Preliminary Study to Investigate Variation in Flooding Tolerance Across Six *Rhododendron viscosum* (L.) Torr. Subpopulations

By Alexander Q. Susko, Steven McNamara, and Stan C. Hokanson—Minneapolis, MN

Abstract

The ability for ornamental shrubs to withstand periodic poorly drained and waterlogged soils greatly improves their utility in the landscape. Many cultivars of deciduous azaleas (*Rhododendron* subg. *Hymenanthes* sect. *Pentanthera* (G.) Don), while ornamental and cold hardy, are poorly characterized for adaptations to flooded or waterlogged soils often present in commercial or residential landscapes. We present research illustrating the flooding tolerance of one species, *Rhododendron viscosum* (L.) Torr., a deciduous azalea that occurs naturally in flooded sites throughout many parts of the southern and southeastern United States. Periodic inundation of seedling root zones was conducted over a 53-day period to simulate severe flooding events. *Rhododendron viscosum* maintained vigor and growth based on accumulated root and shoot biomass despite periodic flooding. However, significant variation in the flooding response existed between subpopulations collected in four states in the southeastern US. Other notable responses to flooding included leaf discoloration and changes in root architecture. This research suggests that some *R. viscosum* subpopulations are tolerant of periodic inundation, and would serve as good places to identify parents for the breeding of future deciduous azalea cultivars with improved tolerance of flooded or waterlogged soils.

Introduction

In many urban and suburban landscapes, woody plants are frequently subjected to flooding stress due in part to heavy clay soil composition, soil compaction, excessive runoff from buildings and paved surfaces, or poor drainage designs.[1] Flooding stress manifests in many ways and can include root system decay, chlorosis due to poor nutrient uptake from the soil, and disease infestation of stressed plants.[2] Briefly, such issues can include limited phosphorous uptake under flooded conditions and increased susceptibility to Phytophthora root rot disease. Woody plants with poor flooding tolerance, even if not killed outright through the processes mentioned above, will lose vigor over time and underperform in a landscape setting. The ability to tolerate poorly drained or waterlogged soils is, therefore, a valuable selection trait when developing new woody landscape plant cultivars.

Many woody plant species and cultivars are known to have vigorous growth despite being subjected to periodic flooding, and thus are frequently recommended for landscape sites subjected to flooding.[3] However, the genus *Rhododendron* has long been regarded as a taxon whose cultivation requires exacting soil requirements, including avoidance of heavy, wet soils.[4] Only one study to date has indirectly assessed flooding tolerance in *Rhododendron* taxa. Krebs (2013) rated 24 elepidote rhododendron (*R.* subg. *Hymenanthes*) cultivars and species to determine growth responses following periodic flooding stress and *Phytophthora cinnamomi* Rands exposure in a field experiment. The species *R. hyperythrum* was identified as possessing resistance to *P. cinnamomi* under flooded conditions.[5] Though this study quantified the impact of flooding on phytophthora susceptibility and plant survival, it is not known how tolerant North American deciduous azaleas (*R.* subg. *Hymenanthes* sect. *Pentanthera*) are of flooded conditions in the absence of disease pressure. Within North American deciduous azalea germplasm, the species *Rhododendron viscosum*, commonly known as swamp azalea, is frequently observed growing along riverbanks and wetlands where periodic inundation occurs.[6,7] *Rhododendron viscosum* has also proven to be sufficiently cold hardy and ornamental for utilization as a parent in breeding *R.* sect. *Pentanthera* interspecific hybrids (Northern Lights Series) developed for cultivation in the upper Midwestern United States.[8] Thus, *R. viscosum* potentially represents a source of germplasm for developing hardy new azalea cultivars with improved tolerance to poorly drained soils. Characterizing the variation for flooding tolerance existing within *R. viscosum* will facilitate development of new varieties better adapted to wet soil conditions.

Herein, we present the results of a preliminary experiment designed to investigate variation in the flooding tolerance of *R. viscosum* seedlings originating from six subpopulations found in four states in the southeastern U.S (Figure 1; Table

▼ Figure 1—Map showing the approximate location of *R. viscosum* subpopulations sampled for this study.

