Plant Breeding and Evaluation

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A Historical Summary of Plant Material Evaluations by SERA-IEG 27

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Significance to Industry: Independent testing of new plant material at a large number of locations is crucial before a new plant is introduced. The members of SERA-IEG 27 (Nursery and Landscape Systems) evaluate plant material then compile results for dissemination. This testing program and the dissemination of results offer a guide to green industry professionals attempting to select the best plants for production and landscape use.

Nature of Work: The Southern Extension and Research Activities/Information Exchange Group 27 (SERA-IEG-27) for Nursery and Landscape Systems was initiated in 1994. This group is composed of research and extension faculty working in the commercial nursery and landscape disciplines from fourteen 1862 land grant universities in the southeastern United States. In addition, horticulture faculty from the USDA – National Arboretum are members. Universities represented are Texas A&M, Oklahoma State, LSU, Arkansas, Auburn, Mississippi State, Georgia, Florida, Tennessee, Kentucky, Clemson, North Carolina State, West Virginia, and Virginia Tech.

The goal of the group is to evaluate landscape plant material across many different areas of the southern United States. During the annual meeting held each summer, members determine which plants will be tested by the group. Plants for evaluation are then given a code indicating state of origin and numerical order of entry into the testing program. Next, plants are supplied as liners or one gallon containers to cooperators who grow the plant to landscape size before planting out.

From one to three plants of each selection are supplied to the cooperators. In SERA-IEG-27, both herbaceous perennials and woody plants are evaluated. Depending on the plant, the evaluation period can range from 2 to 10 years. Results of evaluation are disseminated through educational programs and publications of the participating states.

Results of Work: To date 40 different plant taxa have been evaluated or are being evaluated currently by cooperators in SERA-IEG 27. Plants officially evaluated in the program are show in Table 1. Dunwell et al. (1) and Lindstrom (2) have previously reported on this plant evaluation program effort. Lindstrom (2) documented in 2004 that evaluations have been completed on the plants listed below. These reports summarize the growth, flowering and other aesthetic qualities as well as adaptability in the test locations.

- *Rhododendron* 'GulfRay' MS04-01
- *Cornus mas* 'Spring Glow' NC96-01
- *Acer oliverianum* ssp. *formosanum* GA97-01
- *Styrax japonicum* 'Emerald Pagoda' NC98-01
- *Pittosporum heterophyllum* AL98-01
- *Cephalotaxus harringtonia* 'Berry College Selection' TN98-01
- *Conradina canescens* FL98-01
- *Lagerstroemia* 'Chickasaw' DC99-01
- *Lagerstroemia* 'Pocomoke' DC99-02
- *Plumbago auriculata* 'Hullabaloo' TX99-01
- *Turnera ulmifolia* TX99-02
- *Lonicera* × *americana* TX99-03
- *Ipomoea carnea* ssp. *fistulosa* TX99-04
- *Illicium mexicanum* 'Aztec Fire' NC99-01
- *Iris* 'Churchill Downs' KY99-01
- *Iris* 'Kentucky Derby' KY99-02
- *Hemerocallis* 'Octavia' Series KY99-03
- *Hemerocallis* 'Happy Returns' KY99-03
- *Hemerocallis* 'Milano' Series KY99-03
- *Bulbine canescens* TX00-01
- *Stachys coccinea* AR01-01
- *Conradina canescens* 'SPH' AR01-02

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Table 1. Historical listing of ornamental plants selected for landscape evaluation by SERA-IEG 27 with listing of faculty introducing.

Rhododendron 'GulfRay' MS04-01 (Sam Laiche, 1994)
Cornus mas 'Spring Glow' NC96-01 (Tom Ranney, 1996)
Acer oliverianum ssp. *formosanum* GA97-01 (John Ruter, 1997)
Styrax japonicum 'Emerald Pagoda' NC98-01 (Tom Ranney, 1998)
Pittosporum heterophyllum AL98-01 (Ken Tilt, 1998)
Cephalotaxus harringtonia TN98-01 (Will Witte, 1998)
Conradina canescens FL98-01 (Gary Knox, 1998)
Lagerstroemia 'Chickasaw' DC99-01 (Margaret Pooler, 1999)
Lagerstroemia 'Pocomoke' DC99-02 (Margaret Pooler, 1999)
Plumbago auriculata 'Hullabaloo' TX99-01 (Mike Arnold, 1999)
Turnera ulmifolia TX99-02 (Mike Arnold, 1999)
Lonicera ×*americana* 'Pam's Pink' TX99-03 (Mike Arnold, 1999)
Ipomoea carnea ssp. *fistulosa* TX99-04 (Mike Arnold, 1999)
Illicium mexicanum 'Aztec Fire' NC99-01 (Tom Ranney, 1999)
Iris 'Churchill Downs' KY99-01 (Win Dunwell, 1999)
Iris 'Kentucky Derby' KY99-02 (Win Dunwell, 1999)
Hemerocallis 'Octavia' Series KY99-03 (Win Dunwell, 1999)[Z]
Hemerocallis 'Happy Returns' KY99-03 (Win Dunwell, 1999)
Hemerocallis 'Milano' Series KY99-03 (Win Dunwell, 1999)[Y]
Bulbine caulescens TX00-01 (Mike Arnold, 2000)
Michelia skinneriana FL00-01 (Gary Knox, 2000)
Stachys coccinea AR01-01 (Jon Lindstrom, 2001)
Conradina canescens 'SPH' AR01-02 (Jon Lindstrom, 2001)
Magnolia x 'Jon Jon' FL01-01 (Gary Knox, 2001)
×*Sinocalycalycanthus raulstonii* 'Hartlege Wine' NC02-01 (Tom Ranney, 2001)
Daphniphyllum macropodum DC02-01 (Margaret Pooler, 2002)
Forsythia 'Fairy-Land' TN03-01 (Donna Fare, 2003)
Forsythia 'Minikin' TN03-02 (Donna Fare, 2003)
Forsythia 'Tinkle Bells' TN03-03 (Donna Fare, 2003)
Forsythia 'Pygmy-Red' TN03-04 (Donna Fare, 2003)
Betula nigra 'Summer Cascade' NC03-01 (Tom Ranney, 2003)
Amsonia 'SPH' AR03-01 (Jon Lindstrom, 2003)
Buddleja 02-25-142 AR04-01 (Jon Lindstrom, 2004)
Viburnum luzonicum FL04-01 (Gary Knox, 2004)
Buddleja 03-05-045 AR05-01 (Jon Lindstrom, 2005)
Ceanothus x *delilianus* 'Gloire de Versailles' AR05-02 (Jon Lindstrom, 2005)
Pinus ayacahuite AL05-01 (Ken Tilt, 2005)
Taxodium distichum seedling selections TX07-01 (Mike Arnold, 2007)
Callicarpa dichomata 'Duet' TN07-01 (Donna Fare, 2007)
Syringa 'Betty Ross' TN07-02 (Donna Fare, 2007)

[Z] The 'Octavia' series daylilies included 'Cherry Doll', 'Exotic Marble', 'Marble Model', 'Glow', and 'Orchid'.
[Y] The 'Malino' series daylilies included 'Maraschino', 'Rocket', and 'Violet Mark'.

Preliminary Efforts to Induce Polyploidy in *Cryptomeria japonica*

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Index Words: photoinhibition, tetraploid, Japanese cedar

Significance to the Industry: Cryptomerias, or Japanese cedars, offer an alternative to Leyland cypress due to their limited susceptibility to bagworm infestations (9); however, in full sun they exhibit a browning in winter that can be undesirable (personal observation). The development of a Japanese cedar that does not exhibit winter browning could make it a more viable option for use as a screen or specimen.

Nature of Work: *Cryptomeria* D. Don is a monotypic genus comprised solely of *C. japonica* (L. f.) D. Don and its two varieties: *C. japonica* var. *japonica* and *C. japonica* var. *sinensis* Miquel. *Cryptomeria* is native to China (var. *sinensis*) and Japan (var. *japonica*) and is an important timber tree of the latter, reaching heights of 36 to 46 m in the wild (3), but is typically 15 to 25 m in gardens (3).

Winter browning of Japanese cedar occurs through the conversion of chloroplasts to chromoplasts during winter (5). This transition takes place only in sun-exposed leaves during periods of low temperature, indicating that photoinhibition likely plays a role (5). Plants use several mechanisms to deal with excess light during periods of low temperature when Calvin cycle activity is limiting: reduction of chlorophyll, pH dependent xanthophyll cycle, increased levels of carotenoids, and production of antioxidants or reactive oxygen species (ROS) scavenging enzymes. Japanese cedars, Japanese black pines, and Japanese cypress with leaves that did not exhibit premature withering, shedding, or dying have been shown to have higher levels of superoxide dismutase (SOD) activity than trees that did exhibit this behavior (7). Tetraploid forms of Japanese cedar had six times the SOD activity of other trees in the study (7). This is consistent with the increased enzyme activity reported elsewhere in polyploids. DeMaggio and Lambrukos (4) reported that peroxidase activity per cell increased 3.5 to 4 times with increase in ploidy of gametophytes of the fern *Todea barbara* (L.) Moore. The objective of the current study was to begin to formulate a protocol to develop a tetraploid form of *Cryptomeria japonica* in order to increase antioxidant enzyme activity and prevent winter browning.

