Rooting Response of Deciduous Azaleas, *Rhododendron* Section *Pentanthera*, Stem Cuttings to Mist Regimes and Media Mixes

by

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Abstract

Rooting of stem cuttings of Escatawpa azalea, *Rhododendron austrinum* (Small) Rehder 'Escatawpa', was evaluated on four harvest dates in six media mixes under two mist regimes. Data was collected at 2 week intervals from 6 to 12 weeks after date of stick. Cuttings subjected to the lower mist duration (9 s) produced consistently superior root development than those subjected to the higher mist duration (12 s) both at the same 10 min interval. All media mixtures consisted of 50% pine bark by volume. In the series of media mixes evaluated, the remaining 50% of volume progressed from 50% perlite to 50% peat in 10% increments. The media mixture 2 perlite : 3 peat : 5 pine bark (by volume), produced superior results earlier in the evaluation schedule than the other media mixtures. The majority of cuttings were rooted at 6 weeks, but rooting percentages increased as harvest date progressed from 55% at 42 days to 89% at 84 days after cuttings were stuck. Harvest date was a repeatedly important factor in the analysis of media and mist allowing for meaningful evaluation of the schedule of development of cuttings in different growing conditions within our results. To document the variation across taxa to these propagation protocols, cuttings from 20 cultivars of deciduous azaleas were given the lower mist regime and stuck in the best performing media mixture. Under these protocols, tetraploids and complex multi-generational hybrids achieved superior root ratings compared to diploids, straight species selections, and F-1 hybrids.

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AS	air space
AU	Auburn University
BD	bulk density
DAS	days after sticking
DA	Davis Arboretum
IBA	indole-3-butyric acid
MM	media mixture
NAA	naphthaleneacetic acid
K-IBA	Potassium based indole-3-butyric acid
TC	tissue culture
TP	total porosity
WHC	water holding capacity

Chapter 1

Introduction and Literature Review

The deciduous azaleas of Eastern North America have been heralded as among the finest flowering shrubs since the first written accounts (Bartram, 1794). Propagation difficulty and slow growth has limited this group of plants' availability in the industry (Jones, 2008). These propagation difficulties pose a challenge to maintaining cultivars after development in breeding programs. Scientific and popular literature contain a wide spectrum of recommendations and observations on aspects of vegetative propagation. Research investigating propagation protocols for deciduous azaleas were not addressed in the published literature to a degree that yields a consensus on best management practices (File et al., 2000). There was consensus that it can be difficult to root cuttings of deciduous azaleas (Galle, 1985; Jones, 2008; Skinner, 1961). Success in rooting stem cuttings varied greatly between species, cultivars, and within species (Jones, 2008; Skinner, 1961). Environmental and endogenous factors influencing adventitious root formation include nearly everything affecting plant growth (Pijut et al., 2011). This list includes, but is not limited to hormones, light, oxygen, carbon dioxide, relative humidity, volume and irrigation schedule, growth media pH, physical properties of growth media, and concentration and types of nutrients in the media (Pijut et al., 2011). Seedlings can be raised successfully, but require years to mature to a flowering stage and exhibit wide variability in flower color and form (Skinner, 1954). Vegetative propagation offers a consistent product and less time to first flower (Skinner, 1961).

Types of deciduous azaleas

All deciduous azaleas are in the genus *Rhododendron*, family *Ericaceae*, (Galle, 1985). North America is home to at least 16 species of deciduous azaleas that were potential candidates for this study (Horn, 2005; Kron, 1993; Zhou et al., 2008). The California azalea, *R. occidentale* (Torr. & A. Gray) A. Gray, a western species, and the pinkshell azalea, *R. vaseyi* A. Gray, from the upper Blue Ridge Mountains, do not perform well in the Southeastern U.S. where this research was conducted (Towe, 2004). The Santee azalea, *R. eastmanii* Kron & Creel, has a restricted range (Horn, 2005), and was not available for this study in the necessary quantity. The roseshell azalea, *R. prinophyllum* (Small) Millais, is only marginally hardy at the site of study (Towe, 2004). Only four of the 16 species, *R. arborescens* (Pursh) Torr., *R. flammeum* (Michx.) Sarg., *R. austrinum* (Small) Rehder, and *R. canescens* (Michx.) Sweet, were the topic of accessible research on cutting propagation (File et al., 2000; Jones, 2008; Knight et al., 2005; Lewis and Sizemore, 1978).

Rooting recommendations for Exbury azaleas, a group of complex hybrid azaleas developed in Europe with mostly North American species, were reported on more frequently (Brydon, 1964; Fairweather, 1971; Henny, 1963; Mylin, 1982; Nienhuy, 1980). A plant that is difficult to clone is less likely to be propagated and maintain its genetic integrity than an easy to propagate plant. Exbury azaleas were bred to have large flowers in bright colors. The ability to develop adventitious roots on stem cuttings may have also been a trait that made these the most widely propagated of deciduous azalea types in the world. The cultivars *R.* ×‘Gibraltar’, *R.* ×‘Klondyke’, and *R.* ×‘Chetco’ were used in the breeding program of the Auburn

Azalea series (Smitherman, unpublished report). Henry Skinner, former Director of the U.S. National Arboretum, worked extensively with native azaleas and gave a rare commentary on the comparative difficulty in cutting propagation (Skinner, 1961). He divided the species into three groups. The most difficult to propagate were *R. prinophyllum*, and *R. periclymenoides* (Michx.) Shinners. The easiest to propagate were *R. serrulatum* (Small) Millias, *R. viscosum* (L.) Torr., *R. atlanticum* (Ashe) Rehder, *R. oblongifolium* (Small) Millias, *R. canescens, R. austrinum*, *R. alabamense* Rehder, and *R. arborescens*. In the intermediate group were *R. prunifolium* (Small) Millias, *R. flammeum*, *R. cumberlandense* Braun, and *R. calendulaceum* (Michx.) Torr.. Individual plants that are more stoloniferous or from the southern range of a species were more likely to root than individuals without those characteristics within a species (Skinner, 1961). Sommerville (1998) reported a wide range of cutting rooting ability of material he attempted to propagate, and within most species, there were more difficult and less difficult to root individuals.

Within native deciduous species, the majority of evaluated specimens have diploid (2n=26), or tetraploid (2n=52) chromosome number based on molecular weight and chromosome counts under a microscope (Li, 1957; Zhou et al., 2008). Twelve native species were reported diploid, and four were tetraploid (Zhou et al., 2008). Three of four tetraploid species fall into Skinner's easiest to propagate group (Skinner, 1961; Zhou et al., 2008). Kehr (1996) reported many beneficial effects of polyploidy in plants. Larger, heavier textured flowers, which last longer than others, may be the most valuable horticultural quality imbued by polyploidy. Larger leaves and thicker twigs also are noteworthy, though they are of lesser value to the plant's quality than flower characteristics. Difference in rooting ability between the two ploidy levels was not reported (Kehr, 1996).

In addition to naturally occurring species, approximately 1,450 deciduous azalea cultivars were reported to exist in 1985 (Galle, 1985). It is unknown how many of these cultivars still exist, and this count does not include cultivars named over the last 33 years. Galle (1985) states that over 500 varieties of Ghent hybrids were grown in the 1800's, but only 100 were reported in his publication because many have been lost, and only approximately 25 of the 100 were still grown in 1985. Galle (1985) lists thousands of azalea cultivars, of the 1,450 deciduous azalea cultivars reported, only 115 are listed in the group of Eastern American selections and hybrids. A recent survey conducted by the Auburn University (AU) Davis Arboretum (DA) compiled collections reports from 19 public gardens that hold significant indexed collections of deciduous azaleas and documented 334 (Thompson, unpublished report).

Hybrids were 45% (151/334) of the reported cultivars. Selections made from just four species (*R. austrinum*, *R. calendulaceum, R. flammeum*, and *R. viscosum*) constituted another 31% (102/334) of reported cultivars. The remaining 24% (81/334) of the reported taxa represented the other 11 species of native eastern azalea species (Thompson, unpublished report). While many more cultivars exist in cultivation, these represent the plants maintained with long-term institutional commitment, and reportable records. These qualifications insure accessibility and improve the likely hood of survival of the plants beyond the life of a single grower. Despite intense breeding, the scarcity of cultivars is a predictable result of difficulty in vegetative propagation.

The Alabama azalea was first described by Dr. Charles Mohr (1883) as *Azalea* *nudiflora alba*. The species documented range was centered on the state of Alabama, but can be found in scattered locations in the four adjacent states of Florida, Georgia, Tennessee, and Mississippi (Towe, 2004). It is described as a frequently stoloniferous plant of low to medium height in the wild, exceeding 2 m in cultivation and on more favorable sites (Galle,1985). It is also described as an usually non rhizomatous shrub to small tree growing 3–5 m tall (Kron, 1993), highlighting the lack of consensus on even the most basic aspects of native azaleas (personal observation). In the wild, it occurs on dry, steep, rocky hillsides and open woodlands where it blooms midway through the deciduous azalea bloom season (Kron, 1993). The flowers have the fused corolla typical of the family. Sympetalous flowers are borne on a shortened raceme, or truss, which is typical of the genus. Alabama azaleas have 6 to 12 white flowers per truss, terminating in five petal lobes. The central lobe is the widest, and is typically strongly recurved with a yellow blotch (Kron, 1993). Atypical flowers can have blushes of pink, diffuse yellows, or be completely white (Galle, 1985). The species was routinely reported as diploid, having two complete sets of chromosomes; n=26 (Jones, 2008). The flowers were described as having a distinct and pleasant lemony fragrance. Small stature, fragrance, and tolerance of xeric conditions are characteristics of the Alabama azalea that were recognized as desirable for the Auburn Azalea Breeding Program (R.O. Smitherman, unpublished report).

Individuals propagated from the parent plant accessioned at the DA as 2008143 are referred to as *R. alabamense* 'CCP'. This plant was collected from Colbert County's Cane Creek Preserve in Northwest Alabama. Property owners Jim and Faye Lacefield have gone to extensive efforts to document plants on their property and have put the more than 800 acres into a land trust to be managed by The Nature Conservancy of Alabama. The population of *R. alabamense* present within their property and the adjacent woodlands were estimated to exceed over 1 million individuals (Jim Lacefield, personal communication). This is the largest population of the species reported anywhere within the species range, though populations do achieve greater densities at other locations (R.O. Smitherman, unpublished report). *R. alabamense* 'CCP' is a single individual collected from this population because its foliar and floral characteristics and growth habit and were determined to be typical for the species by Dr. Renford Oneil Smitherman. Dr. Smitherman was a professor emeritus of Auburn University who spent years collecting *R. alabamense* and other species for the Auburn Azalea Breeding Program. Hundreds of these were donated to the AU DA's deciduous azalea collection for display, research, education, and conservation. *R. alabamense* 'CCP' was collected in the winter of 2009 and transplanted directly into the Arboretum collection (R.O. Smitherman, unpublished report).

The second *R. alabamense* evaluated was the result of selective breeding. *R. alabamense* 'Smitty' is a cultivar resulting from a cross made and grown out by Dr. R. O. Smitherman, who was known as Smitty to most people with whom he interacted. This is a cross between *R. alabamense* 'Varnadoe's Yellow' and a vigorous large flowered selection of *R. alabamense* of undocumented origin. Dr. Smitherman produced seedlings from the cross and selected this individual because it retained both the light yellow flower color of 'Varnadoe's Yellow', and the vigorous growth habit and large flower size of the other *R. alabamense* parent plant (R.O. Smitherman, unpublished report).

The second species evaluated was a selection of the Piedmont azalea, *R. canescens* 'Glenn Ave.'. The Piedmont azalea is the most common native azalea, and it grows in a variety of habitats across its range (Towe, 2004). It has one of the widest distributions of any native deciduous azalea, occurring from North Carolina to Florida in the Eastern US, and west to Texas and Arkansas. It is also among the most commonly available species, with hybrid cultivars the most available group of deciduous azaleas for purchase (Galle, 1985). Though more available than other species, the origin of plant material is typically undocumented. This specimen was collected from a population within the city limits of Auburn, AL, offering local customers an option to preserve local genotypes in their landscapes (R.O. Smitherman, unpublished report). Mark Catesby (1731) first described this species. Traits include a large plant size exceeding 5 m, with 6 to 19 fragrant white to dark pink flowers per truss appearing early in the bloom season (Kron,1993). It can tolerate wet sites and is typically found along streambanks, though it can also be found on dry ridges (Galle, 1985, Towe, 2004). It is consistently reported as diploid (Jones, 2008). This species was evaluated for vegetative propagation using various mist intervals and rooting hormone concentrations (File et al., 2005). It had higher rooting percentages than the tetraploid *R. austrinum*, using 0 ppm to 7500 ppm K-IBA. *R. canescens* rooted at 95% when receiving mist 4 s every 6 min, and 70% when receiving 4 s every 12 min compared to 75% and 60% for *R. austrinum* at the same two respective mist intervals.

The third species cultivar evaluated was *R. flammeum* 'Big Red'. This is a selection of the Oconee azalea made from the wild in Macon County Georgia by Dr. Smitherman. It is low growing, highly stoloniferous, and has large dark red flowers in the mid-late season (R.O. Smitherman, unpublished report). The Oconee azalea has a limited range in Georgia and South Carolina, but can be quite variable in habit and flower form across its range (Galle, 1985). It is typically non-stoloniferous, growing 1–3 m tall with yellow to red non-fragrant flowers. It is heat tolerant and can grow well in full sun at low elevations, unlike the other red flowering natives, which prefer shade and/or higher elevations. It was described under a variety of names in the late 1700's and early 1800's, with its current agreed upon epithet given in 1917 by Charles Sargent (Galle, 1985). It is reported a diploid by Kron (1993) and Galle (1985), but Jones (2008) found 20% of the *R. flammeum* evaluated were tetraploids, with the other 80% confirmed as diploids. Aspects of vegetative propagation by stem cuttings were evaluated in the Oconee azalea by Jones (2008). He found that the using softwood over semi-hardwood cuttings had the greatest effect on optimal rooting responses in this species, while hedging and higher concentrations of K-IBA also improving the quality of the root system.

