Propagation

Gene Blythe

Section Editor

The Use of Fungicides for Increasing Micropropagation of Native Deciduous *Rhododendron* spp.

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Significance to Industry Improving the rate of success in starting a micropropagation process to increase availability of native *Rhododendron* for wholesale and retail markets.

Introduction *Rhododendron* is the largest genus in the Ericaceae family, consisting of over 1000 species that are native to North America, Western Europe, and Northern Asia. *Rhododendron* spp. include evergreen and deciduous varieties ranging from shrubs to tree-sized plants with flowers blooming from late winter to early summers. They are often used as ornamentals in landscapes and woodlands due to their display of flowers (1); however, these plants are often difficult to find in retail markets**.**

With certain *Rhododendron* varieties being desirable, propagation is of increased interest. Plant propagation is a method of growing new plants from a range of sources; seed, cuttings, and other plant parts. Some plants do not grow from seed at all or are difficult to reproduce vegetatively. Cuttings may be limited by the size of the mother plant from which a limited number of cuttings can be taken (2). Propagating *Rhododendron* by cuttings is the most popular (as well as problematic) technique one can use; this is particularly true for deciduous azaleas. The main problems are initiation of rooting with deciduous azaleas and production of new growth after rooting (3). To overcome these problems, plants are produced from a single parent plant by vegetative cloning (3). One method of cloning is micropropagation.

Micropropagation is the technique of multiplying plant explants, or cultures, in a sterile environment. In this process, clones of desirable plants are produced in a relatively short period of time from stock cultures (4). Often, these stock cultures start from stem cuttings taken from the landscape which can carry with them a number of fungal pathogens with them, adversely affecting shoot survivability. The aim of this study was to evaluate methods of control that can inhibit or eliminate some fungal contaminants when establishing shoot cultures.

Materials and Methods Three fungicides were used in this study: TM (586 ppm), chlorothalonil (Chloro) (Daconil®, BayerCrop Science) (1378 ppm), and triadimefon (Triad) (75 ppm). These rates were based on recommended rates of application (5). Some fungicides were chosen because their success in preliminary trials, such as Chloro (6). Fungicides used in these evaluations are labeled for fungal disease control in *Rhododendron* (5).

The medium used in these trials was a modified E&R medium (7) supplemented with 2 grams of sucrose and 0.6 grams per 100 ml media. Each of seven fungicides were added to portions of the media and the pH was adjusted to 5.0 prior to autoclaving. Media was dispensed into sterile 18 mm test tubes after autoclaving.

This study was carried out on a variety of deciduous azaleas, nicknamed 'Easter Pink' (*R. cumberlandense* X), 'Cumberland' (*R. cumberlandense*), and 'F-L' (*R. cumberlandense* X). The stock materials came from a private property in Lee County, Alabama. Stem cuttings, 4-6 inches long, were taken from stock plants during the first spring flush of growth before hardening of stems. The fresh stem cuttings were put into moist chambers until their transfer to media.

All leaves were removed from cuttings to expose the bud node. Cuttings were put into a 0.82% sodium hypochlorite solution with 0.001% Tween for 15 minutes under agitation. Each of the stem cuttings were cut into 1- to 1.5-cm cuttings, each with at least one bud node; these were maintained in order of removal from the cutting. These node cuttings were then submersed in a 0.82% sodium hypochlorite solution for no more than 2 minutes and rinsed three times in sterile water with 0.001% Tween for 1 minute. Once these cuttings were rinsed, they were placed onto fungicide-amended media. Stem node pieces (apical to basal) were arranged such that, for example, tips would be placed on each fungicide amendment. If the tip of the first stem cutting was placed on control medium, the next stem cutting tip would start on thiophanate-methyl (TM) medium.

Cultures were kept at ambient indoor conditions under fluorescent lamps that cycled on for 13 hours daily. Data was taken for 31 days, at 5- to 7-day intervals, to monitor days on which the buds or contamination developed. Notes were taken on the state of the node cutting: contaminated, browning, or showing bud emergence.

