Effect of Plant Growth Regulators on Growth of Tissue Culture and Cutting-Produced *Hydrangea quercifolia* 'Alice'©

Amy Fulcher
University of Kentucky, Department of Horticulture, N 318 Ag Science North,
Lexington, Kentucky 40546–0091 U.S.A.
Email: afulcher@uky.edu

NATURE OF WORK

Oakleaf hydrangea (*Hydrangea quercifolia* Bartr.) is a native ornamental shrub that has become increasingly popular in recent years. Oakleaf hydrangea is a large shrub growing 6–8 ft or taller and 6 ft or more wide. The inflorescences are exceptionally showy, with creamy-white sepals occurring in panicles up to 12 inches long in the summer. Leaves are large and coarse, turning striking, deep shades of red in the fall.

Hydrangea cultivars are commercially propagated by tissue culture and by cuttings. Tissue culture commonly utilizes auxin and cytokinin in the medium to stimulate and control root and branch development of the cultures. Habituation is a condition that occurs when plant cultures continue to respond to a hormone, often cytokinin, which is no longer supplied. This phenomenon usually occurs after extended exposure to the hormone during tissue culture (Hartmann et al., 1997). Some possible mechanisms for habituation are overproduction of cytokinin (Sun et al., 2003), and increased levels of the cytokinin receptor *CRE1* (Pischke et al., 2006). Habituation has not been extensively researched for woody ornamental plants; however, habituation is reported to persist after propagation for at least two woody plants, apple and blueberry (Debnath, 2007; Zimmerman, 1986). Habituation extending into production is supported by anecdotal information from growers, for example, the common observation by growers that red maples propagated by tissue culture have more branches and are more uniform.

Oakleaf hydrangea often produces one or a few vigorous branches which exhibit apical dominance, suppressing growth of other branches. As a result, the canopy is sparse and asymmetrical and thereby is considered poor quality (Glasgow et al., 1997). Currently, some growers purchase oakleaf hydrangea propagated by tissue culture for putative enhanced branching during production. To the author's knowledge, there are no published studies of habituation or its carryover into production for oakleaf hydrangea.

While pruning is the nursery standard for modifying branch architecture during production, plant growth regulators (PGRs), either exogenously applied plant hormones or inhibitors of endogenous plant hormone, are used in the horticulture trade. Plant growth regulators have been successfully used to enhance branching on shrubs in container production (Keever and Foster, 1990; Poston et al., 2007). The effect of PGRs on enhancing branch development of oakleaf hydrangea is largely unknown. Gibson and Groninger (2007) found that cyclanilide, the active ingredient in Tiberon, increased total branch number on oakleaf hydrangea.

The objectives of this study were (1) to determine if oakleaf hydrangea propagated by tissue culture produces more branches than those propagated by cuttings, (2) to examine the effect propagation technique on plant response to PGR application during production, and (3) to determine if PGRs can mediate the apical dominance observed in container-grown oakleaf hydrangea.

The oakleaf hydrangea cultivar 'Alice' is reported to be easier to produce in containers than some other oakleaf hydrangeas (Dirr, 1998) and is reported to be prone to poor branch development (pers. Commun. Todd Kelly), and therefore was selected for this study. In May 2008, rooted tissue culture and cutting propagated oakleaf hydrangea 'Alice' were potted into 1-gal containers with Barky Beaver Professional Grow Mix without lime. Plants were fertilized with Harrell's 19–4–8, 5–6 month release, 10 g per container on 13 May 2008. On 25–29 July 2008, plants were repotted into 3-gal containers and were top-dressed with Osmocote Plus 15–9–12, 3–4 month, 38 g per container. Cyclic irrigation was provided during the growing season.

Plants were subjected to one of the following branch-inducing treatments on 11 July 2008: Tiberon® (a chemical pinching agent) 100 ppm concentration, one application; Tiberon® 100 ppm concentration, two applications; Exilis® Plus (6BA), 500 ppm, two applications; a water control, one application; and a pruned control (removing the most terminal three nodes on each branch). Second applications were made 4 weeks after the first application. The experiment was completely randomized design with a 2 × 5 factorial arrangement (propagation technique × branch inducing treatment) with 10 replications.