The fourth species cultivar evaluated was a seed-grown Florida azalea, *R. austrinum*, collected by Dr. Smitherman along the Escambia River in Santa Rosa County Florida (Smitherman, unpublished report). The species *R. austrinum* was discovered by Dr. A. W. Chapman before 1865, and formally described as a distinct species by Dr. John K. Small in 1913 (Galle, 1985). It is a large non-stoloniferous shrub to 5 m, blooming early with trusses of 10 to 24 fragrant yellow to gold to orange flowers, often with orange to red tubes (Kron, 1993). The redder shades were often attributed to hybridization with the sympatric, but diploid *R. canescens* (Towe, 2004). Jones' (2008) analysis of ploidy levels found 92% of *R. austrinum* tested were tetraploids, and 8% were diploids. Recent investigations into ploidy levels of populations of yellow and pink azaleas on the Yellow River and Escambia River showed that the assumed *R. austrinum* and *R. canescens* hybrid scenario may not be the case (Miller, 2011). If that were the case, pink flowered azaleas in these populations would be diploid, yellow flowered azaleas would be tetraploid, and intermediate flower forms would be infertile triploids. The population on the Escambia River instead is comprised of fecund stoloniferous plants in a wide range of flower colors that have thus far proven tetraploid (Miller, 2011). The individual selected for evaluation in this experiment blooms light yellow in late February to early March, and was the earliest blooming azalea in the DAˈs collection since it matured. *R. austrinum* was also the subject of vegetative propagation research by File et al. (2000) and Knight et al. (2005), where it produced the highest rooting percentage at the lower evaluated mist volume, and at lower mist interval (see Table 1.1).

The first species used exclusively in the crosses in this experiment was the Cumberland azalea, *R. cumberlandense*. The Cumberland azalea is a low shrub to 2 m tall blooming with 3 to 7 red to orange flowers per truss late in the bloom season (Kron, 1993). This relatively recently described species was surrounded by a bit of taxonomic confusion (Galle, 1985). Lemon (1938) attempted to describe the low-growing, later flowering relative of *R. calendulaceum* as *R. bakeri*, but the type specimen they collected was significantly different from the Cumberland azalea, and is now believed to be a hybrid between *R. flammeum* and *R. canescens* (Kron,1993). Because of this misidentified specimen, the naturally occurring hybrid between *R. flammeum* and *R. canescens* is now referred to as *R. ×bakeri* (Galle,1985). The diploid Cumberland azalea *R. cumberlandense* was accurately described by Braun

(1941). *R. ×bakeri* 'Tom's Pink' is an early blooming, bright pink selection with an orange blotch made by Tom E. Corley from a wild collected seed lot from Georgia (Smitherman, unpublished report).

The second species utilized exclusively in crosses in this experiment is the plumleaf azalea, *R. prunifolium*. This species was first documented in 1897 by R. Harper, and was formally described by Small in (1913) as *Azalea prunifolia*, then as *R. prunifolium* by Millias (1917). It is a tall shrub approaching the size of small tree, exceeding 5 m in height. It blooms late in the year, starting in early summer, with some individuals continuing into late fall. Flowers are non-fragrant, 4 to 7 per truss, with flower colors ranging from salmon to orange to red (Towe, 2004). The plumleaf azalea is consistently reported as diploid (Jones, 2008). It has the smallest range of any species in this experiment, restricted to several counties along the Georgia and Alabama border along the Chattahoochee River and adjacent drainages (Galle, 1985). Natural hybrids of *R. prunifolium* and *R. arborescens* were reported from Lee County Alabama (Keener, 2018). The *R. arborescens* × *prunifolium* cross in this experiment was a controlled cross, hand pollinated by Dr. Smitherman. *R. prunifolium* is a desirable part of the Auburn Azalea Breeding Program because its late bloom time helped fill gaps in the bloom calendar (R.O. Smitherman, unpublished report).

The third species in crosses in this experiment is the sweet azalea, *R. arborescens*. The populations in Alabama represent the southernmost occurrence for this species whose range stretches all the way to Pennsylvania (Kron, 1993). It was originally discovered and described by John Bartram and F. T. Pursh in 1814, and renamed by Torrey in 1824. The sweet azalea grows to more than 6 m and blooms with fragrant white flowers from March to September (Galle, 1985) and 3 to 7 flowers per truss (Kron, 1993). It is consistently reported a diploid (Jones, 2008). The late bloom time and flower fragrance make it an ideal addition to a breeding program (R.O. Smitherman, unpublished report).

The fourth species in the evaluated crosses is *R. oblongifolium*, the Texas azalea. Described by Small (1903), and renamed by Millias (1917), it has an extended summer bloom period with small, fragrant, long-tubed white flowers, with 3 to 14 per truss. Benefits it brings to a breeding program include the ability to tolerate saturated soils, flowering profusely even in deep shade, and its unique irregular bloom habit. While some azaleas floral buds open nearly in unison, or a single truss will begin and finish blooming within a few days, some Texas azaleas were observed to have numerous trusses open uniformly across the plant that will continue to open individual flowers in a truss for weeks. While the effect is not a striking on any given day, it keeps color present in the garden over long periods (R.O. Smitherman, unpublished report).

The final species to discuss from the evaluated crosses is the most recently described taxa of *Rhododendron* in the U.S., the Red Hills azalea, *R. colemanii*. The cross that resulted in the hybrids evaluated here, *R.* ×ˈPatsyˈs Pinkˈ and *R.* ×ˈPatˈs Pinkˈ, was made in 1987 (R.O. Smitherman, unpublished report), more than 20 years before the species was described (Zhou et al., 2008). The species eluded description for so long in part because of its high phenotypic variability in flower form. Flowers in this species can range from less than 2.5 cm to more than 5 cm. Flower form can manifest in a spectrum ranging from a narrow, nearly tubular form with short crisp margined petal lobes to a widely displayed open corolla with reflexed petal tips and a ruffled margin. Color of the flowers ranges from a creamy yellow-orange, to the more common white, to very deep shades of pink. Flowers are fragrant, and the plants are large, exceeding 6 m in height and width. The fact that it is consistently tetraploid was a major indicator that it was not a type of *R. alabamense*, a diploid, as once believed (Zhou et al., 2008).

The final azalea utilized in the evaluated hybrid crosses is not a species, but a hybrid, *R.* × '*Gibraltar*'. Introduced in 1947 by Lionel de Rothchild from his Exbury estate in England, it has become one of the most popular of the Exbury hybrids (Galle, 1985). The Exbury line got its starter material from Knaphill Nursery, which in turn utilized plants developed in Ghent, Belgium around 1825. At the time of these breeding efforts, European botanists were regularly discovering and describing new North American species, so it is possible that misidentified specimens could have made their way into these programs with no name yet apply to them. The Ghent Hybrids combined North American natives *R. calendulaceum*, *R. periclymenoides*, *R. arborescens*, and *R. viscosum*, with the European *R. luteum* Sweet. These hybrids went through multiple subsequent generations of selective breeding. Around 1880, the Asian deciduous azalea species, *R. molle* (Blume) G. Don and *R. japonicum* (Gray) Sur., were added to the gene pool. By this time, breeding programs using these strains were underway in several European countries. From 1880 to 1920, Anthony Waterer Sr. of Knaphill Nurseries in England added *R. occidentale* Torr.& A. Gray, the sole azalea species from the western U.S., to their selective breeding efforts to improve available cultivars of deciduous azaleas. This was the source material for the Exbury azaleas, initially developed between 1920 and 1937, when Rothchild oversaw the planting of over 1 million *Rhododendron* from which the selections continue to be made (Cash, 1986).

This lineage combining at least eight deciduous azalea species in an unreported number of combinations yielded the cultivar *R.* ×ˈGibraltarˈ introduced in 1947. Vegetative propagation of stem cuttings was a challenge at that time as well, and cultivars were maintained through a variety of alternative techniques such as grafting, root cuttings, and through seed collected from plants believed to have fixed lines (Galle, 1985). These fixed lines produced seedlings similar enough to parents to attach the cultivar name and make sales to the public. They carried the name of the cultivar despite the fact that the genetics were not preserved, as they would have been through vegetative propagation. *R.* ×ˈGibraltarˈ is asserted as one of these fixed lines. *R.* ×ˈGibraltarˈ has several traits that have made it a standout among the Exburyˈs. It produces large balled trusses composed of 10 to 12 orange flowers up to 15 cm across. It has a manageable maximum size rarely exceeding 2 m tall and wide (Galle, 1985). It is also resistant to infections of rust (Bir et al., 1981) and mildew (Galle, 1985).

Many positive traits were combined in the cultivar *R.* ×ˈGibraltarˈ, and other Exbury hybrids (R.O. Smitherman, unpublished report). Much of the selective breeding took place in England at a latitude of approximately 51 °N. Generations of selection pressures in a region where summer temps are milder than the Southern U.S., specifically the approximately 32 ° N latitude of Auburn, Alabama, had created a hybrid series built on a gene pool where half of the species were native to the southeast, yet the plants that resulted from the effort are not hardy here. To breed heat tolerance into *R.* ×ˈ*Gibraltar*ˈ and combine desirable traits of other native species, Auburnˈs gardeners spent decades breeding and selecting hybrids of their own (R.O. Smitherman, unpublished report).

The F1 hybrids and complex hybrids are crosses hand pollinated and selected by R.O Smitherman, D.R. Rouse, and T.E. Corley in their home gardens in Auburn, Alabama between 1984 and 2011. Selected specimens from the breeding program were donated to AU DA from 2008–2014 where they were planted as part of the permanent collections. The species evaluated in this study were also donated by Dr. Smitherman during this period. Crosses between *R.* ×ˈ*Gibraltar*ˈ and *R. austrinum* produced some of the breederˈs favorite plants. They received cultivar names that paid homage to their hometown, and place of employment. *R.* ×ˈAubieˈ, *R.* ×ˈAuburn Tigerˈ, and *R.* ×ˈPlainsmanˈ are siblings resulting from the cross *R. austrinum* × *R.* ×ˈGibraltarˈ. *R.* ×ˈWar Eagleˈ resulted from the cross *R.* ×ˈGibraltarˈ × *R. austrinum.* *R.* ×ˈLatheˈ is an open pollinated seedling of *R.* ×ˈWar Eagleˈ. *R.* ×ˈPatsyˈs Pinkˈ and *R.* ×ˈPatˈs Pinkˈ are siblings produced by the cross *R. colemanii* × *R.* ×ˈGibraltarˈ (R.O. Smitherman, unpublished report).

There is a naturally occurring hybrid *R.* ×*bakeri* resulting from *R. flammeum* × *canescens,* the reciprocal cross, and multigenerational back crosses. Its origin was a wild cottection from an unknown location by T.E. Corley (R.O. Smitherman, unpublished report), so it is included with the wild collected cultivars in comparisons between them, F1 hybrids, and complex hybrids. Parentage of all F1 hybrids and ploidy of species and hybrids are listed in Table 1.2. *R. prunifolium* × *R.* ×ˈGibraltarˈ is the only cross between a diploid and a tetraploid. Because it is predicted to be a triploid, it will be excluded from analysis based on ploidy levels.

Propagation methods

There are several ways to create new plants from existing ones (Bir, 1992).

Growing seedlings is a good way to generate large numbers of plants. Azalea's propensity to hybridize freely is known to result in a diversity of flower colors and shapes, as well as variation in growth rates and plant habits within a single seed lot. This may be desirable for a breeder attempting to create a new plant, but it will take years to go from sowing seeds to knowing what the form of the flower and plant will be. In horticulture, clones are preferable because they offer a reliably uniform product to market that can reliably meet design specifications. Seedling variation causes reduced predictability in the nursery setting as well. Cloned vegetative material is true to type, mature, and results in less time from propagation to floral initiation (Dirr, 1987).

There are several techniques used to clone azaleas. Tip layering, air layering, and grafting, produce success in propagation, but the necessary time and space and stock plants required are greater than the requirements of stem cuttings and tissue culture to produce the same number of plants. Divisions and root cuttings can also be successful, but collecting the propagation material may reduce the productivity of the stock plants, and does not yield large numbers of propagules for production (Galle, 1985). Propagation of stem cuttings is recognized simultaneously as the most popular and problematic technique to propagate deciduous azaleas (Brown, 2017). Dirr (1987) calls it the most fascinating and frustrating area of plant propagation. Rooting stem cuttings can create dozens of propagules per plant, yet still leave stock plants healthy enough for sale or continual use as mother plants, (Maarten van der Geissen, personal communication). If the scale of production needed is closer to hundreds or even thousands of propagules, tissue culture is a cost effective alternative (Dirr, 1987).

Tissue culture (TC) is a viable option for large-scale propagation, but cost to initiate can be prohibitive (Galle, 1985). The cost of building a tissue culture lab stretches from thousands to millions of dollars in establishment costs (Dirr, 1987). The other option is to contract a TC lab to initiate cultures and provide rooted plants. The process to go from a single plant to a cloned propagule can be quite challenging (Brown, 2017). Different species can require variation in any number of aspects in the process, even different cultivars within a given species can have different results when factors are adjusted. Steps in the process typically include pre-propagation, establishment of explants, maintaining explants and maximizing shoot proliferation, rooting, and hardening off. During *in vitro* micro-propagation, sterile cultures can become contaminated (Brown, 2017). This is why the process occurs in a lab and not a greenhouse. Specialty equipment such as autoclaves and fume hoods are required to reduce contamination, but aseptic techniques and attention to detail are still crucial to success in TC (Dirr, 1987). In addition to threat of contamination at every interaction with the culture, each plant's recipe for scheduling and ratios of plant growth regulators in media as well as adjustment to various levels of response to auxins and cytokinins means getting a cultivar established in TC requires time, facilities and experise to achieve success (Brown, 2017). It also requires a demand sufficient to justify the expenditures associated with the process. The production of thousands of saleable plants is an easy way to reconcile the costs (Dirr, 1987). Multiplication of species on the brink of extinction has also long been viewed as an important fit for TC in conservation horticulture (Mathur, 1970). Shoot culture is effective at multiplying a single genetic individual, however it is limited for preservation of genetic diversity. In a case such as the AU

DA's when hundreds of individual genets need only to be multiplied by 10's instead of 1,000's, rooting of stem cuttings though difficult, still may be the best option (personal observation).