Subpop.	State	Abb.	%Org.	Latitude	Longitude	$N_{families}$	$N_{seedlings}$	Precip (in)
Ouachita 1	AR	Oua	3	34°44'34.57"N	94° 6'28.87"W	2	34	50.7
Ouachita 2	AR	Oua2	7	34°41'6.27"N	94°19'5.38"W	2	32	52.9
Angelina	TX	Ang	7	31° 4'33.51"N	94°16'32.65"W	2	29	56.2
DeSoto	MS	Des	20	31° 8'42.37"N	89°14'11.49"W	2	36	59.2
Apalachicola	FL	Apa	15	30°25'10.22"N	84°27'42.22"W	2	38	59.2
Ocala	FL	Oca	40	29°33'55.89"N	81°52'17.51"W	2	39	52.8

▲ Table 1—Location, state, latitude, longitude, percent soil organic matter (%Org.), number of families used, and total number of seedlings tested and annual precipitation in inches for the *R. viscosum* subpopulations tested.

1). Specifically, we measured the response of seedlings to three cycles of root zone inundation and drainage imposed over 53 days, to mimic severe flooding events in a cultivated landscape. As a measure of flooding impact, we measured cumulative shoot and root dry mass and the relationship between the two in seedlings derived from the six *R. viscosum* subpopulations. Finally, we discuss these results in the context of adaptation to flooding and breeding for flooding tolerance in *Rhododendron* sect. *Pentanthera* germplasm.

Materials and Methods

Seedlings derived from the six *R. viscosum* subpopulations were used to estimate the variation for flooding tolerance in the species (Figure 1, Table 1). Locations for germplasm collections were chosen to maximize variation for soil type according to maps available through the NRCS with exact locations of possible collection sites narrowed using online herbarium records.[9,10] Subpopulations were identified within US National Forests during the summer of 2014 under the auspices of plant collection permits. We returned to the *R. viscosum* subpopulations between October 24th and 31st of 2014 to collect open pollinated seed capsules from individual plants identified previously (hereafter seed collected from each individual plant is referred to as maternal half sib families). A more detailed description of the identification and collection of subpopulations have been described.[6] Percent soil organic matter and average annual precipitation values (http://www.ncdc.noaa.gov/cdo-web/) for each collection location are presented in Table 1.

Seed from these wild collected, maternal half sib families was germinated at the University of Minnesota Horticultural Research Center (HRC) in Excelsior, MN during February of 2015 on milled sphagnum moss under 24-hour fluorescent lighting. In May of 2015, seedlings from the maternal half sib families were transplanted into 1" vented plug trays containing a 90% sieved pine bark and 10% perlite growing medium. Seedlings were fertilized as needed throughout the 2015 growing season with 200 ppm N Peter's Excel liquid solution (Grace-Sierra CO., Milipitas, CA). Seedlings were vernalized in a greenhouse maintained at 4.5 °C during the winter of 2015-2016. In April of 2016, seedlings were transplanted into 3x8" square plastic pots with large drainage openings (Anderson Die & Manufacturing, Portland, OR) containing the same growing medium. Seedlings were fertilized monthly between April and July of 2016 with 200

ppm N Peter's Excel solution as they became established in the pots prior to initiating the flooding experiment.

The flooding experiment consisted of two treatments, flooded and non-flooded, with 12 maternal half-sib families (two from each subpopulation) replicated twice in each treatment (Figure 2). The total number of seedlings available for testing per maternal half-sib family ranged between 29 and 39 (Table 1). Similarly-sized seedlings from each maternal half-sib family were randomly assigned to treatments and spatially randomized within each of the treatment replicates. Prior to initiation of treatment, 5 seedlings per subpopulation were harvested to provide an initial determination of seedling root and shoot dry mass as described below.

The flooding treatments were initiated on July 25th, 2016. Flooding was achieved by filling a large plastic tub containing the individual pots with water to the point where it covered the pots to the top of the potting media (Figure 2).

▼ Figure 2—Experimental set up on July 25th, 2016. The two treatments are shown flooded, in plastic tubs (1st and 3rd groups), and non-flooded (2nd and 4th groups) with two replicates each.