Number of branches 6 inches or longer, total number of branches (any branch greater than 0.5 inches in length), width 1 (width at the widest point), width 2 (width perpendicular to the widest point), and height were all measured on 5 Sept. 2008. A growth index was calculated from the height and width data. Growth Index = [(height + average width)/2]. A quality rating, 1–5, was assigned to each plant (1-sparse, asymmetrical branching, surface of substrate not covered by the plant to, 5-full, symmetrical branching, 100% of the surface of the container covered by the plant) (Fig. 1). Data were subjected to statistical analyses, ($\propto = 0.05$; SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

There was a significant interaction for branch inducing treatment and propagation technique for branches 6 inches or longer (Table 1). Plants propagated by tissue culture had significantly greater number of branches 6 inches and longer than those propagated by cuttings. Branch number for plants treated with water controls averaged 6.3 for tissue culture propagated plants and 3.7 for cutting propagated plants (Table 1, Fig. 2). For all dependent variables except height and growth index, the branch inducing treatments had a significant effect. There was a significant effect of propagation technique on branches 6 inches or longer, height, width 1, and quality.

For tissue culture-propagated plants, the number of branches 6 inches and longer was significantly greater for pruned plants, than those treated with Exilis Plus, but not the water control or either of the Tiberon treatments (Table 1, Fig. 3). For plants produced by cuttings, pruned plants had a significantly greater branch number than plants subjected to the other treatments (Table 1, Fig. 4). For tissue culture propagated plants, Tiberon treated plants (either one or two applications) had significantly greater total branch number than Exilis Plus or pruned plants, but

Table 1. Effect of plant growth regulators on *Hydrangea quercifolia* 'Alice' from two propagation techniques.

Propagation technique	Branch treatment	6 in. + branch	Total branch	Height (in.)	Width 1 (in.)	Width 2 (in.)	Growth index	Quality
Cutting	Water	3.7 b[z]	10.9 b	19.8	29.1	23.4 ab	23.0	2.3 b
	Tiberon 1×	4.8 b	16.3 ab	17.0	30.0	21.5 ab	21.4	2.4 b
	Tiberon 2×	5.2 b	21.3 a	17.5	30.1	20.0 ab	21.3	2.4 b
	Exilis Plus	5.3 b	13.3 b	18.0	28.0	25.2 a	22.3	2.4 b
	Prune	7.6 a	11.0 b	22.1	25.8	19.8 b	22.4	3.9 a
Tissue culture	Water	6.3 ab	15.0 ab	22.1	26.7ab	21.2 ab	23.6	3.05 ab
	Tiberon 1×	6.4 ab	17.9 a	20.9	28.1a	19.2 ab	22.3	3.3 ab
	Tiberon 2×	5.8 ab	18.3 a	20.6	27.3a	21.7 ab	22.6	2.7 b
	Exilis Plus	5.0 b	12.1 b	20.3	28.7a	22.8 a	23.1	2.3 b
	Prune	7.4 a	11.5 b	22.5	22.8b	18.5 b	21.5	3.94 a
Source	DF	F statistic	F statistic	F statistic	F statistic	F statistic	F statistic	F statistic
Prop.Tech.	1	5.92**	0.00[ns]	8.14**	4.97*	2.35 ns	1.42 ns	4.43*
Branch Trt.	4	6.73***	8.93***	1.90 ns	4.68**	4.18**	0.68 ns	9.02***
Water Control[y]	-	25.27***	-	-	-	-	-	-
Prop. Tech. × PGR	4	2.83*	0.95ns	0.51 ns	0.80 ns	0.73 ns	0.54 ns	1.11ns

[z]Means followed by the same letter within a column within a propagation technique were not significantly different (Tukey's HSD ∝ = 0.05).
[y]Pairwise comparison of water controls of cutting and tissue culture propagated plants.

Figure 1. Quality rating scale for oakleaf hydrangea 'Alice'.