Attention to detail can improve success rates with stem cutting propagation of deciduous azaleas, but rates of improvement are still genotype dependent (Apine and Kondratovičs, 2005a). Stem cuttings lose moisture once separated from the root system of the stock plant from which they were harvested, so minimizing transpiration loss is vital to maintaining cutting turgor (Grange and Loach, 1983). Stock plant treatments including hedging, forcing, and light exclusion have shown inconsistent degrees of success across multiple types of azaleas (Apine and Kondratovičs, 2005a; Jones, 2008). It was recommended to collect cuttings from turgid, vigorous, juvenile, non-diseased terminal shoots early in the morning and immediately get cuttings into a cool, moist, dark place until the cuttings can be further processed (Bir, 1992). It was repeatedly shown that the addition of synthetic auxins such as indole-3-butyric acid (IBA) or naphthaleneacetic acid (NAA) induced a greater rooting response (Dirr, 1987). A comparison of IBA and NAA treatments on another Ericaceous plant, *Arbutus andrachne,* found IBA to be more effective (Al-Salem and Karam, 2001). Potassium based IBA (K-IBA) was evaluated at six rates ranging from 0 ppm to 10,000 ppm (File et al., 2000). Higher rates correlated to increased root length and a superior visual root rating. At K-IBA concentrations over 8,000 ppm, there was a correlation with mist interval in which the lower mist interval resulted in increased number of roots (File et al., 2000).

Lignification

A range of calendar dates from March to June have been recommended for taking cuttings for the propagation of deciduous azaleas (Apine and Kondratovičs, 2005a; Apine and Kondratovičs, 2005b; Bir, 1992; Skinner, 1961). While rooting success is a major concern, another factor growers of deciduous azaleas must consider is that new growth needs to start before plants go dormant for the winter to increase their chances of leafing out the following spring (Galle,1985). Using softwood cuttings early in the year allows more time for this new growth to develop, though semi-softwood cuttings harvested later in spring can be encouraged to break buds with the addition of artificial light over the summer (Galle, 1985). A comparison of four cultivars of deciduous azaleas found that early softwood cuttings did not root as well as those taken just six days later (Nawrocka-Grzeskowiak and Grzeskowiak, 2003). Cuttings were collected on five dates with 6 day intervals ranging from 29 May to 22 June. The earliest softwood cuttings did not root at all, and for some cultivars neither did the most lignified group collected on 22 June. The third and fourth collection dates rooted more than 50% better than the harvests before and after it, displaying a standard bell curve skewing towards the latter of the five collection dates. The authors concluded that the highest rooting success correlated with the existence of the primary xylem tissue and the decrease in rooting response corresponds to the development of the secondary xylem tissue (Nawrocka-Grzeskowiak and Grzeskowiak, 2003). Rather than relying on a calendar date, physiological cues based on degree of lignification was suggested to be a more important determinants of the best time to take cuttings to achieve the highest success rates (Jones, 2008). Nawrocka-Grzeskowiak and Grzeskowiak (2003) used cultivars that flower simultaneously and drew conclusions relative to bloom time, though the degree of lignification they documented has wider application for species with varying bloom times. Juvenile tissues tend to root more readily than mature tissues, though they can be susceptible to water stress and loss of turgor (Preece, 2003).

Researchers and growers stress the importance of paying attention to the degree of cutting stem lignification to see if it is at the optimal developmental stage for propagation (Jones, 2008; Nawrocka-Grzeskowiak and Grzeskowiak, 2003; Maarten van der Geissen, personal communication). There were a number of indicators that authors recommend observing. A grower at Roadview Farm Nursery states that suitable shoots will be slightly firm, 6 inches in length, still hairy, and just about to snap when bent over double (Nienhuy, 1980). Another suggested cuttings should be taken on soft wood once new growth has ceased (Bir, 1992). A third describes the physiology of a proper cutting as green, before the lower portion becomes hard and brown, and before the apical bud is evident (Brydon, 1964). Research on rooting success based on lignification does exist for a few types of deciduous azaleas (Jones, 2008; Nawrocka-Grzeskowiak and Grzeskowiak, 2003). A comparison of softwood and semi-hardwood cuttings, several concentrations of rooting hormones, and different stock plant treatments, found that softwood cuttings of *R. flammeum* had a mean rooting of 85%, but semi-hardwood cuttings rooted at 35% or less. Semi-hardwood cuttings of *R. austrinum* generated contradictory results in the same experiment (Jones, 2008).

Another experiment at the University of Latvia correlated lignifications to five stages in the flowering process of a group of cultivars (Nawrocka-Grzeskowiak and Grzeskowiak, 2003). This is not applicable to several of the North American native species because some of them flower before their leaves and shoots emerge, and others flower several months after leaves and shoots emerge (Towe, 2004). The paper does link these stages in lignification with a significant physiological transition; the development of the secondary xylem within the stem (Nawrocka-Grzeskowiak and Grzeskowiak, 2003). They report that across all cultivars within their experiment, cuttings rooted poorly if taken too early or once lignification was high. They found stems that had not developed secondary xylem, or in which it was minimal, had the highest ratings on their root rating scale (Nawrocka-Grzeskowiak and Grzeskowiak, 2003).

Mist

Once a cutting is removed from its parent plant, it must be provided with sufficient moisture or it will wilt and die (Dirr, 1987). Various methods have been employed to maintain humidity around cuttings. The Wardian case is a glass topped terrarium that was invented to serve this purpose in 1829 (Preece, 2003). Closed humidity structures encouraged diseases like *Botrytis*, causing failures in cuttings despite ample humidity. Other methods included reducing leaf surface area, maintaining soil moisture, providing shade, and various other coverings to limit desiccation. The first published account of success using a spray chamber for propagation was by Raines (1940), though other growers simultaneously experimented with other fog and mist systems. The systems allowed for enough airflow to reduce pathogens common in the closed humidity boxes, while keeping fine moisture droplets at or near the leaf surface by constantly spraying the cuttings and propagation area (Preece, 2003). The modern standard for providing this moisture is an intermittent mist system, which allows greater control through scheduling of the mist to lower the leaf-to-air vapor pressure gradient, slowing down moisture loss through leaf transpiration (Pijut et al., 2011). A consistent recommendation for success in cutting propagation of deciduous azaleas is the reduction of mist overnight (Bir, 1992; Jones, 2008; Knight et al., 2005; Somerville, 1998). This reduces diseases that can affect leaves (Bir, 1992). It was also recommended that the interval between applications of mist should be increased as cuttings begin to develop roots (Bir, 1992; Grampp, 1976). At this point in a cutting's development, it has been kept alive long enough to begin to support itself (Bir, 1992).

Scientific research into rooting deciduous azalea stem cuttings have dealt with intermittent mist in a variety ways. File et al. (2000) evaluated the difference in application frequency of an equal volume of mist. When cuttings received more frequent application with a shorter duration, 4 s every 2 min, they had 67% success rooting *R. austrinum* versus a 75% success rate when mist was applied less frequently with a longer duration; 8 s every 4 min. Knight et al. (2005) investigated the effects of differences in total volume of mist applied to *R. austrinum* and *R. canescens* using two different intervals, 6 min and 12 min, with a duration of 4 s of mist at each. Both species had a higher rooting percentage and better rooting response at 4 s every 6 min. In another study, mist was a constant with four changes in intervals over the course of each day (Jones, 2008). At night, cuttings in all his experiments received mist for 8 s every 240 min. For 4 h in the morning, mist was applied at 8 s every 20 min. During the 8 h in the middle of the day, the rate was 8 s every 10 min. For the 4 h in the evening, of the rate was 8 s every 15 min, and this led back to the night rate (Jones, 2008). These authors maintained their mist schedule from the time the cuttings were stuck until the end of the study when data was collected (File et al., 2000; Jones, 2008; Knight et al., 2005).

Media

The rooting media is a mixture of soil-like particles intended to fulfill the needs of container grown plants (Bir, 1992). These needs are nutrients, water, gas exchange, and physical support of the cutting and developing root system (Argo, 1998). Research into ideal potting media has been developing since the 1950's when container production in greenhouses and nurseries emerged as a common practice in the industry (Davidson et al., 2000). An ideal rooting media will be available, affordable, provide good air/water relationships, and yield reproducible positive results (Dirr, 1987). The literature contains a variety of media recommendations for rooting deciduous azaleas. There is a noticeable divide between recommendations from European growers of Exbury azaleas, which rely heavily on locally available peat (Nienhuy, 1980), and the azalea growers and researchers in the Southeastern United States where pine bark is locally available (Boyer, 2008; File et al., 2000; Jones, 2008; Knight et al., 2005). Recommendations from Exbury growers includes pure peat (Nienhuy, 1980), 2 peat : 1 sand (by volume) (Nawrocka-Grzeskowiak and Grzeskowiak, 2003), 1 peat : 1 perlite (by volume) (Mylin, 1982), 1 peat : 1 sand : 1 perlite (by volume) (Brydon, 1964), and the specific recommendation of 3 sharp sand and grit: 1 Irish peat moss (by volume) (Fairweather, 1971). Research at North Carolina State University on *R. flammeum* and *R. austrinum* utilized a 2 peat: 1 perlite (by volume) (Jones,2008). Sommerville (1998) suggested 2 pine bark: 1 peat: 1 sand (by volume). The other end of the spectrum is the media used in research at Mississippi State University consisting entirely of screened pine bark fines (File et al., 2000; Knight et al., 2005). Timing for fertilization was recommended to be as soon as rooting begins (Maarten van der Geissen, personal communication). Estimates for time to rooting out ranged from 4 to 10 weeks after the date cuttings were stuck (Knuttel and Addison, 1984; Mylin, 1982; Nienhuy, 1980).

Some media recommendations have fertilizer incorporated, but others wait until rooting to apply fertilizers (File et al., 2000). A benefit of adding nutrients to propagation media is so that they will be available to the plant when roots emerge from the cutting (Galle, 1985). Researchers have opted to mix in micronutrients and apply macro nutrients, for example pine bark amended with 2.96 kg/m^3 dolomitic limestone, and 0.89 kg/m^3 Micromax (File et al., 2000). Rates of 20N-8.8P-16.6K (Mylin, 1982) or 20N-4.4P-8.3K (File et al., 2000) were used for post rooting macro nutrient applications. Maarten van der Geissen (personal communication) recommended 3 bark : 1 peat : 1 perlite (by volume) amended with Micromax at 0.89 kg/m^3, dolomitic lime at 1.19 kg/m^3, gypsum at 1.19 kg/m^3, and 20N-3.06P-8.3K at 5.93 kg/m^3. Bottom heat was also utilized, where media temperatures approaching 85°F were said to encourage the best rooting (Sommerville, 1998).

In the Southeastern United States, pine bark is available, affordable, and effective (Dirr, 1987). It is a byproduct of locally widespread forestry where it can be Hammer milled and screened to produce a product that has been utilized in the nursery industry for decades (Pokorny, 1987). An advantage over other organic materials is its relatively long-term stability in the container, where it maintains the air space (AS) within a mix, while providing water available to the roots thanks to significant amounts of micropores on the surface and within the bark (Pokorny, 1987). It was shown that rooting percentages as high as 95% can be achieved in pure pine bark media (Knight et al., 2005).

Sphagnum peat is sold as compressed, dehydrated plant material that is composed mostly, but not exclusively, of mosses from the genus *Sphagnum* (Dirr, 1987). It is a complex media component that can vary greatly depending on its origin (Giraux et al., 1999). The geographic origin could be from acidic bogs around the world. This will in part dictate the botanical origin of the peat, which can affect its chemical properties. Physical properties are dependent on degree of decomposition (Giraux et al., 1999) and processing (Dirr, 1987). Milled sphagnum peat moss is available and effective, but expensive. It has excellent water holding capacity (WHC) and fine particles that encourage fibrous root growth (Dirr, 1987).

Perlite exists naturally as a glassy volcanic rock (Silber et al., 2010). Horticultural expanded perlite is created by crushing and heating the aluminum-silica rock to a temperature that causes the fragments to expand rapidly into lightweight particles (Dirr,1987). Like pine bark, it is structurally stable in a mix, however, unlike pine bark, its interior pore spaces are sealed. Root growth can be coarse in perlite alone (Dirr, 1987). Perlite is inert with little buffering capacity or cation exchange (Giraux el al., 1999). More recent research has shown that while this is initially true, growing a plant in a mix with perlite can affect the observable dissolution of elements in a media, especially phosphorous and calcium, as well as altering the surface charge of perlite (Silber et al., 2010). Articles were reviewed that recommend mixtures of peat and perlite for rooting deciduous azaleas (Mylin, 1982; Brydon, 1964), but this review of the literature did not yield evidence of studies in which peat and perlite mixtures were evaluated as components of rooting media for deciduous azaleas. Mixtures of peat and perlite can be used as a media to root many woody species in a variety of systems (Giraux et al., 1999). An experiment with *Arbutus andrachne* compared rooting response in media mixtures containing peat and perlite, finding increased rooting response correlating directly with increased percentages of perlite in the mix (Al-Salem and Karam, 2001).

Understanding the properties of media components are important, but understanding the dynamic nature of how the relationship between air, water, and solids affect other properties of the media benefits greatly from observation and evaluation of several additional aspects of media (Argo, 1998). A key property in this relationship is the total porosity (TP) of the media. Typical mineral soils can be expected to have around 50% solids and 50% pore space. A soil less media can have more than 90% pore space (Argo, 1998). In an evaluation of eight medias withTP ranging from 58.55% to 84.05%, it was found that there was no significant difference in rooting percentage at the two ends of the porosity spectrum (Bilderback and Lorscheider, 1995).

There are many other factors that can affect the success of a rooting media including settling of material, particle size, moisture release characteristics, and evaporation at the container surface (Argo, 1998). These relationships become even more complex once you incorporate the need irrigate sufficient to maintain a wet leaf surface without over wetting the media (Rein et al., 1991). Studies have revealed that media air and water content are greater determiners of rooting success than the media's physical properties. Propagation failure can be caused by the lack of oxygen in an over-wet media. Despite their lack of roots and resistance to water absorption through the stem surface, an under-wet media can affect the water economy of leafy cuttings, reducing turgidity, and rooting success. The loss of turgidity is evidence of water stress. Limiting water stress increases the possibility of a stem cutting producing adventitious roots. Limiting water stress is primarily addressed by maintaining high humidity around the leaves of a cutting, but proper wetness of the rooting media must be managed (Rein et al., 1991).