Results and Discussion 'Easter Pink' shoots were established on May 13. Stem pieces on the TM- and Triad-amended media produced the first buds on day 7. After day 21, browning of stem pieces was observed on these same two media, but two of the stem pieces on TM medium had buds on day 31 (Table 1). Stem pieces of this variety on non-amended control medium had contamination by day 7 of culture. Of the 8 original shoots on the TM medium, two produced buds by day 31. Among all tested fungicides, the highest percent survivability was 38% with TM-amended medium.

'Cumberland' shoots were established on May 13. Stem pieces on TM-, Triad-, and Chloro-amended media showed the first bud break on day 5 (Table 2). However, only stem pieces on the TM-amended media retained a bud through day 31. Stem pieces of this variety on the non-amended control media were contaminated by day 5. Of the 5 original shoots in the TM medium, two produced buds by 31 days. The amendment that resulted in the highest percent survivability (40%) was TM.

'F-L' shoots were established on May 13. All shoot pieces produced a bud by day 5 regardless of the amendment, and at least one remained without contamination until day 31 (Table 3)**.** The control was the first to produce leaves on day 21 while the TM

amendment allowed production of leaves on day 31. Browning of shoot pieces was not observed until day 21 with the Triad amendment. Of the 4 original shoots in each amendment; 1 produced a bud in the non-amended control, 3 with TM, 2 with Chloro, and 1 with Triad. Of the 4 original shoots with TM, three produced shoots at the end of the 31 days. The amendment that achieved the highest survival of shoot pieces (75%) was TM. A consistent trend was observed with bud development on TM-amended media with these three *Rhododendron* varieties.

Differences among varieties were noted relative to the different fungicide amendments. Depending on the variety of *Rhododendron*, TM and Chloro were the most successful fungicides in the trials based on the results displaying the highest survival percentage on day 31. While there were similarities between varieties, consistency with micropropagation success with any one fungicide was not observed. Further experiments will seek to narrow down these relationships to determine the best fungicide amendment for use with individual *Rhododendron* varieties.

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Table 1. Numbers of shoot pieces of *Rhododendron* 'Easter Pink' surviving without contamination on fungicide-amended media at varying intervals.

	Days after start						
	0	7	13	21	26	31	Buds
Control	6	0	0	0	0	0	0
Thiophanate-methyl	8	5	5	3	3	3	2
Chlorothalonil	9	2	1	1	1	1	0
Triadimefon	6	2	2	2	0	0	0

Table 2. Numbers of shoot pieces of *Rhododendron* 'Cumberland' surviving without contamination on fungicide-amended media at varying intervals.

	Days after start						
	0	7	13	21	26	31	Buds
Control	4	0	0	0	0	0	0
Thiophanate-methyl	5	4	2	2	2	2	2
Chlorothalonil	4	2	0	0	0	0	0
Triadimefon	4	1	0	0	0	0	0

Table 3. Numbers of shoot pieces of *Rhododendron* 'F-L' surviving without contamination on fungicide-amended media at varying intervals.

	Days after start						
	0	7	13	21	26	31	Buds
Control	4	3	2	1	1	1	1
Thiophanate-methyl	4	3	3	3	3	3	3
Chlorothalonil	4	3	2	2	2	2	2
Triadimefon	4	4	3	2	2	2	1

Stem Cutting Propagation of Aromi Hybrid Deciduous Azaleas

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Index Words Exbury azaleas, rooting, indole butyric acid, nursery production

Significance to Industry Aromi hybrid deciduous azaleas introduce rare color combinations and fragrance in azaleas that adapt well to the heat and humidity of the southeastern U.S. Growers can expect at least 50% rooting for the Aromi hybrids 'Great Balls of Fire', 'Lemonade', and 'Radiant Red' using IBA during rooting of terminal stem cuttings. Rooting percentages can be increased using increasing IBA concentration and rooting durations up to 150 days, and liners suitable for stepping up into larger containers can easily be produced from cuttings in one growing season. Successful propagation of azaleas in this series should allow growers to expand production of these fragrant, deciduous azaleas.

