Cutting Propagation of Azaleas Using Hot Water Treatments to Control Pathogens©

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Azalea web blight, caused by certain binucleate species of *Rhizoctonia*, occurs yearly on some azalea cultivars during nursery production in the southern and eastern U.S.A. Azalea shoots collected for cutting propagation can harbor the pathogen, thus allowing the disease to be perpetuated during the cutting propagation process. A previous study demonstrated that submerging *Rhizoctonia*-infested stem pieces of 'Gumpo White' azalea in 122 °F (50 °C) water for 21 min could eliminate the pathogen without causing damage to leaf tissue. The present study determined that this hot water treatment can be used safely for cuttings of twelve commonly grown azalea cultivars without causing detrimental leaf damage or adversely affecting root development.

INTRODUCTION

Sanitation is a proven and cost-effective approach for limiting the entry of pathogens into a propagation facility (Daughtrey and Benson, 2005; Jones et al., 2001; William-Woodward and Jones, 2001). Hot water treatment is a method of sanitation that can be used for seeds, bulbs, vegetables, fruits, and other plant products (Lurie, 1998; Miller, 2005; USDA-ARS, 2004); however, the technique has not previously been reported for use with stem cuttings.

In our previous study (Copes and Blythe, 2009), hot water treatment was the only sanitation method tested that eliminated *Rhizoctonia* from azalea stem pieces that had been inoculated and colonized by *Rhizoctonia* AG P; soaking stem pieces in selected chemical disinfestants or fungicides was ineffective. *Rhizoctonia* was eliminated (not recovered) from stem pieces submersed in 122 °F (50 °C) water for 21 min and in 131 °F (55 °C) water for 6 min. In that study, terminal leafy cuttings of 'Gumpo White' azalea were treated with the same hot water treatments to evaluate possible damage to leaf tissue, following the assumption that necrosis of more than 25% of the leaf area would result in reduced rooting and lower plant quality. Minor leaf damage resulted on cuttings for the times required to eliminate the pathogen at 122 °F and 131 °C. However, the margin of error in time between killing the pathogen and severely damaging leaf tissue was narrower at 131 °F than at 122 °F. Severe leaf damage occurred when cuttings were submerged in 131 °F water for longer than 13 min, while only minor damage occurred when cuttings were submerged in 122 °F for 40 min.

The first objective of the present study was to determine if hot water treatment [122 °F (50 °C) water for 20 min] would damage leaf tissue and/or reduce root development on cuttings of twelve commonly grown cultivars of azalea. The second objective was to evaluate effects of this hot water treatment when extended from 20 to 80 min on cuttings of the 12 azalea cultivars.

MATERIALS AND METHODS

Terminal cuttings were collected from nursery-grown container plants of 12 azalea taxa ['Conleb' (Autumn Embers™ Encore azalea), 'Fashion', 'Gumpo White', 'Hardy Gardenia', 'Hershey's Red', 'Macranthum Roseum' (syn. 'Macrantha Pink'), 'Midnight Flare', 'Red Ruffles', 'Renee Michele', 'Roblel' (Autumn Debutante™ Encore azalea), 'Watchet', and *R. formosa*] during May and June 2009. In Expt. 1, cuttings were not treated or treated by complete submersion in 122 °F water for 20 min using a temperature-controlled hot water bath. In Expt. 2, cuttings were submerged in 122 °F water for 20, 40, 60, or 80 min. In both experiments, cuttings were inserted in a peat and pine bark substrate in 72-cell trays and placed under intermittent mist in a greenhouse for approximately 7 weeks. Leaf damage was evaluated within 2–7 days after hot water treatment using a scale of 0 (no damage) to 4 (all tissue damaged). At the end of each experiment, cuttings were evaluated for root development [using a scale of 0 (no rooting) to 5 (full, symmetrical root system covering the surface of the substrate plug)].

RESULTS AND DISCUSSION

In Expt. 1, cuttings of nine of the twelve cultivars exhibited no leaf tissue damage following submersion in 122 °F water for 20 min compared with nontreated cuttings (Table 1). Leaf damage on cuttings of the remaining three cultivars was minor and not associated with reduced root development (Tables 1 and 2). Cuttings of 'Fashion' azalea had slightly less root growth on hot-water-treated cuttings compared with nontreated cuttings, although root systems likely would have been comparable after a few more weeks of growth. There was indication of some greater root development on cuttings of 'Conleb' (Autumn Embers™ Encore azalea), 'Hershey's Red', and 'Midnight Flare' azalea receiving the hot water treatment compared with nontreated cuttings, although increased root development was not a focus of this study.