Cuttings were collected from *C. japonica* ‘Yoshino’ on 7 June 2007. Oryzalin treatments were applied, basal ends were re-cut and treated with 3,000 ppm K-IBA, and set in 4 peat : 3 perlite under mist with 60% shade. Oryzalin was supplied in the form of Surflan® at rates of 0, 20, 40, and 60 µM and durations of 0, 12, 24, and 48-h. Oryzalin was applied using 1% agar to cap the meristems for the respective treatment times. An untreated control was included as a standard to determine the effect on survival of treatments and agar caps, respectively.

On 4 December 2007 survival data was recorded. Cuttings were considered dead if the treated meristem did not survive, even if the cutting had rooted and lateral shoots emerged. To evaluate efficacy of treatments for inducing polyploidy, plants were observed for phenotypes indicative of polyploidy in Japanese cedar (1) and stomata were measured. The experimental design was a two-way full factorial design with four replicates. The two factors investigated were oryzalin concentration and duration of treatment. Data were subjected to ANOVA using SAS (SAS Institute, Cary, N.C.). All main effects and interactions were included in the model statement.

Results and Discussion: Analysis revealed that there was no significant effect of concentration (P = 0.09) or duration (P = 0.39) of oryzalin treatment on survival of cuttings. The block effect was not significant (P = 0.12) so it was included in the error degrees of freedom. Since there were no significant main effects, data are presented as means ± SE. Surviving plants have not displayed morphology typical of tetraploids, such as thickened, twisted needles exhibiting the gigas effect (1). Furthermore, there was no difference in size of stomata between any treatments tested. For example, control plants and plants treated with the highest concentration applied for the longest duration (60 µM for 48-h) had stomata with a mean diameter of 26.3 µm and 25.6 µm, respectively. Since the combination of highest oryzalin concentration applied for the longest duration did not produce any phenotypic effects and there was not greater mortality than the control, it appears that the concentrations and/or durations used were too low to induce polyploidy. The duration may need to be increased due to the large genome size of *C. japonica*. Hizume et al. (6) determined Japanese cedar has a 2C DNA content of 22.1 pg. This is an order of magnitude larger than many other woody angiosperms. Diploid *Rhododendron* 'Fragrant Affinity', for instance, has a 2C DNA content of 1.6 pg (2). Further experiments need to be conducted to determine the appropriate concentration. A higher concentration may be necessary to efficiently induce polyploidy since Sanford (8) reported that optimal treatments for inducing polyploidy approach 50% mortality. Since the untreated control had a mean survival of 52%, the optimal treatment (LD_{50}) would result in 26% survival.

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IRD800 Fluorescent Dye may Alter AFLP Primers' Annealing in Flowering Cherries

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Index Words: *Prunus* species, *L. esculentum,* DNA Fingerprinting, AFLP Primer Pairs.

Significance to industry: Compared to conventional breeding, the use of molecular markers is more efficient, saving development costs and reducing the time necessary for plant evaluation and release of new cultivars. Amplified fragment length polymorphism (AFLP*)* marker's based profiling can benefit the nursery industry by reducing costs of genetic characterization and increasing the efficiency of developing new types. As important garden ornamentals for all seasons, beauty can be found in inflorescence, foliage and bark of the Japanese flowering cherries. There is a great confusion over their naming and identification due to the abundance in species. Therefore, development of high through-put genetic identification methods for reliable selection of commercially suitable cherries is imperative. This research illustrates the implications of using fluorescent labeled AFLP markers for flowering cherry accessions.

Nature of Work: Molecular studies that identify different species help preserve biodiversity, lead to the development of phylogenetically more accurate taxonomic classifications and ultimately contribute to a unified classification for all the ramifying lines of life. Japanese flowering cherries are important nursery plants and ideal candidates for true-to-type identification via DNA-fingerprinting (7). The flowering group of cherries is comprised of some 200 species in the genus *Prunus* and all have pink or white flowers with a delicate sweet scent (4). These trees were a gift of friendship to this country from the people of Japan (6). The literature on taxonomic classification of flowering cherry is very limited; in fact, cultivated flowering cherries have uncertain parentage (7). Molecular markers' polymorphism is a very useful tool to assess genetic distances among flowering cherries since these are based on DNA analysis (8, 9). AFLP is a popular molecular marker analysis technique, gives superior assay efficiency indices and can be used without prior genome information (3, 10). In this study, AFLP markers with or without fluorescent labels were used for genetic characterization of selected flowering cherry accessions. Implications of using infra-red-dye (IRD) 800 (Li-Cor Inc., Lincoln, NE) labeled AFLP primers, by comparing DNA amplification from flowering cherry and tomato genomes via labeled and unlabelled primers, are discussed.

DNA samples from six flowering cherry (*Prunus*) accessions, namely *P. incisia* 'February Pink 104 ER', *P.* spp. 'Snow Fountain', *P. incisa*, *P. serulata*, *P. maximowicizii* '62 ER', and *P.* spp 'Fudan Zakara 1 ER' as well as six heirloom tomato (*Lycopersicon*

esculentum) varieties, i.e., Russian, Marizol Red, Andrew Rahart Jumbo, Brandy Wine, Tidwell German and Brimmer were isolated utilizing DNeasy Plant Mini Kit (Qiagen, Santa Clara, CA) and grinding matrix along with Bio Fast Prep System (Q. Biogene, Irvine, CA). AFLP System Analysis Kit (Invitrogen™ Life Technologies, Carlsbad, CA) was used for subsequent assays. In some assays, unlabeled primers designed for *Eco*RI adaptors were replaced with fluorescent dye IRD-800 (Li-Cor Inc., Lincoln, NE) labeled primers. AFLP generated DNA fragments were then separated by sequencing gel electrophoresis using an automated DNA analyzer (Global IR² DNA Analyzer and Sequencer, Li-Cor Inc., Lincoln, NE, USA). Using Saga™ Generation 2- AFLP® Analysis (Li-Cor Inc., Lincoln, NE, USA) software, AFLP profiles were analyzed to generate binary-code (0/1) reports on markers' data. TreeCon-Dendrogram (Scanalytics Inc., Fairfax, VA, USA) software was used to analyze the data to deduce genetic relationships between these accessions and varieties.

Results and Discussions: For selective AFLP amplifications, 16 primers are used to anneal to AFLP adaptors and ligated plant sequences (1). Eight of these primers are designed for *Eco*RI adaptors (E-AAC, E-AAG, E-ACA, E-ACC, E-ACG, E-ACT, E-AGC, and E-AGG) and eight primers are designed for *Mse*I adaptors (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT). All 64 primer-pair combinations consisting of eight *Eco*RI and *Mse*I primers each were used for selective AFLP amplification. Production of AFLP markers without fluorescent labels has been reported for flowering cherries (5) and tomatoes (1). In this study, unlabeled primers designed for *Eco*RI adaptors were replaced with fluorescent dye IRD-800 (Li-Cor Inc., Lincoln, NE) labeled primers. AFLP markers were thus produced for genetic characterization of selected flowering cherry accessions and heirloom tomato varieties.
Sixteen un-labeled AFLP primer-pair combinations have been reported for the six Japanese flowering cherry accessions used in this study (5). When unlabeled primers designed for *Eco*RI adaptors (E-primers) were replaced with fluorescent dye IRD-800 (Li-Cor Inc., Lincoln, NE) labeled primers, 19 AFLP primer pairs were identified that provided the most amplification for each accession (Table 1). However, of these pairs, seven were in common with the previous report (5) and 12 depicted selective amplifications only when unlabelled E-primers were used (Table 2). Out of a total of 64 combinations, there are 62 pairs recommended for AFLP fingerprinting of tomatoes (1). Thus, for comparison, labeled E-primers were also used on six heirloom tomato varieties and 20 pairs were found suitable for AFLP amplification including the 2 pairs (E-AAG/M-CAT and E-AAG/M-CTG) that don't provide discernible amplification with unlabelled E-primers (Table 3). Incorporation of fluorescent dye molecules to primers have been reported to alter their annealing to templates (2, 11). The use of labeled primers for comparative AFLP profiling of genomes helps automated analyses, but should be adopted only after standardizing protocols with a labeling dye. To graph genetic distances among flowering cherry accessions and tomato varieties, 447 and 310 fluorescent-labeled AFLPs were used, respectively (Figures 1, 2). Therefore, if pre-standardized protocols are used, AFLP is a robust method for correctly identifying and tracking the source of genotypes and properly deducing evolutionary relationships.

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Table 1: The 19 AFLP primer pairs[1] (while E-primers labeled with IRD-800[2]) that depicted selective amplification of the DNA marker's in *P. incisa* 'February Pink 104 ER', *P.* spp. 'Snow Fountain', *P. incisa*, *P. serulata*, *P. maximowicizii* '62 ER,' and *P.* spp 'Fudan Zakara 1 ER'.
E-AAC/M-CAA, E-AAC/M-CAG, E-AAG/M-CAA, E-AAG/M-CAT, E-ACA/M-CAA, E-ACA/M-CAC, E-ACA/M-CAG, E-ACA/M-CTC, E-ACC/M-CAA, E-ACC/M-CAG, E-ACG/M-CAA, E-ACT/M-CAC, E-AGC/M-CAA, E-AGC/M-CAG, E-AGC/M-CAT, E-AGG/M-CAA, E-AGG/M-CAC, E-AGG/M-CAG, E-AGG/M-CTT
[1]Each pair consists of primers designed for both *Eco*R I (E) and *Mse* I (M) adaptors. The three selective nucleotides of each primer are shown. [2] Primers designed for *Eco*RI adaptors and labeled with fluorescent infra-red-dye (IRD) 800 (Li-Cor Inc., Lincoln, NE).

