ABSTRACT

HEBERT, CARY JOSEPH. Seed Germination of Five Populations of *Rhododendron vaseyi*, Ploidy Manipulation of Rhododendron 'Fragrantissimum Improved' and the Effects of S-ABA on the Physiology and Marketability of Various Container-Grown Taxa During Short-Term Desiccation. (Under the direction of Dr. Anthony V. LeBude).

Seeds of five populations of *Rhododendron vaseyi* A. Gray (pinkshell azalea), representing a sampling of the entire native distribution of the species, were germinated at 25C (77F) or an 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness), 8, 12, or 24-hr. The response to light and temperature of all populations was similar. Light was required for germination regardless of population or temperature. As photoperiod increased, germination increased for all populations with the alternating temperature partially compensating for the light requirement. The highest cumulative germination for all populations ranged from 51% to 67% and was achieved at 30/20C with a 24-hr photoperiod. These germination percentages, although not high, were due in part to rigorous grading of the seeds prior to initiation of the study suggesting seed viability of *R. vaseyi* is inherently low.

Rhododendron 'Fragrantissimum Improved' is an attractive cultivar with showy, fragrant flowers, but has limited potential for breeding as it is a sterile wide hybrid. Protocols for *in vitro* regeneration and polyploid induction were developed for this cultivar as a means of vegetative propagation and to potentially restore fertility and enhance ornamental traits. Combinations of TDZ (0, 2, 10, 15 µM) and NAA (0, 5, 10 µM) were used to induce shoot

regeneration from leaves. Shoot regeneration was optimized (96% of leaf segments produced shoots) using 8.8 µM TDZ and 10 µM NAA. To induce polyploidy, regenerative callus was treated with 7.5, 15, 30, 60 or 90 µM concentrations of the mitotic inhibitor oryzalin for 1, 3, 5, 7 or 14 days, in various combinations. Oryzalin significantly affected survival and shoot regenerative capacity. A percentage of homogenous, tetraploid shoots were recovered from treatments of 30 µM oryzalin for 1 (13%) and 3 (13%) days and 7.5 µM oryzalin for 7 (20%) and 14 (7%) days.

Reduced post harvest care of woody plant material in mass retail settings can decrease the number of days plants remain marketable. If plants are sold on consignment for growers, reduced sales can lead to poor profitability. This study investigated the effect of spray applications of s-abscisic acid (s-ABA) (ConTego™, Valent Biosciences Corp.) to increase the number of days of marketability for various woody taxa in a simulated retail setting. In the first stage of the study, various well-watered container-grown taxa were treated with a spray application of either 0, 1000 or 2000 $mg \cdot L^{-1}$ s-ABA and water was withheld. Daily, desiccation symptoms were recorded to determine if plants had reached the critical wilting point and thus became unmarketable. Marketability was increased approximately 2-3 days for plants treated with 2000 $mg \cdot L^{-1}$ s-ABA treatment compared to nontreated plants. In the second stage, marketability and physiology of *Ligustrum japonicum* 'Recurvifolium' (wavy leaf privet) was monitored after plants were treated with spray applications of 0, 500, 1000, 1500 or 2000 $mg \cdot L^{-1}$ s-ABA. Stomatal conductance (g_s)

declined for all plants depending on the concentration applied. Plants treated with s-ABA had lower g_s rates and remained marketable longer than nontreated plants. All plants fully recovered to pretreatment g_s rates provided they were rewatered immediately upon reaching the critical wilting point. Therefore, spray applications of s-ABA can increase shelf life of select woody ornamentals.

Seed Germination of Five Populations of *Rhododendron vaseyi*, Ploidy Manipulation of *Rhododendron* 'Fragrantissimum Improved' and the Effects of S-ABA on the Physiology and Marketability of Various Container-Grown Taxa During Short-Term Desiccation

by
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A thesis submitted to the Graduate Faculty of
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BIOGRAPHY

Cary Joseph Hebert was born on September 24, 1982 in Hammond, LA. Cary spent most of his childhood in Monroe, LA. Cary attended private, Catholic schools for most of his life, going to Jesus the Good Shepherd Elementary for kindergarten through 8th grade and then attending Catholic high schools. He started out at St. Frederick's High School in Monroe, LA, but had to switch schools after his father was transferred back to Baton Rouge for his job. In Baton Rouge Cary started out at Catholic High School, an all-boys school run by the Jesuits, for his junior year and then transferred to Bishop Sullivan High School, now St. Michael the Archangel, for his senior year.

Cary began his college education at Louisiana State University in Baton Rouge, LA in 2001 and received his B.S. in Horticultural Sciences in December of 2006. His college days were some of the best of his life and he grew and matured as a person thanks to the people that he met there and the experiences that he accrued. One of the experiences that influenced his decision to go to graduate school the most was an internship he completed at Berkshire Botanical Garden in Stockbridge, MA during the summer of 2005. His time spent at the botanical garden taught him valuable career skills and a desire for achievement.

Cary began his work at NCSU on a Master of Horticultural Science degree under the guidance of Dr. Anthony LeBude. His time was divided between the piedmont and Appalachian Mountains, spending the semesters in Raleigh, NC attending classes and the summers in Mills River, NC conducting research. He was happy to be able to split his time

between the more fast-paced life in Raleigh and more relaxing mood of life in the mountains. He thoroughly enjoyed his time spent in North Carolina and knows that his academic pursuits have prepared him well for whatever career path he may choose to take.

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Chapter 1

Seed Germination of Five Populations of *Rhododendron vaseyi*: Influence of Light and Temperature

(In the format appropriate for submission to the Journal of Environmental Horticulture)

Seed Germination of Five Populations of *Rhododendron vaseyi*:

Influence of Light and Temperature

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Abstract

Seeds of five populations of *Rhododendron vaseyi* A. Gray (pinkshell azalea), representing a sampling throughout the native range of the species, were germinated at 25C (77F) or an 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness), 8, 12, or 24-hr. Germination was recorded every 3 days for 30 days. The response to light and temperature of all populations was similar. Light was required for germination regardless of population or temperature. As photoperiod increased, germination increased for all populations with the alternating temperature partially compensating for the light requirement. The highest cumulative germination for all populations ranged from 51% to 67% and was achieved at 30/20C with a 24-hr photoperiod. These germination percentages, although not high, were due in part to rigorous grading of seeds collected across a broad range of plants and growing conditions prior to initiation of the study suggesting seed viability of *R. vaseyi* is inherently low.

Index words: Ericaceae, native plants, pinkshell azalea, population ecology, sexual propagation.

Significance to the Nursery Industry

Quantitative data are presented concerning the influence of light and temperatures of 25C (77F) or an 8/16-hr thermoperiod of 30/20C (86/68F) on germination of seeds of *Rhododendron vaseyi* from five populations in western North Carolina. Seeds collected from

plants across the entire native distribution respond similarly to light and temperature treatments. Careful cleaning and grading techniques combined with a liberal application of seeds to a germination medium can compensate for low viability and help produce a uniform stand of seedlings. Small seed size plus the light requirement for germination dictates the seeds should be simply dusted on the surface of a germination medium utilizing an 8/16-hr thermoperiod of 30/20C (86/68F) with continuous light (a 24-hr photoperiod). These conditions should maximize germination with germination beginning between 9 and 12 days and nearing completion by 24 days.

Introduction

Rhododendron vaseyi A. Gray (pinkshell azalea) is a rare, deciduous, ericaceous species endemic to Watauga, Avery, and Mitchell Counties in northwest North Carolina and Transylvania, Jackson, and Macon counties in the southwest portion of the state at elevations above 914 m (3000 ft) (15). Found primarily in moist woodlands near mountain springs and streams, native populations of *R. vaseyi* are confined to a relatively small region endemically, but appear abundantly within this range.

In May to June, *R. vaseyi* produces attractive, pink to sometimes white, woodsy smelling flowers in corymbs. Flowers appear prior to leaf development in 5-10 cm (2-4 in) wide clusters of 3 to 15 flowers (8). Five to seven stamens are produced, which are more than other deciduous *Rhododendron* L. spp. (azaleas) native to the southeast United States, but fewer than the 10 stamens produced in flowers of most evergreen *Rhododendron* L. spp.

(rhododendrons) native to the same area (9). The corolla tube is noticeably shorter than most other native deciduous species within the genus (11). The attractive clusters of fragrant spring flowers, excellent deep burgundy fall color, and exfoliating bark of *R. vaseyi* make it an appealing landscape plant, especially popular among native plant enthusiasts. *Rhododendron vaseyi* is typically sold by local or mail order nurseries that propagate the plants by seeds or to a lesser extent by stem cuttings.

The official germination testing protocol for *Rhododendron* spp. requires germination for 21 days using an 8/16-hr thermoperiod of 30/20C (86/68F) with 8 hr of light daily during the high temperature portion of the cycle or a constant 25C (77F) with 8 hr of light daily (1). Seed germination protocols were reported previously for two populations of *R. vaseyi* (10, 19). For *R. vaseyi*, LeBude et al. (10) and Walker et al. (19) studied the effect of a constant temperature of 25C (77F) or an 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness), ½, 1, 2, 4, 8, 12 or 24-hr. Both studies utilized seeds from the same population collected from the northernmost range of the species and LeBude et al. (10) also included another population from the southernmost range. In each study, light was required for germination and germination at each temperature increased with increasing photoperiod. With continuous light, seeds from the population common to both studies, as well as the additional population included by LeBude et al. (10), had total germination of 50% at 30/20C (86/68F) and 31% at 25C (77F). The light requirement was not surprising since seeds of many ericaceous species, including *Rhododendron*, have a light requirement for germination (3, 4, 12). Prior to conducting their

studies Walker et al. (19) and LeBude et al. (10) subjected seeds to rigorous grading in attempts to achieve the highest germination possible. Despite these efforts, maximum germination of 50% led Walker et al. (19) and LeBude et al. (10) to hypothesize that low overall germination for *R. vaseyi* might be due to inherently low viability. However, before such a hypothesis can be accepted, a sampling of seeds from the entire native distribution would need to be tested. This would provide evidence that low germination percentages result from inherently poor seed viability. Therefore, the objective of this research was to determine the influence of light and temperature on seed germination of *R. vaseyi* representing the entire distribution of the species.

Materials and Methods

Mature seed capsules (fruit) were collected in Fall 2007 from five native populations of open-pollinated plants of *R. vaseyi* in western North Carolina. Populations 1 and 2 in the present study were the same populations utilized by LeBude et al. (10) and Walker et al. (19).

On November 3, capsules were collected from Pilot Mountain in Transylvania County (lat. 35°16'23.60"N, long. 82°52'2.17"W) [mean elevation = 1539 m (5050 ft)] and along the ridgeline between Jackson and Transylvania counties at the intersection of Highway 215 and Charley's Creek Road (lat. 35°16'12.37"N, long. 82°55'16.47"W) [mean elevation = 1295 m (4250 ft)]. These were pooled and designated Population 1. Capsules for Population 2 were collected the same day from plants growing adjacent to the parking lots of the Lynn Cove Viaduct Visitors Center (lat. 36° 5'25.58"N, long. 81°48'52.37"W) and Rough

Ridge (lat. 36° 5'49.68"N, long. 81°47'57.00"W) at mileposts 303 and 300 [mean elevation = 1330 m (4364 ft)], respectively, of the Blue Ridge Parkway. Capsules were also collected from plants growing alongside hiking trails and adjacent forests and all capsules were pooled. On October 22, capsules were collected along Highway 107 (lat. 35° 4'37.13"N, long. 83° 4'0.67"W) [mean elevation = 1054 m (3460 ft)] south of Cashiers, NC, pooled, and designated Population 3. For Population 4, capsules were collected and pooled October 22 from the Southern Appalachian Highlands Reserve (lat. 35° 7'51.41"N, long. 82°57'42.57"W) [mean elevation = 1067 m (3500 ft)]. Capsules for Population 5 were collected and pooled November 2 from the overlooks of John Rock, Fetterbush, and Devil's Courthouse (lat. 35°18'15.49"N, long. 82°53'16.62"W) along the Blue Ridge Parkway between mileposts 419 and 422 [mean elevation = 1655 m (5429 ft)].

Following collection, capsules of all populations were dried at 21C (70F) for 10 days after which seeds were released gently using a rolling pin. Chaff and other debris were removed using sieves and a dissecting microscope; cleaned seeds were graded further under a dissecting microscope to remove abnormal and damaged seeds and any debris not removed by previous cleaning. Graded seeds were stored in darkness at 4C (39F) in sealed glass vials until the germination study was initiated.

On January 7, 2008, graded seeds were removed from storage and placed in covered 9-cm (3.5 in) diameter glass petri dishes. Each dish contained two pre-soaked germination blotters moistened with tap water. After placement of seeds in the petri dishes, half the dishes for each population were designated for germination at 25C (77F) and the other half

for germination at an 8/16-hr thermoperiod of 30/20C (86/68F), which was determined to be the optimal thermoperiod treatment in previous germination studies (10, 19). All dishes were placed in black sateen cloth bags and the seeds were allowed to imbibe overnight at 21C (70F). The following day, the bags were randomized within two growth chambers [C-chambers (18)] each set at the appropriate temperature. Temperatures within chambers varied ± 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected daily to the following photoperiods: 0 (total darkness), 8, 12, or 24-hr. Since previous studies with *R. vaseyi* determined that a 24-hr photoperiod produced the highest germination at 25C (77F) and 30/20C (86/68F) (10, 19), the present study utilized only four photoperiods to reduce the number of experimental units. All photoperiod treatments, with the exception of 0 and 24 hr began at 8 A.M. daily and these coincided with the transition to the high temperature portion of the cycle for the 30/20C (86/68F) thermoperiod. Growth chambers were equipped with cool-white fluorescent lamps providing a photosynthetic photon flux (400-700 nm) of approximately 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.2 klx) as measured outside the dishes at dish level with a cosine-corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE).

Daily photoperiod treatments were regulated by removal and placement of the petri dishes into black sateen cloth bags. Petri dishes for the 24-hr photoperiod treatment remained continuously unbagged in the chambers. Dishes for the 0-hr (total darkness) treatment remained bagged throughout the experiment and all germination counts and moistening of the blotters for this treatment were performed in a dark room utilizing a

fluorescent lamp equipped with a #122 Roscolux green diffusion filter (Rosco Laboratories, Inc., Stamford, CT). Germination blotters were kept moist with tap water throughout the experiment. Seeds showing signs of decay were removed from the dishes when recording data.

The experiment was a split plot with temperature as the main plot and light and population as the subplot arranged factorially. Within a temperature regime, photoperiod and population combinations were replicated four times with a replication consisting of a petri dish containing 100 seeds. Germination counts were recorded every 3 days for 30 days and germinated seeds were removed from the dishes. A seed was considered germinated when radicle emergence was ≥ 1 mm (0.04 in) in length. Percent germination was calculated as the mean of four replications per treatment. Data were subjected to analysis of variance (ANOVA) procedures (PROC GLM) and regression analysis (PROC REG) where appropriate to determine relationships between temperature, photoperiod, and population (17).

Results and Discussion

Seeds of all five populations of *R. vaseyi* required light for germination regardless of temperature (Figs. 1 and 2). This is consistent with previous reports of seed germination of this species (10, 19), as well as other species of *Rhododendron* and members of the Ericaceae (2, 3, 4, 12).