Photo Alexander Susko

The flooding was maintained for 36 hours for each inundation. Subsequent inundations occurred August 1st through August 4th and August 22nd through 26th (36 hours for each inundation). Between inundation treatments, seedlings were removed from the water, allowed to drain and then watered daily as needed. Seedlings in the non-flooded treatment were watered daily as needed throughout the duration of the experiment. Seedlings from both replicates of the flooded and non-flooded treatments were harvested on September 14th and 15th, 2016 at 52-53 days following the start of the initial inundation period. Seedlings were removed from the containers and the roots were gently washed free of growing medium. Seedlings were labeled, placed in paper bags, and dried for 72 hours at 70°C.

Following drying, plants were partitioned into roots and shoots by cutting the stem just above the location of the first lateral root. Shoot and root dry mass were determined with an electronic balance and recorded in milligrams. Data were analyzed in Rstudio v.3.2.1, using analysis of variance (ANOVA) and least significant difference (LSD) tests to determine the significance of the observed differences between subpopulations and treatments. Root and shoot mass data required a base-2 logarithmic transformation (log2) prior to analysis, as the variation between root and shoot masses was not equal across seedlings with different sized root systems.

Results and Discussion

Pre-experiment ANOVAs of dried root and shoot mass data revealed no significant differences among subpopulations with

Initial Shoot Mass				
Source of Variation	df	Mean Square	F value	P value
Subpopulation	5	2.52	2.02	0.11
Residuals	30	1.25		

Initial Root Mass				
Source of Variation	df	Mean Square	F value	P value
Subpopulation	5	4.12	1.98	0.11
Residuals	30	2.12		

▲ Table 2—ANOVA for pre-flooding *R. viscosum* seedling shoot, root dry mass.

initial dried root and shoot mass ANOVA p-values of 0.11, indicating that seedling root and shoot mass sizes were statistically equivalent at the start of the experiment (Table 2). After the experiment, flooding alone did not significantly affect *R. viscosum* shoot (p = 0.34) or root (p = 0.41) dry masses in this experiment (Table 3, Table 4). This result suggests *R. viscosum* germplasm appears to have some tolerance for flooding.
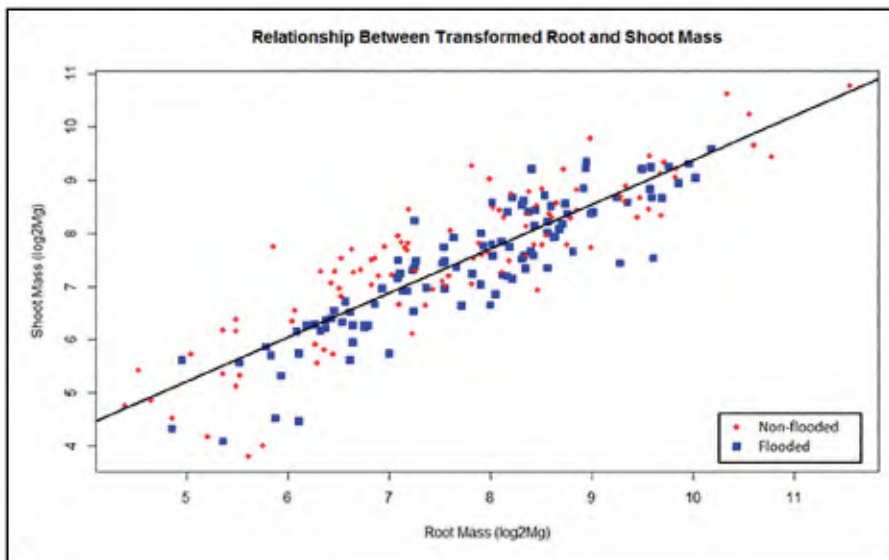
We were also interested in examining whether the flooding treatment had any impact on the correlation between dried root and shoot mass. The relationship between dried shoot and root mass within both treatments was linear and highly correlated (r = 0.87) with no difference in the degree of correlation, or distribution of points around the trendline, between the flooded and non-flooded treatments (Figure 3). A correlation between dry shoot and root mass indicates that there is no substantial effect of the flooding treatment on how the *R. viscosum* seedlings allocated growth to the roots vs. shoots based on mass alone. Based on this highly linear trend, there appeared to be no limit on root or shoot growth induced by the flooding treatment.