Nature of Work Exbury azaleas are hybrids resulting from crosses among deciduous *Rhododendron* species of the east and west coast of the United States, Europe, and Asia (1, 2, 3). Parent plants include the U.S. species *Rhododendron arborescens*, *R. viscosum*, *R. nudiflorum*, *R. calendulaceum*, and *R. occidentale* (1); the European parent *R. luteum*; and the Asian species *R. molle* and *R. japonicum*. Exbury hybrids, one of five sub-groups of the Knap Hill hybrids, were grown throughout Europe and many areas of United States, particularly the east and northeast, beginning as early as the mid 1930's. Exbury hybrids lack the heat tolerance to perform well in the Southeastern United States.

Dr. Eugene Aromi began breeding azaleas in the 1970s, successfully making over 1000 interspecific crosses (Maarten van der Giessen, pers. comm.). Some of his better known hybrids resulted from interspecific crosses between the European bred Exbury hybrids and native *Rhododendron* species of the Southeastern United States in an attempt to capture rare color combinations, while instilling greater heat tolerance (4). Aromi azaleas have fragrance in association with flower colors that typically are not associated with fragrance (such as orange and red), more flower color combinations, larger blooms, and greater heat tolerance than Exbury hybrids.

Aromi azaleas, like native U.S. species of deciduous azaleas, are generally propagated vegetatively by terminal softwood stem cuttings treated with a K-IBA solution (Maarten van der Giessen, pers. comm.). Prior research conducted with propagation of deciduous azaleas gave conflicting results regarding K-IBA concentrations. Maarten van der Geissen, a nurseryman and friend of the late Dr. Aromi, states that using a 2500 ppm solution provides the most favorable results for most Aromi azaleas. Dirr and Heuser (5) reported that 4000 ppm is the ideal concentration for U.S. native species, while Knight (6)

concluded that 7500 ppm is the optimal K-IBA concentration for Aromi parent plants *Rhododendron austrinum* and *R. canescens*. The objective of this research was to compare the effect of IBA concentration on the rooting of stem cuttings of three cultivars of Aromi hybrid deciduous azaleas.

Terminal softwood stem cuttings (4-5 in length) were taken from the Aromi hybrid azalea cultivars 'Lemonade', 'Radiant Red', and 'Great Balls of Fire' on May 12, 2015. The basal 1 inch of each cutting was treated for 5 sec with one of seven water soluble solutions of IBA ranging from 2,500 ppm to 10,000 ppm in 1,250 ppm increments, before sticking in Dillen 1801 cells. The substrate consisted of 3 fine pine bark: 1 peat: 1 perlite amended per cu. yd. with 1.5 lb Micromax, 2 lb dolomitic lime, 2 lb gypsum, and 10 lb Nutricote 20–7–10 270-day controlled release fertilizer. Intermittent mist was applied for 10 sec every 6 min for the first 10 days, 10 sec every 15 min for the following 40 days, and 10 sec/ 30 min until the experiment was terminated at 150 days after treatment (DAT). Cuttings were arranged in a greenhouse in Auburn, AL in a randomized complete block design with six blocks and six cuttings per treatment per cultivar per block. Subjective root ratings were collected using a 0-5 scale: 0- dead, 1- callused, 2- roots initiated, 3- light rooting, 4- medium rooting, 5- heavy rooting. Rooting and subjective root ratings were collected three times at 50-day intervals on two cuttings per treatment per cultivar per block at each date. The length of the two longest roots per cutting was measured 100 DAT. Data were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Results and Discussion Among all concentrations and rooting durations, 76% of cuttings of 'Radiant Red' azalea rooted, which was greater than the 51% and 52% rooting of 'Great Balls of Fire' and 'Red Lemonade' azaleas, respectively, which were similar (Table 1). Rooting increased linearly with increasing K-IBA concentration, ranging from 55 to 74%. As expected, rooting percentages increased over time, from 34% at 50 days after treatment (DAT) to 56% and 88% at 100 and 150 DAT, respectively. This linear increase in rooting percentages over time has practical implications in that most growers typically do not leave deciduous azalea cuttings in propagation for as long as 150 days. A similar trend and percentages were found with callusing of treated stems, suggesting that if the cutting callused, then rooting occurred (data not shown).