For each temperature, germination increased as a function of photoperiod for all populations (Figs. 1 and 2). Regardless of the population, germination after day 9 was generally always greatest for the 24-hr photoperiod for each temperature (Fig. 1). In addition, there was a significant interaction between population, temperature, and photoperiod (ANOVA not presented). At day 30, germination at 25C (77F) for seeds subjected to continuous light was greatest for Population 2 (45%), followed by Population 3 (38%), Population 1 (33%), Population 5 (32%), and Population 4 (30%) (Fig. 1A-E). However, at day 30 for seeds subjected to the same photoperiod but a 30/20C (86/68F) thermoperiod, germination was greatest for seeds of Population 5 (67%), followed by Population 2 (63%), Population 3 (59%), Population 4 (54%) and Population 1 (51%) (Fig. 1F-J). Despite the interaction between population, temperature, and photoperiod, germination was highest for all photoperiods at 30/20C (86/68F) compared to the same photoperiods at 25C (77F), with the exception of the 0-hr photoperiod, of which no germination occurred.

The alternating temperature of 30/20C (86/68F) partly compensated for the light requirement for germination at photoperiods < 24 hr. For example, at 30/20C (86/68F), each population had higher cumulative germination at day 30 for the 12-hr photoperiod than at day 30 for the 24-hr photoperiod at 25C (77F) (Fig. 2A-H). The extent of this difference in germination between the two photoperiods and temperatures depended on the population. For example, Population 5 had a difference in germination of 20% (55% vs. 35%) between these conditions, whereas Population 3 had a difference of 2% (40% vs. 38%) at the same

conditions (Fig. 2C, E, H, and J). An alternating temperature partially compensating for the light requirement of *R. vaseyi* has been reported previously (10, 19), as well as for other species and genera (2, 3, 4, 6, 12, 14).

Due to the significant interactions for temperature, photoperiod, time (days), and population (ANOVA not presented), cumulative germination was regressed on photoperiod within each population by temperature for each 3-day interval. Data for total darkness were included in regression analyses to insure sufficient degrees of freedom when testing both the linear and quadratic terms of photoperiod.

At 30/20C (86/68F), germination occurred by day 9 in the 12- and 24-hr photoperiods for Populations 2 and 4. By day 9 at 25C (77F) germination occurred in the 8-hr photoperiod for Populations 2 and 4 and in the 24-hr photoperiod for Populations 2, 3, 4, and 5. By day 9 no germination was observed for Populations 1 and 3 regardless of temperature or photoperiod and germination was not noted until day 12. By day 15 at 25C (77F), germination for all populations had a similar linear relationship with photoperiod, and by day 21 all populations shared a similar linear plus quadratic relationship (Table 1, Fig. 2). At 30/20C (86/68F), the response of all populations to light was less uniform than at 25C (77F). Populations 1, 3 and 4 had similar linear responses to photoperiod after day 15 (the slope of the linear regression was 2 for each population) (equations not presented). In contrast, Population 2 had a linear plus quadratic relationship (the slope of the linear portion of the curve was 8), indicating more seeds germinated per hour increase of light. Population 5 had

similar linear response (slope = 2) to photoperiod as Populations 1, 3, and 4, but also had a linear plus quadratic response (slope of the linear portion was 6) similarly to Population 2.

Mean germination of the five populations at 30/20C (86/68F) for the 24-hr photoperiod at day 30 was 59% ±7.7% SD, indicating the populations were very similar in viability, but there were differences in vigor as reflected by differences in the rate of germination. When subjected to similar temperature and photoperiod conditions, seeds of one provenance of *R. catawbiense* Michx. (Catawba rhododendron) germinated approximately 3 days earlier on days 6 to 9 and with cumulative germination of 98% compared to two other provenances which germinated on days 9 to 12 and with cumulative germination of 80% and 90% (16). Rowe et al. (16) concluded differences in germination between provenances were due to seed vigor rather than inherent differences between provenances with respect to their responses to light or temperature. In the present study, seeds from Populations 2 and 5 germinated initially three (Population 5) or four (Population 2) times as fast as the other three populations at 30/20C (Fig. 1). Even though seeds of Population 2 had a greater initial germination rate than seeds of Population 5, cumulative germination for Population 2 was 63% compared to 67% in Population 5. The rate of germination of Populations 2 and 5 was unique when compared to the other populations, but may represent greater seed vigor in these populations than any inherent fundamental physiological differences compared to the other three populations.

Generally, the germination responses to light and temperature herein of all five populations were similar and results are similar to previous work reported for two of the five

populations of *R. vaseyi* by LeBude et al. (10) and Walker et al. (19). Populations 1 and 2 in the current study are the same populations investigated by LeBude et al. (10) and Walker et al. (19). For both LeBude et al. (10), overall germination at 30/20C (86/68F) with a 24-hr photoperiod was 45% and 50% for Populations 1 and 2, respectively, compared to 51% and 63%, respectively, for the current study (Fig. 1F and G). Mean peak cumulative germination of approximately 65% for Populations 2 and 5 at 30/20C for the 24-hr photoperiod is only nominally higher than the 50% germination reported by LeBude et al. (10) and Walker et al. (19) (Fig. 1G and J). The relatively low germination percentages observed by LeBude et al. (10) and Walker et al. (19) could have resulted from poor seed viability caused by such environmental factors as rainfall or temperature during seed development (7).

It is possible low seed viability of *R. vaseyi* may be due to its limited range, and methods to estimate viability prior to sowing would be useful. For fragmented populations of the prairie wildflower, *Silene regia* Sims (royal catchfly), Menges (13) found populations > 150 individuals had uniformly high germination percentages compared to smaller populations which had lower and more variable germination percentages. In small populations there is a greater effect of genetic drift and genetic erosion on the frequency of alleles and it is possible this, combined with inbreeding depression within the population, may have an adverse effect on seed viability and vigor (5, 13). Despite its limited natural range, *R. vaseyi* appears to occur in relatively large numbers within specific habitats scattered throughout this range. Seeds in the present study were collected from at least 50 individual plants of *R. vaseyi* per population and were pooled from a number of locations within the

vicinity of each population. The actual number of plants in each population is currently unknown, but nevertheless, research studying genetic diversity of the different populations of *R. vaseyi* within its natural range could determine the extent of genetic diversity within the species and yield clues as to whether the species is affected by inbreeding depression or genetic drift. Alternatively, crosses within and between populations could be made to determine if germination increases when populations are outcrossed.

At the conclusion of this study on day 30, remaining nongerminated seeds in petri dishes of each population at 30/20C (86/68F) with a 24-hr photoperiod were examined under a dissecting microscope. This was done to determine if these remaining seeds were possibly viable which might indicate whether this temperature/photoperiod treatment was sufficient to elicit maximum germination. The vast majority of the remaining seeds were observed to be decayed and empty and obviously not capable of germination. Very few of the remaining seeds were nondecayed and of these seeds the majority did not appear to have any nutritive tissue also indicating they were not viable. Thus, the authors are confident that germinating seeds of *R. vaseyi* at an 8/16-hr thermoperiod of 30/20C (86/68F) with a 24-hr photoperiod is optimum for germination and 30-day germination illustrated in Figs. 1 and 2 with a 24-hr photoperiod is the maximum that was attainable under the environmental conditions of which these seeds were germinated. These moderate germination percentages reflected rigorous and time consuming grading of seeds prior to initiating this research, and without such effort, germination would have been much lower. These findings support the hypothesis that seed viability of *R. vaseyi* is inherently low. Although seeds of *R. vaseyi* are not difficult to

germinate, development of simple and less time consuming procedures than those described herein to grade seeds would be useful to propagators.

As reported previously, seeds of *R. vaseyi*, like many species of *Rhododendron* are quite small (4) being 1 to 1.5 mm (0.04 to 0.06 in) in length (10). Small seed size plus the light requirement for germination dictates that seeds should be simply dusted on the surface of a germination medium utilizing an 8/16-hr thermoperiod of 30/20C (86/68F) with continuous light. These conditions should maximize germination with germination beginning between 9 and 12 days and nearing completion by 24 days.

Literature Cited

1. Association of Official Seed Analysts. 1993. Rules for testing seeds. Journal of Seed Technology 16:1–113.
2. Arocha, L.O., F.A. Blazich, S.L. Warren, M. Thetford, and J.B. Berry. 1999. Seed germination of *Rhododendron chapmanii*: Influence of light and temperature. J. Environ. Hort. 17:193-196.
3. Blazich, F.A. and D.B. Rowe. 2008. *Rhododendron* L., rhododendron and azalea. p. 943-951. *In*: F.T. Bonner and R.P. Karrfalt (Editors). The Woody Plant Seed Manual. Agr. Hdbk. 727. U.S. Dept. Ag. Forest Serv., Washington, DC (Also online at <http://www.nsl.fs.fed.us/nsl_wpsm.html>.)
4. Blazich, F.A., S.L. Warren, J.R. Acedo, and R.O. Whitehead. 1991. Seed germination of *Leucothoe fontanesiana* as influenced by light and temperature. J. Environ. Hort. 9:72-75.
5. Ellstrand, N.C. and D.R. Elam. 1993. Population genetic consequences of small population size: Implications for plant conservation. Annu. Rev. Ecol. Systematics 24:217-242.
6. Erfmeier, A. and H. Bruelheide. 2005. Invasive and native *Rhododendron ponticum* populations: Is there evidence for phenotypic differences in germination and growth? Ecography 28:417-428.
7. Fenner, E. 1991. The effects of parent environment on seed germinability. Seed Sci. Res. 1:75-84.
8. Kral, R. 1983. Ericaceae: *Rhododedron vaseyi* Gray. U.S. Dept. Agr. Forest Serv.

Southern Regional Tech. Publ. R-8. 2:854-858.

9. Lance, R. 2004. Woody Plants of the Southeast United States: A Winter Guide. Univ. Georgia Press, Athens.
10. LeBude, A.V., F.A. Blazich, L.C. Walker, and S.M. Robinson. 2008. Seed germination of two populations of *Rhododendron vaseyi*: Influence of light and temperature. J. Environ. Hort. 26:217-221.
11. Leypoldt, B. 1993. *Rhododendron vaseyi*: The pinkshell azalea. J. Amer. Rhododendron Soc. 47:118.
12. Malek, A.A., F.A. Blazich, S.L. Warren, and J.E. Shelton. 1989. Influence of light and temperature on seed germination of mountain laurel. J. Environ. Hort. 7:161-162.
13. Menges, E.S. 1991. Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biol. 5:158-164.
14. Probert, R.J., R.D. Smith, and P. Birch. 1985. Germination responses to light and alternating temperatures in European populations of *Dactylis glomerata* L. New Phytol. 99:305-316.
15. Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. Univ. North Carolina Press, Chapel Hill.
16. Rowe, D.B., F.A. Blazich, S.L. Warren, and T.G. Ranney. 1994. Seed germination of three provenances of *Rhododendron catawbiense*: Influence of light and temperature. J. Environ. Hort. 12:155-158.

17. SAS Institute, Inc. 2001. SAS/STAT User's Guide; Release 9.1.3 Edition. SAS Inst., Inc., Cary, NC.

18. Thomas, J.F., R.J. Downs, and C.H. Saravitz. 2004. Phytotron procedural manual for controlled-environment research at the Southeastern Plant Environment Laboratory. NC Agr. Res. Serv. Tech. Bul. 244 (Revised). Accessed July 9, 2007. <http://www.ncsu.edu/phytotron/manual.pdf>.

19. Walker, L.C., A.V. LeBude, F.A. Blazich, and J.E. Conner. 2006. Seed germination of pinkshell azalea (*Rhododendron vaseyi*) as influenced by light and temperature. Proc. SNA Res. Conf., 51st Annu. Rpt. p. 370-373.

Table 1. Influence of photoperiod on cumulative seed germination of five populations (pop.) of *Rhododendron vaseyi* for days 3 to 30. Data for the 0 hr photoperiod treatment were included in the analysis. This table can be used to determine the statistical significance for the relationships shown in Fig. 2.

			Time (days)									
Temp.	Pop.	Photoperiod[z]	3	6	9	12	15	18	21	24	27	30
25C	1	L	-	-	-[y]	NS	*	*	*	*	*	*
		Q	-	-	-	NS	*	*	*	*	*	*
	2	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	*	*	NS	NS	*	*	*	*
	3	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	*	*	*	NS	*	*	*	*
	4	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	NS	NS	*	*	*	*	*	*
	5	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	NS	*	NS	*	*	*	*	*
30/20C	1	L	-	-	-	NS	*	*	*	*	*	*
		Q	-	-	-	NS	NS	NS	NS	NS	NS	NS
	2	L	-	-	-	*	NS	NS	NS	NS	NS	NS
		Q	-	-	-	*	*	*	*	*	*	*
	3	L	-	-	-	NS	*	*	*	*	*	*
		Q	-	-	-	NS	NS	*	*	*	*	*
	4	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	*	NS	*	NS	NS	NS	NS	NS
	5	L	-	-	NS	*	*	*	*	*	*	*
		Q	-	-	NS	NS	NS	NS	NS	*	*	*

[z]NS,* Nonsignificant or significant ($P < 0.10$) linear (L) or quadratic (Q) response, respectively.

[y]For all populations no germination occurred by day 6 and for some populations no germination occurred by day 9 depending on temperature and photoperiod.

Figure 1. Influence of light and temperature on seed germination of five populations of *R. vaseyi*. (A) Population 1, (B) Population 2, (C) Population 3, (D) Population 4, and (E) Population 5 germinated at 25C (77F) with daily photoperiods (L) of total darkness (L-0), 8-hr (L-8), 12-hr (L-12), or 24-hr (L-24). (F) Population 1, (G) Population 2, (H) Population 3, (I) Population 4, and (J) Population 5 germinated at an 8/16-hr thermoperiod of 30/20C (86/68F) utilizing the same photoperiods as for 25C (77F). Each symbol is mean germination of four replicates (petri dishes) each containing 100 seeds. The legends in (A) and (F) apply to (B-E) and (G-J), respectively.