Data collection

Several methods were employed by researchers to quantify the success of rooting azalea cuttings. Similar rooting scales were used by most of the recent publications, but there is not a standard. File (2000) used a visual rating scale with a range from 1 to 5 in which 1 was unhealthy, and 5 extremely healthy. Knight et al. (2005) used a visual scale from 0 to 5 with 0 being dead, and 5 being extremely healthy. Nawrocka-Grzeskowiak and Grzeskowiak's (2003) scale was based on cubic centimeters of root volume ranging from 1 to 6 with 1 indicating few roots, and 6 indicating a root-ball volume greater than 180.1 cm^3. Jones (2008) calculated and reported root system size index in square centimeters that ran from 0 to approximately 30 cm^3. Additional parameters reported included rooting percentage, cutting growth, root number, average root length (of three longest roots) (Knight et al., 2005), number of dead cuttings, number of healthy non-rooted cuttings, number of healthy, rooted cuttings (Nawrocka-Grzeskowiak and Grzeskowiak, 2003), number of roots, and length of roots (File et al., 2000). Data were collected at 49 d (Jones, 2008), 68 d (Knight et al., 2005), 102 d (File et al., 2000), or approximately 330 d (Nawrocka-Grzeskowiak and Grzeskowiak, 2003). The wide spectrum of data collection dates provided a snapshot of root development, but no published research provided information on the progression of root development over time in deciduous azalea cuttings.

Objective

The objectives of this research was to determine a successful mist regime and media mix for a single deciduous azalea cultivar, and apply those protocols to a variety of cultivars to document variation in responses across groups. Success was qualified by superior performance in root length, area, rating, and rooting percentage. This research also documents information on the progression of root development of deciduous azalea cuttings over time in multiple cultivars, media, and mist conditions.

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Table 1.1. Mist treatments and rooting percent of *Rhododendron austrinum* cuttings in three experiments.

DAS[z]	Regime	Interval (min)	Duration (s)	Mist (s/d)	Rooted (%)	Evaluated (DAS)	Author
1-42	High	10	12	864			Thompson et al.
43-84	High	10	9	648	84	70	2018
1-42	Low	10	8	576			Thompson et al.
43-84	Low	10	6	432	90	70	2018
1 - 68	Fixed	4	2	1020	67	68	File et al.
1 - 68	Fixed	8	4	1020	75	68	2000
1 - 68	High	4	6	440	75	68	Knight et al.
1 - 68	Low	4	12	220	60	68	2005

[z]DAS = days after sticking cuttings.

Table 1.2. Reported counts of deciduous azalea, *Rhododendron* section *Pentanthera*, cultivars maintained in North American public gardens.[z]

Species	Cultivars	Gardens providing survey data
alabamense	10	Arnold Arboretum, Harvard University
arborescens	10	Atlanta Botanical Garden
atlanticum	11	Bartlett Arboretum & Gardens
austrinum	34	Bellefontaine Cemetary and Gardens
calendulaceum	19	Brooklyn Botanic Garden
canescens	12	Connecticut College Arboretum
colemanii	3	Davis Arboretum, Auburn University
cumberlandense	12	Dawes Arboretum
eastmanii	1	Highstead Arboretum
flammeum	29	Huntsville Botanical Gardens
periclymenoides	12	Jenkins Arboretum
prinophyllum	0	Missouri Botanical Garden
prunifolium	6	Mt. Cuba Center
vaseyi	4	NC Arboretum
viscosum	20	NY Botanical Garden
x - hybrids	151	Polly Hill Arboretum
Grand Total	334	Tyler Arboretum
		Univ. of BC Bot Gardens
		US Natl. Arboretum

[z]Survey conducted 2017 by the Davis Arborteum, Auburn University

Chapter 2

Rooting response of stem cuttings of *Rhododendron austrinum* 'Escatawpa' to mist regimes and medias

Abstract

Rooting of Escatawpa azalea, *Rhododendron austrinum* (Small) Rehder 'Escatawpa', stem cuttings were evaluated on four harvest dates in six media under two mist regimes. Cuttings received 8 s (low) or 12 s (high) mist duration at 10 min intervals for the first 42 d. Thereafter, mist durations was reduced by 25% to 6 s (low) or 9 s (high) mist duration at the same interval. Data were collected at 2 week intervals from 6 to 12 weeks after date of stick. Cuttings given the low mist duration produced consistently superior root development than those given the high mist duration. All media mixtures consisted of 50% pine bark by volume. The remaining 50% of volume progressed from perlite to peat in 10% increments. The media comprised of a 2 perlite : 3 peat : 5 pine bark ratio (by volume), produced superior results earlier in the evaluation than the other media. At the final harvest there was no difference between the 2 perlite : 3 peat : 5 pine bark ratio (by volume) and the 1 perlite : 4 peat : 5 pine bark ratio (by volume). The majority of cuttings were rooted at 6 weeks. Rooting percentages increased at each harvest date from 55% at 42 d to 89% at 84 d after sticking. Harvest date was a repeatedly significant factor in the analysis of media and mist duration allowing for meaningful evaluation of the timing of development of cuttings in different growing conditions in our results. Providing more time and less mist resulted in superior results as measured by root length, area and root ratings.

Introduction

Scientific and popular literature contain a wide spectrum of recommendations and observations on vegetative propagation of deciduous azaleas, *Rhododendron* section *Pentanthera*. Few species have research-based propagation protocols in the literature (File et al., 2000). Vegetative propagation offers a consistent product and less time to first flower (Skinner, 1961). There is consensus that it is difficult to root cuttings of deciduous azaleas (File et al., 2000; Galle, 1985; Jones, 2008; Skinner, 1961). The effects of mist were evaluated (File et al., 2000; Knight et al., 2005), but only grower suggestions for best media choices for rooting cuttings of deciduous azaleas were reported, and no existing research investigated how the two interact.

File et al. (2000) evaluated the difference in application frequency of an equal mist volume. When cuttings received more frequent applications with a shorter duration, 4 s every 2 min, rooting success was 67% in *R. austrinum* versus a 75% success rate when mist was applied less frequently with a longer duration of 8 s every 4 min. Knight et al. (2005) researched the effects of differences in total mist volume applied to *R. austrinum* and *R. canescens* at two different intervals, 6 min or 12 min, and a 4 s mist duration at each. Both species had a higher rooting percentage and better rooting at 6 min (Knight et al., 2005). When rooting woody stem cuttings, it is recommended that mist should be reduced once most of the cuttings have begun to develop roots (Bir, 1992; Grampp, 1976; Maarten van der Geissen, personal communication).

A variety of media recommendations for rooting deciduous azaleas was reported. European growers of Exbury azaleas, rely heavily on locally available peat

(Nienhuy, 1980), but the azalea growers and researchers in the Southeastern United States utilize the locally available pine bark (Boyer, 2008; File et al., 2000; Jones, 2008; Knight et al., 2005). Research at North Carolina State University on *R. flammeum* and *R. austrinum* used a 2 peat : 1 perlite (by volume) media (Jones, 2008). Sommerville (1998) used a 2 pine bark : 1 peat : 1 sand (by volume). Media used in research at Mississippi State University consisted entirely of screened pine bark fines (File et al., 2000; Knight et al., 2005). Maarten van der Geissen (personal communication) recommended 3 bark : 1 peat : 1 perlite (by volume) amended with Micromax at 0.89 kg/m^3, dolomitic lime at 1.19 kg/m^3, gypsum at 1.19 kg/m^3, and a 20N-3.06P-8.3K type 270 Nutricote at 5.93 kg/m.

In the Southeastern United States, pine bark is available, affordable, and effective (Dirr, 1987). It is acidic and contributes to the air space (AS) in a media mixture where its large particle size is not prone to compaction (Argo, 1998). Rooting percentages up to 95% were reported in *R. canescens* in pure pine bark media (Knight et al., 2005). Sphagnum peat is sold as compressed, dehydrated plant material composed mostly, but not exclusively of mosses from the genus *Sphagnum* (Dirr, 1987). It is a complex media component that can vary greatly depending on its origin (Giraux et al., 1999). Peat has excellent water holding capacity (WHC) and fine particles that encourage fibrous root growth (Dirr, 1987). Perlite is derived from a naturally occurring glassy volcanic rock (Silber et al., 2010). Horticultural expanded perlite is created by crushing and heating the aluminum-silica rock to a temperature that causes the fragments to expand rapidly into lightweight particles (Dirr, 1987). Perlite is inert with little buffering capacity or cation exchange capacity (Giraux et al., 1999). Growers recommend mixtures of peat and perlite for rooting deciduous azaleas (Mylin, 1982; Brydon, 1964).

Studies have shown that air and water content in media are greater determiners of rooting success than the media's physical properties (Rein et al., 1991). Propagation failure can be caused by the lack of oxygen in an over wet media. Despite a cutting's lack of roots and resistance to water absorption through the stem surface, an under wet media can affect the water economy of leafy cuttings, reducing turgidity, and rooting success. Limiting water stress is accomplished by maintaining high humidity around the leaves of a cutting, but moisture must also be managed with proper wetness of the rooting media.

The cultivar *R. austrinum* 'Escatawpa' was chosen based on its availability, rooting potential, and its contribution to the reproducibility of the research. This cultivar is selection of a species reported to be among the easiest to root (Skinner, 1961). It has been established in the trade for decades, can be sourced from multiple nurseries, and is grown in publicly accessible living collections. Seed grown azaleas can exhibit variation in numerous traits including rooting ability even within species (Sommerville, 1998). Published research on rooting response to amounts of mist for *R. austrinum* (File et al., 2000; Knight et al., 2005) does not identify where within the species' spectrum of rooting ability their source material falls. When the seconds of mist per day is calculated for these two experiments, it can be seen that there is a gap in the evaluated amount of mist supplied (Table 2.1). This experiment investigates the possibility that a higher rooting percentage could be achieved with *R. austrinum* by setting amounts of mist per day in between those documented previously. The objective is to evaluate the rooting response on stem cuttings of Escatawpa azalea, *Rhododendron austrinum* 'Escatawpa', to two mist regimes and six media mixtures (MM) on four harvest dates.

Materials and methods

This study was conducted at the Paterson Greenhouse Facility, Auburn University (AU) campus in Auburn, AL. The source of cuttings were plants established in the ground at the AU Davis Arboretum, AU campus in Auburn, AL. Cutting collection began at 0600 HR on the day they were stuck. Material was collected as pre-semi-hardwood cuttings. This stage of development was characterized as occurring after the secondary xylem had begun to form, but before it is fully developed. The field test used to determine this degree of lignification was that the cuttings were flexible, but rigid enough to snap when bent past a 90° angle. Cuttings were collected at a length of approximately 15 cm, misted with water from a spray bottle, sealed in plastic bags, and transported in a cooler with an icepack. Coolers were transported directly to the Paterson Greenhouse Facility for processing and sticking in media.

Cuttings were processed and stuck inside a propagation greenhouse. The greenhouse roof glazing was a double walled inflated 0.15 mm polyethylene with 38% shade cloth. Sidewalls are constructed of clear corrugated polycarbonate. The greenhouse cooling engaged at 76° F both day and night. There are four cooling stages separated by 2° F each. The first three cooling stages consist of a series of fans and vents with stage four, the evaporative cooling system initiating at 82° F. Thermometers to record minimum and maximum temperatures were monitored inside mist tents. The minimum recorded temperature was 71° F overnight 26 June 2017. The maximum recorded temperature was a daytime temp of 90° F, on 2 July 2016.

Mist tents were constructed on expanded metal benches inside a propagation greenhouse using a PVC frame with 0.15 mm (6 mil) translucent polyethylene plastic on the tops and sides. The tops were designed with a 5 cm gap in the gable to allow airflow. The bench surface was porous expanded metal to allow airflow and water drainage. Within each mist zone, six deflector type mist nozzles (Ein dor 809 Series,Tavlit, Yavne, Israel) were mounted on 30 cm tall risers and operated at approximately 50 psi. These were arranged in a rectangular pattern so that all flats in the experiment were inside the rectangle receiving uniform double coverage. An automatic controller (Mist Master 11, Superior Controls Inc., Valencia, CA) maintained the mist schedule.

Standard 1204 inserts (STI-1204, T.O. Plastics, Clearwater, MN) were placed in mesh bottom trays with side openings, and uniformly filled with media, then placed under mist for 3 d prior to the sticking of cuttings to ensure the media has uniform and sufficient moisture. A cell in each of the 12 packs were 6 cm in height, tapered, straight sided, with 70 cm^3 volume. The six media ratios contained 50% screened and aged pine bark fines, and ratios of peat moss (BPF fine milled sphagnum, Berger, Quebec, Canada) and coarse perlite (Sunshine premium grade, Sun Gro Horticulture, Agawam, MA). The perlite to peat moss ratios in the medias progressed from 0 to 50% by volume (Table 2.2) so that MM1 was a 1 pine bark : 1 perlite (by volume) mix, MM6 was a 1 pine bark : 1 peat moss (by volume) mix, and the ratios in between progress in increments of 10% between these. All media were amended with 0.59 kg micronutrient fertilizer (Micromax, Scott's Company Inc., Maryville, OH); 1.18 kg dolomitic lime; 2.95 kg gypsum; 3.54 kg 15N-3.93P-9.96K controlled release fertilizer (Osmocote Plus, Scott's Company Inc., Maryville, OH) per cubic meter.

Processed cuttings were 10 cm in length from the cut base to the base of the most apical visible petiole with the lower two-thirds of the stem stripped of leaves. The remaining terminal leaves were trimmed to a surface area of approximately two-thirds their original size to reduce transpiration loss and leaf abscission. This was accomplished by bunching the remaining leaves on each cutting and making a single cut across the top of the whorl taking care that the terminal buds were not removed. Cuttings were trimmed to final specifications immediately before dipping in a 5,000 ppm K-IBA solution to a depth of 4 cm for 5 s and stuck into the media to a 5 cm depth and were then randomly distributed into the mist tent.

Cuttings received 8 s (low) or 12 s (high) mist duration at 10 min intervals for the first 42 days after sticking (DAS). Thereafter, mist duration was reduced by 25% to 6 s (low) or 9 s (high) mist duration at the same interval. Mist was applied from 0630 HR until 1830 HR. The two mist duration treatments were randomly assigned to six mist zones within the greenhouse.