Cultivar and IBA concentration main effects were significant for root length measurements, but not rooting duration (Table 2). Root lengths of 'Radiant Red' azalea, measured 100 DAT, were 86 and 51% greater than those of 'Great Balls of Fire' and 'Lemonade' azaleas, respectively. Root length increased linearly, up to 75%, with increasing rates of K-IBA, regardless of cultivar.

Cultivar by IBA concentration and cultivar by rooting duration interactions were significant for the subjective root rating (Table 3). The average root ratings for 'Great Balls of Fire' and 'Radiant Red' azaleas increased linearly, while that of 'Lemonade' azalea changed quadratically with increasing IBA concentration. The average root rating for 'Lemonade' azalea was greater at the lowest and highest concentrations than at intermediate concentrations. 'Radiant Red' had the highest average root rating among cultivars at all

IBA concentrations and rooting durations, except 3750 ppm IBA, when it was similar to that of 'Lemonade. At all other concentrations and all rooting durations, the average ratings were similar for cuttings of 'Great Balls of Fire' and 'Lemonade'.
These results indicate that 'Radiant Red', 'Great Balls of Fire' and 'Lemonade' Aromi hybrid azaleas can be rooted using K-IBA at 2500 to 10,000 ppm, with increased rooting percentages using higher concentrations and leaving cuttings in propagation for up to 150 days. Liners suitable for stepping up into larger containers can easily be produced from cuttings in one growing season using these methods.

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Table 1. Effect of cultivar, IBA concentration, and rooting duration on the rooting of stem cuttings of three cultivars of Aromi azaleas.[z]

Cultivar	Rooted cuttings (no.)	IBA concentration (ppm)	Rooted cuttings (no.)	DAT[y]	Rooted cuttings (no.)
Great Balls of Fire	128b[x]	2500	59[w]	50	86[v]
Radiant Red	191a	3750	60	100	141
Lemonade	130b	5000	58	150	222
		6250	65	Sig.[u]	Q*
		7500	61		
		8750	66		
		10000	80		
		Sig.	L***		

[z]The cultivar, concentration, and rooting duration main effects were significant at α = 0.05; no interactions were significant.
[y]Days after treatment.
[x]Counts of cuttings rooted out of 252. Comparisons among cultivars (lower case in column) using estimate statements at α = 0.05.
[w]Counts of rooted cuttings out of 108.
[v]Sum of rooted cuttings for the three cultivars out of 252.
[u]Significant (Sig.) linear (L) or quadratic (Q) trends (in columns) using orthogonal polynomials at α = 0.05 (*) or 0.001 (***).

Table 2. Effect of cultivar and IBA concentration on the root length of stem cuttings of three cultivars of Aromi azaleas.[z].

Cultivar	Root length[y] (cm)	IBA concentration (ppm)	Root length (cm)
Great Balls of Fire	3.5b[x]	2500	3.6
Radiant Red	6.5a	3750	4.1
Lemonade	4.3b	5000	2.8
		6250	4.5
		7500	5.4
		8750	6.4
		10000	6.3
		Sig.	L***[w]

[z]Only data from 100 days after treatment were analyzed. The cultivar and concentration main effects were significant at α = 0.05; the interaction was not.
[y]Length (cm) of two longest roots on each cutting in.
[x]Least squares means comparisons among cultivars (lower case in column) using the Shaffer-simulated method at α =0.05.
[w]Significant (Sig.) linear (L) trend (in column) using orthogonal polynomials at α =0.001 (***).

Table 3. Effect of cultivar, IBA concentration, and rooting duration on the subjective root rating of stem cuttings of three cultivars of Aromi azaleas.[z]

	Cultivar				Cultivar		
Conc.[y] (ppm)	Great Balls of Fire	Radiant Red	Lemonade	DAT[x]	Great Balls of Fire	Radiant Red	Lemonade
2500	1b[w]	3a	1.5a	50	1b	2a	1b
3750	1b	3.5a	2a	100	1b	4a	1b
5000	1b	3a	1b	150	3b	4a	2.5b
6250	1.5b	3a	1b	Sig.v	Q***	L***	L***
7500	1b	4a	1b				
8750	2b	4a	1b				
10000	2.5b	4a	3b				
Sig.	L***	L***	Q*				

[z]The cultivar by concentration and cultivar by rooting duration interactions were significant for subjective root ratings at α = 0.05. Subjective root ratings scale: 0-dead, 1-callused, 2-roots initiated, 3-light rooting, 4-medium rooting, 5-heavy rooting.
[y]Conc. = concentration.
[x]Days after treatment.
[w]Medians are reported. Comparisons among cultivars (lower case in rows) using estimate statements at α = 0.05.
[v]Significant (Sig.) linear (L) or quadratic (Q) trends (in columns) using orthogonal polynomials at α = 0.05 (*) or 0.001 (***).