Table 2: The 12 AFLP primer pairs[1] that didn't reveal discernible amplification with unlabelled E-primers (Holcombe et al., 2007[2]) but depicted selective amplification of the DNA marker's in *P. incisa* 'February Pink 104 ER', *P.* spp. 'Snow Fountain', *P. incisia*, *P. serulata*, *P. maximowicizii* '62 ER,' and *P.* spp 'Fudan Zakara 1 ER' when IRD-800[3] labeled primers designed for *Eco*RI adaptors were used.
E-AAC/M-CAA, E-AAG/M-CAA, E-ACA/M-CAG, E-ACA/M-CTC, E-ACC/M-CAG, E-ACG/M-CAA, E-ACT/M-CAC, E-AGC/M-CAT, E-AGG/M-CAA, E-AGG/M-CAC, E-AGG/M-CAG, E-AGG/M-CTT
[1]Each pair consists of primers designed for both *Eco*R I (E) and *Mse* I (M) adaptors. The three selective nucleotides of each primer are shown. [2] Holcombe et. al. 2007 (9). [3]Primers designed for *Eco*RI adaptors and labeled with fluorescent infra-red-dye (IRD) 800 (Li-Cor Inc., Lincoln, NE).

Table 3: The 20 AFLP primer pairs[1] (while E-primers labeled with IRD-800[2]) that depicted selective amplification of the DNA maker's in six heirloom tomato (*Lycopersicon esculentum*) varieties; Russian, Marizol Red, Andrew Rahart Jumbo, Brandy Wine, Tidwell German and Brimmer.
E-AAC/M-CTC, E-AAC/M-CTT, E-AAG/M-CTC, E-AAG/M-CTG, E-AAG/M-CTT, E-AAG/M- CAT, E-ACA/M-CAA, E-ACG/M-CAC, E-ACG/M-CAG, E-ACG/M-CAT, E-ACT/M-CAC, E-ACT/M-CAG, E-ACT/M-CAT, E-AGC/M-CAA, E- AGC/M-CAC, E-AGC/M-CAG, E-AGC/M-CAT, E-AGG/M-CAA, E-AGG/M-CAC, E-AGG/M-CAG
[1]Each pair consists of primers designed for both *Eco*R I (E) and *Mse* I (M) adaptors. The three selective nucleotides of each primer are shown. [2]Primers designed for *Eco*RI adaptors and labeled with fluorescent infra-red-dye (IRD) 800 (Li-Cor Inc., Lincoln, NE).

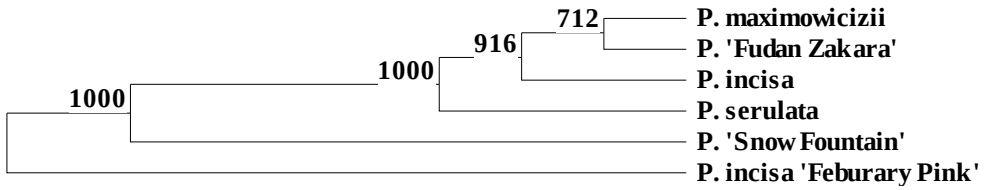

Figure 1: Genetic distances among six Japanese flowering cherry (*Prunus* spp.) accessions as depicted by TreeCon-Dendogram (Scanalytics Inc., Fairfax, VA) based analysis of 447 polymorphic AFLP markers. UPGMA (unweighted pair-group mathematical average) method for transformed distance estimation was used to graph the genetic similarities in the tree topology. Bootstrap values (at tree nodes) are shown for 1000 UPGMA searches.

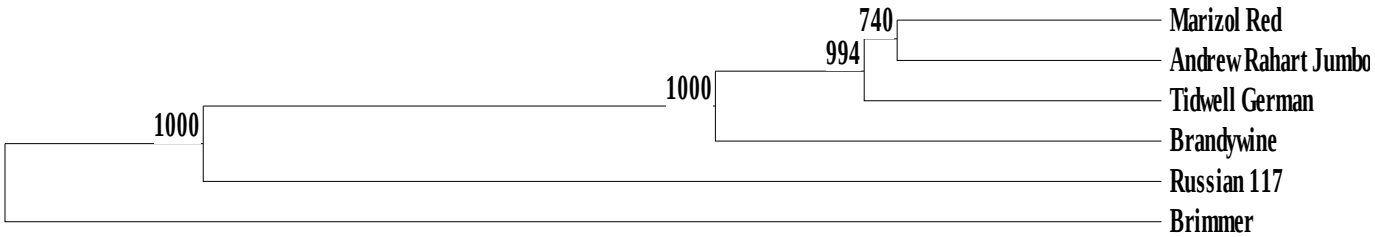

Figure 2: Genetic distances among in six heirloom tomato (*Lycopersicon esculentum*) varieties as depicted by TreeCon-Dendogram (Scanalytics Inc., Fairfax, VA) based analysis of 310 polymorphic AFLP markers. UPGMA (unweighted pair-group mathematical average) method for transformed distance estimation was used to graph the genetic similarities in the tree topology. Bootstrap values (at tree nodes) are shown for 1000 UPGMA searches.

Sterility Seen in Backcross Population of *Buddleja*

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Index Words: Ornamental plant breeding, Backcross, Interspecific hybrid, Butterfly bush, Sterility

Significance to Industry: Along with increasing panicle size, backcrossing divergent *Buddleja* L. species and hybrids may induce sterility as seen in a BC_1F_1 population of *B*. *davidii* Franch. 'Empire Blue' × *B*. *indica* Lam. backcrossed to *B*. *davidii* 'Empire Blue'. The sterility will limit further hybridization; however, if superior hybrids are identified, this method of inducing sterility would prove to be an asset.

Nature of Work: Most of the 100 plus *Buddleja* species, including those in section Buddleja, have dehiscent capsules which allow for rapid seed dispersal. This rapid seed dispersal, combined with a high germination percentage, has allowed *Buddleja davidii*, the most popular species in the industry, to colonize disturbed areas (2, 4). Despite these problems, butterfly bush remains a popular landscape plant, as there are few plants that can match its long bloom time and ability to attract butterflies.

There are a number of underutilized species that would allow for the introgression of important traits into *B*. *davidii*. These traits include flower color, leaf pubescence for pest resistance, and a non-dehiscent fruit to reduce invasiveness. Recent hybridization efforts have been successful in generating plants with altered fruit morphology by using *B*. *indica* from sect. Nicodemia (Tenore) Leeuw. (3).

A problem with *B*. *indica* in hybridization is that a number of undesirable characters (including smaller panicle size) can be found in the progeny. Progeny from such crosses have proven to be highly fertile (3), allowing backcross hybridization to incorporate more favorable traits from *B*. *davidii* back into the hybrid. The aim of this experiment was to evaluate progeny of a *B*. *davidii* 'Empire Blue' × *B*. *indica* cross after backcrossing it to *B*. *davidii* 'Empire Blue' to see if the plants generated would be of greater ornamental value through increased panicle size, while retaining the oak-like foliage and non-dehiscent fruit from *B*. *indica*.

Results and Discussion: Viable seed were obtained from the reciprocal backcrosses, and 43 seedlings resulted. Traits such as height and width showed marked variation within the seedling population, as mature plants in the field reached heights of 0.2 to 1.2 m and widths of 0.2 to 1.4 m, which were intermediate to *B*. *davidii* 'Empire Blue' and 01-37-661. Flower, leaf, and panicle morphology was intermediate of the parents. Variation among progeny for flower and leaf morphology was limited but did differ in color.

Surprisingly, all progeny from the crosses appeared to be sterile following two years of field evaluation, even though the original F1 hybrid was highly fertile. The BC_1F_1 hybrids were surrounded by multiple *Buddleja* species and hybrids allowing for open pollination, but no seed set was observed. Attempts to cross plants back to *B. davidii* 'Empire Blue' and 01-37-661 were unsuccessful.

In order to understand what might be affecting fertility, the original hybrid and backcross progeny were subjected to flow cytometric analysis. Among the backcross progeny, DNA content showed variability. Changes in chromosomes and genome organization could have been prompted by wide hybridization resulting in abnormal chromosome arrangements, transposons, or genomic rearrangements including loss, mutation, or suppression of duplicate genes affecting DNA amount (1, 5, 6). At present it is unclear as to the exact cause of the abnormal DNA content seen in the original hybrid and backcross progeny, but it can be concluded that sterility is likely the result of it. Observed lack of fruit and seed set in the backcross population was an obvious characteristic of female sterility, which could be influenced by unsuitable genome compatibility, lack of maturity in ovule development, or from physiological problems not allowing for fertilization.

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Golden Camellias from Guangxi, China

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Index Words: Breeding, Camellia, Clone, Cultivar, Germplasm, Golden Tea, Guangxi, Hybrid, Ornamentals, Species.