Data recorded for each cutting were rooted or unrooted, root length (mm), root area (cm^2), and root rating (0 to 5). Rooted or unrooted and root length were recorded on the harvest date, and root area and root rating were recorded later using photographs taken of each cutting on the harvest dates. The root rating system was established upon completion of the first year of data collection to encompass the entire spectrum of root development observed during the experiment.

On each harvest date, 25% of the cuttings from each media ratio within the six mist zones were randomly selected for destructive harvests. Data were collected at 42, 56, 70, and 84 DAS. Each cutting was removed from its cell pack and loose media was shaken away. The roots were dunked in a water bath five times to loosen and wash away additional media. A cutting was rooted if it had roots greater than or equal to 1 mm in length, or unrooted if they were dead, showed no evidence of root initiation, or had roots less than 1mm in length. Root length was recorded from the point of root tissue emergence from the stem cutting to the root tip. Measurements of the three longest lateral roots with independent stem attachments were recorded. Secondary and tertiary branching of lateral roots were not evaluated. Cuttings were then photographed using a digital SLR camera (Nikon D-80/ AF-S Nikor 18-135 mm, Nikon Inc., Melville, NY) set to 24 mm attached to a bracketed horizontal camera mount. Cuttings with roots spread were photographed against a white backdrop with black 1 cm gridlines along with their label denoting media and mist regime, and a paper note with the length of the three longest roots recorded.

After the final harvest date, the 0 to 5 root rating scale was established based on the range of rooting responses observed in the photographic record (Fig. 1) created during the first year of data collection. The scale was based on the cutting's rooting success where a 0 was a dead cutting, and a 5 was a fully formed root system, uniformly reaching the walls of the cell pack consisting of roots from lateral roots arising from multiple attachment points on the stem (Table 2.3). The root area of each cutting was evaluated by counting all squares on the grid more than 50% obscured by roots. The average length of the three longest roots used in statistical analysis.

Physical and chemical properties of the media were also measured. Electrical conductivity (EC) and pH were monitored using the pour through method (Wright, 1986). Leachate from each media and mist regime were collected on each harvest date from the containers of cuttings being evaluated. A bench top multi-parameter meter (Thermo Scientific Orion Star A215 pH/Conductivity, Thermo Fisher Scientific, Waltham, MA) was used to measure pH and EC. Physical properties of the media were measured using procedures described by Bilderback et al. (1982) using the NCSU porometer. AS, WHC, total porosity (TP) and bulk density (BD) were calculated. Particle distribution was measured by passing an air-dried sample through 12.7, 9.50, 6.35, 3.35, 2.36, 2.0, 1.7, 1.4, 0.5, 0.25, 0.10, or 0.05 mm sieves with particles that were less than 0.5 mm collected in a pan. Sieves were placed in a sieve shaker (8" Roto Tap RX-29, WS Tyler, Mentor OH) and shaken for 3 min (278 oscillations per min, 159 taps per min). All media in the particle distribution evaluation were weighed on a digital scale (Adventurer Pro Precision Balance AV3102C, OHAUS, Pine Brook, NJ). Weights of media particles collected in the sieves were separated into three categories for analysis. The sieves in the coarse category were 12.7, 9.50, 6.35, 3.35, and 2.36 mm. Sieves in the medium, category were 2.0, 1.7, and 1.4 mm. Sieves in the fine category were 0.5, 0.25, 0.10, 0.05 mm, and the fines that fell into the pan at the bottom of the stack.

This experiment was conducted from 1 June through 24 August 2016, and 30 May through 22 August 2017. On both years, for mist (high, low) n=3. For substrate (MM1 – MM6), in 2016, n=4, and in 2017 n=8. For harvest (42-84 DAS), n=4 on both years. A total of 576 cuttings were evaluated in 2016, and 1152 cuttings were evaluated in 2017.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The treatment design was a 3-way factorial of media, mist duration, and harvest date. The experimental design was a split plot with media and mist duration in the main plot and harvest date in the sub-plot. Year was included in the model as a random variable. For average root length and root area, differences among medias were determined using the simulated method. Differences in mist durations were determined using F-tests. Linear and quadratic trends over harvest dates were examined using qualitative-quantitative regression models. Root rating was analyzed using the multinomial probability distribution and the Laplace method. All significances were at $\alpha = 0.05$.

Results

Only the DAS main effect for rooted or unrooted was significant. Media ratio and mist duration did not affect whether a cutting rooted. Rooting percentages reached 55% at 42 DAS, 78% at 56 DAS, 86% at 70 DAS, and 89% at 84 DAS (data not shown). The media ratio by harvest interaction for root length were significant (Table 2.4). The media ratio by DAS interaction was significant. MM1, MM3, MM5, and MM6 showed increasing linear trends in mean root length over DAS while MM2 showed a quadratic trend, but no trend was found for MM4. Among the DAS there was no difference at 42 or 54 DAS. MM4 and MM5 had the longest roots, at 70 and 84 DAS. MM2 had the shortest root length at 70 DAS and MM6 had the shortest rootlength at 84 DAS. The effect of mist duration by DAS on root length was significant. Mist duration did not result in different root lengths at 42 DAS. Roots were longer under the low mist duration at 56, 70, and 84 DAS.

The analysis of root rating and root area did not reveal a significant difference in results until 70 DAS. Root length in contrast showed differences from the first evaluation. At 42 DAS, the mean root length in MM6 was 27.4 mm longer than, the mean root length in MM1. MM6 maintained the superior rank through all 4 harvest dates. MM6 was not different from the other peat mixes, MM4 and MM5, at two points in the evaluation (Table 2.4).

Analysis of root area (Table 2.5) showed mist duration and DAS have significant interaction. It was not different at 42 DAS or 56 DAS, but at 70 and 84 DAS the lower mist regime produced superior results. On all four harvests, root area was greater for the low mist duration. Media and DAS did not have significant interactions until 70 and 84 DAS. Superior results were documented at 70 DAS and 84 DAS in MM4, MM5, and MM6. MM4 and MM 5 produced superior root area measurements at 70 DAS, and 84 DAS. MM1, MM2, MM3, and MM6 had similar results at 84 DAS, with a range of mean root areas separated by just 0.6 cm^2. MM4 had a mean root area 1.95^2 cm larger than the mean of those four, and MM5's mean root area was 3.35^2 cm larger.

In the analysis of root ratings, the media by harvest interaction and the duration main effect were significant. The low mist duration yielded a superior median root rating compared to the higher mist duration (Table 2.6). There was no difference in results of the harvest media interaction at 42 DAS and 56 DAS. MM4 produced a superior root rating at 70 DAS. At 84 DAS, MM4 and MM5 had superior root ratings.

Chemical monitoring of leachate showed variation, but remained within acceptable parameters. EC monitoring showed differences in the durations, media, and harvest main effects (Table 2.7). The pH monitoring yielded similar results (Table 2.8), showing separation of means among media mixes and mist durations. The only main effect where a media was found to be non-acidic was the evaluation of media mixes where perlite heavy mixes, MM1 and MM2, had a 7.0 pH. Among mist durations, harvests, and the four other media mixes, the mean pH was always acidic.

Analysis of physical properties of the media also showed that the mixes fall within acceptable parameters for propagation media. Particle distribution analysis (Table 2.9) showed Fine particles composed a significantly greater portion of the mix in peat heavy MM5 and MM6.

Results from the evaluation of specific physical properties based on data collected from the porometer showed clear trends in AS, WHC, and TP (Table 2.10). AS was highest among the perlite heavy mixes, MM1 and MM2. The peat heaviest mix, MM6, had more than 10% less AS. It also had 16% more WHC and 5.9% more TP than MM1. There was little variation in BD among media mixes and the reported amounts were within expectations for a container media (Argo, 1998).

Discussion

The rooting percentage increased to the final harvest. The final rooting percentage was 89%. Previous studies of *R. austrinum* by File (2000) and Knight (2005) resulted in a maximum rooting percentage of 75%. Their studies evaluated cuttings at 68 DAS, most comparable to our 70 DAS evaluation, at which time we had 86% of cuttings rooted. File (2000) provided cuttings with 1020 seconds of mist per day, and Knight (2005) 440 to 220 s/d. By setting the average s/d for both the high and low mist regimes evaluated in this experiment in between the amount of mist in these two experiments, it allowed us to see if there was a more optimal mist regime between these two (Table 2.1). While media and mist within this study did not affect the rooting percentage, it repeatedly affected the quality of the root system developed after rooting as evaluated by root rating, area, and length. Differences in the characteristics of the root systems developed in the two regimes of this study, providing insight into the effects of the durations of mist on multiple aspects of root development in azalea liners. The evaluation of mist regimes showed that the lower mist duration resulted in superior root area and root rating.

The greatest mean root length was achieved in MM6, but MM6 did not produce cuttings that received superior root ratings. On the 70 DAS and 84 DAS, MM4, MM5, and MM6 yielded the greatest root area. Root length and area are clear indicators of root development, but the root rating system is incorporated to evaluate the best mist regime for producing quality liners for continuing the production cycle past the evaluation period. The evaluation and analysis of media mixtures showed that for Escatawpa azalea, the three mixtures with more peat than perlite (MM4, MM5, and MM6) produced superior results in root length, area and rating. The same three medias all produced superior root area and root rating results at 84 DAS. Only MM4 produced superior results in all three measures before the final harvest date. MM4 and MM5 produced a median root rating of 5 at the final harvest. They were the only two media mixes that produced a median rating equivalent to a fully formed liner in this experiment. Media mixes showed linear trends in the increase of their root length and root ratings, so it is possible that if the evaluations had continued past 84 days, that the root rating of 5 could have been achieved in other media mixes as well.

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Table 2.1. Mist treatments and rooting percent of *Rhododendron austrinum* cuttings in three experiments.

DAS[z]	Regime	Interval (min)	Duration (s)	Mist (s/d)	Rooted (%)	Evaluated (DAS)	Author
1-42	High	10	12	864			Thompson et al.
43-84	High	10	9	648	84	70	2018
1-42	Low	10	8	576			Thompson et al.
43-84	Low	10	6	432	90	70	2018
1 - 68	Fixed	4	2	1020	67	68	File et al.
1 - 68	Fixed	8	4	1020	75	68	2000
1 - 68	High	4	6	440	75	68	Knight et al.
1 - 68	Low	4	12	220	60	68	2005

[z]Days after sticking (cuttings)

Table 2.2. Media mixtures.

Media mix	Pine bark	Perlite	Peat moss
MM1	50[z]	50	0
MM2	50	40	10
MM3	50	30	20
MM4	50	20	30
MM5	50	10	40
MM6	50	0	50

[z]Percent by volume

Table 2.3. Root rating scale descriptions based on visual attributes.

Rating	Descriptions
0	Dead
1	Live, non-rooted
2	Roots present, but with no or minimal secondary branching
3	Secondary root branching density capable of retaining some media after washing
4	Profuse secondary and tertiary root branching retains media after washing, roots partially fill a 70 cm^3 cell
5	Profuse secondary and tertiary root branching retains media after washing, roots uniformly fill a 70 cm^3 cell

Table 2.4. Average root length (mm) on stem cuttings of *Rhododendron austrinum* 'Escatawpa'.[z]

Media ratio[x]	DAS[y] 42	56	70	84	Sign.[w]
1:1:0	3.4ns[v]	12.8ns	24.7bc	40.2bc	L***
5:4:1	2.0	9.4	19.0c	43.0bc	Q*
5:3:2	3.2	11.6	25.6bc	40.6bc	L***
5:2:3	5.6	16.9	36.3a	46.2ab	NS
5:1:4	3.5	13.3	28.8ab	51.1a	L***
1:0:1	3.4	11.6	22.7bc	38.0c	L***

Mist duration	DAS[y] 42	56	70	84	Sign.[w]
High[u]	2.9ns[t]	10.3b	22.9b	37.7b	Q**
Low[s]	4.2	15.0a	29.4a	48.7a	Q***

[z]The media by harvest and duration by harvest interactions were significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Media ratios of pine bark : perlite : peat (by volume).

[w]Not significant (NS) or significant linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*), < 0.01 (**), or 0.001 (***).

[v]Least squares means comparisons among medias (lower case in columns) using the simulated method at $P < 0.05$.

[u]12 s mist 1-42 DAS, 9 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[t]Least squares means comparisons between durations (upper case in rows)using F-tests at $P < 0.05$.

[s]8 s mist 1-42 DAS, 6 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

Table 2.5. Root area (cm^2) on stem cuttings of *Rhododendron austrinum* 'Escatawpa'.^{z}

Media ratio^x	DAS^y 42	56	70	84	Sign.^{w}	Mist duration	DAS 42	56	70	84	Sign.
1:1:0	0.1ns^v	1.1ns	4.4bc	9.0b	Q**	High^u	0.1^t	0.9	4.2b	8.9b	Q***
5:4:1	0.1	0.8	3.1c	9.6b	Q***	Low^s	0.2	1.4	5.8a	11.4a	Q***
5:3:2	0.1	1	5.0bc	9.3b	Q**						
5:2:3	0.2	1.8	7.5a	11.2ab	L***						
5:1:4	0.2	1.2	6.0ab	12.6a	Q***						
1:0:1	0.1	0.9	4.1bc	9.1b	Q***						

^{z}The media by harvest and duration by harvest interactions were significant at P < 0.05.

^{y}DAS = days after sticking cutting.

^{x}Media ratios of pine bark : perlite : peat (by volume).

^{w}Significant linear (L) or quadratic (Q) trends using model regressions at P < 0.01 (**) or 0.001

^{v}Least squares means comparisons among medias (lower case in columns) using the simulated method at P < 0.05.

^{u}12 s mist 1-42 DAS, 9 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

^{t}Least squares means comparisons between durations (upper case in rows)using F-tests at P <

^{s}8 s mist 1-42 DAS, 6 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

Table 2.6. Root rating of stem cuttings of *Rhododendron austrinum* 'Escatawpa'.[z]

Media ratio[x]	DAS[y] 42	56	70	84	Sign.[w]	Mist Duration	
1:1:0	2ns[vu]	2ns	3b	4b	L***	High[t]	2b[r]
5:4:1	1	2	2c	4b	Q**	Low[r]	3a
5:3:2	2	2	3b	4b	L***		
5:2:3	2	2	4a	5a	L***		
5:1:4	2	2	3b	5a	L***		
1:0:1	1	2	3b	4b	L***		

[z]The media by harvest interaction and the duration main effect were significant at P < 0.05.