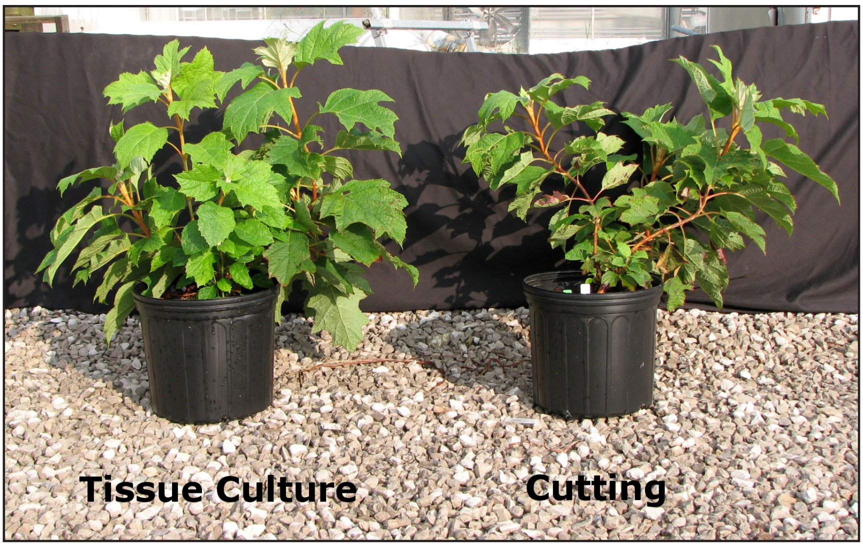

Figure 2. Effect of propagation method on branches six inches or longer.

Figure 3. Effect of tissue culture propagation on oakleaf hydrangea.

Figure 4. Effect of cutting production on oakleaf hydrangea.

Figure 5. Poor coverage of the container surface by many plants treated with PGRs.

not more than the water control. For cutting produced plants, plants receiving two applications of Tiberon had a greater number of total branches than plants treated with water, pruning, or Exilis Plus. However, plants sprayed twice with Tiberon exhibited substantial phytotoxicity (bright yellow chlorosis) rendering plants unmarketable (data not shown). The effect of Tiberon and Exilis Plus on total branch number and phytotoxicity of cutting produced plants is consistent with results of Gibson and Groninger (2007).

Width 1 was significantly greater for both Tiberon treatments and Exilis Plus than for pruned plants on tissue culture propagated plants. For both cutting and tissue culture propagated plants, width 2 was significantly less on plants pruned as compared to those treated with Exilis Plus.

The growth index was not affected by the branch-inducing treatments; however, there was a disparity in branch architecture and plant uniformity. Many plants subjected to treatments other than pruning had a few dominant, horizontal branches, creating an asymmetrical canopy, like that described by growers. Often the surface of the substrate was not covered by branches and/or foliage (Fig. 5). The quality rating was used as an indicator of branch architecture and overall plant appearance. For cutting propagated plants, plants subjected to pruning had a higher quality than those treated with other treatments. For tissue culture propagated plants, plants subjected to the pruning treatment had a higher quality than those treated with Tiberon two times or Exilis Plus, but not higher than Tiberon one time or the water control.

Future evaluations are necessary to determine the total effect of the treatments on oakleaf hydrangea 'Alice'. At the time of writing, the plants were actively growing and, therefore, their response to the treatments may be in flux. Also, a carryover of the treatments into the 2009 growing season is possible.

Based on these data, there was an advantage to tissue culture propagation. Tissue culture propagated plants had more branches 6 inches or longer than cutting propagated plants. Pruning, rather than applying PGRs, was more effective at generating branches 6 inches long or longer for cutting-propagated plants and significantly increased quality. There appeared to be no advantage to pruning or applying PGRs to tissue culture propagated plants. Additionally, PGRs did not mediate the apical dominant growth habit of oakleaf hydrangea 'Alice'. Based on these results, growers may want to select tissue culture propagated oakleaf hydrangea 'Alice' to enhance branching and quality while reducing labor costs.

Ackowledgments. Appreciation is expressed to the Kentucky Horticulture Council and IR-4 Woody Ornamental Branching program for financial support, A. McGill and Son Nursery, Bayer Environmental Sciences, and Fine Americas, Harrell, Inc. and The Scott Co. for supplies, and to Leah Dougherty, Evie Kester, Angela Schoergendorfer, E. Michelle Senn, Theo Steele, and Bonka Vaneva for their assistance.

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