Although *R. viscosum* appears to be tolerant to flooding, it must be recognized that adaptability to waterlogged conditions varied depending on the subpopulation in which the seedlings originated. Subpopulation mean root and shoot dry masses varied significantly following the experiment (p < 0.001) in a linear model accounting for subpopulation, treatment, and replicate effects on dried shoot masses (Table 3). Detection of significant variation for flooding tolerance at the subpopulation level indicates that some populations would serve as better sources of flooding tolerance for breeding than others. Significant differences between subpopulation dried shoot mass means were detected and are demarcated at a p-value of 0.05
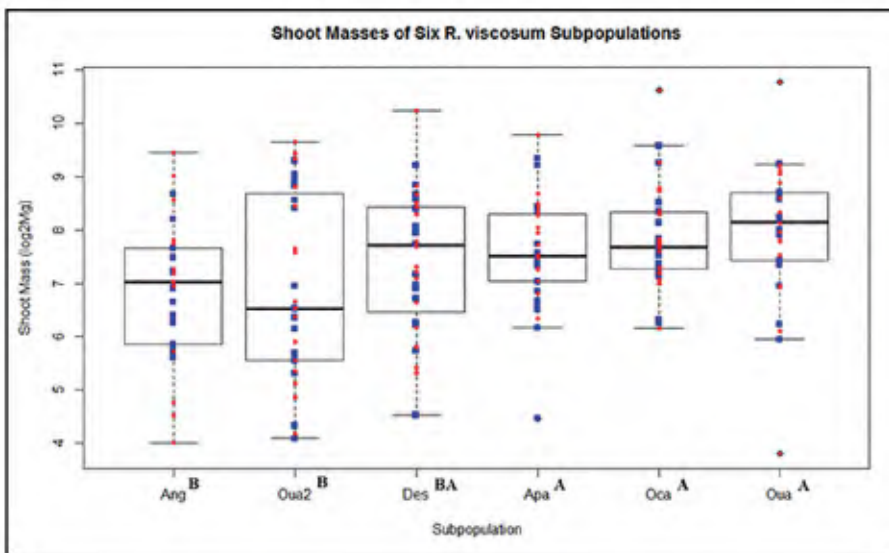
▼ Table 3—ANOVA results of *R. viscosum* final shoot mass data.

Source of Variation	df	Mean Square	F value	P value
Subpopulation (S)	5	6.967	4.463	<0.001 ***
Treatment (T)	1	1.43	0.92	0.34
Replicate (R)	1	6.95	4.45	0.04 *
S x T	5	0.40	0.26	0.94
S x R	5	2.40	1.60	0.16
T x R	1	2.54	1.63	0.20
S x T x R	5	0.74	0.48	0.79
Residuals	184	1.56		

Signif. codes: p-value < 0.001, '***'; 0.01, '**'; 0.05, '*'; 0.1, '.'

Relationship Between Transformed Root and Shoot Mass

Shoot Masses of Six R. viscosum Subpopulations

▲ Figure 4— Boxplots and distributions of shoot dry mass data by subpopulation. Red circular points indicate observations from the non-flooded treatments, while blue square points indicate observations from the flooded treatments. Subpopulations are ordered left to right with increasing mean log^{2}Mg shoot mass, with unique superscripts denoting significantly different means at alpha = 0.05.

▼ Table 4—ANOVA results of *R. viscosum* final root mass data.

Source of Variation	df	Mean Square	F value	P value
Subpopulation (S)	5	7.79	4.72	<0.001 ***
Treatment (T)	1	1.11	0.67	0.41
Replicate (R)	1	7.78	4.71	0.03 *
S x T	5	0.60	0.36	0.87
S x R	5	3.39	2.06	0.07 .
T x R	1	4.82	2.92	0.09 .
S x T x R	5	2.68	1.62	0.16
Residuals	184	1.65		

Signif. codes: p-value < 0.001, '***'; 0.01, '**'; 0.05, '*'; 0.1, '.'