[y]DAS = days after sticking cutting.

[x]Media ratios of pine bark : perlite : peat (by volume).

[w]Significant linear (L) or quadratic (Q) trends using qualitative/quantitative model regressions at P < 0.01 (**) or 0.001 (***).

[v]Rating system from 0 being dead to 5 being a fully rooted cutting

[u]Differences among medias (lower case in columns) were estimated at P < 0.05.
ns = not significant.

[t]12 s mist 1-42 DAS, 9 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[s]Difference between mist durations (lower case in column) using main effect F-test at P < 0.05.

[r]8 s mist 1-42 DAS, 6 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

Table 2.7. Electrical conductivity (mS/cm) of six media mixes on four harvest dates under two mist regimes.[z]

Mist duration		Media[y] ratio		DAS[x]	
High[w]	253.9a[v]	1:1:0	235.0b[u]	42	199.5
Low[t]	235.7b	5:4:1	214.3b	56	231.6
		5:3:2	280.8a	70	251.9
		5:2:3	241.9ab	84	296.2
		5:1:4	252.9ab	Sign.[s]	L***
		1:0:1	243.9ab		

[z]The duration, media and harvest main effects were significant at $P < 0.05$.

[y]Media ratios pine bark : perlite : peat (by volume).

[x]Days after sticking cutting.

[w]12 s mist 1-42 DAS, 9 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[v]Least squares means comparisons between durations (lower case in column)using main effect F-tests at $P < 0.05$.

[u]Least squares means comparisons among medias (lower case in column) using the simulated method at $P < 0.05$.

[t]8 s mist 1-42 DAS, 6 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[s]Significant (Sign.) linear (L) trend using model regression at $P < 0.001$ (***).

Table 2.8. pH of six media mixes on four harvests under two mist regimes.[z]

Mist duration		Media ratio[y]		DAS[x]	
High[w]	6.7b[v]	1:1:0	7.0a[u]	42	6.7
Low[t]	6.8a	5:4:1	7.0a	56	6.8
		5:3:2	6.8b	70	6.7
		5:2:3	6.7bc	84	6.9
		5:1:4	6.7bc	Sign.[s]	L**
		1:0:1	6.6c		

[z]The duration, media and harvest main effects were significant at $P < 0.05$.

[y]Media ratios pine bark : perlite : peat (by volume).

[x]Days after sticking cutting.

[w]12 s mist 1-42 DAS, 9 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[v]Least squares means comparisons between durations (lower case in column)using main effect F-tests at $P < 0.05$.

[u]Least squares means comparisons among medias (lower case in column) using the simulated method at $P < 0.05$.

[t]8 s mist 1-42 DAS, 6 s mist 42 - 84 DAS at 10 min intervals from 0630 HR until 1830 HR

[s]Significant (Sign.) linear (L) trend using model regression at $P < .001$ (***).

Table 2.9. Particle distribution of six media mixtures.

Media ratio[z]	Coarse	Medium	Fine
1:1:0	61.4a[y]	29.5b	9.1b
5:4:1	60.0a	31.0ab	9.0b
5:3:2	56.8ab	32.0ab	11.2ab
5:2:3	59.8ab	28.4b	11.8ab
5:1:4	52.1b	34.0a	13.9a
1:0:1	55.1ab	30.4ab	14.5a

[z]Media ratios bark : perlite : peat (by volume).

[y]Least squares means comparisons among medias (lower case in columns) using the simulated method at $P < 0.05$.

Table 2.10. Physical properties of six media mixtures.

Media ratio[z]	AS[y]	WHC[x]	TP[w]	BD[v]
1:1:0	35.5a[u]	42.4d	77.9c	0.1276a
5:4:1	35.9a	43.9d	79.5bc	0.1223ab
5:3:2	30.0b	48.8c	78.8bc	0.1237ab
5:2:3	28.9bc	53.2b	82.1ab	0.1229ab
5:1:4	30.3b	54.6ab	85.0a	0.1125b
1:0:1	25.4c	58.4a	83.8a	0.1164ab

[z]Media ratios bark : perlite : peat (by volume).

[y]AS = air space

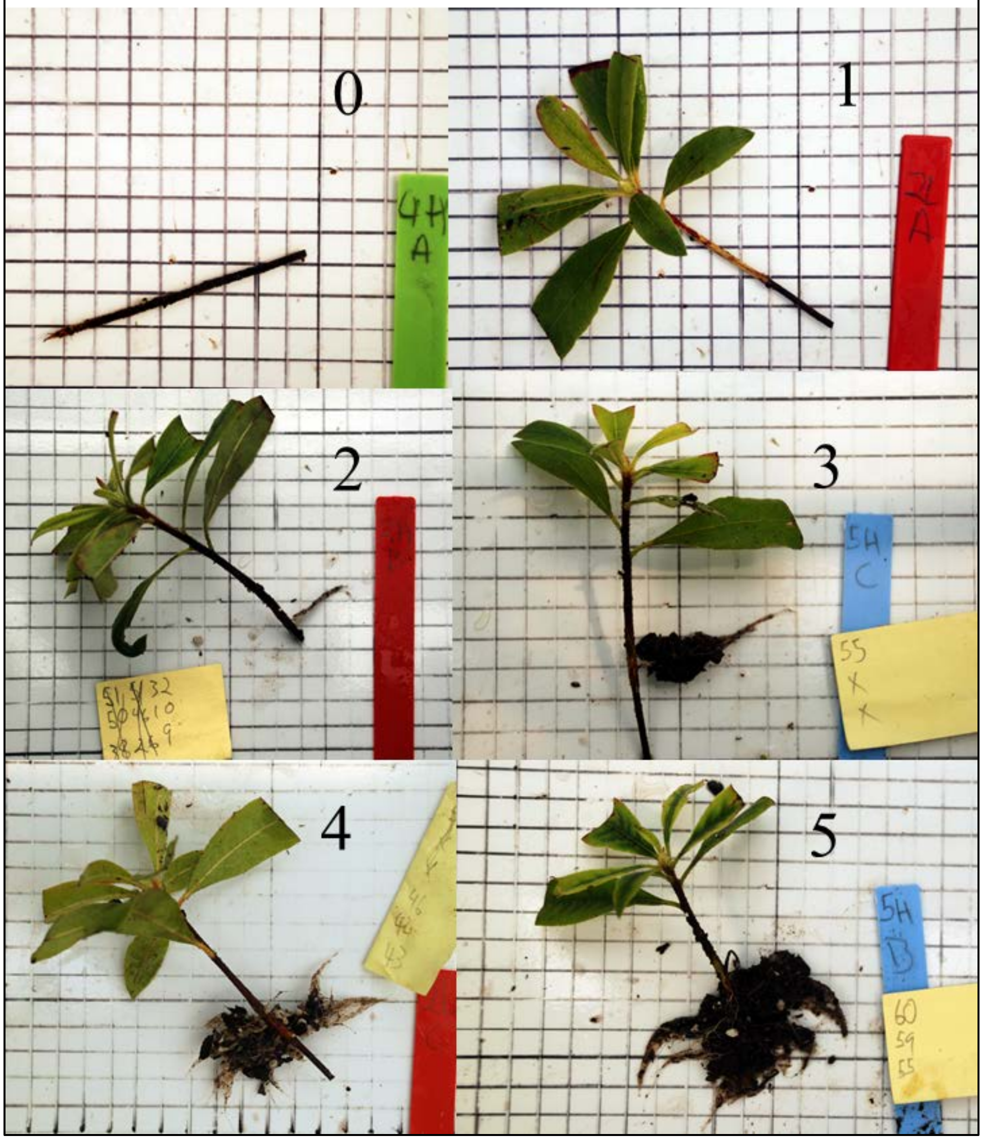
[x]WHC = water holding capacity

[w]TP = total porosity

[v]BD = bulk density

[u]Least squares means comparisons among medias (lower case in columns) using the simulated method at $P < 0.05$.

Figure 1. Azalea root rating scale 0-5



Chapter 3

Evaluation of effect of cultivar, ploidy, and origin on rooting of stem cuttings of deciduous azaleas, *Rhododendron* section *Pentanthera*

Abstract

This study was conducted at the Paterson Greenhouse Facility, Auburn University in Auburn, Alabama to evaluate rooting of stem cuttings of 20 cultivars of deciduous azaleas, *Rhododendron* section *Pentanthera*. Within cultivars, differences in ploidy level and cultivar origins were examined. There were three different origins (wild collected, F1 hybrids, and complex hybrids) and two ploidy levels (diploid, and tetraploid). Cuttings from all cultivars were placed in a mist tent within a greenhouse receiving intermittent mist applications, stuck in a 3 peat : 2 perlite : 5 pine bark (by volume) media mix. Data on root length, root area, root rating (0 to 5), and rooting (yes/no) were collected at 2 week intervals from 6 to 12 weeks after the date of stick. Difference were found in responses among the cultivars, showing the varied rooting response among the deciduous azaleas. Under these propagation conditions, tetraploids and complex hybrids had better results than diploids, wild collected selections, or F1 hybrids. Further evaluation of varied propagation protocols could achieve improved rates of success in rooting the diploids, wild collected selections, and F1 hybrids.

Introduction

The deciduous azaleas of Eastern North America have been heralded as among the finest flowering shrubs since their first written accounts (Bartram, 1794). Difficult propagation and slow growth has limited availability in plant markets and interest from the nursery industry (Jones, 2008). Scientific and popular literature contain a wide spectrum of recommendations and observations on aspects of vegetative propagation. Research based propagation protocols for deciduous azaleas were not addressed in the published literature to a degree that yields a consensus on best management practices with the exception of just a few species (File et al., 2000). There is consensus that it can be difficult to root cuttings of deciduous azaleas (File et al., 2000; Galle, 1985; Jones, 2008; Skinner, 1961). Success in rooting stem cuttings varies greatly between species, cultivars, and even within species (Jones, 2008; Skinner, 1961). Rooting cuttings is not the only propagation option. Seedlings of deciduous azaleas can be raised successfully, but require 3 to 7 years to mature to a flowering stage and exhibit wide variability in flower color and form (Skinner, 1954). Vegetative propagation offers a consistent product and less time to first flower (Skinner, 1961). Rooting stem cuttings produces true to type clones without reducing the vigor of the parent plant.

The goal of this research was to determine if a mist regime and media mix found to be successful for rooting stem cuttings of a single cultivar of deciduous azalea will have equivalent success when applied to 20 cultivars (Thompson, 2018). An initial evaluation of mist regimes and media mixes was based on the ability of the wild collected tetraploid Escatawpa azalea, *Rhododendron austrinum* (Small) Rehder 'Escatawpa', to produce adventitious roots on terminal stem cuttings. This experiment utilizes the mist regime and media mix that produced superior results. The cultivar *R. austrinum* 'Escatawpa' was chosen for the experiment that determined the baseline media and mist protocols based on its availability, rooting potential, and its contribution to the reproducibility of the research. The 20 cultivars that were chosen for comparative analysis in this experiment and represent different areas on the spectrum of deciduous azaleas in the collection of Auburn University (AU) Davis Arboretum (DA), in Auburn, AL. In this experiment, we restricted the potentially very wide representation of the spectrum evaluated by only utilizing cultivars that had the same degree of stem lignification once the experiment was ready to commence. We compared the ability of a variety of deciduous azalea cultivars to produce adventitious roots on terminal stem cuttings over an 84 d period under a single mist regime in a single media mix.

Objectives of this project are to increase the knowledge base that facilitates propagation of deciduous azaleas for the nursery industry, conservation, and long term safeguarding of specific genotypes in botanical collections by documenting rooting response, analyzing any observed variation between the types of deciduous azaleas. Documenting the rooting response on stem cuttings of 20 cultivars of deciduous azaleas is not an intended to produce the necessary protocols to propagate the evaluated cultivars, but rather to distinguish between those that respond well to an established set of mist and media protocols and those that do not.

Materials and Methods

This study was conducted at the Paterson Greenhouse Facility, AU, AU campus in Auburn, AL. The source of cuttings were plants established in the ground at the AU DA, AU campus in Auburn, AL. Cutting collection began at 0600 HR on the day they were stuck. Material was collected as pre-semi-hardwood cuttings. This stage of development was characterized as occurring after the secondary xylem had begun to form, but before it was fully developed. The field test used to determine this degree of lignification was that the cuttings were flexible, but rigid enough to snap when bent past a 90° angle. The starting date for the experiment was based on a sufficient amount of cutting material reaching this developmental stage. Cuttings were collected at a length of approximately 15 cm, misted with water from a spray bottle, sealed in plastic bags, and transported in a cooler with an icepack. The coolers were transported directly to the Paterson Greenhouse Facility for processing and sticking in media.

Cuttings were processed and stuck inside a propagation greenhouse. The greenhouse roof glazing was a double walled inflated 0.15 mm polyethylene with 38% shade cloth. Sidewalls are constructed of clear corrugated polycarbonate. The greenhouse cooling system engaged at 76° F both day and night. There are four cooling stages separated by 2° F each. The first three cooling stages consist of a series of fans and vents with stage four, the evaporative cooling system initiating at 82° F. Thermometers to record minimum and maximum temperatures were monitored inside mist tents. The minimum recorded temperature was 71° F overnight 26 June 2017. The maximum recorded temperature was 90° F, on 2 July 2016.

Mist tents were erected inside a propagation greenhouse on expanded metal greenhouse benches using a PVC frame with 0.15 mm (6 mil) translucent polyethylene plastic on the tops and sides. The tops were designed with a 5 cm gap in the gable to allow air flow. The bench surface was porous expanded metal to allow airflow and water drainage. Within each mist zone, six deflector type mist nozzles (Ein dor 809 Series,Tavlit, Yavne, Israel) were mounted on 30 cm tall risers and operated at approximately 50 psi. These were arranged in a rectangular pattern so that all flats in the experiment were inside the rectangle receiving uniform double coverage. An automatic controller (Mist Master 11, Superior Controls Inc., Valencia, CA) maintained the mist schedule.