From Lagoon to Liner: Development of a Transplant Media

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Index Words Substrate, swine lagoon waste, bioassay, swine lagoon compost

Significance to Industry A seedling germination media was produced from a blend of 15:85 (v/v) swine lagoon solids and ground peanut hulls using an in-vessel compost reactor. Composting was accelerated (requiring 3 days once within optimum temperature range) with the closed reactor. Composts are stable (do not continue to heat and decompose) after maturity; a minimum of 11 days is recommended for compost maturation. Composts produced were consistent from batch to batch.

Nature of Work There are 8.7 million hogs in North Carolina alone (1). Much of the waste from these hogs is captured in anaerobic lagoons composed of a manure slurry washed from the animals' pens. Locally available swine lagoon solids (SLS) are an attractive option for horticultural plant production as they may provide a portion, or all, of a plant's nutrient requirements when mixed into growing media (2). However, the electrical conductivity (EC) levels of SLS amended media can be high enough to damage plant roots (3). Composting the SLS with a carbon source may reduce the soluble salts and produce a media that can be useful for horticultural plant production. Nine percent of U.S. peanut production occurs in North Carolina, making peanut hulls another large waste component for the state (4) and requiring significant bulk-waste disposal for the peanut industry (5). Utilizing SLS composted with peanut hulls as a transplant media may be a means of dispersing concentrated nutrients in a cost-effective, environmentally conscientious manner, as well as producing a media suitable for horticultural transplant production.

There are several considerations for determining whether a compost can be utilized in plant production: the degree of stability or maturity (which implies a stable organic matter content and the absence of phytotoxic compounds), pH, EC, water-holding capacity, and air-holding capacity, nutrient content, nutrient availability, and lack of excessive heavy metals (6). The objective of this study was to evaluate the physical and chemical suitability of composted SLS and ground peanut hulls for use as a transplant media

A compost was developed using 15:85 (v/v) SLS and peanut hulls. This 15:85 SLS to peanut hull ratio provided a C:N ratio of 23.7:1, which was close to the standard composting guidelines for C:N ratio (25:1) (7). The SLS were dredged from a lagoon on Ingold Farms in Garland, NC (Murphy Brown, LLC, Warsaw, NC) and dewatered in a geotextile bag for 2 years. The SLS were removed from the bag on December 14, 2015,

taken to the composting facility (Murphy Brown, LLC, Warsaw, NC), and placed under shelter on January 4, 2016. Peanut hulls were obtained from a peanut processing facility (Golden Grove Inc., Warsaw, NC). The peanut hulls were ground and sieved through a 1/8-inch (3.175-mm) screen (Massey Ferguson 15 Grinder Mixer, Duluth, GA). The ground peanut hulls and SLS were spread in layers on a concrete pad and mixed with a front end loader until the SLS were evenly distributed. Three cubic yards (2.3 cubic meters) of the mixture were loaded into an in-vessel compost reactor equipped with a thermometer (Figure 1). Three gallons (11.4 L) of water were added to the reactor. Temperature was recorded once in the morning and once in the afternoon. When the temperature reached 131F (55C), the temperature was monitored twice daily until the temperature fell below 131F (indicating that composting was complete) (7)

Two batches of compost were completed and dumped in separate piles to mature. Batch 1 (B1) was composted in the reactor for 8 days: 3 days to reach optimum temperature, 3 days at optimum temperature, and 2 days to allow cooling and further composting. Batch 2 (B2) was composted for a total of 10 days: 2 days to reach optimum temperature, 3 days at optimum temperature, and 5 days to allow cooling and further composting. Following composting, composts were removed from the reactor and piled under shelter to allow resting for maturation