Significance to Industry: New plants are new blood for the ornamental nursery industry. With each trip to China, I am amazed by the new plant materials she can offer. Although golden camellias are not new to our ornamental world, the diversity of plant materials, especially new species discovered in recent years, is phenomenonal. These plants are distributed in a narrow basin of the upper Rongjiang River in Guangxi, China. After the first golden camellia was discovered in 1948, only two more species were collected in next 30 years. From 1979 to 2001, a total of 40 new species had been identified. Today, taxonomists do not agree with each other on the legitimacy of each species. For horticultural professionals, these variations are worth being explored as new cultivars. Detailed investigations with a focus on potential cold tolerant clones and possible hybrids should be conducted. Future collaboration on plant exploration, introduction, breeding, and better management of golden camellias with our Chinese colleagues in the field of ornamentals is highly recommended.

Nature of Work: *Camellia japonica* and *C. sansanqua* are well-known ornamental plants for formal and informal gardens. Since so many professional and amateur gardeners have collected, bred, selected, and introduced new cultivars, more than 2,000 cultivars derived from these two species are on the market today (4). In recent years, new species with great ornamental potential, such as *C. azalea* and *C. tamdaoensis*, have brought a lot of attention to this genus (5, 9). Also, breeding of cold hardy germplasm has yielded many new cultivars and extended Camellia cultivation areas to USDA zone 6 (2). The U.S. National Arboretum (8) used *C. oleifera* 'Lu Shan Snow' as a parent and introduced more than a dozen ornamental camellia cultivars with improved cold tolerance. Golden camellias (yellow camellias) are beautiful ornamental plants with great market potential. After the first species was published in 1948, only two more species were collected in the next 40 years. In the last 20 years, however, more than 40 new golden camellia taxa were documented (Table 1). The driving force to study golden camellias is their great ornamental market potential and medicinal applications.

Camellia naturally occurs only in southeastern Asia countries. However, wild golden camellias could only be found in China and Vietnam. In addition to one isolated species in Yunnan (China) and two disjunct species in Guizhou (China), all of the other species are in the upper Rongjiang river ranges (Table 2). The geographical location is latitude $21^0$30'– $22^0$55'N, longitude $107^0$36'– $108^0$33'E, and elevation from 100 to 450 (1,100) meters. The climate is a tropical monsoon type with dry and snow-free winters and wet and hot summers. The annual mean temperature is 22.3^0C (72.2^0F). The highest average temperature is 32.7^0C (90.8^0F) in July and the lowest average temperature is 17.2^0C (63.0^0F) in January. The annual precipitation is 1326.0 mm (52.2"). From April to September, the monthly precipitation is more than 100 mm (4"). The annual relative humidity is 79%. The main soil type is acidic red clay with a pH of 4.5-5.5. Golden camellias can also grow with slightly basic soil with a pH of 6.5-7.5. In the past ten years, we have investigated both wild and cultivated golden camellias in Guangxi and adjacent provinces in China. The objective of this paper is to share the preliminary results of these natural species, which should be helpful in plant introduction and breeding.

Results and Discussion: One of the natural species delineation criteria is its geographic location. Gao et al. (5) accepted 34 species and one cultivar. Among them, 32 species are distributed in the upper Rongjiang river ranges, including China (Guangxi) and Vietnam. This portion of Rongjiang River occupies about 600 km (378 miles) long and 300 km (190 miles) wide. It is hard to understand that more than 90% of golden camellias occurred in this area. The legitimacy of these golden camellia species should be further investigated.

Taxonomic confusion of golden camellias is reflected in Table 1. Three well-known taxonomists have only agreed upon eight species and one variety. The other 30 or more taxa are still in question! Authors had reviewed some publications of new golden camellia plants and learned that the nomenclature did not follow the "International Code of Botanical Nomenclature" and "International Code of Nomenclature for Cultivated Plants". Secondly, morphological variations under different niche climates contributed to the taxonomic confusion. It is possible that some published species may actually be variants within a species. For horticultural professionals, these natural variations may be evaluated in our research plots and gardens, and may lead to development of new cultivars. Although only one cultivar and two hybrid cultivars were documented, there is tremendous potential to explore selected new cultivars of golden camellias from their natural habitats.

Breeding of new golden camellias was initiated more than 20 years ago. The focus of the breeding work is to transfer the intense yellow pigmentation to some cold hardy Camellia species and cultivars (1). Huang and Zhang (6) reported that they successfully crossbred *C. nitidissima* with *C. japonica* and *C. sansanqua* and obtained two cultivars, 'Multiply' and 'Xinzi'. In the future, breeding work should focus on cold hardiness of golden camellias to bring these beautiful plants from controlled environments to outdoor landscapes.

Production of golden camellias is very successful. From seed germination and rooting of cuttings, to grafting (on young and old stock plants), thousands of new plants were generated each year in Guangxi. We visited some camellia collection sites and found species, such as *C. nitidissima*, that could overwinter in Zhejiang and Hunan provinces (USDA zone 7-8) with minor winter damage. With some winter protection, we can probably grow some golden camellias along the US southeastern coast.

Germplasm collection, breeding, and conservation of golden camellias are underway. If you have the chance to visit Guangxi, China in late March to early May, be sure to visit Collection of Golden Camellia in Guangxi Academy of Forestry (Nanning, Guangxi), Golden Camellia Park (Nanning, Guangxi), and Golden Camellia Natural Reserves (Fangcheng, Guangxi). The bright golden flowers are breathtaking. After careful examination, you would find that the diversity of flower texture, sizes, and floral structures are phenomenal. It is possible that we will crossbreed these plants with some other species and produce some new desirable cultivars for our landscapes. Further plant exploration, collection, and molecular-aided studies will ensure our success. We look forward to collaborating with you and bringing better golden camellias to our daily gardens.

Acknowledgement: Financial support from Guangxi Academy of Forestry, Hunan Provincial Government, University of Maine, and Central South University of Forestry and Technology is appreciated. Great thanks should go to the field guides and staff in Golden Camellia Park (Nanning, Guangxi) for their support and help.

Supporting Online Material:
http://www.umaine.edu/maineplants/TalkDZ/SNA08GoldTea.pdf
Slide presentation for the 53rd SNA Research Conference.

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Table 1: Taxonomic treatments of golden camellias from Gao et al. (5), Ming (7), and Chang (3).

No.	Gao et al. (2005)	Ming (2000)	Chang (1998)
01	C. achrysantha	= C. petelotii	C. achrysantha
02	**C. aurea**	**C. aurea**	**C. aurea**
03	(C. chrysantha)	= C. petelotii	=C. nitidissima
04	(C. c. var. longistyla)	= C. petelotii	
05	**C. chrysanthoides**	**C. chrysanthoides**	**C. chrysanthoides**
06	C. crassiphylla	(Vietnam)	(Vietnam)
07	C. cucphuongensis	(Vietnam)	(Vietnam)
08	**C. euphlebia**	**C. euphlebia**	**C. euphlebia**
09	(C. e. var. yunnanensis)	= C. fascicularis	
10	**C. fascicularis**	**C. fascicularis**	**C. fascicularis**
11	C. flava	(Vietnam)	(Vietnam)
12	C. flavia f. polypetala	= C. flavida	
13	**C. flavida**	**C. flavida**	**C. flavida**
14	n/a	C. f. var. patens	
15	C. grandis	n/a	C. grandis
16	C. huana	C. huana	C. liberofilamenta
17	C. hulungensis	(Vietnam)	(Vietnam)
18	**C. impressinervis**	**C. impressinervis**	**C. impressinervis**
19	C. 'innominata'	n/a	n/a
20	C. leptopetala	n/a	C. leptopetala
21	C. liberofilamenta	= C. huana	C. liberofilamenta
22	C. limonia	= C. indochinensis	
23	C. l. f. obovata	= C. indochinensis	
24	n/a	C. i. var. tunghinensis	C. tunghinensis
25	(C. longganensis)	= C. flavida	C. longganensis
26	(C. l. var. grandis)	= C. flavida	C. longganensis
27	C. l. var. patens)	= C. f. var. patens	
28	C. longruiensis	= C. flavida	
29	C. longzhouensis	= C. chrysanthoides	
30	**C. micrantha**	**C. micrantha**	**C. micrantha**
31	C. multipetala	= C. f. var. patens	n/a
32	C. nitidissima	= C. petelotii	C. nitidissima
33	**C. n. var. microcarpa**	**C. n. var. microcarpa**	**C. n. var. microcarpa**
34	(C. n. var.phaeopubisperma)	= C. petelotii	C. nitidissima
35	C. parvifolia	n/a	n/a
36	C. parvipetala	= C. micrantha	C. micrantha
37	C. petelotii	C. petelotii	n/a
38	C. pingguoensis	C. pingguoensis	n/a
39	C. p. var. terminalis	C. p. var. terminalis	= C. terminalis
40	C. ptilosperma	= C. flavida	
41	**C. pubipetala**	**C. pubipetala**	**C. pubipetala**
42	(C. quinqueloculosa)	= C. f. var. patens	
43	C. rosmannii	(Vietnam)	(Vietnam)
44	C. tamdaoensis	(Vietnam)	(Vietnam)
45	C. tianeensis	= C. huana	n/a
46	C. tunghinensis	= C. i. var. tunghinensis	C. tunghinensis
47	C. wumingensis	= C. f. var. patens	
48	C. xiashiensis	= C. chrysanthoides	= C. parvipetala

Table 2: Distribution of golden camellias in the world (5).