◀ Figure 3— Relationship between dry root and shoot masses following the flooding experiment. Coefficient of correlation (r) = 0.87. Red circular points indicate observations from the non-flooded treatments, while blue square points indicate observations from the flooded treatments.

(Figure 4). Dried root mass means displayed a similar trend between subpopulations regarding ranking based on means and the lack of differentiation between flooded and non-flooded treatments, but possessed more significant differences based on the LSD test overall (Figure 5).

Differences in soil organic matter within subpopulations could also be responsible for some of these differences in flooding tolerance noted. We used a soilless potting media with high organic matter (90%) in these experiments. This mix was chosen because it is the optimal azalea growing mixture used in the breeding program and it allowed us to control for soil variability during the experiment. However, in regards to organic content, this mix was not representative of the soils from the locations where the subpopulations originated, which contain between 3% (Ouachita) and 40% (Ocala) organic matter (Table 1).[6] Soils with high levels of organic matter often retain water for longer periods than the low organic matter, fast draining alluvial soils found in the Ouachita 1 and 2, Angelina subpopulations. Seedlings from the Ouachita, Ouachita2, and Angelina subpopulations in the flooded treatment displayed discolored (reddened) foliage at the conclusion of the 53-day experiment relative to the seedlings from the same subpopulations in the non-flooded treatment (Figure 6). Seedlings from the Desoto, Apalachicola, and Ocala subpopulation in the flooding treatments

exhibited little to no reddening and looked similar to non-flooded seedlings (Figure 6). The foliage reddening observed is similar to the fall color seen in these subpopulations and is also a common symptom of stress in numerous plant taxa under drought or flooding conditions.[11] Such discoloration could stem specifically from nutrient deficiencies, such as a lack of available phosphorous, induced by changes in the soil redox potential as oxygen becomes scarce.[12] Generally speaking, phosphorous can become limited in soils which are subject to periodic flooding as the redox potential fluctuates and causes the transition of phosphorous to insoluble forms during dry periods, manifesting in phosphorous deficiency.[12]

Such deficiencies can discolor foliage and reduce the vigor of the plant over time and could explain the discoloration observed in our experiment, although nutrient analysis of plant tissue and potting media would be necessary to confirm this.

Differences were also observed between subpopulations for root architecture. Root systems from the Desoto, Apalachicola, and Ocala seedlings in the flooded treatment appeared to grow and branch profusely just below the surface of the media (Figure 7); a response not noted in the other subpopulations. These root systems were not necessarily more massive, (Figure 2), but they were observed to have
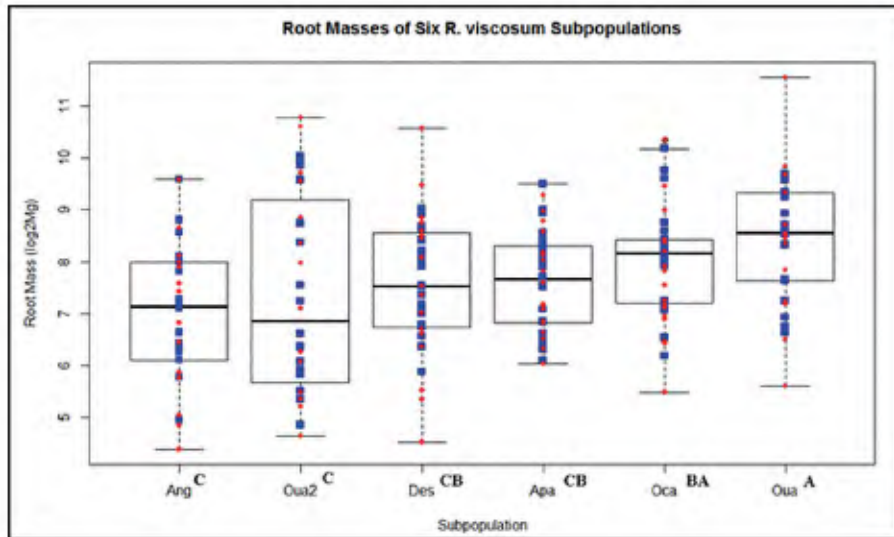

▲ Figure 5—Boxplots and distributions of root dry mass data by subpopulation. Red circular points indicate observations from the non-flooded treatments, while blue square points indicate observations from the flooded treatments. Subpopulations are ordered left to right with increasing mean $\log^2$Mg root mass, with unique superscripts denoting significantly different means at alpha = 0.05.