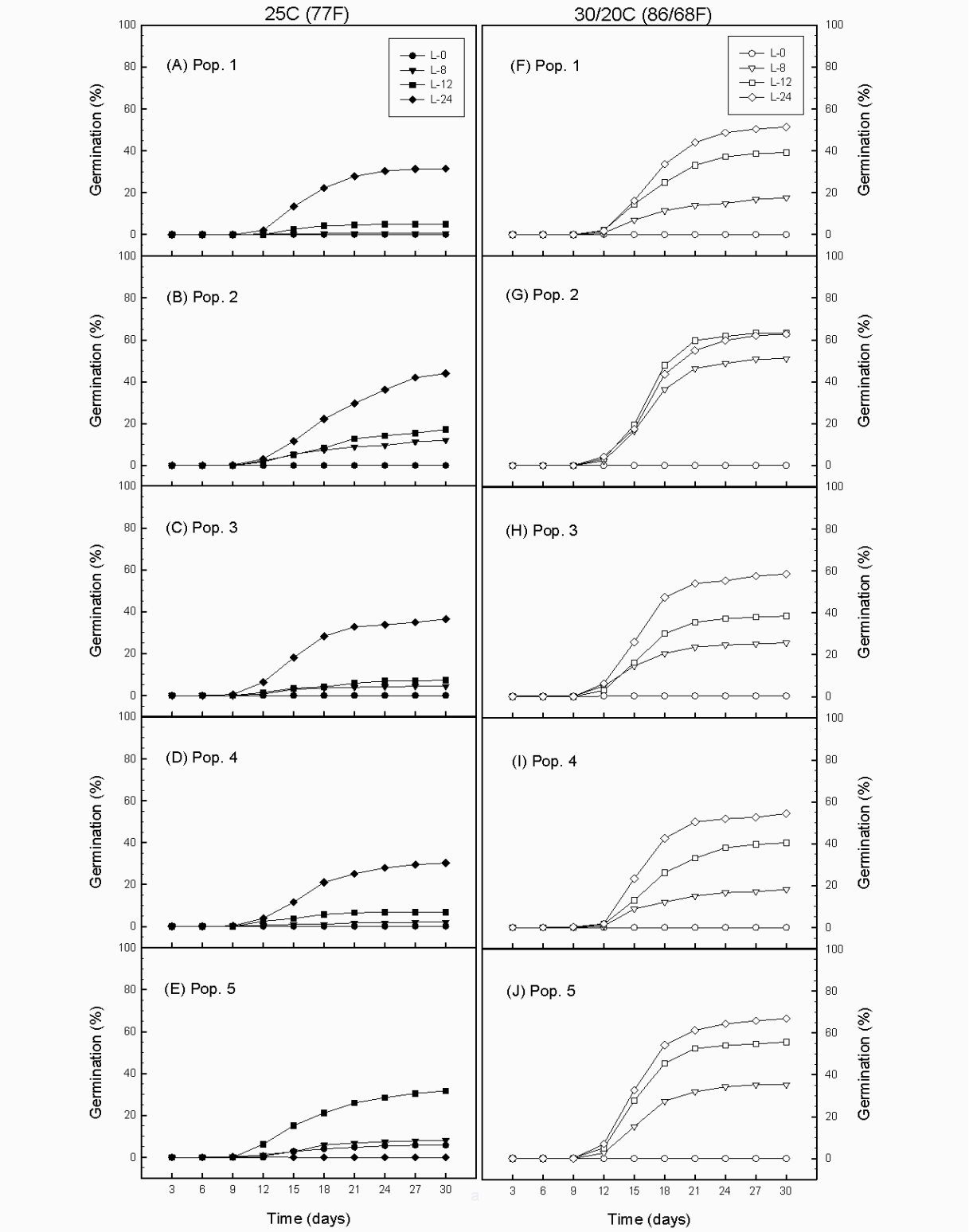
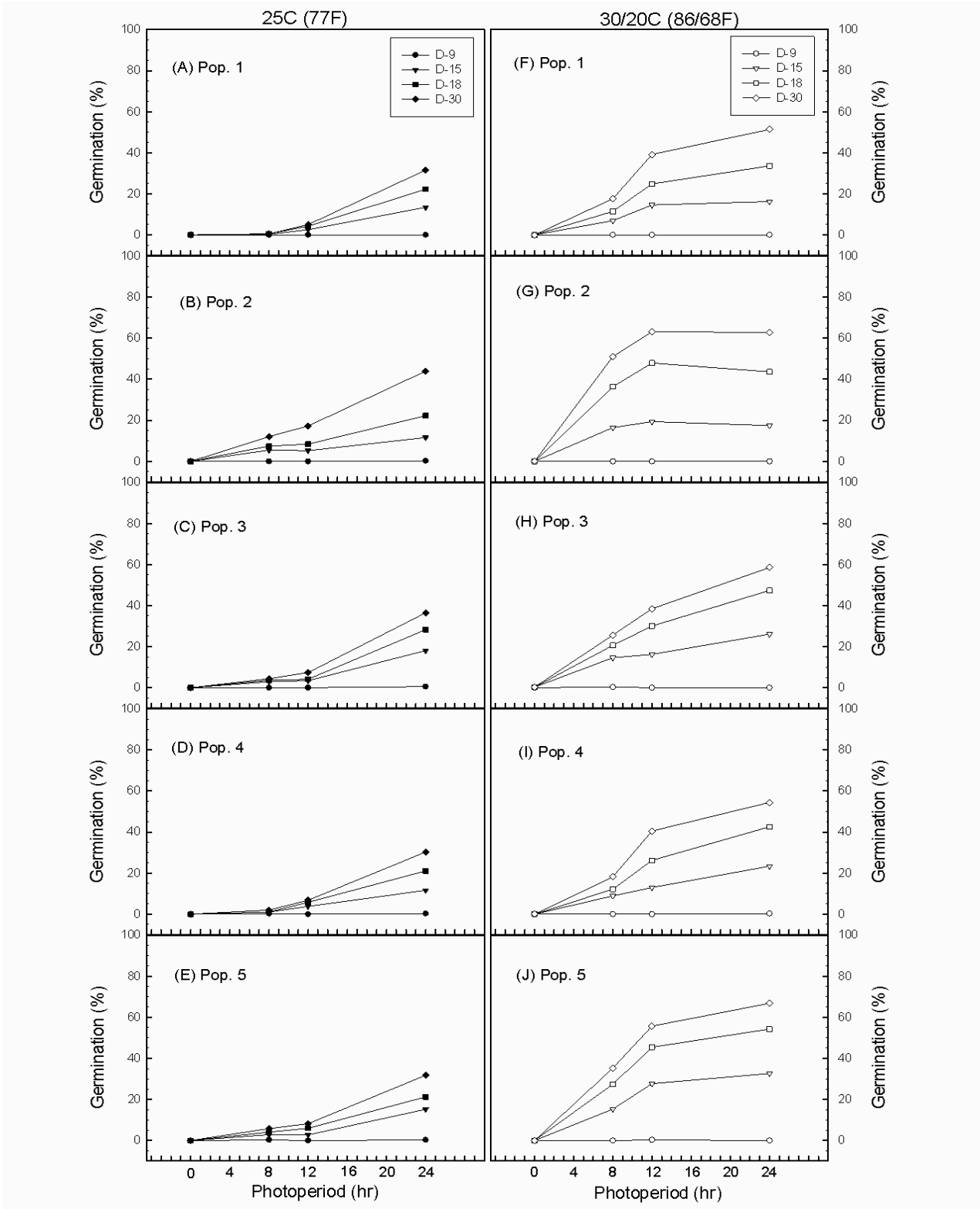

25C (77F)
30/20C (86/68F)
(A) Pop. 1
(B) Pop. 2
(C) Pop. 3
(D) Pop. 4
(E) Pop. 5
(F) Pop. 1
(G) Pop. 2
(H) Pop. 3
(I) Pop. 4
(J) Pop. 5
L-0
L-8
L-12
L-24
Germination (%)
Time (days)

Figure 2. Cumulative seed germination of five populations of *R. vaseyi* as influenced by photoperiod at days (D) 9 to 30. (A) Population 1, (B) Population 2, (C) Population 3, (D) Population 4, and (E) Population 5 germinated at 25C (77F) with daily photoperiods of total darkness (0), 8, 12, or 24-hr. (F) Population 1, (G) Population 2, (H) Population 3, (I) Population 4, and (J) Population 5 germinated at an 8/16-hr thermoperiod of 30/20C (86/68F) utilizing the same photoperiods as for 25C (77F). Each symbol is mean germination of four replicates (petri dishes) each containing 100 seeds. The legends in (A) and (F) apply to (B-E) and (G-J), respectively. Data for days 3, 6, 12, 21, 24, and 27 were omitted since germination did not occur until day 9, germination for day 12 was similar to day 15, and days 21, 24, and 27 were similar to day 30. Statistical significance of the lines in each graph is presented in Table 1.


25C (77F)
30/20C (86/68F)
(A) Pop. 1
(B) Pop. 2
(C) Pop. 3
(D) Pop. 4
(E) Pop. 5
(F) Pop. 1
(G) Pop. 2
(H) Pop. 3
(I) Pop. 4
(J) Pop. 5
D-9
D-15
D-18
D-30
Germination (%)
Photoperiod (hr)
0
4
8
12
16
20
24
0
20
40
60
80
100

Chapter 2

In Vitro Regeneration and Polyploid Induction of *Rhododendron* 'Fragrantissimum Improved'

(In the format appropriate for submission to HortScience)

In Vitro Regeneration and Polyploid Induction of *Rhododendron* 'Fragrantissimum Improved'

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In Vitro Regeneration and Polyploid Induction of *Rhododendron* 'Fragrantissimum Improved'

Additional index words. Polyploidy, Shoot Organogenesis, Oryzalin, Thidiazuron (TDZ), 6-(γ,γ-dimethylallylamino) purine (2iP), indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA)

Abstract. *Rhododendron* L.'Fragrantissimum Improved' is an attractive cultivar with showy, fragrant flowers, but has limited potential for breeding as it is a sterile wide hybrid. Protocols for in vitro regeneration and polyploid induction were developed for this cultivar as a means of propagation and to potentially restore fertility and enhance ornamental traits. Combinations of TDZ (0, 2, 10, 15 μM) and NAA (0, 5, 10 μM) were used to induce shoot regeneration from leaves. Shoot regeneration was optimized (96% of leaf segments produced shoots) using 8.8 μM TDZ and 10 μM NAA. To induce polyploidy, regenerative callus was treated with 7.5, 15, 30, 60 or 90 μM concentrations of the mitotic inhibitor oryzalin for 1, 3, 5, 7 or 14 days, in various combinations. Oryzalin significantly affected survival and shoot regenerative capacity. A percentage of homogenous, tetraploid shoots were recovered from treatments of 30 μM oryzalin for 1 (13%) or 3 (13%) days and 7.5 μM

oryzalin for 7 (20%) or 14 (7%) days. These new allotetraploids will be evaluated for ornamental traits and fertility and potentially incorporated into future breeding programs.

The genus *Rhododendron* includes diverse species and phenotypes with a broad range of ornamental characteristics and environmental tolerances. These traits make the genus appealing for breeding novel cultivars for use in the landscape. *Rhododendron* 'Fragrantissimum Improved' is a unique wide hybrid between *R. edgeworthii* Hook. and *R. formosum* Wallich. var. *formosum* (American Rhododendron Society, 2009) that is an improvement on the leggy and sprawling cultivar *R.* 'Fragrantissimum', which has been in the nursery industry for over 100 years. 'Fragrantissimum Improved' exhibits a compact growth habit, attractive exfoliating bark, lush evergreen foliage, and clusters of large, white blushed-pink, pleasantly fragrant flowers. These ornamental traits are highly desirable for breeding and development of an improved cold hardy cultivar. Like many wide hybrids, however, *R.* 'Fragrantissimum Improved' is sterile (T.G. Ranney, personal experience).

Hybrid sterility or chromosomal sterility can result from structural differences in chromosomes between species, thus preventing proper alignment during Metaphase I of meiosis. This can prevent the formation of viable gametes due to the presence of univalents and laggard chromosomes (Contreras et al., 2007). Contreras et al. (2007) also found laggard chromosomes and bivalent bridges during cell division in the sterile wide hybrid *R.* 'Fragrant Affinity', which lead to infertility. In many cases, fertility can be restored in sterile wide hybrids by doubling the number of chromosomes to produce allotetraploids. Allotetraploids have homologous pairs of chromosomes that allow for disomic pairing and formation of

balanced gametes during meiosis (Lu and Bridgen, 1997; Olsen, 2006; Ramsey and Schemske, 2002; Ranney, 2006).

Allopolyploids have been successfully induced in many genera, including *Buddleia* L., *Lilium* L., *Nerine* Herb. and *Syringa* L. (Rose et al., 2000a; Rose et al., 2000b; Van Tuyl et al., 1992). In addition to restored fertility, allotetraploids often possess improved ornamental characteristics such as thicker, darker colored leaves, larger flowers and improved pest or disease resistance, which makes them desirable to breeders (Comai, 2005; Ranney, 2006). Several studies have shown that allotetraploids can also be developed successfully in rhododendrons (Jones et al., 2008; Pryor and Frazier, 1968; Sakai et al., 2004). Further, the development of allotetraploids of *R.* 'Fragrant Affinity' improved pollen viability and led to the restoration of fertility (Contreas et al., 2007).

In vitro regeneration protocols provide an excellent mechanism for the manipulation of ploidy level, mutation treatment, and transgenic applications. In vitro shoot regeneration protocols have been developed for several *Rhododendron* species and hybrids belonging to diverse subsections including: *R. canadense* (L.) Torr., *R. mucronulatum* Turcz., *R. schlippenbachii* Maxim., *R. yedoense* var. *poukhanense* (Lev.) Nakai, *R.* 'Boule de Neige', and *R.* 'Gibraltar' (McCowen and Lloyd, 1982); *R. ponticum* L. (Almeida et al., 2005); *R. simsii* 'Helmut Vogel' (Mertens et al., 1996); *R. catawbiense* 'English Roseum' (Sicuranza and Mitkowski, 2007); and *R.* P.J.M. Group (McCowen and Lloyd, 1982; Preece and Imel, 1991). The majority of these species and hybrids belong to the subgenera *Hymenanthes*, *Pentanthera*, and *Tsutsusi* and represent various sections and subsections with only *R.*

mucronulatum and *R.* PJM Group representing subgenus *Rhododendron* (subsections *Rhodorastra* and *Caroliniana*) (American Rhododendron Society, 2009). The parents of *Rhododendron* 'Fragrantissimum Improved' are members of subgenus *Rhododendron* subsection *Edgeworthii* (*R. edgeworthii*) and subsection *Maddenia* (*R. formosum* var. *formosum*). We have found no published work on tissue culture protocols for rhododendron species in subsection *Edgeworthia* or subsection *Maddenia.*

In vitro shoot regeneration from Rhododendron leaves is most commonly stimulated by a combination of cytokinins, 6-(γ,γ-dimethylallylamino) purine (2iP) or zeatin, and auxins, indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA). To maximize shoot regeneration from leaves, 2iP and zeatin are typically required in high concentrations that often exceeding 50 μM (Iapichino and Chen, 1995; Iapichino et al., 1992; Mertens et al., 1996; Tomsone and Gentere, 2003). In comparison, thidiazuron (TDZ) has been an effective cytokinin in some species and can be used in lower concentrations, often making it more efficient (Bates et al., 1992; Huetteman and Preece, 1993). Thidiazuron has been successfully used at lower concentrations in several *Rhododendron* species (Preece and Imel, 1991; Samyn et al., 2002). For *R*. P.J.M. Group, Preece and Imel (1991) found TDZ to be effective at low concentrations with 51.6 shoots obtained on media supplemented with 10 μM TDZ and 1 μM IBA compared to 20.8 shoots with 50 μM 2iP and 10 μM IBA.

Efficient in vitro regeneration systems provide an ideal platform for the manipulation of ploidy levels. Early polyploidization experiments employed the chemical colchicine for mitotic inhibition. However, oryzalin is often preferred to colchicine due to its reduced

toxicity, higher affinity to plant tubulins, effectiveness at lower concentrations and higher survival of plantlets (Hansen and Anderson, 1996; Sree Ramulu et al., 1991; Väinölä, 2000; van Tuyl et al., 1992). Oryzalin has also been used successfully for in vitro ploidy manipulation in several genera, including *Buddleia*, *Hypericum* L., *Miscanthus* Andersson, and *Rosa* L. (Dunn and Lindstrom, 2007; Kermani et al., 2003; Meyer, 2009; Petersen et al., 2003; Rose et al., 2000). However, there have been few studies investigating in vitro ploidy manipulation in *Rhododendron*. In an intersubgeneric hybrid of an evergreen and deciduous azalea the highest tetraploid formation (84.6%) occurred when explants were treated with 300 μM oryzalin for 48 hours (Sakai et al., 2004). In another study utilizing one evergreen and two deciduous *Rhododendron* hybrids the highest percentage of tetraploids (18.2%) were formed when explants were treated with 150 μM oryzalin for 24 hours (Väinölä, 2000).

Development of an efficient in vitro regeneration system for *R.* 'Fragrantissimum Improved' would provide a desirable propagation methodology for this cultivar and related species and an avenue for additional ploidy maniupulation. Development of an allopolyploid of this cultivar could further enhance ornamental traits and restore fertility. Thus, the objectives of this study were to 1) establish an efficient protocol for in vitro shoot regeneration from leaves of *R*. 'Fragrantissimum Improved' and 2) develop an oryzalin-mediated protocol for polyploid induction in 'Fragrantissimum Improved'.

Materials and Methods

Plant material. Recently expanded leaves of *Rhododendron* 'Fragrantissimum Improved' were collected from greenhouse-grown stock plants maintained at the Mountain Horticultural Crops Research Station (MHCRS), Mills River, N.C. Leaves were rinsed under tap water for 4.5 h and then surface sterilized for 25 min using a 20% (v/v) solution of commercial bleach (5.25% NaClO) containing two drops of Tween 20 and agitated periodically. Prior to transfer to the sterile culture medium, plants were rinsed three times in distilled water for 5 min per rinse.