Country	Province	Number of taxa	Percentage (%)
China	Guangxi	22	62.8
China	Yunnan	1	2.9
China	Guizhou	2	5.7
Vietnam	Vinh Phuc	4	11.4
Vietnam	Ninh Binh	1	2.9
Vietnam	Quang Ninh	1	2.9
Vietnam	Lang Son	2	5.7
China/Vietnam	Guangxi/Lang Son	2	5.7
Total		35	100

Fertility of Neopolyploid *Rhododendron* and Occurrence of Unreduced Gametes in Triploid Cultivars

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Index Words: Pollen Viability, Polyploidy, Unreduced Gamete

Significance to Industry: Polyploidy, the condition of having multiple sets of chromosomes, has important implications for plant breeding and can influence ornamental characteristics, crossability, plant vigor, and gene expression. Polyploidy can also have a profound influence on reproductive biology, including fertility. The potential for utilizing polyploids in a breeding program is dependent upon fertility of specific taxa. A greater understanding of fertility mechanisms in polyploid *Rhododendron* and information on fertility of specific clones will better allow breeders to utilize polyploids in plant improvement programs, ultimately leading to the development of improved cultivars for the nursery industry.

Nature of Work: Fertility of newly developed polyploids (neopolyploids) can vary considerably and is influenced by their specific origins (5). Polyploids that arise from within a single species are referred to as autopolyploids. Autopolyploids may lack fertility due to the presence of multiple homologous chromosomes that can result in multivalent pairing and unequal segregation in meiosis (4). Polyploids that arise from hybrids between species are referred to as allopolyploids. Allopolyploids are often fertile due to nonrandom, disomic pairing between two distinct sets of chromosomes from the two parental species. In many cases, however, polyploids fall somewhere between an autopolyploid and an allopolyploid; where there is partial chromosome homology resulting in a combination of disomic and polysomic pairing, often referred to as segmental allopolyploids (4).

Moreover, fertility can also be affected by the number of chromosome sets. Many times, odd-ploidy cytotypes are found to be highly infertile, if not sterile (4). Such is the case with triploids within the genus *Rhododendron*. Infertility of triploids results from the fact that three sets of chromosomes cannot be divided evenly during meiosis, yielding unequal segregation of the chromosomes often resulting in aneuploid gametes or meiotic failure. Many triploids possess desirable ornamental characteristics in growth and flower morphology, yet the reproductive biology of triploids in *Rhododendron* is not well understood. Unreduced gametes may be associated with triploids (6) and can be utilized for breeding and as bridges for gene transfer between polyploid levels (4). The pollen structure in *Rhododendron* is typically a tetrad of grains that are tightly grouped at maturity; however, the structure of unreduced pollen is a mixture of larger dyads and monads. The objectives of this project were to: 1) evaluate the effect of increased ploidy level on pollen fertility of selected *Rhododendron* and 2) evaluate pollen fertility of naturally occurring triploids found in the genus.

Comparing fertility between ploidy levels. To study the influence of increased ploidy level on fertility, neopolyploids and their progenitor taxa were chosen, based on prior work by Jones et al. (2), to compare the pollen viabilities between ploidy levels within the same genotype. All pollen was collected at anthesis from plants at the Mountain Horticultural Crops Research Station, dried at 70ºF (~21ºC) for 24 hrs., and stored at -13ºF (-25ºC) until testing. Pollen was placed on glass microscope slides, and the grains were stained with 1% acetocarmine (w/v) for 15 minutes. Pollen grains that stained a distinct red-pink color were scored as viable (5). The tetrad nature of *Rhododendron* pollen required each individual grain in the tetrad to be analyzed. Each tetrad has the potential to contain four viable grains. The experimental design was a randomized complete block with ten replicates blocked by day over a time of one week. A minimum of 50 tetrads were randomly selected and analyzed per replicate. Pollen was observed at 300× using a light microscope (Nikon Eclipse 80i, Nikon, Melville, NY). Pollen viability percentages were calculated and the data were subjected to analysis of variance and pairwise means comparisons between ploidy levels for a given genotype (LSMEANS option, PROC GLM; SAS version 8.02, SAS Institute., Cary, N.C.; SAS Institute, 1988).

Triploid fertility. The presence of dyad and/or monad pollen grains was utilized to study the existence of unreduced gametes in confirmed triploid taxa (2). Pollen was collected at anthesis, and the frequency of viable, unreduced gametes in the triploid taxa was determined using pollen staining as described above and observed under a light microscope at 300×. The experimental design was a randomized complete block with 5 replicates blocked over time. At least 50 sporads (tetrads, dyads, or monads) were randomly selected and analyzed per replicate. Pollen was considered viable and unreduced if there was a well-stained monad or dyad and the pollen diameter was visibly larger (>120%) than normal. The frequency of unreduced gametes was determined using the equation (3):

Unreduced pollen frequency = [(2 × # of dyads) + (# of monads)] / (# of total grains).

Data were then subjected to analysis of variance and means compared using least significant differences (LSD) (PROC GLM; SAS version 8.02, SAS Institute., Cary, N.C.; SAS Institute, 1988).

Results and Discussion: Comparing fertility between ploidy levels. Pollen grains were readily apparent as being stained or unstained. Pollen viabilities for all taxa ranged from 1.5 – 63.8 % (Table 1). There was a significant effect of ploidy level (P<0.001), genotype (P<0.0001), and a ploidy-genotype interaction (P<0.0001) on fertility. The significant interaction indicated the effect of polyploidy on fertility depended on genotype. The results demonstrated that fertility of polyploid *Rhododendron* can be highly variable and that neopolyploids may have enhanced or reduced fertility depending on the genotype. The effect of polyploidy on fertility most likely results from the level of homology among the chromosome sets and subsequent impacts on chromosome pairing during meiosis. Individuals of autopolyploid origin generally displayed reduced fertility compared to their progenitors. The induced tetraploid form of *R. fortunei* is a prime example of low fertility associated with an induced auotpolyploid.

Reduced pollen fertility of the octoploid, *R.* 'Fragrant Star', is likely the result of the plant's autoallopolyploid background in which polysomic pairing most likely occurs among the 4 homologs from each of the parental species. Increased fertility was observed in polyploids of probable allopolyploid background most likely resulting from disomic pairing of chromosomes at meiosis. The potential to enhance fertility through polyploid induction is evident in the case of *R.* 'Fragrant Affinity'. The diploid form is the result of a wide inter-subgeneric cross and exhibits little to no fertility. Through the creation of an allopolyploid from the diploid progenitor cultivar, male and female fertility were effectively restored (1). In cases where no effect of increased ploidy on fertility was observed, the complex hybrids and induced tetraploids most likely functioned as segmental allopolyploids with moderately high fertility regardless of ploidy level.
Triploid fertility. Viable dyad and monad grains were observed in pollen samples from triploid taxa, ranging from 0.2 to 5.3% (Table 2), indicating the presence of unreduced pollen. The increased size of unreduced pollen diameter was also clearly evident. There was a significant effect (P<0.0001) of taxa on the percentage of unreduced pollen. *Rhododendron* 'Red Wing' had the highest percentage of unreduced pollen at 5.3%, followed by *R.* 'Hallelujah' at 2.9%, while the remaining taxa were similar with less than 1.1% unreduced gametes. In studies of *Vaccinium* spp., also in the *Ericaceae*, significant differences in frequencies of unreduced pollen among taxa have also been reported (3). The production of unreduced pollen by triploid taxa indicated the potential for utilizing certain taxa in breeding programs. The greater frequency of unreduced pollen found in *R.* 'Red Wing' and 'Hallelujah' may allow for successful hybridizations given adequate numbers of pollinations.

Overall, the influence of polyploidy on fertility in *Rhododendron* is highly variable and appears to be influenced by the ploidy level, degree of homology among chromosomes, and in the case of triploids, the frequency of unreduced gamete formation. A greater understanding of fertility mechanisms in polyploid *Rhododendron* and information on fertility of specific clones will allow breeders to better utilize polyploids in plant improvement programs.

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Table 1. Pollen viability of polyploid *Rhododendron* and progenitor taxa.

Taxa	Genotype	Ploidy Level	Viability (%)[1]	Contrast[2]
'Nova Zembla'	1	2*x*	33.4 ± 1.8	NS[3]
'Super Nova'	1	4*x*	30.4 ± 2.5	
'Vulcan'	2	2*x*	48.0 ± 1.3	NS
'Vulcan Tetra'	2	4*x*	46.5 ± 6.4	
'Snowbird'	3	4*x*	63.8 ± 3.5	*P*<0.0001
'Fragrant Star'	3	8*x*	16.4 ± 1.5	
'Fragrant Affinity'	4	2*x*	1.5 ± 0.2	*P*<0.0001
'Fragrant Affinity Tetra'	4	4*x*	19.9 ± 2.3	
R. fortunei	5	2*x*	47.4 ± 4.0	*P*<0.0001
R. fortunei 'Tetra'	5	4*x*	7.7 ± 1.8	
'PJM'	6	2*x*	31.6 ± 1.4	*P*<0.0001
'Northern Starburst'	6	4*x*	47.0 ± 1.3	
'The Honourable Jean Marie de Montague'	7	2*x*	28.7 ± 2.8	*P*<0.0001
'Briggs Red Star'	7	4*x*	11.0 ± 1.1	
'Weston's Aglo'	8	2*x*	19.6 ± 0.9	*P*<0.0001
'Bubblegum'	8	4*x*	58.8 ± 3.2	

[1]Values represent means ± SEM for 10 replications.
[2]Contrast represents LSD, $_{0.05}$ mean separations between common (highlighted) genotypes of different ploidy levels.
[3]NS = Not significant.