▼ Figure 6—Experimental layout for the flooding treatment, showing *R. viscosum* seedlings organized by subpopulation, replicate, and treatment. Seedlings were re-organized for photographing after being randomized within replicates during the duration of the flooding study.

▲ Figure 7—*R. viscosum* seedling from the Ocala subpopulation showing densely matted, branched rooting habit near the media surface.
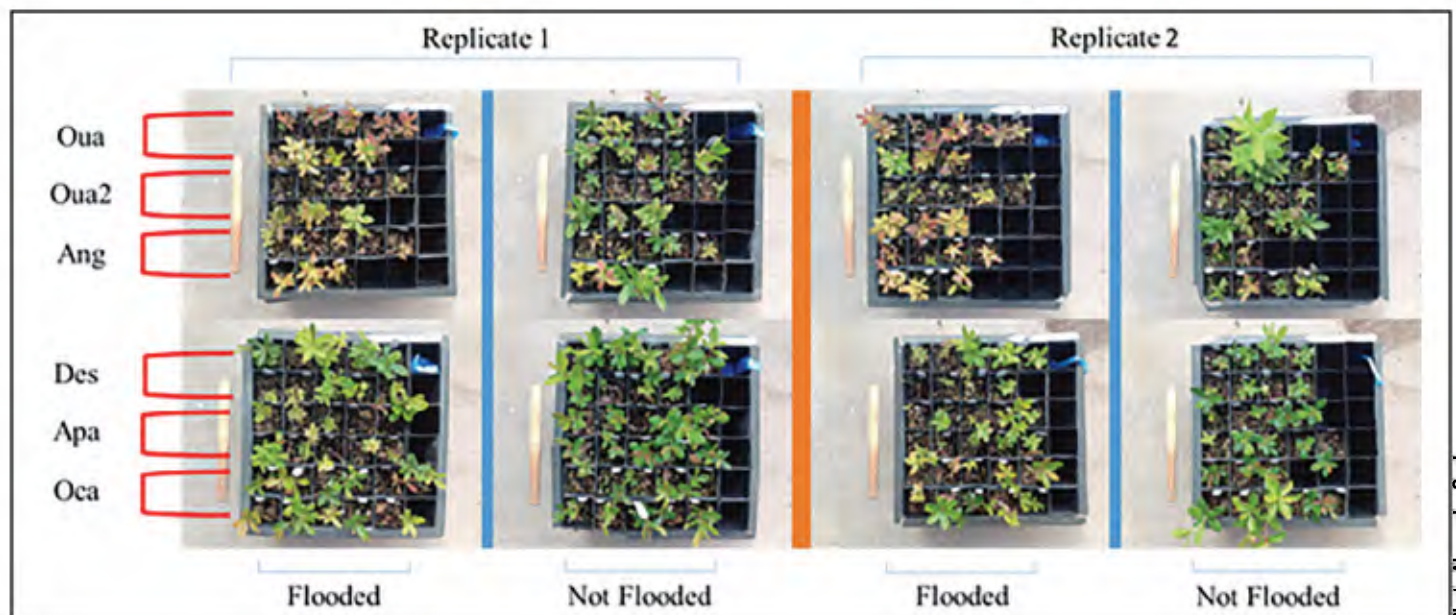
produced more new roots. Roots from seedlings from the Ouachita, Ouachita2, and Angelina subpopulations were noticeably more woody and thick following the experiment compared to the other subpopulations. Root branching and proliferation in response to flooding has been observed in other woody plant taxa, where elevated levels of ethylene in flooded roots increase auxin production and subsequent root branching.[13,14] Such differences in root architecture across *R. viscosum* subpopulations merit further study to determine what if any role they play in flooding response.