In Expt. 2, results from submersing stem cuttings in 122 °F water for 20 to 80 min indicated that cultivars do vary in sensitivity of leaf tissue and rooting response to hot water treatment (Tables 3 and 4). As in earlier experiments with 'Gumpo White' azalea (Copes and Blythe, 2009), submerging cuttings in 122 °F water for 40 min did not severely damage leaf tissue (with ratings of mostly less than 3) on any of the twelve cultivars tested. Submerging cuttings for 60 min or longer increased the likelihood that cuttings would be severely damaged or killed (Table 3). Approximately one-half of the cultivars showed no significant reduction in root development with increasing duration of hot water treatment (Table 4).

Since stem cuttings only need to be submerged for 21 min in 122 °F water to eliminate the pathogen, even the most heat-sensitive of the twelve cultivars could be accidentally submerged for 40 min without hurting the cuttings. Based on published (but limited) research, many other pathogens may survive this heat treatment, while only a few pathogens, including some types of propagules of *Pythium* and *Phytophthora*, may be detrimentally affected by this heat treatment. Research would be needed to determine which pathogens can be killed by heat, if the depth of pathogen structures within plant tissue affects pathogen mortality, and whether different types of heat sources are similarly as effective as hot water.

LITERATURE CITED

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Table 1. Numbers of terminal cuttings of 12 azalea cultivars exhibiting no leaf tissue damage when treated or not treated by submersion in 122 °F water for 20 min, inserted in a pine bark and peat medium in plug trays, and placed under intermittent mist (n = 36). Leaf damage was assessed within 2 to 7 days after treatment.[Z]

Rhododendron taxa	Nontreated	Treated	*p*-value[Y]
'Conleb' (Autumn Embers™ Encore azalea)	36	35	0.5000
'Fashion'	35	32	0.1785
formosa (syn. 'Formosum')	32	31	0.5000
'Gumpo White'	36	34	0.2465
'Hardy Gardenia'	33	30	0.2391
'Hershey's Red'	36	33	0.1197
'Macrantha Roseum' (syn. 'Macrantha Pink')	35	20	<0.0001
'Midnight Flare'	35	31	0.0993
'Red Ruffles'	35	31	0.0993
'Renee Michele'	33	29	0.1535
'Roblel' (Autumn Debutante™ Encore azalea)	33	36	1.0000
'Watchet'	31	29	0.3765

[Z]Leaf tissue damage, when it occurred, was minor.

[Y]*p*-values for tests of increased leaf damage with use of heat treatment (alternative hypothesis) based on Fisher's exact test (lower-tailed). Small numbers ($p < 0.10$) indicate a statistically significant difference in response between nontreated and treated cuttings.

Table 2. Numbers of terminal cuttings of 12 azalea cultivars exhibiting full, symmetrical root development when treated or not treated by submersion in 122 °F water for 20 min, inserted in a pine bark/peat medium in plug trays, and maintained for 7 weeks under intermittent mist (n = 36).[Z]

Rhododendron taxa	Nontreated	Treated	p-value[Y]
'Conleb' (Autumn Embers™ Encore azalea)	29	35	0.9975
'Fashion'	28	16	0.0037
formosa	31	34	0.9467
'Gumpo White'	35	34	0.5000
'Hardy Gardenia'	36	36	1.0000
'Hershey's Red'	7	15	0.9899
'Macrantha Roseum'	35	34	0.50000
'Midnight Flare'	32	36	1.0000
'Red Ruffles'	33	35	0.9427
'Renee Michele'	35	34	0.50000
'Roblel' (Autumn Debutante™ Encore azalea)	34	36	1.0000
'Watchet'	34	33	0.5000

[Z]Root development on all other cuttings was acceptable; no cuttings produced small root systems or failed to root.
[Y]p-values for tests of decreased root development with use of heat treatment (alternative hypothesis) based on Fisher's exact test (lower-tailed). Small numbers ($p<0.10$) indicate a statistically significant difference in response between nontreated and treated cuttings.

QUESTIONS AND ANSWERS

Douglas Justice: Were the microorganisms like *Rhizoctonia* actually killed by the hot water treatment? The temperature of the hot water (122 °F) doesn't seem that hot.

Gene Blythe: It doesn't seem particularly hot, but they were completely killed by that treatment. We've tried higher and lower temperatures for the hot water, but water at 122 °F is effective and seems to be the safest to use with most cuttings.

Mike Bone: Could you elaborate on the design of the "tube with holes" used to hold the cuttings and to keep them submerged?