Table 2. Percent unreduced gametes in selected triploid *Rhododendron* taxa.

Taxa	Viable Unreduced Gametes (%)[1]
'Hallelujah'	2.87 ± 0.55 B
'Red Wing'	5.31 ± 0.81 A
'Taurus'	1.09 ± 0.13 C
'White Ruffles'	0.65 ± 0.19 C
Azaleodendron 94-28/2	0.45 ± 0.19 C
Azaleodendron 94-28/3	0.98 ± 0.27 C
Azaleodendron 94-28/7	0.60 ± 0.23 C
Azaleodendron 94-28/14	0.23 ± 0.01 C

[1]Values represent means ± SEM. Means followed by a different letter are significantly different at *P*<0.05.

Selection for Early Blooming in Crapemyrtles

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Index Words: *Lagerstroemia,* ornamental breeding, flowering

Significance to Industry: Crapemyrtles (*Lagerstroemia indica* L. and related interspecific hybrids) are highly desirable landscape plants in the southern United States because of ease of production and culture, striking flower colors and long-lasting blooms, tolerance to biotic and abiotic stresses, a range of plant habits, and exfoliating bark (2). Flowering usually begins as early as mid-June and continues through the end of September (2,5), although a range of bloom periods has been observed (1). The Razzle Dazzle™ series includes five dwarf *Lagerstroemia* cultivars that flower in mid- to late summer (4). Studies by Pounders et al. (3) showed genetic control for early flowering in *Lagerstroemia.* The development of dwarf cultivars with earlier flower initiation and long bloom period would enhance the horticultural value of this genus.

Nature of Work: In spring 2005, 23 *Lagerstroemia* seedlings from 3 populations began flowering approximately 75 days after initial seed sowing. This study was conducted to determine whether these plants would express the same early flowering trait when they were more mature, to determine initial flowering time for progeny from these plants, and to determine length of bloom period in these plants. All three populations were derived from selections made by Dr. Michael A. Dirr in the University of Georgia crapemyrtle breeding program. Population 1 originated from open-pollinated seed of breeding line dwarf #7, which was a selection from an open-pollinated population of 'Pocomoke'. Population 2 was derived from offspring of a cross between two cultivars, 'Cherry Dazzle™' and 'Raspberry Dazzle™', and population 3 came from a cross between breeding line #35-6 (also selected from an open-pollinated population of 'Pocomoke') and breeding line dwarf #7. Seed were sown in a research greenhouse in January, 2005. When plants reached a height of approximately 7 cm, it was noted 23 seedlings from these three populations had begun to flower. These 23 plants were selected and grown in 2005 to evaluate flower and leaf color, plant growth habit, amount of flower production, and pest resistance. In fall 2005, 11 of these plants produced small quantities of seed. This open-pollinated seed was sown in 2006. From this seed, 75 seedlings were selected based on dwarf plant growth habit, were transplanted to 3-gallon containers and were grown on the same nursery pad area as their parent plants. In the fall of 2006, open-pollinated seed was collected from the 20 plants that set seed from among the original 23. In 2007, this seed was sown, 240 seedlings were selected based on dwarf plant growth habit, transplanted to 3-gallon containers and grown on the same nursery pad. These populations allowed us to evaluate and compare date of first flower and bloom period in physiologically mature plants that had flowered for three years, in their mature open-pollinated offspring that had flowered for two years, and in

open-pollinated populations derived from the original 23 plants that were flowering the first year from seed. In 2007, all plants in this study were evaluated twice weekly for date of first flower and date of last flower, and at the end of the growing season were also evaluated for seed production and leaf retention.

Results and discussion: The plants initially selected for early seedling flowering did not express this trait in subsequent years. In 2006 (data not shown), the average flowering date for these plants was July 5, and no early flowering was observed in the 75 selected offspring. In 2007 the average flowering date for the original plants and their two-year-old offspring from the original plants was the same (Table 1). From the initial 23 selections the earliest flowering were two plants from population 1 that flowered in early June in both 2006 and 2007. In 2007, these two plants both started flowering on June 13. However, neither plant produced dwarf offspring with early flowering. Two-year-old offspring from the first plant had an average first flower date of July 2 and year-old offspring averaged July 23. Neither date was significantly different from the overall average of all plants in the study. Offspring from the second plant flowered on similar dates. Overall, first flower date of female parent plants was a poor predictor of their open-pollinated offspring performance among mature plants (r=0.33). Selection of female parent plants based on rank showed no correlation with rank of flowering date for their offspring (r=0.00). Female parent phenotype cannot be used in these populations to predict average date of first flower in these segregating populations. Flower duration was weakly correlated between female parent and two-year-old offspring (r=0.55). However rank correlation could not be used to select female plants whose open-pollinated offspring would exhibit desirable traits. Older plants retained leaves longer than 1-year-old plants. The negative correlations for leaf retention between the oldest plants and their 1-year-old offspring suggest that this may be an artifact of maturity rather than a trait under strong genetic control in these populations.

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Table 1. Mean values for four *Lagerstroemia* plant characteristics in original plants and selections from open-pollinated offspring populations, along with correlation and rank correlation values.

Characteristic	Original plants (3-years-old)	2-year-old offspring	1-year-old offspring	Correlation (r) between original plants and 2-year old offspring	Rank correlation (r) between original and 2-year old offspring	Correlation (r) between original plants and 1-year old offspring	Rank correlation (r) between original and 1-year old offspring
Date of first flower	July 2a	July 2a	July 28b	0.33^{NS}	0.00^{NS}	0.36^{NS}	0.47*
Bloom duration	76a	68a	58b	0.55*	0.45^{NS}	0.13^{NS}	0.08^{NS}
Seed production rating	3.2a	3.2a	3.3a	0.52^{NS}	0.40^{NS}	0.08^{NS}	0.04^{NS}
Leaf retention rating	2.6a	2.8a	3.2b	0.00^{NS}	-0.04^{NS}	-0.34^{NS}	-0.39^{NS}

Values within a row followed by the same letter are not different at *P*>0.05 based on LSD

^{NS} and * indicate correlation coefficients that are not sigificant or significant at $P \leq 0.05$, respectively

Seed production rating based on a 1-5 scale, with 1 being sterile and 5 highly prolific

Leaf retention rating based on a 1-5 scale, with 1 having no leaf loss and 5 having no leaf retention

Evaluation of a 15-year-old Carolina Silverbell Provenance Trial

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Index Words: *Halesia carolina*, *Halesia tetraptera, Halesia parviflora,* Styracaceae, Germplasm

Significance to Nursery Industry: Carolina silverbell (*Halesia carolina* L.) is an underutilized flowering tree, native to woodland habitats across the southeastern United States. Much variation has been documented in floral, fruit, and growth habit characteristics across its range. Understanding this variation is necessary for selecting improved cultivars for the nursery industry and for forming the basis of a *Halesia* breeding program. Significant correlations with latitude for morphological characters in a provenance trial of *H. carolina* indicate significant variation exists for important ornamental traits necessary to develop new silverbells for the nursery industry.

Nature of Work: Carolina silverbell (*Halesia carolina* L. Styracaceae) is a small, multi-stemmed to large, single-trunked tree native to the southeastern United States, from eastern Oklahoma to the Coastal Plain of the Carolinas, and southern Ohio to northern Florida (1). As the name implies, flowers are bell-shaped, typically white to rose-pink, and occur prior to, or as, new foliage emerges, and are the primary ornamental feature of the genus. Fruits are ellipsoid to clavate shaped, four-winged corky drupes that persist on the tree throughout fall and winter, and are often present at flowering. Although a half-a-dozen cultivars exist (1), they are not common in cultivation. *Halesia carolina* and *H. diptera* J.Ellis (two-wing silverbell) are the two currently recognized species native to North America, with a third species (*H. macgregori* Chun.) native to China (2,3). Taxonomic investigations into the former *H. carolina* complex [syn. *H. monticola* (Rehder) Sarg.*, H. parviflora* Michx.*,* and *H. tetraptera* J.Ellis], demonstrated that the previous division of *H. carolina* into several species or infraspecific divisions was not warranted, as taxonomically relevant characters showed continuous variations correlated with latitude: as latitude increases, flower and fruit characters generally increase (2). As these characters are relevant ornamental features and selection criteria for plant breeding programs, we tested this clinal pattern of variation to latitude for *H. carolina sensu latu* against our germplasm collection to 1) verify observations by Fritsch and Lucas (2) on plants growing in a common garden experiment and 2) evaluate the collection for identifying elite parents to initiate a *Halesia* breeding program.

A large germplasm collection of *H. carolina*, collected over the species range, was planted at the U.S. Department of Agriculture (USDA) Plant Introduction Station, in Glenn Dale, Maryland (38°57’50” N, 76°48’15”W). The provenance trial consisted of

half-sib families sown from open pollinated seeds from selected trees in seven states (AL, GA, OK, NC, SC, TN, WV) beginning in 1992. The bulk of the plantings occurred between 1995 and 1997, although some accessions were planted as late 1999. Planting design was completely randomized by collection locality (county, state), with uneven replication (locality) and subsamples (seedlings per collection). Latitude and longitude were determined from collection notes or assigned based on county centroids. Data collected was based on the methods of Fritsch and Lucas (2); only fruit data is presented, and includes: FRLENGTH, fruit semi-width at 1/3 (FRWIDTHA) and 2/3 (FRWIDTH B) of fruit length from the distal end, FRWIDTHDIF [(FRWIDTHA - FRWIDTH B)/ FRWIDTHA], fruit wing width at 1/3 (WINGA) and 2/3 (WINGB) of fruit length from the distal end, and WINGDIF [(WINGA - WINGB)/WINGA]. Height and diameter-at-breast height (DBH) were measured, and habit recorded (scored as 1=multi-stem, no dominant stem, 2= multi-stem, with dominant stem, or 3=single stem). All characters were subject to pairwise comparisons with latitude using Pearson's correlations with significant character correlations used to test for partial correlations.