Shoot regeneration. The base growth medium was MS basal salts (Murashige and Skoog, 1962) supplemented with 10 μM MS vitamins, 30 $g \cdot L^{-1}$ sucrose, 0.1 $g \cdot L^{-1}$ myo-Inositol, 0.1 $g \cdot L^{-1}$ MES Monohydrate, and 8 $g \cdot L^{-1}$ agar (pH 5.75-5.80 prior to autoclaving). Media was supplemented with 0, 5, 10, 15 or 20 μM TDZ in combination with either NAA or IAA at 0, 2.5, 5, 7.5 or 10 μM. Cultures were incubated at 23 ± 2 °C in complete darkness and the number of segments producing callus tissue and number of segments producing shoots was recorded after 4 weeks. Each set of TDZ by auxin treatments was treated as a separate experiment with a completely randomized factorial design (5 rates of TDZ × 5 rates of auxin = 25 total hormone treatment combinations) with replicates consisting of petri dishes receiving the same hormone combination. There were 8 replications (petri dishes) per treatment combination, each with 5 subsamples (leaf segments). Data was analyzed using ANOVA and multiple regression procedures (PROC GLM, SAS version 9.1; SAS Inst., Cary, N.C.).

Polyploid induction. Two experiments were conducted to determine an effective concentration and duration of oryzalin treatment on callus survival and polyploid induction in regenerated shoots. Shoot organogenic callus used in all experiments was maintained on basal media containing 5 µM TDZ and 10 µM NAA. In the first experiment, calli were submerged in a liquid MS medium supplemented with 0, 30, 60 or 90 µM oryzalin for 1, 3 or 5 days on a reciprocating shaker. A stock solution of 1 mM oryzalin was dissolved in ethanol, filter sterilized and added to cooled autoclaved media. In the second experiment, calli were submerged in 0, 7.5 or 15 µM oryzalin for 7 or 14 days using the same technique. After treatment with the mitotic inhibitor, plants were transferred and washed in liquid MS media for 24 h. Finally the callus cultures were grown on solidified MS media supplemented with 5 µM TDZ and 10 µM NAA at 25 °C in complete darkness and survival was measured after 4 weeks. Surviving calli were placed onto an Anderson's elongation media (Anderson, 1984) containing 10 µM 2iP and 1 µM IBA and supplemented with 10 µM MS vitamins, 30 $g \cdot L^{-1}$ sucrose, 0.1 $g \cdot L^{-1}$ myo-Inositol, 0.1 $g \cdot L^{-1}$ MES Monohydrate and 8 $g \cdot L^{-1}$ agar (pH 5.50 prior to autoclaving). There were 6 replications, each with 5 callus pieces (approximately 1 cm^2), for each treatment (1 petri dish per treatment), arranged in a completely randomized design. Analysis of variance (ANOVA) was used to determine treatment main effects and interactions and multiple regression techniques were used to determine relationships between variables (PROC GLM, SAS version 9.1; SAS Inst., Cary, N.C.).

Determining tissue ploidy level. Holoploid, 2C DNA content (i.e., DNA content of the entire nonreplicated, chromosome complement) and associated ploidy level was

determined through flow cytometry (Lysák et al., 1998). Approximately 4 months after treatment, leaves (approximately 0.5 cm^2) were collected from newly elongated shoots, finely chopped and incubated in 0.4 mL nuclei extraction buffer for 1 to 2 min at 25 °C. The preparation was then filtered using Partec CellTrics® disposable filters to remove debris. Nuclei were stained with 1.5 mL 4', 6-diamidino-2-phenylindole (DAPI) staining buffer and incubated again at 25 °C for 1 to 2 min. The preparation was analyzed for relative DNA content using a flow cytometer (Partec PA-I, Partec). The genomic size of *R.* 'Fragrantissimum Improved' was determined using an internal standard of *Pisum sativum* L. 'Ctirad' with a known genome size of 8.76 pg (Greihuber et al., 2007). Ploidy level was determined by comparing peak position of diploid *R.* 'Fragrantissimum Improved' from untreated controls with the peak position of each sample. Three shoots (subsamples) were chosen randomly from each replicate of the 0, 7.5, 15, and 30 µM oryzalin treatments and analyzed for ploidy level. All data were subjected to ANOVA and multiple regression analysis (Proc GLM, SAS version 9.1; SAS Instit., Cary, N.C.).

Results and Discussion

Shoot regeneration. Leaf segments formed limited callus and shoots on media containing TDZ in combination with IAA. For the IAA treatments, only the combinations 5 µM TDZ and 10 µM IAA (0.06%), 5 µM TDZ and 2.5 µM IAA (0.03%), and 15 µM TDZ and 10 µM IAA (0.06%) produced shoots (data not shown). This is consistent with previous observations that IAA did not promote organogenesis in *R.* 'Little John' (D.H. Touchell,

personal communication). In contrast, studies on *R. ponticum* L. subsp. *baeticum* (Boiss. & Reut.) Hand.-Mazz. showed IAA (11.4 μM) to be an effective auxin source for shoot regeneration from both apical shoots and nodal segments (Almeida et al., 2005). Due to the limited shoot formation on media containing IAA, our analysis focused on the effect of NAA and TDZ on callus formation and shoot regeneration.

Regression analysis showed that TDZ and NAA concentrations, and their interaction significantly affected callus and shoot formation ($P<0.01$). For both the percentage of segments forming callus (Figure 1) and shoots (Figure 2), there was a significant quadratic response for TDZ and NAA concentrations as well as their interaction. The predicted optimal concentration for shoot production in *R.* 'Fragrantissimum Improved' was 8.8 μM TDZ in combination with 10 μM NAA with 96% of callus producing shoots (Figure 2). In comparison, other studies have found used higher or lower levels of cytokinins to induce shoot formation, suggesting in vitro responses may be specific to sub groups or species. For example, floral explants of *Rhododendron* 'Irina' produced the maximum number of shoots per explant using a combination of 73.8 μM 2iP, 14.8 μM IBA, and 9.1 μM TDZ (Tomsone et al., 2004). In contrast, Preece and Imel (1991) found low concentrations of TDZ (0.1 μM) combined with 1.0 μM IBA to be optimal for shoot regeneration from leaves of *R.* P.J.M. Group.

Induction of polyploids. *Rhododendron* 'Fragrantissimum Improved' had a 2C DNA content of 1.42 pg ± 0.16 (mean ± SEM, n=10) which is consistent with 2C DNA contents of diploids (Jones et al., 2007).

In the first experiment, the effect of higher concentrations for shorter duration was investigated. Percentage of surviving calli was affected by oryzalin concentration, duration, and their interaction ($P<0.0001$) (Table 1). At 0 and 30 μM concentrations, a duration of 5 days reduced callus survival compared to days 1 and 3. At concentrations of 60 or 90 μM, however, survival was low (less than 35%) regardless of duration (Table 1). Callus that survived exposed to 60 μM and 90 μM oryzalin failed to produce shoots. Oryzalin concentration influenced the percentage of diploid shoots produced ($P<0.03$). The effect of oryzalin concentration on the number of mixoploid and homogenous tetraploids, however, was not significant. In addition, treatment duration did not influence ploidy level. As a result there were no significant differences between treatments for the number of diploid, mixoploid or tetraploid shoots recovered. For this experiment, 2 tetraploids (13.13%) was the maximum number attained with 30 μM oryzalin for 3 days (Table 1).

In a second experiment, the influence of lower concentrations of oryzalin combined with longer treatment durations was investigated. An interaction between oryzalin concentration and treatment duration resulted in a significant decline in survival in the 15 μM oryzalin, 14 day duration treatment ($P=0.04$). There was a significant effect of oryzalin concentration on the induction of polyploidy ($P < 0.05$) with both mixoploid and tetraploid shoots resulting from 7.5 μM oryzalin treatments. Three tetraploids (20%) were produced in the 7.5 μM oryzalin for 7 days (Table 2).

R. 'Fragrantissimum Improved' appears highly sensitive to oryzalin with only minimal survival obtained at the highest oryzalin concentration (90 μM) and polyploid

induction occurring at concentrations as low as 7.5 μM (Table 1 and 2). In comparison Sakai et al. (2004) found that explants from an interspecific hybrid between an evergreen (*R. kiusianum* Makino x *R. eriocarpum* Nakai) and a deciduous azalea (*R. japonicum* f. *flavum* Suringer) survived oryzalin concentrations of 30, 150, and 300 μM oryzalin for up to 72 h. In a study of an intersubgeneric *Rhododendron* hybrid, Sakai et al. (2004) determined the optimal treatment for tetraploid induction to be 300 μM oryzalin for 48 h yielding 84.6% tetraploids. Similarly, Väinölä (2000) found that three *Rhododendron* hybrids could tolerate 150 μM for 48 h, with the highest percentage of tetraploids (18.2%) occurring after 24 h.

The differences in sensitivity to oryzalin observed between *R*. 'Fragrantissimum' and other *Rhododendron* hybrids may be attributed to the growth and morphology of the different tissues used. Mitotic inhibitors such as oryzalin work by arresting cell division in actively growing tissues and their effectiveness is dependent on their ability to penetrate those tissues. For example, Kermani et al. (2003) found that in *Rosa* 'Thérèse' Bugnet a higher proportion of tetraploids (66%) was obtained after treatment of thin nodal segments with 5 μM oryzalin for 1 day compared to shoots treated with 5 μM oryzalin for 14 days (40%). The higher success rate in the thin nodal segments was attributed to a more efficient penetration of oryzalin to the dividing cells. For *Rhododendron* hybrids, high concentrations of oryzalin were required to penetrate nodal sections to reach meristematic tissues and induce polyploidy (Väinölä, 2000). In comparison, for *R*. 'Fragrantissimum' an effective shoot regeneration system allowed developing meristems to be directly exposed to low concentrations of oryzalin to induce polyploidy.

In conclusion, effective in vitro shoot regeneration and polyploidy induction protocols were developed for *R.* 'Fragrantissimum Improved'. These procedures provide a foundation for in vitro regeneration and ploidy manipulation on other *Rhododendron*, particularly in subgenus *Rhododendron* and subsections *Edgeworthia* and *Maddenia*. The polyploids produced in this study will be grown out to maturity to further evaluate ornamental traits and fertility and potentially incorporate these in future breeding programs.

Literature Cited

Almeida, R., S. Gonçalves, and A. Romano. 2005. In vitro micropropagation of endangered *Rhododendron ponticum* L. subsp. *Baeticum* (Bossier and Reuter) Handel-Mazzetti. Biodiversity and Conservation 14:1059-1069.

American Rhododendron Society: Members worldwide. ©1998-2009. Description of *Rhododendron* 'Fragrantissimum Improved'. November 4, 2009, <http://www.rhododendron.org/descriptionH_new.asp?ID=865>.

Anderson, W.C. 1984. A revised tissue culture medium for shoot multiplication of rhododendron. J. Amer. Soc. Hort. Sci. 109: 343-347.

Bates, S., J.E. Preece, N.E. Navarrete, J.W. Van Sambeek, and G.R. Gaffney. 1992. Thidiazuron stimulates shoot organogenesis and somatic embryogenesis in white ash (*Fraxinus Americana* L.). Plant Cell Tiss. Organ Cult. 31:21-29.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Natl. Rev. Genet. 6:836-846.

Contreras, R.N., T.G. Ranney, and S.P. Tallury. 2007. Reproductive behavior of diploid and allotetraploid *Rhododendron* L. 'Fragrant Affinity.' HortScience 42:31-34.

Dunn, L., and J.T. Lindstrom. 2007. Oryzalin-induced chromosome doubling in *Buddleja* to facilitate interspecific hybridization. Hortscience 42:1326-1328.

Greihuber, J., J. Doležel, M.A. Lysák, and M.D. Bennet. 2005. The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. Ann. Bot. 95: 255-260.

Hansen, N.J.P., and S.B. Anderson. 1996. In vitro chromosome doubling potential of colchicine, oryzalin, trifluralin, and APM in *Brassica napus* microspore culture. Euphytica 88:159-164.

Huetteman, C.A., and J.E. Preece. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss. Organ Cult. 33: 105-119.

Iapichino, G., S. McCulloch, and T.H.H. Chen. 1992. Adventitious shoot formation from leaf explants of Rhododendron. Plant Cell Tiss. Organ Cult. 30:237-241.

Iapichino, G., and T.H.H. Chen. 1995. Genotypic effects on plant regeneration from leaf segments of Rhododendron. Advances in Horticultural Science. 9:170-172.

Jones, J.R., T.G. Ranney, and T.A. Eaker. 2008. A novel method for inducing polyploidy in Rhododendron seedlings. J. Amer. Rhod. Soc. 62:130-135

Jones, J.R., T.G. Ranney, and N.P. Lynch. 2007. Ploidy levels and relative genome sizes of diverse species, hybrids, and cultivars of rhododendron. J. Amer. Rhod. Soc. 61:220-227.

Kermani M.J., V. Sarasan, A.V. Roberts, K. Yokoya, J. Wentworth, and V.K. Sieber. 2003 Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. Theor. Appl. Genet. 107:1195-1200.

Lu, C., and M.P. Bridgen. 1997. Chromosomal doubling and fertility study of *Alstroemeria aurea* x *A. caryophyllaea*. Euphytica 94:75-81.

Lysák, M.A., M. Doleželová, J.P. Horry, R. Swennen, and J. Doležel. 1998. Flow cytometic analysis of DNA content in *Musa*. Theor. Appl. Genet. 98: 1344-1350.

McCown, B.H., and G.B. Lloyd. 1982. A survey of the response of *Rhododendron* to in vitro culture. Plant Cell Tiss. Organ Cult. 2:77-85.

Mertens, M. 1996. In vitro regeneration of evergreen azalea from leaves. Plant Cell Tiss. Organ Cult. 45:231-236.

Meyer, E. 2009. Evaluation, propagation, and improvement of gordonieae trees and an interspecific *Hypericum* hybrid. Thesis. North Carolina State University. Online. <http://www.lib.ncsu.edu.www.lib.ncsu.edu:2048/theses/available/etd-03122009-151304/>

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:472-497.

Olsen, R.T., T.G. Ranney, and Z. Viloria. 2006. Reproductive behavior of induced allotetraploid× *Chiltalpa* and in vitro embryo culture of polyploidy progeny. J. Amer. Soc. Hort. Sci. 131:716-724.

Petersen, K.K., P. Hagberg, and K. Kristiansen. 2003. Colchicine and oryzalin mediated chromosome doubling in different genotypes of *Miscanthus sinensis*. Plant Cell Tiss. Organ Cult. 73:137-146.

Preece, J.E., and M.R. Imel. 1991. Plant regeneration from leaf explants of *Rhododendron* 'P.J.M. Hybrids' [sic]. Sci. Hort. 48:159–170.

Pryor, R. L., and L.C. Frazier. 1968. Colchicine-induced tetraploid azaleas. HortScience 3:283-286.

Ramsey, J., and D.W. Schemske. 2002. Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. 33:589-639.

Ranney, T.G. 2006. Polyploidy: From evolution to new plant development. Comb. Proc. Intl. Plant Prop. Soc. 56:137-142.

Rose, J.B., J. Kubba, and K.R. Tobutt. 2000a. Chromosome doubling in sterile *Syringa vulgaris* x *S. pinnatifolia* hybrids by in vitro culture of nodal explants. Plant Cell Tiss. Organ Cult. 63:127-132.

Rose, J.B., J. Kubba, and K.R. Tobutt. 2000b. Induction of tetraploidy in *Buddleia globosa.* Plant Cell Tiss. Organ Cult. 63:121-125.

