficacy of ethephon on vegetative annuals. *HortTechnology* 14:83–87.
- Townsley-Brascamp, W. and N.E. Marr. 1995. Evaluation and analysis of consumer preferences for outdoor ornamental plants. *Acta Hort.* 391:199–206.
- Zhou, T.S. and N. Hara. 1988. Development of shoot in *Hydrangea macrophylla* I. terminal and axillary buds. *Bot. Mag. Tokyo* 101:281–291.