Finally, in looking at these shoot and root mass results, it is worth noting that seed from all subpopulations but Apalachicola was collected from plants growing adjacent to or near bodies of water.[6] Signs of flash flooding and periodic inundation, including bent *R. viscosum* plants and debris in branches, abounded in all except the Apalachicola subpopulation.[6] However, this Apalachicola subpopulation did receive high annual rainfall relative to the other subpopulations (Table 1). These observations from the wild suggest that some *R. viscosum* populations are subjected to periodic inundation or increased annual precipitation, and may thus be more tolerant to flooding.

In summary, *R. viscosum* germplasm evaluated in this trial does not appear to be significantly affected by flooding when measured by root and shoot dry mass. Nonetheless, there exists variation in vigor among subpopulations as measured by root and shoot dry masses when subjected to periodic flooding conditions. These results suggest *R. viscosum* may serve as a good potential source for flooding tolerance

for deciduous azalea breeding. We stress that these results are preliminary: It is also possible that genetic variation for other traits beyond root and shoot growth that could influence flooding tolerance in *R. viscosum* exist. Additional research on flooding tolerance in *R. viscosum* should focus on these more complex responses and analyze a larger number of maternal half sib families per subpopulation. It will also be imperative to compare the flooding tolerance of *R. viscosum* to other commercially available cultivars in order to determine the potential for improvement that could be gained from the germplasm. Such efforts would broaden our understanding of flood tolerance in *R. viscosum* and potentially facilitate development of more durable *Rhododendron* sect. *Pentanthera* cultivars.

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Steven McNamara, Research Scientist, University of Minnesota Horticultural Research Center, Excelsior, MN.

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Acknowledgements

For the Record

On page 31 in Part II of Barbara Bullock's article in the Summer 2017 issue, *R.* 'Lohengrin' was incorrectly identified as a Glenn Dale hybrid. Author Bullock has sent in this correction: "It's an old Kaempferi cultivar from the 19th century that Morrison planted probably for comparison reasons. (As stated in the text, p. 29, column 2, 2nd paragraph.)"

Also, the caption to Figure 13 on p. 46 should have read: Rick Bauer (l) presented J. Jackson (r) with a commemorative President's gavel for his service 2013-2017.

Recognizing Generous ASA Members

By Paul Beck, Treasurer

I would like to recognize and thank the following members who made donations to the Operating Fund of the Azalea Society of America in 2016-2017 by paying dues in excess of the $30 annual regular amount or by making direct payments to the ASA. We have three categories for donations – **Contributing** (payment of $25 to $99 for the year); **Supporting** ($100 to $199), and **Endowing** ($200 or more). My apologies if I missed anyone.

Contributing Members

Fred & Sandra Anderson	Doug Leonard & Jane Tripp
Faith & Gerald Bange	William & Peggy Meyers
Dave & Virginia Banks	Jerry O'Dell
Sam & Ginger Burd	Barbara Stump
Vincent J. Ciolino	David & Joan Taylor
Gina & Richard Hebert	Mike & Debbie White
Bob & Martha Kelly	

Supporting Members

Dan & Barbara Krabill
Greg Peterson & Robb Akridge

Endowing Members

Paul & Carolyn Beck

Recognizing Other Donations

In addition to the donations as part of membership renewal, the ASA has also received the following special donations, for which we are very grateful.

Operating Fund: Lloyd & Margaret Willis, in honor of Paul & Carolyn Beck. Budne & Diane Reinke, in memory of Francis Louer.

The **2016 ARS/ASA Joint Convention** donated a very generous $5,000 contribution to the Operating Fund. A big THANK YOU to the convention committee for this donation.

Azalea Research Fund: Fred Anderson, Dave & Virginia Banks, Hale & Susan Booth, Mark Crawford, Jerry O'Dell, David & Joan Taylor, Margaret Vogel

The **Azalea Research Fund** also received a very generous $2,000 donation from the Dolan Gardens Foundation.

The **Great Gardens of America** donated $2,100 to support an unspecified purpose. The BOD will be looking at how best to use this donation.

The **George Harding Memorial Garden** donated $3,200 to help continue the legacy and memory of George W. Harding.