Sakai, K., I. Miyajima, K. Ureshino, Y. Ozaki, and H. Okubo. 2004. Oryzalin-induced allotetraploids of an intersubgeneric hybrid between evergreen and deciduous azaleas. J. Fac. Agr., Kyushu Univ. 49:293-299.

Samyn, G., S. De Schepper, and E. Van Bockstaele. Adventitous shoot regeneration and appearance of sports in several azalea cultivars. Plant Cell Tiss. Organ Cult. 70: 223-227.

Sicuranza, J., and N.A. Mitkowski. 2007. The production of callus, shoot, and rooted plantlets of *Rhododendron catawbiense* 'English Roseum' from florets. HortScience 42:410–411.

Sree Ramulu, K., H.A. Verhoeven, and P. Dijkhuis. 1991. Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. Protoplasma 160:65-71.

Tomsone, S., D. Gertnere, and D. Novikova. 2004. The influence of thidiazuron on shoot regeneration and proliferation of rhododendrons in vitro. Acta Universitatis Latviensis ser. Biology. 676:239-242.

Väinölä, A. 2000. Polyploidization and early screening of *Rhododendron* hybrids. Euphytica 112:239-244.

van Tuyl, J. M., B. Meijer, and M.P. van Diën. 1992. The use of oryzalin as an alternative for colchicine in in vitro chromosome doubling of *Lilium* and *Nerine*. Acta Hort. (ISHS) 325:625-630.

Table 1. Effects of short duration, high concentration oryzalin treatments on survival and polyploidy induction in *Rhododendron* 'Fragrantissimum Improved' callus cultures.

Concentration (μM)	Duration (Days)	Survival (%)[z]	Ploidy Level (%)		
			2x	Mix[y]	4x
0	1	100 A	100 A	0 A	0 A
	3	87 A	100 A	0 A	0 A
	5	81 B	100 A	0 A	0 A
30	1	100 A	80 AB	7 A	13 B
	3	98 A	80 AB	7 A	13 B
	5	55 C	60 B	40 B	0 A
60	1	24 D	-	-	-
	3	35 D	-	-	-
	5	20 D	-	-	-
90	1	14 E	-	-	-
	3	0 F	-	-	-
	5	0 F	-	-	-

[z] Means followed by different letters within columns are significantly different, LSD $P<0.05$.

[y] Mixaploid (cytochimera) tissue.

"-", Shoots failed to regenerate from callus.

Table 2. Effects of long duration, low concentration oryzalin treatments on survival and polyploidy induction in *Rhododendron* 'Fragrantissimum Improved' callus cultures.

Concentration (μM)	Duration (Days)	Survival (%)[z]	Ploidy Level (%)		
			2x	Mix[y]	4x
0	7	100 A	100 A	0 A	0 A
	14	90 A	100 A	0 A	0 A
7.5	7	98 A	70 B	10 A	20 B
	14	83 A	87 AB	7 A	7 A
15	7	100 A	100 A	0 A	0 A
	14	40 B	100 A	0 A	0 A

[z] Means followed by different letters within columns are significantly different, LSD $P<0.05$.

[y] Mixaploid (cytochimera) tissue.

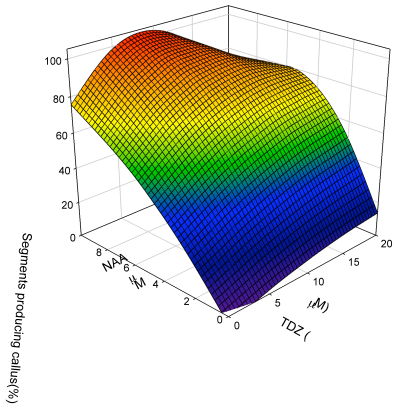

Figure 1. Percent of leaf segments producing callus in response to NAA and TDZ concentrations. Percent of segments producing callus = -4.2 +(1.5*TDZ) -(0.03*TDZ^2) +(11.8*NAA) -(0.4*NAA^2) +(0.5*TDZ*NAA) -(0.004*TDZ^2*NAA^2); $P<0.0001$; $r^2 = 0.56$

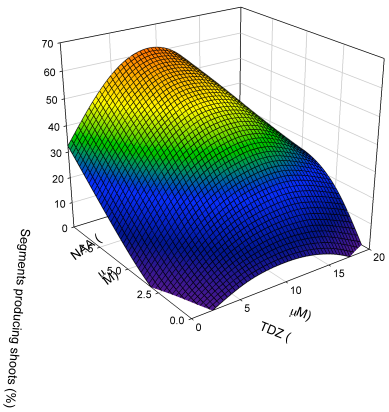

Figure 2. Percentage leaf segments producing shoots in response to NAA and TDZ concentrations. Percent of leaf segments producing shoots = -1.34 + (1.708*TDZ) - (0.107*TDZ^2) -(1.988*NAA) +(0.47*NAA^2) +(0.76*TDZ*NAA) -(0.0039*TDZ^2*NAA^2); $P<0.0001$; $r^2 = 0.62$

Chapter 3

Effects of S-ABA on the Physiology and Marketability of Various Container-Grown Taxa During Short-Term Desiccation

(In the format appropriate for submission to the Journal of Environmental Horticulture)

Effects of S-ABA on the Physiology and Marketability of Various Container-Grown Taxa During Short-Term Desiccation

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Abstract

Reduced post harvest care of woody plant material in mass retail settings can decrease the number of days plants remain marketable. If plants are sold on consignment for growers, reduced sales can lead to poor profitability. This study investigated the effect of spray applications of s-abscisic acid (s-ABA) (ConTego™, Valent Biosciences Corp.) to increase the number of days of marketability for various woody taxa in a simulated retail setting. In the first stage of the study, various well-watered container-grown taxa were treated with a spray application of either 0, 1000 or 2000 $mg \cdot L^{-1}$ s-ABA and water was withheld. Daily, desiccation symptoms were recorded to determine if plants had reached the critical wilting point and thus became unmarketable. Marketability was increased approximately 2-3 days for plants treated with 2000 $mg \cdot L^{-1}$ s-ABA treatment compared to nontreated plants. In the second stage, marketability and physiology of *Ligustrum japonicum* 'Recurvifolium' (wavy leaf privet) was monitored after plants were treated with spray applications of 0, 500, 1000, 1500 or 2000 $mg \cdot L^{-1}$ s-ABA. Stomatal conductance (g_s) declined for all plants depending on the concentration applied. Plants treated with s-ABA had lower g_s rates and remained marketable longer than nontreated plants. All plants fully recovered to pretreatment g_s rates provided they were rewatered immediately upon reaching the critical wilting point. Spray applications of s-ABA can increase shelf life of select woody ornamentals.

Index words

critical wilting point, Contego®, nursery crops, s-ABA, stomatal conductance, stem water potential

Significance to the Nursery Industry

S-ABA is a practical, safe and affordable chemical used to improve the shelf-life of woody plant material marketed in retail settings (2). Spray applications of 1000 to 2000 $mg \cdot L^{-1}$ s-ABA increased shelf-life of plant material approximately 2-3 days after water was withheld. Potentially, the compound could be applied to plants at a nursery before they are shipped to retail locations to increase the number marketable days. Alternatively, since s-ABA application allows plants to maintain a higher water status for a longer period of time without injury, plants may be able to be shipped lighter, thus reducing transportation costs. Additionally, the compound has potential to be used for lowering irrigation frequency during a short period of production because plants can withstand short periods of desiccation without injury. This might compromise growth in the short term, but would utilize limited water resources during times of drought and preserve plant material for continued production when water resources return. These possibilities need further testing at nurseries and in retail settings.

Introduction

In recent years there has been an increase in the number of customers purchasing ornamental plants from mass retail outlets compared to independent garden centers (35, 36). Unfortunately, it is often difficult to give consistent, optimal care to plants marketed in any large, commercial, retail setting. Reduced irrigation frequency can limit the marketability of container-grown plants to a few days or a few weeks. In some mass retail platforms, growers are paid on consignment, thus, reimbursement for shrinkage (e.g., unsold plants lost to poor post delivery care) is not recovered by growers. It is estimated that over 60% of the gardening public purchases their plant material from large retail markets (36).

Valent Biosciences, Corp. has developed and formulated an all-natural abscisic acid compound (s-ABA) (ConTego™, Valent Biosciences Corp.) which has been shown to prolong shelf life in vegetable transplants including *Capsicum annuum* L. (pepper), *Cynara scolymus* L. (artichoke), and *Lycopersicon esculentum* Mill. (tomato), as well as annuals such as *Antirrhinum majus* L. (snapdragon), *Impatiens wallerana* Hook. f. 'Blitz Orange' (impatiens), *Petunia* x *hybrida* Hort. Vilm-Andr. 'Royal Pearls' (petunia), and *Tropaeolum majus* L. (nasturtium), and woody ornamentals including Gardenia 'August Beauty' (gardenia), *Hydrangea* Endless Summer™ (French hydrangea), *Malus sargentii* Rehd. (Sargent's crabapple), Nandina 'Gulfstream' (nandina), *Nerium oleander* L. (oleander), *Ulmus parvifolia* Jacq. (lace bark elm), and *Viburnum plicatum* var. *tomentosum* Thunb. (doublefile viburnum) (2, 4, 5, 7, 14, 18, 27). The compound is applied as a spray or drench to plants prior to shipment to retailers to extend shelf life and preserve marketability. Once

applied, the compound increases the number of days before desiccation symptoms appear, for example wilting, compared to plants that have not received the compound. Thus, plants remain marketable for a longer period of time in retail settings while receiving minimal irrigation.

Initial experiments using drench applications found that s-ABA increased the days of marketability for a wide range of container grown woody taxa (5, unpublished data). Despite being effective at lower concentrations, drench applications apply more volume of water per square meter, are difficult to apply or are less efficient than spray applications, and may not integrate as smoothly as spray applications into existing production systems. Stamps and Chandler (28) reported that s-ABA concentration was negatively correlated with both transpiration rate and cumulative water loss when applied as a spray to container-grown cultivars of *Hibiscus rosa-sinensis* L. (hibiscus). Total hours of marketability was cultivar dependent but was increased suggesting that spray applications of s-ABA are effective in increasing number of days of marketability in container-grown tropical plants during periods of short term desiccation.

The mechanism of improved short-term desiccation tolerance is thought to be closure of stomata by the application of s-ABA. Although not exogenously applied, ABA concentration in the xylem sap of field-grown corn was found to be correlated with stomatal conductance (30). Typically synthesized in the root system and transported to the stomata, ABA plays an important role in the communication of drought stress signals from the roots in drying soil through the xylem (6, 8, 9, 10, 19). Abscisic acid also plays other roles in various

short-term plant responses, such as fruit and leaf abscission, but it also induces long-term responses such as reduced mean stomatal conductance and stomatal dimensions, as well as increasing the density of stomata and mean water use efficiency (6, 12, 19, 21). In a study comparing the physiological effects of ABA analogs to ABA, Flores and Körffling (11) found similar decreases in stomatal pore width and transpiration rate in both the ABA analog and ABA treated plants as compared to the controls. Sharma et al. (26) found tissue concentrations of the ABA analog 8' Acetylene ABA Methyl Ester in tomato seedlings were significantly higher in root-dip applications as compared to foliar applications, however, both application methods resulted in decreases in cumulative water use. Trejo et al. (31) also found that stomatal closure was highly sensitive to method of ABA application with both the cuticle and mesophyll cells posing as significant barriers either by forming an obstruction to osmotic diffusion (cuticle) or through metabolism into inactive forms (mesophyll). For spray applications, presumably, the plant absorbs s-ABA through the stomata, which affects similar physiological changes as when the chemical is transported from the roots.

In this study we hypothesize that the concentration of s-ABA negatively impacts transpiration. Higher concentrations decrease transpiration more substantially than lower concentrations, thus lengthening the time it takes to reach the critical wilting point. Lower concentrations of s-ABA reduce transpiration slightly, therefore, water is still lost, but at a faster rate than if higher concentrations of s-ABA were applied. Nevertheless, the degree to which stomatal conductance recovers to pre-drought, pre-treatment conditions is unknown. Therefore, the main objectives of the present study were to evaluate the effect of spray

applications of s-ABA on the number of days to critical wilting point (CWP) for select woody ornamental taxa, and the effect of s-ABA on gas exchange and recovery of gas exchange parameters to pre-desiccation rates for *Ligustrum japonicum* 'Recurvifolium.'

Materials and Methods

Determining days to critical wilting point of various container grown woody taxa. Thirty plants of five taxa were separated into two runs of two or three taxa each. Taxa were tested separately by a randomized complete block design. Each test contained 10 blocks with a single plant for each treatment in each block (10 blocks x 3 treatments=30 plants per taxa). Plants in both runs were watered to container capacity before being treated with s-ABA under a clear polyethylene covered structure at McCorkle's Nursery in Dearing, GA. The first run, conducted on May 19, 2009, included *Hydrangea macrophylla* 'Bailmer' Endless Summer® Blushing Bride™ (12 L) and *Rhododendron* 'Roblel' PP#16278 Autumn Debutante™ (26.5 L). The second run, conducted on June 8, 2009, included *Loropetalum chinense* var. rubrum 'Sizzling Pink' (12 L), *Trachelospermum jasminoides* var. *pubescens* 'Madison' (19 L) and *Lagerstroemia* 'GAMAD I' PP#16917 Cherry Dazzle™ (26.5 L). Container plants were treated with spray applications of either 0 (water control), 1000 or 2000 $mg \cdot L^{-1}$ s-ABA (Contego®, Valent BioSciences Corp., Ocoee, Fl.) supplemented with the addition of 0.47 $mL \cdot L^{-1}$ (0.05%) CapSil® (Aquatrols Corporation of America, Inc., Cherry Hill, N.J.) and applied until runoff using a diaphragm pump backpack sprayer (model 473-D, Solo Inc., Newport News, Va.) through a wide-angle, single, fan-spray nozzle at a

pressure of 14 psi. After treatment application, water was withheld for the remainder of the experiment. Within 24-48 h after treatment, plants were delivered via covered tractor trailer to the Mountain Horticultural Crops Research Station (MHCRS, Mills River, N.C.). Plants were scored daily for marketability between 0900 and 1100 HR by determining if they had reached the critical wilting point (CWP). Critical wilting point was inferred from plants being unable to recover turgidity unless rewatered. Plants were designated as unmarketable upon reaching CWP or if discoloration (yellow foliage) was present on over 25% of the plant. All plants of a particular species were rewatered when all plants of that species had reached CWP and marketability was recorded again the following day to denote recovery.

Determine the effect of s-ABA applications on gas exchange of container grown Ligustrum japonicum 'Recurvifolium' during short term desiccation. Two trials, approximately 6 weeks apart, were conducted. For each trial, plants in 12 L containers were watered to container capacity 1 hr before treatment with s-ABA. In the first trial, beginning on May 19, 2009, plants were treated under a clear polyethylene covered structure at McCorkle's Nursery in Dearing, Ga. prior to shipment to the MHCRS. Plants were treated with spray applications of either 0 (water control), 500, 1000, 1500 or 2000 $mg \cdot L^{-1}$ s-ABA at a rate of 3 qt/100 ft^2 (0.306 $L \cdot m^{-2}$) supplemented with the addition of 0.47 $mL \cdot L^{-1}$ (0.05%) CapSil using the same equipment described above. After treatment, water was withheld for the remainder of the experiment. Upon arrival at the MHCRS, plants were moved to a clear polyethylene covered structure equipped with fans and a cooling system set to 72-78F (22-26C) day and 68-72F (20-22C) night temperatures. Daily, between 0900 and 1100 HR, plants were

assessed for their marketability by determining whether or not they had reached CWP. After all plants in each block reached CWP, that block was rewatered and marketability was recorded the following day.