Results and Discussion: Fruit morphological characters exhibited weak but significant positive correlations with latitude, ranging from r=0.147 to 0.190 (Table 1). Fruit morphological characters exhibited stronger correlations with other fruit characters, from r= -0.467 to 0.742. Weak significant positive correlations also occurred between growth and fruit characters, with r=0.109 to 0.180. As expected, there were positive correlations between growth parameters for height and DBH (r=0.876) but weaker for height and habit (r=0.195), as well as DBH and habit(r=0.143). In general, fruit characters exhibited weak positive correlations with growth, such that taller plants tended to have single-trunks with larger DBH, but no consistent correlations with fruit shape (Table 1). Partial correlations were calculated for each character that showed significant correlations with latitude, to test whether these variables were independent from non-significant characters (Table 1). FRWIDTHA, FRWIDTHB, WINGA, WINGB, and habit in partial correlation with latitude produced similar positive correlation coefficients as the prior analysis, indicating that fruit size increases with increasing latitude, and that plant habit tends towards single-trunk trees with increasing latitude. The lack of significant correlations between height and DBH for latitude (Table 1) may be due to the extended planting schedule of the original provenance trial. However, using habit as a proxy for ultimate height, the weak significant correlation between habit and latitude may indicate that given time, plant height may show the same positive correlation with latitude, as suggested by Fritsch and Lucas(2). After 13 years, some of the oldest plantings are 7.4 m (24.3 ft) tall (e.g. latitude 35°09'16") while 10-year-old plantings had a mean height of 5 m (16.4 ft) (data not shown). While the provenance trial included half-sib families from seven states across five degrees of latitude (Table 2), our collection represents mainly Appalachian populations at the mid-latitudes of the range (formerly *H. tetraptera*), and does not include collections from lower latitudes in southern Alabama and Georgia, and northern Florida that are representative of the small-flowered, multi-stemmed, clavate fruit populations of *H. carolina* (formerly *H. parviflora*) that could have strengthened our correlations with latitude.

Our results are in general agreement with Fritsch and Lucas (2) who found significant correlations with latitude and suggested that small, multi-stemmed trees at low latitudes would transition to large, single-trunk trees at higher latitudes. Our provenance trial served as a common garden experiment to test this, resulting in a weak positive correlation for habit with latitude. Correlations for the other traits indicate that they are under genetic control and can be selected for in a breeding program. Further analysis will test whether flower size is correlated with fruit size and latitude to determine ideal parents for breeding.

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Table 1. Pairwise Pearson's correlations for morphological and growth characters of a provenance trial of Halesia carolina. Upper right portion, Pairwise comparision (n=446 to 469, depending on comparison). Lower left portion, partial correlations for significant characters with latitude holding other characters constant (n=440 per comparison). *, **, *** denotes $P < 0.05$, 0.01, and 0.001, respectively.

	Latitude	FRLENGTH	FRWIDTHA	FRWIDTHB	FRWIDTHDIF	WINGA	WINGB	WINGDIF	Height	DBH	Habit
Latitude	---	-0.062	0.166***	0.163***	-0.017	0.190***	0.147***	0.073	0.038	-0.011	0.178***
FRLENGTH		---	0.259***	0.009	0.288***	0.171***	-0.057	0.239***	0.109*	0.111*	0.016
FRWIDTHA	0.180***		---	0.674***	0.318***	0.658***	0.440***	0.269***	0.146**	0.091	0.070
FRWIDTHB	0.173***		0.976***	---	-0.463***	0.473***	0.742***	-0.165***	0.081	0.046	0.042
FRWIDTHDIF					---	0.162***	0.430***	0.538***	0.50	0.031	0.031
WINGA	0.174***		0.715***	0.695***		---	0.416***	0.580***	0.180***	0.128***	0.117*
WINGB	0.201***		0.749***	0.749***		0.949***	---	-0.467***	-0.022	-0.031	-0.019
WINGDIF								---	0.179***	0.138***	0.125***
Height									---	0.876***	*
DBH										---	0.143*
Habit	0.140**		0.031	0.036		0.035	0.028				---

Table 2. Mean height, DBH, and habit for combined half-sib *Halesia carolina* families according to latitude of original parent trees. Habit scored as 1= multi-stemmed, no dominant stem; 2= multi-stem, with dominant stem; and 3=single stemmed.

County, State	Latitude	n (families, individuals)	Height m (ft)	DBH cm (in)	Habit
Fayette, WV	38°02'28"	1, 20	5.6 (18.4)	7.9 (3.1)	2.7
Cocke, TN	35°56'53"	1, 4	3.0 (9.8)	2.9 (1.2)	2.3
Sevier, TN	35°48'12"	5, 12	3.7 (12.1)	4.3 (1.7)	2.3
Burke, NC	35°46'45"	3, 21	5.2 (17.1)	8.2 (3.2)	2.7
Buncombe, NC	35°37'15"	1, 1	5.7 (18.7)	6.4 (2.5)	3.0
Haywood, NC	35°32'26"	2, 8	4.5 (14.8)	6.0 (2.3)	3.0
Swain, NC	35°29'17"	6, 41	5.1 (16.7)	7.3 (2.9)	2.8
Cherokee, NC	35°28'23"	3, 14	5.2 (17.1)	7.3 (2.9)	2.4
Rutherford, NC	35°23'45"	2, 34	5.5 (18.0)	7.7 (3.0)	2.7
Jackson, NC	35°15'50"	4, 15	5.6 (18.4)	7.7 (3.0)	2.6
Macon, NC	35°09'45"	6, 16	6.0 (19.7)	7.9 (3.1)	2.7
Polk, TN	35°09'16"	6, 91	7.4 (24.3)	11.3 (4.5)	2.5
Clay, NC	35°04'27"	3, 12	3.6 (11.8)	3.6 (1.4)	2.4
Oconee, SC	34°45'53"	2, 11	3.6 (11.8)	4.7 (1.8)	2.3
Cherokee, AL	34°45'09"	2, 3	5.3 (17.4)	7.4 (2.9)	2.7
McCurtain, OK	34°19'10"	1, 3	1.8 (5.9)	1.5 (0.6)	1.0
St. Clair, AL	33°41'22"	11, 106	5.6 (18.4)	8.4 (3.3)	2.4
Columbia, GA	33°33'38"	1, 15	5.7 (18.7)	8.9 (3.5)	2.5
Jefferson, AL	33°32'40"	5, 39	5.1 (16.7)	7.7 (3.0)	2.4
Bibb, AL	33□02'23"	2, 4	4.9 (16.1)	6.1 (2.4)	1.5

In-vitro Polyploid Induction of *Rudbeckia spp.*

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Index Words: Mitotic Inhibitor, Flow Cytometry, Polyploidy, Chromosome Doubling, Tissue Culture, *Rudbeckia maxima, Rudbeckia hirta, Rudbeckia subtomentosa*

Significance to the Industry: The genus *Rudbeckia* contains many diverse, adaptable, and desirable ornamental species. The development of new polyploids, through chromosome doubling, may increase ornamental characteristics, expand breeding opportunities, and restore fertility in sterile hybrids – ultimately leading to the development of improved cultivars. In-vitro treatments, ranging from 15 to 60 µM oryzalin over 3 to 5 days, were effective at inducing polyploidy, depending on taxa. New tetraploids of *R. maxima, R. subtomentosa*, and a novel interspecific hybrid were successfully developed and will be evaluated for ornamental characteristics and utilized in an ongoing breeding program to create improved hybrids.

Nature of the Work: The genus *Rudbeckia* contains approximately 30 species of annuals, biennials and perennials easily distinguished by their signature colorful ray corollas and disk shaped receptacles. *Rudbeckia maxima* is one of the tallest species in the genus, reaching over seven feet, and is noted for its blue-green foliage and large inflorescences with prominent cones. *Rudbeckia subtomentosa* has vibrant yellow florets and is a reliable perennial with broad adaptability and disease resistance (1). *Rudbeckia subtomentosa* 'Henri Eilers' is a unique cultivar with attractive quilled ray florets. The hybrid H062 is a cross between the durable perennial *R. subtomentosa* and showy annual *R. hirta*, developed at NC State University, but appears to be sterile. Polyploidy occurs naturally in *R. fulgida* and *R. hirta* species (3). The creation of tetraploids in *R. maxima*, *R. subtomentosa* 'Henri Eilers,' and H062 could enhance ornamental traits, facilitate hybridization with other tetraploids, and restore fertility in the interspecific hybrid H062. Oryzalin has been found to be an effective chromosome doubling agent, but optimal treatments (including dose and duration of exposure) vary for different species (2). The objectives of this study were to evaluate the efficacy of varied dosages and durations of oryzalin exposure as an in-vitro chromosome doubling treatment for *Rudbeckia spp*. and to develop new tetraploid clones for use in breeding projects.