Standard 1204 inserts (STI-1204, T.O. Plastics, Clearwater, MN) were placed in mesh bottom trays with side openings, and uniformly filled with media, then placed under mist for 3 days prior to the sticking of cuttings to ensure the media has uniform and sufficient moist. A cell in each of the 12 packs were 6 cm in height, tapered, straight sided, with 70 cm^3 volume. The media ratio contained 50% screened and aged pine bark fines, 30% BPF (Berger, Quebec Canada) fine milled sphagnum peat moss and 20% Sunshine premium grade (Sun Gro Horticulture, Agawam, MA) coarse perlite. All media were amended with 0.59 kg micronutrient fertilizer (Micromax, Scott's Company Inc., Maryville, OH); 1.18 kg dolomitic lime; 2.95 kg gypsum; 3.54 kg 15N- 3.93P- 9.96K controlled release fertilizer (Osmocote Plus, Scott's Company Inc., Maryville, OH) per cubic meter.

Processed cuttings were 10 cm in length from the cut base to the base of the most apical visible petiole with the lower two-thirds of the stem stripped of leaves. The remaining terminal leaves were trimmed to a surface area of approximately two-thirds their original size to reduce transpiration loss and leaf abscission. This was accomplished by bunching the remaining leaves on each cutting and making a single cut across the top of the whorl taking care that the terminal buds were not removed. Cuttings were trimmed to final specifications immediately before being dipped in a 5,000 ppm K-IBA solution to a depth of 4 cm for 5 s and stuck into the media to a depth of 5 cm, and were then randomly distributed into the mist tent.

Cuttings received 8 s of mist duration for the first 42 d of the experiment. Half way through the experiment, mist duration was reduced to align our experiment with the standard practice of reducing the irrigation once rooting had initiated on the majority of cuttings (Bir, 1992; Sommerville, 1998; Maarten van der Geissen, personal communication). Forty-two days into the experiment 100 cuttings were destructively harvested for data collection. Among the observed cuttings, 80 were rooted 20 were unrooted, indicating the majority of cuttings had rooted and it was time to reduce the mist duration. At 42 days after sticking (DAS), the mist duration was reduced by 25% across all treatments. The resulting mist duration was 6 s. The mist interval was 10 min throughout the experiment. Mist was applied from 0630 HR until 1830 HR.

Data recorded for each cutting were rooted or unrooted, root length (mm), root area (cm^2), and root rating (0 to 5). See Fig. 1 for visual reference of the root rating system and Table 3.2 for root rating descriptions. Rooted or unrooted, and root length were recorded on the harvest date. Root area and root rating were recorded after the completion of the experiment using photographs taken of each cutting on the harvest dates. The root rating system was established upon completion of the first year of data collection to encompass the entire spectrum of root development observed during the experiment.

On each harvest date 25% of the cuttings from each cultivar were randomly selected for destructive harvests. Data was collected at 42, 56, 70, and 84 DAS. Each cutting was removed from its cell pack, and loose media was shaken away. The roots were dunked in a water bath five times to loosen and wash away additional media. A cutting was rooted if it had roots greater than or equal to 1 mm in length, or unrooted if they were dead, showed no evidence of root initiation, or had roots less than 1mm in length. Adventitious roots emerged laterally from the stem cutting, so that there is no primary root present, but typically had multiple lateral roots. Root length was recorded from the point of root tissue emergence from the stem cutting to the root tip.

Measurements of the three longest lateral roots with independent stem attachments were recorded. Secondary and tertiary branching of lateral roots were not evaluated as part of root length. Cuttings were then photographed using a digital SLR camera (Nikon D-80/ AF-S Nikor 18-135 mm, Nikon Inc., Melville, NY) set to 24 mm attached to a bracketed horizontal camera mount. Cuttings with roots spread were photographed against a white backdrop with black 1 cm gridlines along with their label denoting media and mist regime, and a paper note with the length of the three longest roots recorded.

After the final harvest date, the 0 to 5 root rating scale was established based on scales utilized in other deciduous azalea rooting evaluations, and the range of rooting responses observed in the photographic record (Fig. 1) created during the first year of data collection in this experiment. The scale was based on the cutting's rooting success where a 0 was a dead cutting, and a 5 was a fully formed root system, uniformly reaching the walls of the cell pack consisting of roots from lateral roots arising from multiple attachment points on the stem (Table 3.2). The root area of each cutting was evaluated by counting all squares on the grid more than 50% obscured by roots. The average length of the three longest roots used in statistical analysis.

Physical and chemical properties of the media were also measured. Electrical conductivity (EC) and pH were monitored using the pour through method (Wright, 1986). We collected three samples of leachate on each harvest date from the media of cuttings being evaluated. A bench top multi-parameter meter (Thermo Scientific Orion Star A215 pH/Conductivity, Thermo Fisher Scientific, Waltham, MA) was used to measure pH and EC. Physical properties of the media were measured using procedures described by Bilderback et al. (1982) using the NCSU porometer. Measurements were used to calculate the air space (AS), water holding capacity (WHC), total porosity (TP) and bulk density (BD). Particle distribution was evaluated by passing an air-dried sample through 12.7, 9.50, 6.35, 3.35, 2.36, 2.0, 1.7, 1.4, 0.5, 0.25, 0.10, or 0.05 mm sieves with particles that were less than 0.5 mm collected in a pan. Sieves were placed in a sieve shaker (8" Roto Tap RX-29, WS Tyler, Mentor OH) and shaken for 3 min (278 oscillations per min, 159 taps per min). All media in the particle distribution evaluation were weighed on a digital scale (Adventurer Pro Precision Balance AV3102C, OHAUS, Pine Brook, NJ). Standard sieve sizes were separated into three categories for analysis. The sieves in the coarse category were 12.7, 9.50, 6.35, 3.35, and 2.36 mm. Sieves in the medium, category were, 2.0, 1.7, and 1.4 mm. Sieves in the fine category were 0.5, 0.25, 0.10, and .05 mm, and the fines that fell into the pan at the bottom of the stack.

This experiment evaluates plants from three categories and two ploidy levels (Table 3.1). Selections of four species of native azaleas are included, representing two cultivars of *R. alabamense*, and one each of *R. austrinum*, *R. canescens* and *R. flammeum*. Cultivar names were assigned to genetically unique individuals for reference within this experiment. Single quotations denoting a cultivar here are not an indication of registration with the Royal Horticultural Society's registry of *Rhododendron* cultivars (Leslie, 2017), or an indication of intent to propagate beyond the duration of this experiment or registered using the names listed here. The only registered cultivar evaluated in this experiment is the complex hybrid *R.* ×'Patsy's Pink'. The complex hybrids were 8 of 20 cultivars, the F1 hybrids made by the Auburn Azalea breeders were 7 of 20 cultivars, and the wild collected plants are were 5 of 20 cultivars. The cultivars evaluated were chosen based on three criteria: proper physiological development at the time the experiment began, a need to know how to propagate them, a desire to represent a diversity of deciduous azaleas.

This experiment was conducted from 30 May through 22 August 2017. For cultivars, n=5. For harvest (DAS) n=4. For ploidy, n=50. Regarding origin, on each harvest, for complex hybrids n= 40. For F1 hybrids, n=35. For wild collected plants, n=25. Over the course of this experiment 400 cuttings were evaluated.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Root length and root area were analyzed using the Gaussian distribution. Cultivar and DAS, ploidy and DAS, and origin and DAS were analyzed as completely randomized designs in 2-way factorial treatment designs. Where residual plots and a significant covariance test for homogeneity indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity. Pair-wise least squares means differences among cultivars and origins were determined using the simulated method. Differences between ploidy were determined using F-tests. Linear and quadratic trends over DAS were determined using simple model regressions in the cases of significant main effects or qualitative/quantitative model regressions in the cases of significant interactions. Root rating was analyzed using the multinomial probability distribution with a cumulative logit link and the Laplace estimation method. Pair-wise comparisons among cultivars were estimated. Rooting yes/no was analyzed using the binary probability distribution. Differences among origins for rooted yes or no were estimated. Percent rooted was yes rooted divided by the total observed. All significances were at α=0.05.

Results

Analysis of root length showed that the cultivar by DAS was a significant interaction. Root length increased linearly from 42 DAS to 84 DAS for 90% of the cultivars, but no trend was found for *R. alabamense* 'CCP' and *R. flammeum* 'Big Red' (Table 3.3). The longest root length occurred in *R.* ×'Patsy's Pink' at all DAS. The shortest occurred in *R. flammeum* 'Big Red' at 42, 56, and 84 DAS, but *R. alabamense* 'CCP' had the shortest 70 DAS. The widest variation in root lengths occurred at 70 DAS, when five different groups occurred across the 20 cultivars. Four cultivars fell into the inferior fifth group: *R. alabamense* 'CCP', *R. flammeum* 'Big Red', *R. cumberlandense* × *R. arborescens,* and *R.* ×*bakeri* 'Tom's Pink'. At 84 DAS, there were three different groups. In the superior group, *R.* ×'Patsy's Pink', *R.* ×'Pat's Pink', and *R. prunifolium* × *R.* 'Gibraltar' had no overlap with the second tier plants, though 14 cultivars had no difference between the first or the second tier group of plants. Only *R. cumberlandense* × *R. arborescens* was in the second tier without overlap, and only *R. flammeum* 'Big Red' was in the third tier. This resulted in *R. alabamense* 'CCP' being the only cultivar having no difference from either the second or third tier groups.

Similar trends emerged in the results of rooting percentages (Table 3.4) where plants that had superior root lengths also had superior rooting percentages. This evaluation however only resulted in three different groups. Non hybrid species cultivars comprise the group with the lowest rooting percentages. Exceptions to this are *R. canescens* 'Glenn Ave.' with 80% rooting and *R. austrinum* 'Escambia River', which had 100% rooting along with several of the hybrid cultivars. The only cultivar that achieved 100% rooting that did not have *R.* ×'Gibraltar' as a parent was *R.*

cumberlandense × *R. oblongifolium*. The only cultivars that did have *R*. ×'Gibraltar' as a parent and did not have 100% rooting were *R.* ×'War Eagle' and *R.* × 'Auburn Tiger'.

The root rating analysis results showed a significant interaction in DAS and cultivar main effects (Table 3.5). Ratings increased linearly with time across the four harvests days evaluated, achieving the maximum root rating by 70 DAS. The analysis also showed separation into three different groups of cultivars. The cultivars that had *R.* ×'Gibraltar' as a parent were all in the superior group as was an F1 hybrid, *R. arborescens* × *R. prunifolium*, that achieved the highest possible rating. In the group with the lowest root ratings, 75% of cultivars were selections of species.

The 20 evaluated cultivars originate from three sources as indicated by Table 3.1. Table 3.6 shows the mean lengths of roots grown by each origin on the four DAS evaluations. There was a significant interaction in the DAS by origin main effects with a linear increase in root length for all origins over time. The wild collected plants had the shortest roots at 42 and 70 DAS. The complex hybrids had the longest roots on all 4 harvest dates. Table 3.7's data on root area reinforces these trends, again separating into three different groups. Origin by DAS also had a significant interaction in this analysis with root area increasingly linearly over time. Wild collected plants had the smallest area in this evaluation only at 70 DAS. They were in the intermediate group on the other 3 harvest dates. F1 hybrids were in the intermediate group for the first three harvests, but joined the group with the largest root area at 84 DAS. Complex hybrids were in the group with the largest root area on all four harvest dates. Root rating also separated into three groups among origins and increased linearly with time for all origins across harvest dates (Table 3.8). Complex hybrids achieved the highest possible median root rating. F1 hybrids were in the intermediate group, superior to wild collected plants.

Deciduous azaleas have two primary ploidy levels as indicated by Table 3.1. Table 3.9 shows the linear trend of increasing root length over time and that the average root length of tetraploid cultivars was 68% greater than diploids. Analysis of root area (Table 3.10) shows that ploidy and DAS have significant interaction. It also shows separation of the means between the two ploidy levels at each DAS evaluation with tetraploids producing superior results every time. In Table 3.11, the linear trend of increasing root ratings is seen again, as is the clear separation between diploids and tetraploids in this experiment. Here tetraploids achieve superior results, this time with the median rating of the diploid cultivars indicating they have only achieved secondary root branching sufficient to hold some media, where tetraploids have developed into a fully formed liner. Finally in Table 3.12 we can see the rooting percentage comparison between the two ploidy levels. Less than a third of the diploid cuttings developed roots over 1mm in length compared to 178 out of 180 rooting among the tetraploid plants.

The media particle distribution was 50% coarse particles, 32% medium particles, and 18% fine particles (data not shown). The media had 41.9% AS, and 38.4% WHC making the TP 80.3% (data not shown). The AS, WHC and TP were inside the parameters discussed by Argo (1998) for normal container porosity. The BD of the mix was 0.15 g/cm^3. The BD results were the expected inverse of the high TP of the media. EC increased over the four harvest dates from 208.7 mS/cm to 228.9 mS/cm to 281.6 mS/cm to 364.0 mS/cm on the final harvest (data not shown), remaining within acceptable parameters. The range of the pH across the four harvest dates was from 6.48 to 6.66 (data not shown). The pH was higher than the recommended range for azaleas, but it clearly did not inhibit root development across all cultivars considering some cultivars had 100% rooting and mean root ratings of 5.

Discussion

The evaluation of 20 cultivars was an effort to observe variation in development of adventitious roots among deciduous azaleas over several weeks. Table 3.3 exemplifies this variation on all the harvest dates, but it is most pronounced at 70 DAS. On this harvest date, five different groups are identified with mean root lengths ranging from 5.9 cm for *R. alabamense* 'CCP' to 69.5 cm for *R.* ×'Patsy's Pink'. Evaluating root rating and rooting percentages allowed for three distinct groups to separate out within the results. All the analysis performed on the cultivars revealed trends within the group of 20 cultivars, warranting further investigation and analysis.

Polyploids are reported to be more vigorous than diploids (Jones, 2008; Miller, 2011), and the concept of hybrid vigor is well known. With the information on the plants origins and ploidy levels already at hand, it seemed a logical way to categorize the cultivars for further analysis of variation between groups. To see if these were distinct separations among these 20 cultivars. Table 3.10 shows the increase in root area between diploids and tetraploids over the four harvest dates. On the first three harvests, the tetraploids median root area was more than double that of the diploids, but on the fourth harvest date, it was only 40% greater. This suggests that the diploids could achieve success under extended production or evaluation periods. A noteworthy caveat is that the plant utilized to establish the base line protocols for mist and media that all these cuttings were subjected to was a tetraploid. A series of evaluations of media and mist preferences for a diploid species could yield protocols that would benefit them more than the ones utilized here.