In Trial 2, beginning on June 20, 2009, plants were shipped to the MHCRS prior to treatment. All treatments and applications were the same as described for Trial 1. Plants were treated and then moved into a clear polyethylene covered structure with cooling fans, but no cooling pads. Environmental settings were similar. The vapor pressure deficit for each trial is shown in Figure 1. Marketability was recorded daily between 0900 and 1100 HR as described previously. Each individual plant was rewatered after reaching CWP, regardless if the other plants in the same block had reached CWP.

Initial gravimetric weight at container capacity was measured daily for each plot using an electronic balance (model FV-30K, A&D Engineering Inc., San Jose, Calif.) between 0900 and 1100 HR until the end of each trial. Stomatal conductance (g_s) and net photosynthesis at ambient conditions (A_{net}) was measured on all plants in either four (Trial 1) or five (Trial 2) randomly chosen blocks between 0900 and 1100 HR daily for 1 to 5, 7, 9, and 11 days after treatment (DAT) in Trial 1 or all DAT in Trial 2. Ambient light readings were recorded prior to each trial to provide a basis for leaf cuvette conditions. Stomatal conductance (g_s) was measured on the most recently expanded leaves using a Ciras-1 portable photosynthesis system (PP Systems, Inc., Amesbury, Mass.) with cuvette conditions set to 25C (77F), PAR 500 μmol m^2 s^{-1}, 350 ppm CO_2, 0.63 kPa VPD. Stem water potential was recorded daily between 0900 and 1100 using a pressure chamber (3005-Series model,

SoilMoisture Equipment Corp., Santa Barbara, Calif.) (25) for plants in Trial 2 only. Data for gas exchange was used to determine any initial effects of s-ABA application and to determine the extent of its effect on plant gas exchange prior to reaching CWP. Gravimetric data were used to monitor cumulative water loss (CWL) from the container and plant system over time.

Determine the length of time necessary for recovery to pre-treatment stomatal conductance levels for Ligustrum japonicum 'Recurvifolium'. This objective utilized the plant material from both trials in the second objective. When all plants in a block in Trial 1 reached CWP they were rewatered. In Trial 2, plants were rewatered immediately upon reaching CWP regardless of the status of other plants within the block. After rewatering in both trials, measurements of marketability and gas exchange were recorded on DAT 1, 3 and 5 for Trial 1 and daily for 7 DAT in Trial 2.

The experimental design for both trials in objective 2 and the recovery portion in objective 3 was a spit-plot with DAT on the main plot and treatment on the subplot. Treatments were arranged randomly within each block in each trial. Trial 1 contained 10 blocks, while trial 2 contained 5 blocks. Each block contained five plants, one plant corresponding to each treatment.

Data analysis. All taxa were treated as separate experiments and the data were analyzed separately. Days to CWP (marketability) for all taxa were subjected to analysis of variance procedures (ANOVA) using the general linear models procedure. When the treatment main effect was significant, means were separated using Tukey's LSD with the probability of a

greater F-value ≤ 0.10. When treatment main effects were significant for *Ligustrum* only, data for days to CWP, gas exchange, Ψ_s, or CWL were regressed on either s-ABA concentration, DAT, or both to determine if the relationship between those variables was significant (23). Data for recovery after rewatering was treated similarly, but separately from data recorded during the dry-down experiments.

Results and Discussion

An application of s-ABA significantly increased days to critical wilting point (CWP) for all species except *Hydrangea* Blushing Bride™ (P=0.11) (ANOVA not presented). *Rhododendron* and *Loropetalum* had an average increase of 2-3 days over the control (Table 1). *Trachelospermum* was generally slow to reach CWP as the nontreated controls remained marketable for 12 days; however, an application of either 1000 or 2000 mg·L^{-1} of s-ABA increased marketability another 4 to 6 days, respectively. For *Lagerstroemia*, days to CWP was extended three days regardless of concentration of s-ABA applied. Although marketability of *Hydrangea* Blushing Bride™ was not affected by spray applications of s-ABA, previous experiments have shown drench applications to be effective in increasing days to CWP for *Hydrangea* Endless Summer® (5) and *Hydrangea macrophylla* 'Dooley' (unpublished data). Generally, hydrangea transpire heavily and wilt frequently, so either the concentration may need to be increased if spray applications are to be effective or the drench application method may be used preferentially for this species. For all species, s-ABA treated

plants recovered full turgidity after rewatering to container capacity and were considered marketable. Nontreated controls remained unmarketable after rewatering.

Treatment with s-ABA also affected days to CWP for *Ligustrum japonicum* 'Recurvifolium'. Overall marketability between the two trials of *Ligustrum* differed by approximately 1.5 days for each treatment (ANOVA not presented). Because there was not a trial by treatment interaction, data were averaged over both trials to show the positive linear relationship between days of marketability and s-ABA concentration (Fig. 2). Approximately 10 days of marketability occurred for plants treated with 2000 $mg \cdot L^{-1}$ s-ABA. This was more than 2 days of additional marketability over the nontreated control and suggests that spray applications of s-ABA can increase shelf life of select container grown woody ornamentals.

Stomatal conductance (g_s) of *Ligustrum* was affected by trial, treatment, DAT, and all interactions between those variables ($P<0.01$) (ANOVA not presented). After treatments were applied and water withheld in each trial, g_s generally declined until rewatering for both treated plants and non treated controls (Figs. 3 and 4). From the beginning of Trial 1 to DAT 1, g_s decreased from an average of 172 $mmol \cdot m^{-2} \cdot s^{-1}$ for all treatments to approximately 50 $mmol \cdot m^{-2} \cdot s^{-1}$ for the nontreated controls and 15 $mmol \cdot m^{-2} \cdot s^{-1}$ for all other treatments (Fig. 3). Stomatal conductance in *Populus cathayana* Rheder and *Populus kangdingensis* Z.Wang and S.L. Tung was also reduced with the application of exogenous ABA under both nonstressed and drought stressed conditions (34). In the present study, from DAT 1 until the nontreated controls reached CWP on DAT 7, g_s declined linearly and at a greater rate than treated plants

as represented by the steeper slope of the regression line. With the exception of the 1000 $mg \cdot L^{-1}$ s-ABA treatment (relationship was not significant) the relationship between g_s and DAT was linear and quadratic for treated plants until DAT 9 when all plants reached CWP. The greater rate of water loss for controls resulted in fewer days to CWP (7 days) compared to all other treated plants (9 days).

For Trial 2, the relationship between g_s and DAT differed depending on the concentration of ABA applied. The relationship was similar for the control and the 500 $mg \cdot L^{-1}$ s-ABA treatment, as g_s declined with a negative linear (-19 and -23 linear coefficients for 0 and 500 $mg \cdot L^{-1}$ s-ABA, respectively) and quadratic relationship (-1.0 and 0.01 quadratic coefficients for 0 and 500 $mg \cdot L^{-1}$ s-ABA, respectively) (Fig. 4). Although not a parallel decline for the nontreated control and 500 $mg \cdot L^{-1}$ s-ABA treatment, g_s differed by approximately 25 and 20 $mmol \cdot m^{-2} \cdot s^{-1}$ at days 1 and 5, respectively, between the two treatments. Plants in both treatments reached CWP on DAT 8. In contrast, g_s had a positive linear and quadratic response for plants treated with either 1500 or 2000 $mg \cdot L^{-1}$ s-ABA. On DAT 1, g_s of plants treated with 2000 $mg \cdot L^{-1}$ s-ABA was approximately 75 $mmol \cdot m^{-2} \cdot s^{-1}$ less than plants treated with 1500 $mg \cdot L^{-1}$ s-ABA. By DAT 5, however, there was no difference between those treatments. Plants in the 1500 ppm treatment reached CWP by DAT 9, whereas those in the 2000 $mg \cdot L^{-1}$ s-ABA treatment reached CWP on DAT 10. The g_s rate of plants treated with 1000 $mg \cdot L^{-1}$ s-ABA had a relationship that appeared intermediate between that of the nontreated controls and 500 $mg \cdot L^{-1}$ s-ABA treatment, and that of plants treated with 1500 and 2000 $mg \cdot L^{-1}$ s-ABA. The g_s rates on Day 1 for the 1000 $mg \cdot L^{-1}$ s-ABA

treatment were similar to the nontreated control and 500 mg·L^{-1} s-ABA, but as DAT progressed, rates were more similar to the 1500 and 2000 mg·L^{-1} s-ABA treatments. Nevertheless, plants treated with 1000 mg·L^{-1} s-ABA reached CWP at DAT 8, which was similar to both the control and 500 mg·L^{-1} s-ABA treated plants.

As the concentration of s-ABA applied increased in Trial 2, there was an increase in the slope of the linear portion of the regression curve. The slope of the linear portion of the curve was -28, -23, -22, -13 and -9 for the nontreated control, 500, 1000, 1500 and 2000 mg·L^{-1} s-ABA treatments, respectively. These relationships between g_s and DAT for each concentration of s-ABA in Trial 2 indicate that s-ABA has an effect on g_s with as little as 500 mg·L^{-1} s-ABA application, but prolonged desiccation tolerance was not imparted to these plants until concentrations were above 1000 mg·L^{-1} s-ABA.

Initial g_s rates on DAT 1 for Trial 2 were over 7 times higher than those in Trial 1. This could be due to where both s-ABA was applied and the environments where water was withheld for each trial. In Trial 1, plants were shipped via tractor-trailer after treatment, and placed within a greenhouse provided with cooling pads and fans at the MHCRS. High temperatures, low humidity and the absence of light experienced during handling and transit (29) could have had a significant effect on g_s values. Stomatal conductance declined in seedlings of *Cucumis sativus* L. (cucumber) and tomato, depending on the concentration of exogenous s-ABA applied and the temperature at which seedlings were stored (33). At a storage temperature of 20C (68F) and in total darkness, all seedlings had the same transpiration rate regardless of the s-ABA concentration sprayed (33). Large reductions in

stomatal conductance have been achieved previously by application of exogenous ABA. In Goreta et al. (13), an application of exogenous ABA to pepper seedlings produced a sharp decline in g_s rates from 864 mmol·m^{-2}·s^{-1} to 390 mmol·m^{-2}·s^{-1} (55% reduction in g_s rate) within 24 hr after treatment. In addition, repeated periods of drought stress have caused physiological changes in stomatal response, which increased sensitivity to exogenous ABA (1). If the plants in Trial 1 had gone through previous cycles of drought stress at the commercial nursery, while plants obtained for Trial 2 had remained under well-watered conditions, this could contribute to the apparent increased sensitivity to ABA in Trial 1. A third trial using the same methods as in Trial 2 was also conducted and the g_s responses were intermediate between Trials 1 and 2; however, most of the data were not publishable due to instrument malfunctions over the course of the entire study.

Due to the large disparity in g_s responses between the two trials in the present study, it is difficult to determine which model (Trial 1 or Trial 2) best describes the general response of g_s in plants after treatment with s-ABA. In Trial 1, g_s was reduced to a similar rate for all treated plants and remained so until the end of the experiment. In contrast, g_s in Trial 2 was reduced depending on the s-ABA concentration and remained distinct until plants reached CWP. These findings support the original hypothesis that s-ABA negatively impacts g_s, and higher concentrations impact g_s more negatively. Yet, it is quite possible that the environmental conditions within which the plants are treated, shipped and ultimately marketed affect the physiological parameters controlling transpiration. In both trials,

however, s-ABA applications reduced gs, and plants receiving higher concentrations of s-ABA tended to have lower g_s and remained marketable longer.

Cumulative water loss (CWL) was affected by s-ABA treatment, DAT, and the interactions of trial by treatment and trial by DAT (ANOVA not presented). Initial water loss (DAT 1-5) was higher in Trial 2 than Trial 1, but became similar at DAT 9-10 for both trials (Fig 5). CWL had a negative linear relationship with s-ABA concentration in Trial 1, indicating that less water loss accumulated over time with higher concentrations (Figure 6). This is consistent with the results reported by Sharma et al. (26) indicating applications of an ABA analog to tomato seedlings resulted in significantly lower cumulative moisture use (CMU) compared to the controls, with CMU decreasing with increasing ABA concentration. Similarly, Kim and van Iersel (17) found that applications of s-ABA to *Salvia splendens* Sellow ex Schult. (salvia) delayed water loss through stomatal closure and lower cumulative water loss rates, increasing marketability by two (250-500 ppm S-ABA) to three days (1000-2000 ppm S-ABA). A subset of data containing just the last day of CWL before plants in both trials reached CWP was analyzed separately (ANOVA not presented). There was no effect of trial or treatment, indicating that all plants, regardless of treatment, accumulated the same amount of water lost (3212 g) before reaching CWP. Davenport et al. (7) reported that applications of a film-forming antitranspirant to *Nerium oleander* resulted in approximately 2 days of additional marketability even though both treated and nontreated plants transpired approximately equal amounts of water over a ten day period. The current study also found

concentrations of s-ABA delayed days to CWP by further delaying water lost through stomates compared to nontreated controls under desiccated conditions.

Net photosynthesis at ambient conditions (A_{net}) was affected by trial, s-ABA treatment, DAT, and all interactions except trial by treatment by DAT (ANOVA not presented). The relationship of A_{net} to treatment and DAT for each trial was very similar to the relationships between g_s with those variables (data not shown). The natural log g_s was highly correlated with A_{net} ($P<0.01$; $R^2=0.92$; data not shown), indicating that the effect of ABA on photosynthesis was largely due to its affect on g_s. Franks and Farquhar (12) found that prolonged treatment of *Tradescantia virginiana* L. (Virginia spiderwort) with ABA under well watered conditions did not affect photosynthetic capacity (CO_2 assimilation rate for any given leaf intercellular CO_2 concentration), but did increase the water use efficiency of plants. In contrast, under drought stressed conditions, Goreta et al. (13) suggested that ABA applied to pepper transplants had nonstomatal effects on photosynthesis because intercellular CO_2 concentrations (C_i) of s-ABA treated plants were the same as nontreated plants, yet A_{net} of s-ABA treated plants was reduced. Popova et al. (22) found a similar effect of ABA on photosynthetic rate of drought stressed barley, but could not pinpoint which physiological process was responsible for the reduction in CO_2 assimilation. Jifon and Syvertsen (15) suggested supraoptimal leaf temperatures and photoinhibition at midday as contributing factors to differences in A_{net} when C_i was similar among treatments. In the present study, C_i was not similar among treatments in either trial (ANOVA not presented). We did not, however, as in Goreta et al. (13) or Popova et al. (22), measure

photosynthesis at midday environmental conditions. Therefore, we can only confirm that at the ambient environmental conditions used to measure photosynthesis in the cuvette in this experiment, between 0900 and 1100 HR, it does not appear that s-ABA affected photosynthesis other than by reducing g_s. It is still possible, however, that s-ABA caused a reduction in A_{net} at midday even though we did not record measurements at that time or under those conditions.

Stem water potential (Ψ_s) in Trial 2 (Ψ_s was not measured in Trial 1) was affected by DAT and treatment but not their interaction (ANOVA not presented). As expected, Ψ_s decreased for all plants after water was withheld and continued to decrease linearly and quadratically until plants reached CWP (Fig 7). The nontreated control and plants treated with 1000 $mg \cdot L^{-1}$ s-ABA had the lowest Ψ_s over all DAT, whereas plants treated with 2000 $mg \cdot L^{-1}$ s-ABA had the highest Ψ_s. Despite decreasing the rate of Ψ_s over DAT, s-ABA applications did not change the Ψ_s values measured at CWP for each treatment. At DAT 8, when plants in the nontreated control reached CWP, Ψ_s was -2.1 MPa, while plants treated with 2000 $mg \cdot L^{-1}$ s-ABA had Ψ_s of -1.2 MPa. When plants in the 2000 $mg \cdot L^{-1}$ s-ABA treatment reached CWP at DAT 10, Ψ_s was -2.1 MPa. All treatments, including the nontreated control, reached a mean of -2.4 MPa at CWP.