A bioassay study was conducted three times with the two different batches of composts following the procedures of the Mulch and Soil Council (8). Each bioassay study used a randomized complete block design with six replications and a two-way factorial of four plant species and two media [the SLS peanut hull compost (SLC) and the control (Jolly Gardener Pro-Line C/P, Jolly Gardener, Poland, ME)]. The indicator species tested followed the soil and mulch council's guidelines (8) and included *Raphanus sativus* 'Cherriette' (radish), *Zinnia elegans* 'Dreamland Red' (zinnia), *Lycopersicum esculentum* 'Moneymaker' (tomato), and *Tagetes* patula 'Janie Deep Orange' (marigold). Five seeds were planted in each 4-inch (65.6 milliliters) square pot and allowed to germinate in a greenhouse (85F day/65F night) (29.4C day/18.3C night) under mist applied for 8 seconds every 10 minutes. Fourteen days after sowing, seedlings were counted and then each pot was thinned to one plant, removed from mist, and watered by hand once a day. After 28 days, plants were recounted. Three bioassay experiments were conducted as shown in Table 1. All variables were subjected to analysis of variance (ANOVA) procedures using the GLM procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) and α=0.05.

Results and Discussion Germination of seedlings of each species, 14 days after sowing, was significantly different for each bioassay (P=0.009) and each media (P=0.045). However, when germination count data were analyzed by media and species, the main effect of bioassay was not significant for the control media, with the exception of tomato (P=0.008), nor was it significant for SLC with the exception of zinnia (P=0.01). This indicates that germination of each species was similar for each batch of compost and days of maturation, indicating that 4 days of maturation may be sufficient. However, growth of seedlings in B1 with 4 days of maturation was somewhat abnormal, with poorly formed roots (personal observation), indicating that 11 days of maturation should be recommended.

Germination of each species at each bioassay were different for each media (Table 2). Each species germinated better in the control media than in the SLC; however, germination of radish, marigold, and tomato were numerically very similar between the two media. Zinnia germination was reduced by 40% when sown in the SLC. This reduction may be due to EC as increasing salinity decreases germination in zinnia (9).

Seedling growth at 28 days after sowing was not impacted by species, thus data were averaged over species. The two-way interaction of media by bioassay was significant for SLC only (*P*=0.003). Seedling growth in bioassays 1 and 2 are statistically similar indicating that composting of B1 and B2 produced similar SLC (data not shown) (Figure 2). For bioassay 1, with B1 compost and 4 days of maturation, media affected seedling growth (*P*=0.008) with the control having a greater number of surviving seedlings than SLC, again indicating that 4 days of maturation was not sufficient (data not shown).

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Table 1. Bioassay experiment and compost maturity.

Bioassay[z]	Compost utilized[y]	Days of maturation[x]
1	Batch 1	4
2	Batch 2	11
4	Batch 1	57

[z] Procedures followed the Mulch and Soil Council's guidelines (8).
[y] Composts were a 15:85 blend (v/v) of swine lagoon solids and ground peanut hulls. Batch 1 was composted for 8 days: 3 days to reach optimum temperature [131F (55C)], maintained at optimum temperatures for 3 days, then cooled in a static pile for 2 days. Batch 2 was composted for a total of 10 days: 2 days to reach optimum temperature, 3 days at optimum temperature, and 5 days cooling in a static pile.
[x] Composts were rested in a static pile to cool and allow curing and maturation.

Table 2. Number of seedlings germinated 14 days after sowing.

Media[z]	Marigold	Radish	Tomato	Zinnia
Control	4.7 a[y]	5.0 a	4.7 a	4.8 a
SLC	4.2 b	4.5 b	4.2 b	2.8 b

[z] Media: commercially available, peat-based product (Control) and 15:85 (v/v) swine lagoon solids:peanut hull compost (SLC)]. N=192.
[y] Means between species with different letters are significantly different from each other based on Tukey's honestly significant difference procedure ($P \leq 0.05$).

Figure 1: In-vessel compost reactor.

Figure 2: Batch 1 (right) and Batch 2 (left) finished composts.