Vegetative stem cuttings may not be a viable option for all cultivars. An experiment designed to find the preferred media and mist regime for *R. flammeum* 'Big Red' may be an exercise in futility, seeing as how its root development was consistently inferior and it rooted at only 25%. For plants that perform this poorly in stem cutting propagation, tissue culture is a worthwhile consideration. Other diploids like the *R. alabamense* cultivars had root initiation success rates of 40% and 55%. If these could be improved by 15% or 20% it may be worth the investigation to benefit the availability of these increasingly rare native plants.

The choice to initiate evaluations of the root system beginning when the majority of cuttings had developed roots greater than 1 mm allowed for data to be recorded across a span of time that includes cutting first beginning to root into their propagation media. The development of the cutting is then followed thru to the point where a majority of cuttings have developed into a fully formed liner. These rooted out cuttings represent a product ready to move on to the next stage of nursery production. Adding the dimension of time to the evaluation allowed for documentation of how different media and mist regimes influence the rates of development of the varying aspects or root development in deciduous azalea cuttings. Observing this difference in rate of development can help inform growers scheduling. A complex hybrid tetraploid azalea may be ready to transplant at 8 weeks, but to reach the root rating of 5, diploid and F1 hybrids may have benefitted from an additional 2 to 4 weeks beyond the 12 week term of this project.

For the tetraploids evaluated in this experiment it is clear that vegetative propagation using stem cuttings is a viable option for nursery production, and the long term continuation of living accessions of azaleas in gardens with greenhouse facilities. Long term continuation of cultivars would naturally select for the ability to develop adventitious roots. This may explain why the complex hybrid cultivars with *R.* ×'Gibraltar' as a parent performed better in this experiment than any other group. Alternatively this is a section pressure that would never be present on a gene pool of wild plants where all reproduction would be through seedling recruitment. This could explain why wild collected plant material was found to be less likely than cultivated plants to produce adventitious roots. Several cultivars that were diploid F1 hybrids performed well in this experiment. *R. cumberlandense* has been grouped with the most difficult to root species, but the F1 hybrids *R. cumberlandense* × *R. arborescens*, and *R. cumberlandense* × *R. oblongifolium* rooted at 85% and 100%. The three lowest rooting percentages belonged to the two *R. alabamense* cultivars and the *R. flammeum* cultivar, but the *R. alabamense* × *R. flammeum* rooted at 85%. Its median root rating was 2.5, but it is possible that a more appropriate media and or mist regime would improve that rating. *R. prunifolium* × *R. alabamense* and *R. prunifolium* × *R. oblongifolium* both produced median root ratings of 4.5, and *R. arborescens* × *R. prunifolium* had the highest possible median root rating of 5.

Propagators should expect to find variation in success rates among deciduous azaleas, and be careful not to approach this subgenus as uniform group that is difficult to root. The diploid wild collected azaleas in this evaluation did not perform well under these conditions. Wild diploids are the most prevalent group of deciduous azaleas, and have likely shaped perception of this group as difficult to propagate. The set of propagation protocols utilized in this experiment were developed for a wild collected species selection of a tetraploid deciduous azalea. Our results indicate that the protocols translated well to other tetraploids including wild collected ones and complex hybrids, and also achieved success in multiple evaluations of some F1 diploid hybrids.

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Table 3.1. Origin and presumed ploidy of 20 cultivars of deciduous azaleas, *Rhododendron* section *Pentanthera*, from the collections at the Davis Arboretum, Auburn University, Auburn, AL.

Cultivars of *Rhododendron*	F1 hybrid	Complex hybrid	Wild collected	tetraploid	diploid
alabamense 'CCP'			X		X
alabamense 'Smitty'	X				X
alabamense × *flammeum*	X				X
arborescens × *prunifolium*	X				X
× 'Aubie'		X		X	
canescens 'Glenn Ave.'			X		X
× 'Auburn Tiger'		X		X	
flammeum 'Big Red'			X		X
cumberlandense × *oblongifolium*	X				X
austrinum 'Escambia River'			X	X	
× 'Lathe'		X		X	
cumberlandense × *arborescens*	X				X
× 'Pats Pink'		X		X	
× 'Patsy's Pink'		X		X	
× 'Plainsman'		X		X	
prunifolium × *alabamense*	X				X
prunifolium × 'Gibraltar'		X		-[z]	-
prunifolium × *oblongifolium*	X				X
× *bakeri* 'Tom's Pink'			X	X	
× 'WarEagle'		X		X	

[z]Ploidy unknown.

Table 3.2. Root rating scale descriptions based on visual attributes.

Rating	Descriptions
0	Dead
1	Live, non-rooted
2	Roots present, but with no or minimal secondary branching
3	Secondary root branching density capable of retaining some media after washing
4	Profuse secondary and tertiary root branching retains media after washing, roots partially fill a 70 cm^3 cell
5	Profuse secondary and tertiary root branching retains media after washing, roots uniformly fill a 70 cm^3 cell

Table 3.3. Root length (cm) on stem cuttings of deciduous azalea, *Rhododendron* section *Pentanthera* , on four harvest dates.[z]

	DAS[y]				
Cultivars of *Rhododendron*	42	56	70	84	Sign.[x]
×'Aubie'	16.9abcd[w]	35.3abcd	48.3abcd	64.2ab	L***
×'Auburn Tiger'	6.9cd	30.9abcd	55.1abc	63.7ab	L***
×'Lathe'	31.7abc	48.8ab	63.4ab	62.6ab	L***
×'Pat's Pink'	44.9a	54.8a	62.9ab	71.1a	L***
×'Patsy's Pink'	38.2ab	55.1a	69.5a	71.5a	L***
×'Plainsman'	17.3abcd	25.7abcd	42.1abcde	50.2ab	L***
×'WarEagle'	8.2bcd	19.4bcd	38.0abcde	62.5ab	L***
alabamense 'CCP'	0.7cd	21.8abcd	5.9e	29.7bc	NS
alabamense 'Smitty'	1.8cd	11.5bcd	15.7de	41.4ab	L**
alabamense × *flammeum*	12.1bcd	13.5bcd	24.6cde	61.7ab	L***
arborescens × *prunifolium*	4.9bc	9.9cd	56.1abc	57.1ab	L***
austrinum 'Escambia River'	10.7bcd	20.5bcd	38.4abcde	50.1ab	L***
canescens 'Glenn Ave.'	12.9abcd	42.3abc	39.7abcde	58.1ab	L**
flammeum 'Big Red'	0.0d	0.8d	8.3e	1.1c	NS
cumberlandense × *arborescens*	3.6cd	5.7cd	10.8e	31.3b	L**
cumberlandense × *oblongifolium*	14.3abcd	12.1bcd	30.0bcde	52.5ab	L**
prunifolium × 'Gibraltar'	27.9abcd	42.7ab	61.9ab	68.8a	L***
prunifolium × *alabamense*	29.2abcd	40.1abc	45.7abcde	65.1ab	L**
prunifolium × *oblongifolium*	21.9abcd	36.3abcd	48.1abcde	52.9ab	L*
×*bakeri* 'Tom's Pink'	9.9bcd	31.8abcd	13.2e	63.0ab	L**

[z]The cultivar by DAS interaction was significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Not significant (NS) or significant (Sign.) linear (L) trends using qualitative-quantitative model regression at $P < 0.05$ (*), 0.01 (**) or 0.001 (***).

[w]Least squares means comparisons among cultivars (lower case in columns) using the simulated method at $P < 0.05$.

Table 3.4. Rooting percentage of deciduous azalea, *Rhododendron* section *Pentanthera* , stem cuttings.[z]

Cultivars of *Rhododendron*	Rooted (%)
×'Aubie'	100a[y]
×'Auburn Tiger'	95a
×'Lathe'	100a
×'Pat's Pink'	100a
×'Patsy's Pink'	100a
×'Plainsman'	100a
×'WarEagle'	95a
alabamense 'CCP'	40c
alabamense 'Smitty'	55bc
alabamense × *flammeum*	85ab
arborescens × *prunifolium*	75ab
austrinum 'Escambia River'	100a
canescens 'Glenn Ave.'	80ab
flammeum 'Big Red'	25c
cumberlandense × *arborescens*	85ab
cumberlandense × *oblongifolium*	100a
prunifolium × 'Gibraltar'	100a
prunifolium × *alabamense*	95a
prunifolium × *oblongifolium*	95a
×*bakeri* 'Tom's Pink'	85ab

[z]The cultivar main effect was significant at $P < 0.05$.

[y]Reported are percentages. Differences among cultivars (lower case in column) were estimated at $P < 0.05$.

Table 3.5. Root ratings of deciduous azaleas, *Rhododendron* section *Pentanthera*, stem cuttings.[z]

Cultivars of *Rhododendron*		DAS[y]	
×'Aubie'	5a[xw]	42	2
×'Auburn Tiger'	4ab	56	3.5
×'Lathe'	5a	70	5
×'Pat's Pink'	5a	84	5
×'Patsy's Pink'	5a	Sign.[v]	L**
×'Plainsman'	4ab		
×'WarEagle'	3b		
alabamense 'CCP'	1c		
alabamense 'Smitty'	2c		
alabamense × *flammeum*	2.5bc		
arborescens × *prunifolium*	5ab		
austrinum 'Escambia River'	3.5b		
canescens 'Glenn Ave.'	4.5ab		
flammeum 'Big Red'	1c		
cumberlandense × *arborescens*	2c		
cumberlandense × *oblongifolium*	3b		
prunifolium × 'Gibraltar'	5ab		
prunifolium × *alabamense*	4.5ab		
prunifolium × *oblongifolium*	4.5ab		
×*bakeri* 'Tom's Pink'	3b		

[z]The cultivar and DAS main effects were significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Rating system from 0 being dead to 5 being a fully rooted cutting.

[w]Reported are medians. Differences among cultivars were estimated at $P < 0.05$.

[v]Significant (Sign.) linear (L) trend using model regression at $P < 0.01$ (**).

Table 3.6. Root length (cm) of cultivars of deciduous azaleas, *Rhododendron* section *Pentanthera* , from three different origins.[z]

	DAS[y]				
Origin	42	56	70	84	Sign.[x]
F1 hybrid	14.4b[w]	19.6b	35.9b	53.4b	L***
Complex hybrid	24.0a	39.1a	55.2a	64.3a	L***
Wild collected	6.0c	21.5b	20.2c	40.6b	L***

[z]The origin by DAS interaction was significant at P < 0.05.

[y]DAS = days after sticking cutting.

[x]Significant (Sign.) linear (L) trends using qualitative-quantitative model regression at P < 0.001 (***).

[w]Least squares means comparisons among origins (lower case in columns) using the simulated method at P < 0.05.

Table 3.7. Root area (cm^2) of cultivars of deciduous azaleas, *Rhododendron* section *Pentanthera*, from three different origins.[z]

	DAS[y]				
Origin	42	56	70	84	Sign.[x]
F1 hybrid	1.67b[w]	3.23b	10.10b	15.97a	L***
Complex hybrid	3.53a	7.78a	16.38a	19.13a	L***
Wild collected	0.37b	3.63b	5.07c	10.00b	L***

[z]The origin by DAS interaction was significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Significant (Sign.) linear (L) trends using qualitative-quantitative model regression at $P < 0.001$ (***).

[w]Least squares means comparisons among origins (lower case in columns) using the simulated method at $P < 0.05$.

Table 3.8. Root rating of cultivars of deciduous azaleas, *Rhododendron* section *Pentanthera* , from three different origins.[z]

Origin		DAS[y]	
F1 hybrid	3b[xw]	42	2
Complex hybrid	5a	56	3.5
Wild collected	2c	70	5
		84	5
		Sign.[v]	L***

[z]The origin and DAS main effects were significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Rating system from 0 being dead to 5 being a fully rooted cutting

[w]Reported are medians. Differences in orgins were estimated at $P < 0.05$.

[v] Significant (Sign.) linear (L) trend using model regression at $P < 0.001$ (***).

Table 3.9. Average root length (mm) of stem cuttings of 2 two ploidy levels of deciduous azalea, *Rhododendron* section *Pentanthera* , cultivars.[z]

Ploidy			DAS[y]	
Diploid	26.1b[x]		42	16.3
Tetraploid	43.9a		56	28.8
			70	40.2
			84	54.7
			Sign.[w]	L***

[z]The ploidy and DAS main effects were significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Difference in ploidy using the main effect F-test at $P < 0.05$.

[w]Significant (Sign.) linear (L) trend using model regression at $P < 0.001$ (***).

Table 3.10. Root area (cm^2) of stem cuttings of two ploidy levels of deciduous azalea, *Rhododendron* section *Pentanthera*, cultivars.[z]

	DAS[y]				
Ploidy	42	56	70	84	Sign.[x]
Diploid	1.1b[w]	3.5b	7.2b	13.3b	L***
Tetraploid	3.2a	7.2a	15.9a	18.1a	L***

[z]The ploidy by DAS interaction was significant at $P < 0.05$.

[y]DAS = days after sticking cutting.

[x]Significant (Sign.) linear (L) trends using qualitative-quantitative model regression at $P < 0.001$ (***).

[w]Means within columns not followed by the same letter are significantly different at $P < 0.05$ using F-tests.

Table 3.11. Root ratings of stem cuttings of two ploidy levels of deciduous azalea, *Rhododendron* section *Pentanthera* , cultivars.[z]

Ploidy		DAS[y]	
Diploid	3b[xw]	42	2
Tetraploid	5a	56	3.5
		70	5
		84	5
		Sign.[v]	L***

[z]The ploidy and harvest main effects were significant at $P < 0.05$.

[y]Days after sticking (cutting)

[x]Rating system from 0 being dead to 5 being a fully rooted cutting

[w]Reported are medians. Difference in ploidy using the main effect F-test at $P < 0.05$.

[v]Reported are medians. Significant (Sign.) linear (L) trend using model regression at $P < 0.001$ (***).

Table 3.12. Rooting percentage of deciduous azalea, *Rhododendron* section *Pentanthera*, stem cuttings.[z]

Ploidy	
Diploid	29b[y]
Tetraploid	99a

[z]The ploidy main effect was significant at $P < 0.05$.

[y]Reported are counts of yes rooted/total observed. Difference in ploidy using the main affect F-test at $P < 0.05$.

<u>Figure 1. Azalea root rating scale 0-5</u>