Percent recovery of g_s to pretreatment rates (%g_s) was affected by trial, DAT and s-ABA treatment and the interaction of Trial by DAT (ANOVA not presented). Mean %g_s for plants in Trial 1 was significantly lower than that of Trial 2 (23% and 81%, respectively). Plants in Trial 1 were not rewatered until all plants within a block reached CWP, therefore,

individual plants (nontreated control or 500 mg·L^{-1}) were at CWP for 1-5 days before rewatering and may have experienced extremely low Ψ_s. As a result, foliar necrosis and scorching occurred on some plants. In Trial 2, individual plants were rewatered the day CWP was reached, which appears to have allowed plants to recover more fully from desiccation. Moreover, %g_s was measured 1, 3, and 5 days after rewatering for plants in Trial 1, whereas, %g_s was measured daily for seven days in Trial 2. Even if %g_s was measured for seven days in Trial 1, plants may not have recovered to pretreatment g_s rates because severely low Ψ_s were experienced. Percentage g_s recovery after rewatering also decreased for kidney bean plants (*Phaseolus vulgaris* 'Yamashirokurosanndosaitou') experiencing decreased Ψ_s (20). Because all plants in Trial 2 reached a similar Ψ_s at CWP and were then rewatered immediately, all plants regained their pretreatment g_s levels. Based on these findings, applications of s-ABA to *Ligustrum* reduced g_s rates while water was withheld, but s-ABA did not impair recovery of g_s provided plants were rewatered immediately after reaching CWP.

In conclusion, results of the study herein suggest that applications of s-ABA to plants can delay desiccation when water is withheld. With the exception of *H. macrophylla* Blushing Bride^{TM}, exogenous ABA applications of 2000 mg·L^{-1} increased days of marketability by approximately 1 to 6 days depending on species. Initially, reduction in stomatal conductance depends on the concentration of s-ABA applied. During the time water is withheld, decreased conductance rates allow plants to maintain both turgidity and a high Ψs, therefore the days to CWP are increased. An application of 500 mg·L^{-1} s-ABA affected

g_s but appeared to have a shortened period of efficacy because plants reached CWP the same day as nontreated controls. An increase in days to CWP was not imparted to plants until s-ABA concentrations were above 1000 mg·L^{-1}. While all *Ligustrum* in Trial 1 treated with s-ABA had similar market life (9 days), in Trial 2, the controls, 500 and 1000 mg·L^{-1} s-ABA treatments remained marketable for 8 days, plants treated with 1500 mg·L^{-1} s-ABA lasted 9 days, and plants in the 2000 mg·L^{-1} treatment remained marketable for 10 days; thus indicating that exogenously applied s-ABA is efficacious in reducing water loss and increasing marketability. The 2000 mg·L^{-1} s-ABA treatment had lower rates of g_s in both trials, accumulated less water loss and had the highest Ψ_s per day over the experiment. As a result those plants remained marketable for longer periods than plants treated with lower concentrations.

Literature Cited

1. Ackerson, R.C. 1980. Stomatal response of cotton to water stress and abscisic acid as affected by water stress. Plant Physiol. 65:455-459.
2. Barrett, J. and C. Campbell. 2006. s-ABA: Developing a new tool for the big grower. Big Grower 1:26-29.
3. Blackman, P.G. and W.J. Davies. 1985. Root to shoot communication in maize plants of the effects of soil drying. J. Exp. Bot. 36:39-48.
4. Blanchard, M.G., L.A. Newton, E.S. Runkle, D. Woolard, and C.A. Campbell. 2007. Exogenous applications of abscisic acid improved the postharvest drought tolerance of several annual bedding plant species. Acta Hort. 755:127-132.
5. Campbell, C.A., J. Conner, and A. LeBude. 2008. Short-term drought tolerance of container-grown ornamental plants in response to abscisic acid (ABA). HortScience 43:1104. (Abstr.)
6. Cornish, K. and J.A.D. Zeevaart. 1985. Abscisic acid accumulation by roots of *Xanthium strumarium* L. and *Lycopersicon esculentum* Mill. in relation to water stress. Plant Physiol. 79:653-658.
7. Davenport, D.C., P.E. Martin, and R.M. Hagan. 1973. Effects of an antitranspirant on water use by highway oleander (*Nerium oleander* L.) plantings. J. Amer. Soc. Hort. Sci. 98:421-425.

8. Davies, W.J., G. Kudoyarova, and W. Hartung. 2005. Relation to other signaling pathways in the detection of soil drying and the mediation of the plants response to drought. J. Plant Growth Regul. 24:285–295.
9. Davies, W.J., F. Tardieu, and C. Trejo. 1994. Update on long-distance signaling: How do chemical signals work in plants that grow in drying soil? Plant Physiol. 104:309-314.
10. Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:55-76.
11. Flores, A. and K. Dörffling. 1990. A comparative study of the effects of abscisic acid and new terpenoid abscisic acid analogs on plant physiological processes. J. Plant Growth Regul. 9:133-139.
12. Franks, P.J. and G.D. Farquhar. 2001. The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. Plant Physiol. 125:935-942.
13. Goreta, S., D.I. Leskovar, and J.L. Jifon. 2007. Gas exchange, water status, and growth of pepper seedlings exposed to transient water deficit stress are differentially altered by antitranspirants. J. Amer. Soc. Hort. Sci. 132:603-610.
14. Hummel, R.L. 1990. Water relations of container-grown woody and herbaceous plants following antitranspirant sprays. HortSicence 25:772-775.

15. Jifon, J.L. and J.P. Syvertsen. 2003. Kaolin particle film applications can increase photosynthesis and water use efficiency of 'Ruby Red' grapefruit leaves. J. Amer. Soc. Hort. Sci. 128:107-112.
16. Jones, R.J. and T.A. Mansfield. 1970. Suppression of stomatal opening in leaves treated with abscisic acid. J. Exp. Bot. 21:714-719.
17. Kim, J. and M.W. van Iersel. 2008. ABA drenches induce stomatal closure and prolong shelf life of *Salvia splendens.* SNA Research Conference Proceed. 53:107-111.
18. Leskovar, D.I. and D. J. Cantliffe. 1992. Pepper seedling growth response to drought stress and exogenous abscisic acid. J. Amer. Soc. Hort. Sci. 117:389-393.
19. Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. 39:299-319.
20. Myashita, K., S. Tanakamaru, T. Maitani, and K. Kimura. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Expt. Bot. 53:205-214.
21. Okhuma, K., L.J. Lyon, F.T. Addicott, and O.E. Smith. 1963. Abscisin II, an abscission accelerating substance from young cotton fruit. Science. 142:1592-1593.
22. Popova, L.P., T.D. Tsonev, G.N. Lazova, and Z.G. Stoinova. 1996. Drought- and ABA-induced changes in photosynthesis of barley plants. Physiol. Plant. 96:623-629.
23. SAS Institute, Inc. 2001. SAS/STAT User's Guide; Release 9.1.3 Edition, SAS Inst., Cary, NC.

24. Sauter, A., W.J. Davies, and W. Hartung. 2001. The long distance abscisic acid signal in the droughted plant: The fate of the hormone on its way from the root to the shoot. J. Exp. Bot. 52:1-7.

25. Scholander, P.F., E.D. Bradstreet, E.A. Hemmingsen, and H.T. Hammel. 1965. Sap pressure in vascular plants. Science 148:339-346.

26. Sharma, N., S.R. Abrams, and D.R. Waterer. 2005. Uptake, movement, activity, and persistence of an abscisic acid analog (8' acetylene ABA methyl ester) in marigold and tomato. J. Plant Growth Regul. 24:28-35.

27. Sharma, N., S.R. Abrams, and D.R. Waterer. 2006. Evaluation of abscisic acid analogs as holding agents for bedding plant seedlings. HortTechnology 16:71-77.

28. Stamps, R.H. and A.L. Chandler. 2005. Effects of s-ABA on water loss and desiccation of containerized *Hibiscus rosa-sinensis*. HortScience 40:1115-1116. (Abstr.)

29. Starman, T.W., S.E. Beach, and K.L. Eixmann. 2007. Postharvest decline symptoms after simulated shipping and during shelf life of 21 cultivars of vegetative annuals. HortTechnology 17:544-551.

30. Tardieu, F., N. Katerji, O. Bethenod, J. Zhang, and W.J. Davies. 1991. Maize stomatal conductance in the field: Its relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. Plant Cell Environ. 14:121-126.

31. Tattini, M., G. Montagni, and M.L. Traversi. 2002. Gas exchange, water relations and osmotic adjustment in *Phillyrea latifolia* grown at various salinity concentrations. Tree Physiol. 22:403-412.
32. Trejo, C.L., W.J. Davies, and P.R. Lucero del Mar. 1993. Sensitivity of stomata to abscisic acid: An effect of the mesophyll. Plant Physiol. 102:497-502.
33. Yamazaki, H., T. Nishijima, and M. Koshioka. 1995. Effects of (+)- s-abscisic acid on the quality of stored cucumber and tomato seedlings. HortScience 30: 80-82.
34. Yin, C., B. Duan, X. Wang, and C. Li. 2004. Morphological and physiological responses of two contrasting poplar species to drought stress and exogenous abscisic acid application. Plant Science 167:1091-1097.
35. Yue, C. and B.K. Behe. 2008. Estimating U.S. consumer's choice of floral retail outlets. HortScience 43:764-769.
36. Yue, C. and B.K. Behe. 2009. Factors affecting U.S. consumer patronage of garden centers and mass-merchandisers. Acta Hort. (ISHS) 831:301-308.

Table 1. Effect of exogenous s-ABA application on the mean days to critical wilting point (CWP) of selected container-grown woody ornamentals. Numbers within columns separated by different letters are significantly different ($P<0.05$). Data points are the mean of 10 blocks.

	Days to Critical Wilting Point				
Treatment ($mg \cdot L^{-1}$)	*Loropetalum chinense* 'Sizzling Pink'	*Lagerstroemia* Cherry Dazzle™ 'GAMAD I'	*Hydrangea macrophylla* Endless Summer® Blushing Bride™	*Rhododendron* Autumn Debutante™	*Trachelospermum jasminoides* var. *pubescens* 'Madison'
0	5.8c	5.3b	8.4a	7.0c	12.1c
1000	6.5b	8.0a	8.8a	9.3b	16.6b
2000	7.0a	8.6a	9.5a	9.9a	18.6a

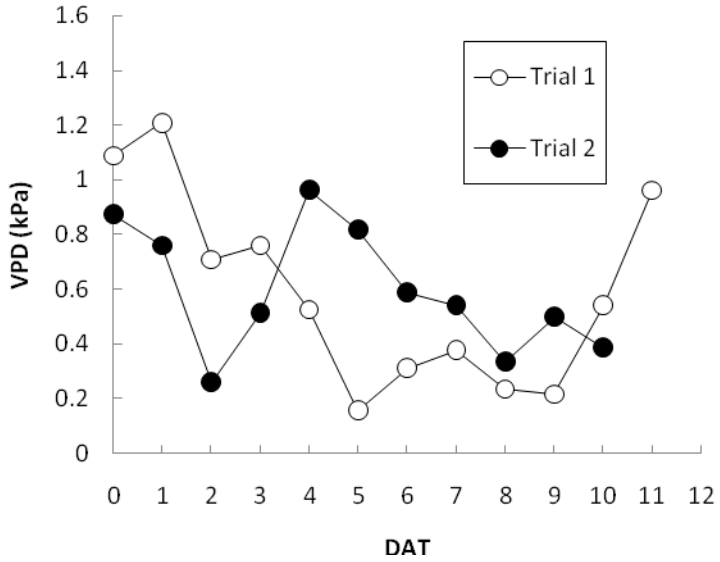

Figure 1. Mean vapor pressure deficit (VPD) between 0800 and 1900 HR for each day after treatment (DAT) for two trials of *Ligustrum japonicum* 'Recurvifolium.'

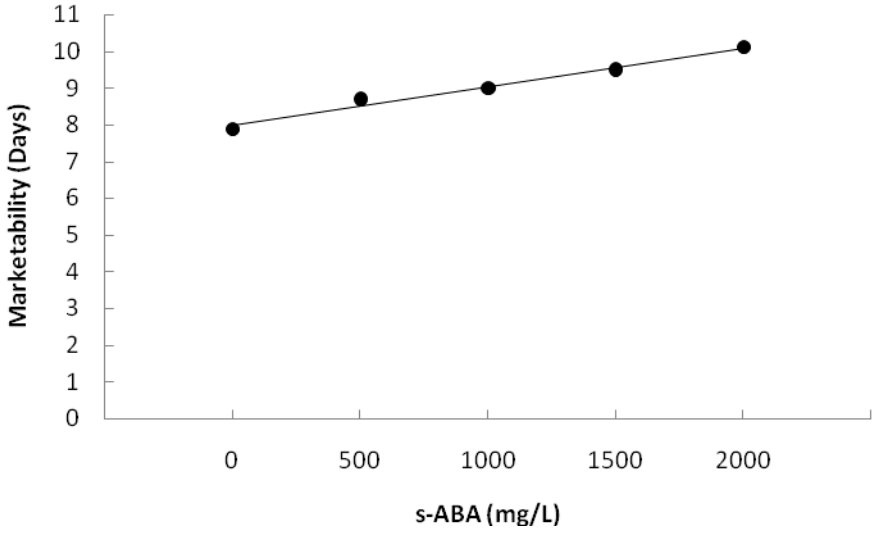

Figure 2. Days after treatment (DAT) with a spray application of 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA to plants of *Ligustrum japonicum* 'Recurvifolium' and water withheld before reaching the critical wilting point and becoming unmarketable. Data points are the mean of two trials and 10 (Trial 1) or 5 (Trial 2) replications. Marketability (DAT) = 7.99 +(0.001*trt); $P<0.01$; $r^2=0.98$

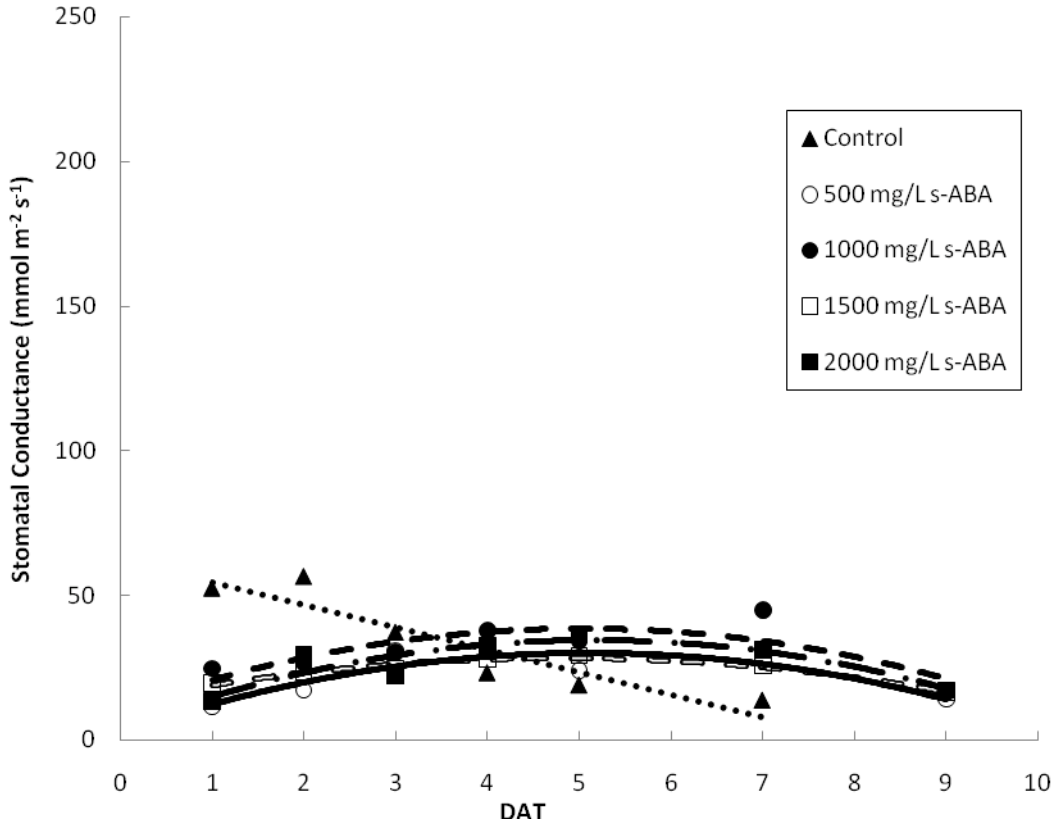

Figure 3. Mean stomatal conductance (g_s) (mmol·m^{-2}·s^{-1}) for each day after treatment (DAT) with a spray application of 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA to plants of *Ligustrum japonicum* 'Recurvifolium' and water withheld before reaching the critical wilting point in Trial 1. Data points are the mean of 10 replications. Trendline designations and regression equations are: g_s (control) = 71.94 -(14.34*day) +(0.83*day^2), P=0.03, r^2 = 0.90; g_s (500 mg·L^{-1} s-ABA) = 2.46 +(10.85*day) -(1.06*day^2), P=0.04, r^2 = 0.79; g_s (1000 mg·L^{-1} s-ABA) = 10.63 +(11.16*day) -(1.11*day^2), P=0.13, r^2 = 0.64; g_s (1500 mg·L^{-1} s-ABA) = 13.20 +(6.34*day) -(0.66*day^2), P=0.0008, r^2 = 0.97; g_s (2000 mg·L^{-1} s-ABA) = 4.44 +(11.63*day) -(1.13*day^2), P=0.04, r^2= 0.79.