Gene Blythe: We used a conventional, laboratory hot water bath that could keep the water at the temperature we set. The experiment was set up so we were only treating six cuttings of a cultivar at a time. The cuttings were put into the plastic tube. You could probably substitute muslin cloth or something like that. We made holes in the side of the plastic tube to be sure water was thoroughly moving around the cuttings while they were submerged in the hot water bath. In a nursery setting the same equipment used for hot water treatment of seeds could be used for the treatment of cuttings. It's important to carefully monitor the water temperature so it stays at or near 122 °F.

Table 3. Median leaf tissue damage ratings for leafy, terminal cuttings of 12 azalea cultivars submerged in 122 °F water for 20 to 80 min, inserted in a pine bark/peat medium in plug trays, and placed under intermittent mist (n = 12). Ratings were assigned within 1 week after treatment using a 0 to 4 scale (0: no damage; 4: complete damage).

	Duration of submersion (min.)				
Rhododendron taxa	20	40	60	80	*p*-value[Z]
'Conleb' (Autumn Embers™ Encore azalea)	0	0	2	3	<0.0001
'Fashion'	0	2	3	4	<0.0001
formosa	2.5	3.5	4	4	<0.0001
'Gumpo White'	0	0	0	1.5	0.0675
'Hardy Gardenia'	0	2	2	3	<0.0001
'Hershey's Red'	0	2	3	3	<0.0001
'Macrantha Roseum'	2	3	4	4	<0.0001
'Midnight Flare'	0.5	2.5	4	4	<0.0001
'Red Ruffles'	0	1.5	3.5	3.5	<0.0001
'Renee Michele'	0	1	2	3	<0.0001
'Roblel' (Autumn Debutante™ Encore azalea)	0	0.5	0	2	<0.0001
'Watchet'	0	0.5	1	2	<0.0001

[Z]*p*-values for tests of nonzero correlation (alternative hypothesis) between duration of submersion and response ratings based on Cochran-Mantel-Haenszel statistics. Small numbers ($p<0.10$) indicate a statistically significant change in response with increasing duration of submersion.

John Low: Since you were able to get complete kill after a 20-min exposure of the cuttings to hot water, why did you look at longer time periods?

Gene Blythe: We were curious to see what kind of damage longer time periods caused to the cuttings.

Jim Conner: Is the azalea blight similar to the camellia blight? Does it affect the flowers by turning them brown or are the two diseases totally different?

Gene Blythe: The azalea web blight kills stems. You'll typically find it toward the center of the plant. In humid conditions you may actually see the hyphae growing on the surfaces of leaves. It can actually look like a spider web, thus the name. It will kill the foliage and, eventually, the stems.

Steve McCulloch: Have you considered using a surfactant in combination with the hot water?

Gene Blythe: No, we haven't looked at that at all.

Table 4. Median rooting response ratings for leafy, terminal cuttings of 12 azalea cultivars submerged in 122 °F water for 20 to 80 min, inserted in a pine bark/peat medium in plug trays, and maintained for 7 weeks under intermittent mist (n = 12). Ratings were assigned on a 0 to 5 scale (0: no rooting; 5: full, symmetrical root development).

	Duration of submersion (min.)				
Rhododendron taxa	20	40	60	80	*p*-value[Z]
'Conleb' (Autumn Embers™ Encore azalea)	5	5	5	4.5	0.1592
'Fashion'	4	3	2.5	0	<0.0001
formosa	5	4	2.5	0	<0.0001
'Gumpo White'	3.5	3.5	3	3	0.1883
'Hardy Gardenia'	4.5	4	3.5	3.5	0.2158
'Hershey's Red'	3	3	2.5	2.5	0.2581
'Macrantha Roseum'	3	3.5	0	0	<0.0001
'Midnight Flare'	5	5	1	0.5	<0.0001
'Red Ruffles'	3	3	3	1	0.0026
'Renee Michele'	4	4	3.5	3	0.0608
'Roblel' (Autumn Debutante™ Encore azalea)	5	5	5	5	0.1223
'Watchet'	5	4.5	3.5	2	<0.0001

[Z]*p*-values for tests of nonzero correlation (alternative hypothesis) between duration of submersion and response ratings based on Cochran-Mantel-Haenszel statistics. Small numbers ($p<0.10$) indicate a statistically significant change in response with increasing duration of submersion.

Nevin Smith: Has anyone looked at the necessary temperatures to kill various common plant pathogens like *Fusarium* and *Botrytis* and *Phytophthora* and *Pythium*?

Gene Blythe: I'm sure that's been done, but I don't have the exact details on that.