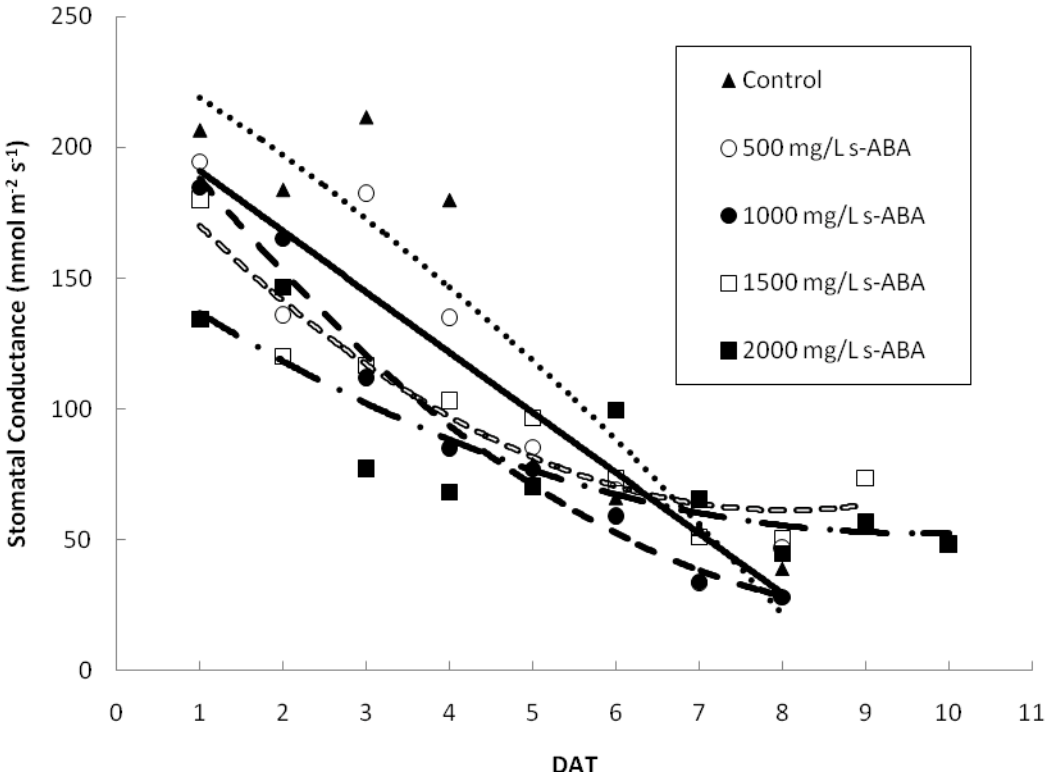

Figure 4. Mean stomatal conductance (g_s) (mmol·m^{-2}·s^{-1}) for each day after treatment (DAT) with a spray application of 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA to plants of *Ligustrum japonicum* 'Recurvifolium' and water withheld before reaching the critical wilting point in Trial 2. Data points are the mean of 5 replications. Trendline designations and regression equations are: g_s (control) = 239.01 -(19.03*day) -(1.02*day^2), P<0.007, r^2 = 0.86; g_s (500 mg·L^{-1} s-ABA) = 214.41 -(23.22*day) +(0.01*day^2), P<0.007, r^2 = 0.86; g_s (1000 mg·L^{-1} s-ABA) = 228.69 -(42.46*day) +(2.18*day^2), P=0.0001, r^2 = 0.98; g_s (1500 mg·L^{-1} s-ABA) =

202.72 -(35.10*day) +(2.18*day^2), P=0.0008, r^2 = 0.91; g_s (2000 mg·L^{-1} s-ABA) = 157.77 -(21.87*day) +(1.14*day^2), P=0.01, r^2 = 0.71.

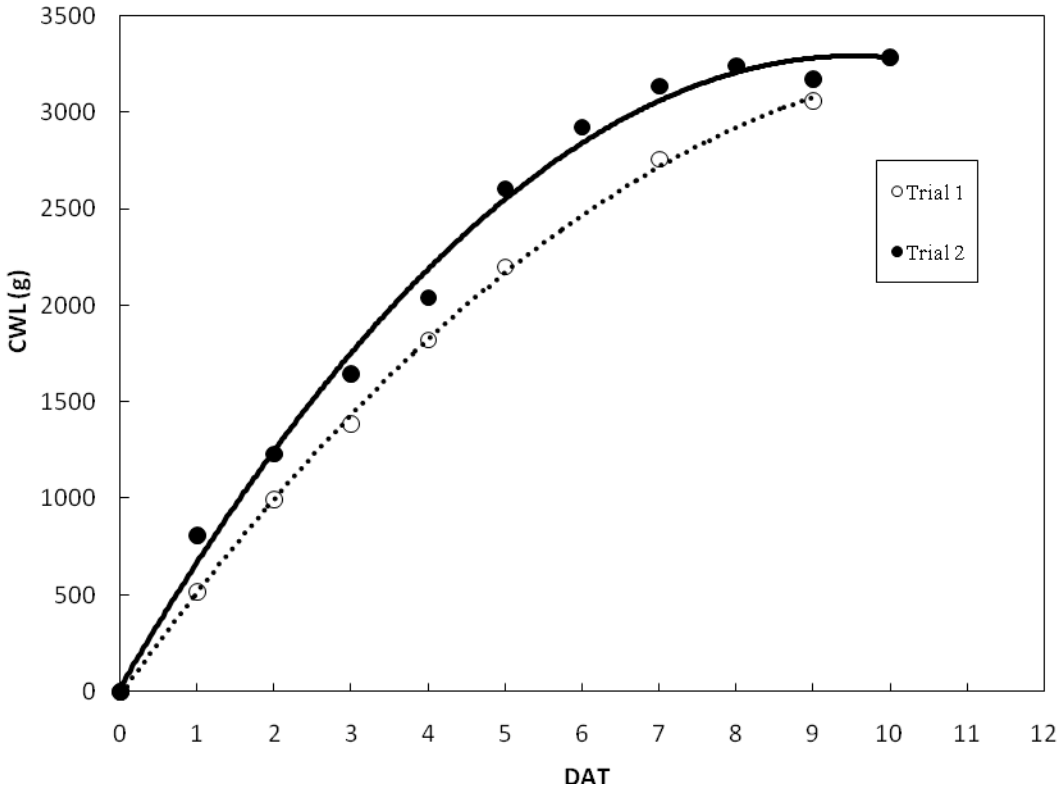

Figure 5. Mean cumulative water loss (CWL) for each day after treatment (DAT) with a spray application of 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA to plants of *Ligustrum japonicum* 'Recurvifolium' and water withheld before reaching the critical wilting point in two trials. Data points are the mean of five treatments and 10 (Trial 1) or 5 (Trial 2) replications. CWL (g) (Trial 1) = -65.29 +(584.59*day) -(26.21*day^2), P<0.01, r^2 = 0.76; CWL (g) (Trial 2) = 82.10 +(656.92*day) -(33.05*day^2), P<0.01, r^2 = 0.88.

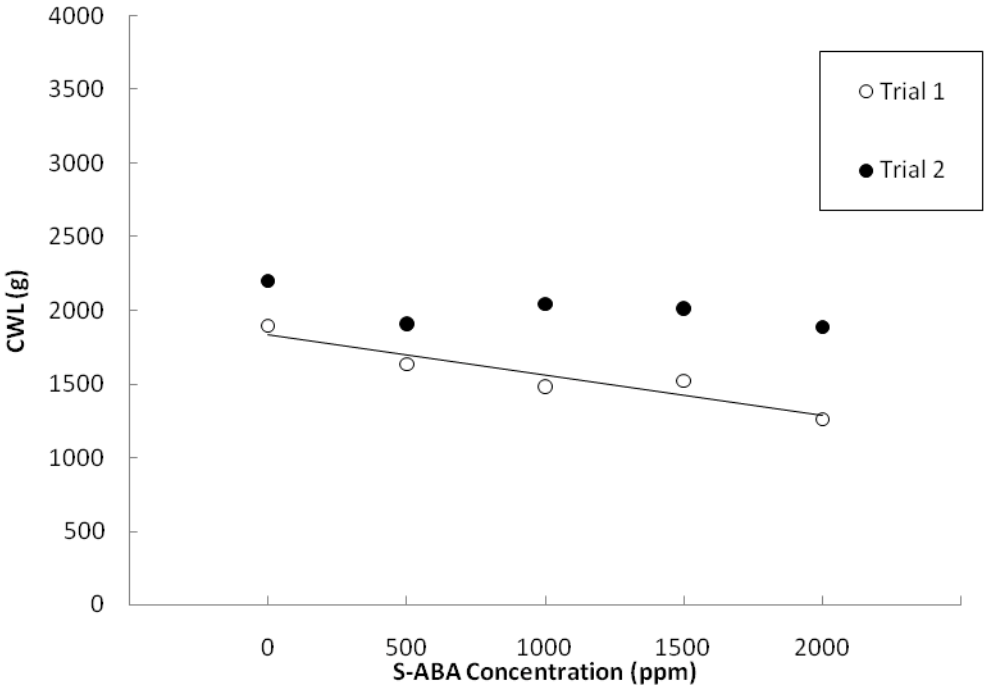

Figure 6. Mean cumulative water loss (CWL) for all DAT until CWP for plants treated with either 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA in two trials. Data points are the mean of 10 replications and nine DAT (Trial 1) or 5 replications and 10 DAT (Trial 2). CWL (g) (Trial 1) = 2200.34 -(0.52*trt) +(0.00008*trt^2), P=0.08, r^2 = 0.92.

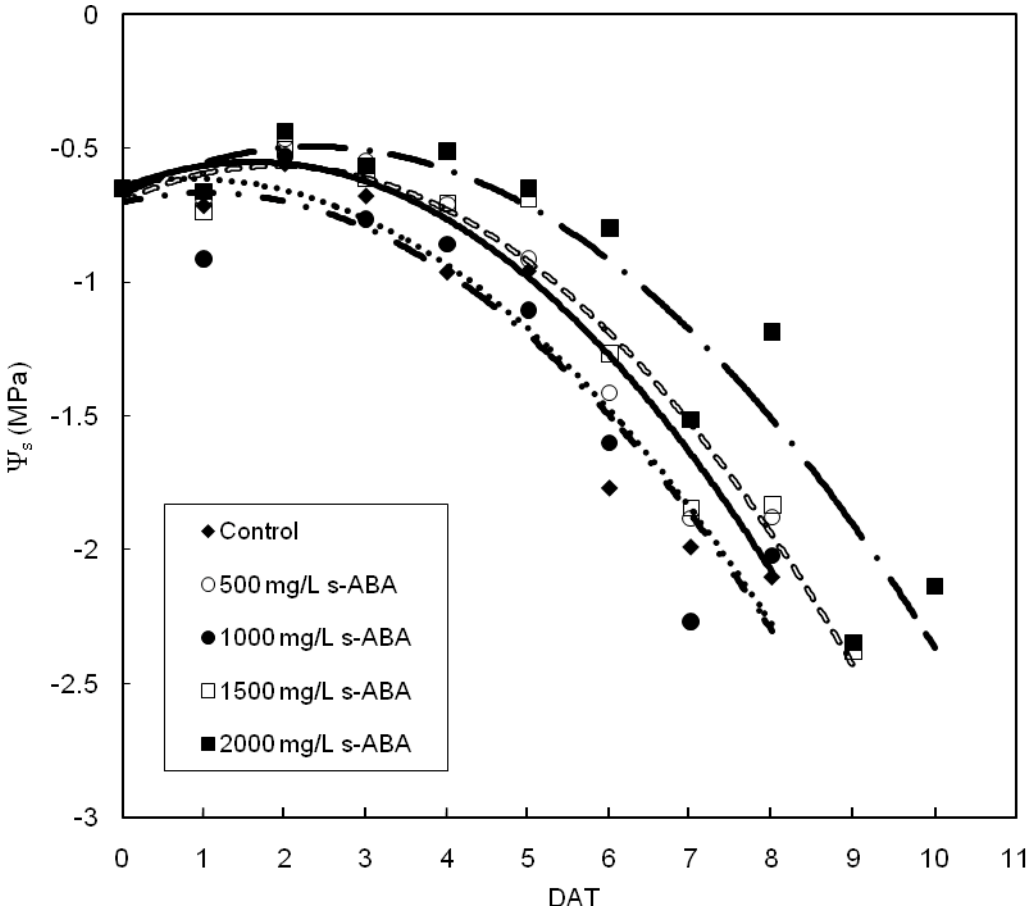

Figure 7. Mean stem water potential (Ψ_s) for each day after treatment (DAT) with a spray application of 0, 500, 1000, 1500 or 2000 mg·L^{-1} s-ABA to plants of *Ligustrum japonicum* 'Recurvifolium' and water withheld before reaching the critical wilting point in Trial 2. Trendline designations and regression equations are: Ψ_s (Control) = 0.65 -(0.07*day) +(0.04*day^2), $P<0.01$, $r^2 = 0.62$; Ψ_s (500 mg·L^{-1} s-ABA) = 0.69 -(0.15*day) +(0.04*day^2), $P<0.01$, $r^2 = 0.71$; Ψ_s (1000 mg·L^{-1} s-ABA) = 0.83 -(0.14*day) +(0.04*day^2), $P<0.01$, $r^2 = 0.69$; Ψ_s (1500 mg·L^{-1} s-ABA) = 0.73 -(0.14*day) +(0.04*day^2), $P<0.01$, $r^2 = 0.82$; Ψ_s (2000 mg·L^{-1} s-ABA) = 0.72 -(0.17*day) +(0.03*day^2), $P<0.01$, $r^2 = 0.72$