Tissue culture: Material for this study was selected from *R. maxima*, *R.* H062, and *R. subtomentosa* 'Henri Eilers' tissue culture collections maintained at the Mountain Horticultural Crops Research and Extension Center, Fletcher, NC. Cultures were maintained on Murashige and Skooge (MS) basal media supplemented with 3% sucrose and 2 µM benzylamino purine (BAP). The pH was adjusted to 5.75 ± 0.03 and the media was solidified with 0.8% agar. Stock cultures were maintained at 23 ^{o}C under

cool white fluorescent lights (130 μmol·m^{-2}·s^{-1}) with a 16 h photoperiod and subcultured every four weeks to create a sufficient population sample size for each taxa. A minimum of 30 shoots (3-5 mm in length) were harvested for oryzalin treatments 20-24 days after subculturing.

Oryzalin treatment: Oryzalin (1 M stock solution, dissolved in 70 % ethanol) was filter sterilized and added to cooled liquid MS media after autoclaving. For *R. subtomentosa*, shoot apices were excised from in-vitro grown plantlets and treated with different concentrations of oryzalin (0, 15, 30, 60, or 90 μM) for 3, 5 or 7 d in a factorial combination. *Rudbeckia maxima* and *R.* H062 were only treated with 0, 60 or 90μm for 3 or 5 d due to lower amounts of available tissue. Shoot apices were placed in baby food jars containing 25 mL of oryzalin solution and placed on an orbital shaker. After treatment, the shoot apices were rinsed in distilled water for ten minutes, three separate times, and placed on shoot regeneration medium (MS media with 2μM BAP). Cultures were then placed under standard culture conditions in a completely randomized experimental design. Each taxa was treated as a separate experiment. Mortality was recorded four weeks after the completion of each treatment. Survival data were analyzed using analysis of variance and LSD means separation (SYSTAT 10, SYSTAT, Chicago, IL).

Flow Cytometry: Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry for each surviving shoot eight weeks after initial treatment. Approximately 0.25 sq. in. (1.6 sq. cm.) of leaf, shoot or callus tissue was chopped with a razor blade in a petri dish containing 400 μL of cold extraction buffer (CyStain UV Precise P, Partec, Münster, Germany). The suspension was filtered through 50-μm nylon mesh and nuclei were stained using 1.6 mL staining buffer containing 4', 6-diamidino-2-phenylindole (DAPI) (CyStain UV Precise P, Partec). The suspension was analyzed using a flow cytometer with fluorescence excitation provided by a mercury arc lamp (PA-I Ploidy Analyzer, Partec). The mean fluorescence of each sample was compared with an internal standard of known ploidy and DNA content. Only samples with sufficient tissue were analyzed; remaining samples will be tested at a later date.

Results and Discussion: Tetraploids were successfully induced in all three taxa, demonstrating that oryzalin is an effective mitotic inhibitor for inducing polyploidy in *Rudbeckia* species (Table 1). Oryzalin has also been used successfully to double chromosome numbers of in-vitro grown shoots of *Buddleja*, *Miscanthus*, *Syringa,* and *Rhododendron* (2, 4, 5, 6, and 7). Oryzalin-treated shoot apices were observed to grow slower than non-treated shoot apices. In some treatments, slow growth limited the amount of material sufficient for ploidy analysis at this time. It was also observed that an increase in exposure and concentration of oryzalin treatments resulted in greater callus and less organized shoot growth in the surviving shoot apices (data not presented).

There was a significant interaction between oryzalin concentration and duration of exposure on shoot apex survival for all three taxa ($P<0.05$). In general, tissues were more sensitive to increasing oryzalin concentrations as duration of exposure increased, resulting in reduced survival at the higher concentration/duration combinations (Table 1). For *R. subtomentosa*, all individuals subjected to the 7 d duration (with the exception of 1 shoot treated with a 30 µM concentration) and/or the 90 µM oryzalin concentration died, indicating that those treatments were excessive. For *R.* H062 and *R. maxima*, all plants died following 5 day treatments of either 60 or 90 µM oryzalin also indicating that those treatments were too extreme.

There were no significant effects of oryzalin dose or duration on the percent of homogeneous tetraploid formation for any of the taxa. However, for *R. subtomentosa*, tetraploids were induced in both the 3 d, 15 and 30µM treatments and in the 5 d, 30 and 60 µM treatments which produced 25, 38, 14, and 22% tetraploids respectively (Table 1). For *R.* H062 a mixaploid and 3 tetraploids (43%) were produced by the 3 d, 60 µM concentration treatment. *Rudbeckia* H062 receiving the 5 d, 60 µM treatment were not large enough to sample for ploidy analysis at this time. Mixaploids, a conglomeration of cells of varying ploidy levels, may result from oryzalin not penetrating or affecting all initial cells and histogenic layers or due to asynchronous cell cycling among initial cells. Only one *R .maxima* tetraploid (6%) was recovered from the 3 d, 60 µM treatment. The remaining three *R. maxima* survivors from the 3 d, 90 µM treatment were not of sufficient size for testing and will be tested at a later date. Lack of clear trends in dose and duration of oryzalin treatments on polyploid induction were also reported for *Rhododendron* hybrids (6) and may reflect the random variation in cell cycles, tissue sensitivity, and chemical penetration.

Results from this study have shown that oryzalin is effective for in-vitro induction of polyploids in *Rudbeckia spp*. In-vitro treatments, ranging from 15 to 60 µM oryzalin over 3 to 5 days, were taxa dependent but effective at inducing polyploidy, while minimizing mortality. Polyploids developed from these studies will be evaluated for ornamental merit and use in ongoing breeding efforts.

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Table 1: Effects of oryzalin treatments on survival and polyploid induction in selected *Rudbeckia* taxa.

Taxa	Concentration (µM)	Duration (Days)	Survival (%)[Z]	Ploidy Level (%) 2x	Mix[Y]	4x
R. subtomentosa 'Henry Eilers'	0	3	100 A	100	0	0
		5	100 A	100	0	0
		7	100 A	100	0	0
	15	3	70 B	75	0	25
		5	-[X]	-[X]	-[X]	-[X]
		7	0 E	-	-	-
	30	3	47 C	63	0	38
		5	20 D	85.71	0	14.29
		7	3 E	-[W]	-[W]	-[W]
	60	3	23 D	100	0	0
		5	23 D	78	0	22
		7	0 E	-	-	-
	90	3	0 E	-	-	-
		5	0 E	-	-	-
		7	0 E	-	-	-
R. H062	0	3	100 A	100	0	0
		5	100 A	100	0	0
	60	3	67 B	50	12.5	37.5
		5	0 D	-	-	-
	90	3	43 C	-[W]	-[W]	-[W]
		5	0 D	-	-	-
R. maxima	0	3	100 A	100	0	0
		5	100 A	100	0	0
	60	3	63 B	94	0	6
		5	0 D	-	-	-
	90	3	17 C	100	0	0
		5	0 D	-	-	-

[Z]Means followed by different letters within columns for a given taxa are significantly different, LSD $P < 0.05$.

[Y]Mixaploid (cytochimera) tissue.

[X]Missing treatment.

[W] Insufficient tissue for testing.

Evaluation of Cuphea Species for Traits of Ornamental Value

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Index Words: *Cuphea splendida* var. *viridiflava, C. pulchara*

Significance to Industry: In recent years several ornamental forms of cuphea have been marketed as summer bedding plants and/or perennials adapted to the heat and humidity of the southeastern U.S. Selection of additional species and hybrids with superior adaption would provide new products for the nursery industry and provide consumers with alternatives to poorly adapted plant material for the South.

Nature of Work: *Cuphea*, a distant cousin of crapemyrtles, is a genus of about 260 species of annual and perennial plants in the Lythraceae family native to warm temperate to tropical regions of the Americas. The species range from low-growing herbs to semi-woody shrubs up to 2 m tall. At least two herbaceous annual species, *C. viscosissima* and *C. carthagensis*, are native to the southern U.S. The most popular ornamental species include the annual species *Cuphea hyssopifola* (False Mexican Heather) and *C. procumbens* as well as the perennial species *C. ignea* (cigar flower), *C. llavea* (batflower), and C. micropetala (3). All these species are tolerant of moderate moisture stress and are easily cultured under summer conditions in the South. With the exception of the short-day flowering *C. micropetala*, the species flower non-stop during the growing season.

Interspecific hybrids between various ornamental species have also resulted in successful landscape cultivars. One of the most popular is 'David Verity', a presumed hybrid of *C. ignea* and *C. micropetala* (Fig. 1). Recently, four cultivars resulting from crosses between *C. llavea* and *C. procumbens* have been introduced from Australia as the "Flamenco Series" which appear to be a marked improvement over either parent (Fig. 2). Preliminary evaluations of various interspecific combinations in *Cuphea* indicate that hybrids between species with different chromosome numbers are possible within the same taxonomic sections but that crosses between sections are not (2).

Results and Discussion: A number of cuphea species and clones, particularly in the *Cuphea* taxonomic sections *Heterodon* and *Melvilla* (1) with various ornamental traits, have been collected for evaluation under environmental conditions prevalent in the Gulf South. Conditions include full sun and overhead irrigation typical of container production environments in the region. Under such conditions, *C. ignea* and *C. cyanea* have grown very poorly or died. A number of species including *C. lutea, C. viscosissima , C. lanceolata, C. wrightii , C. tolucana* and *C. lanceolata* have grown well but flowering traits and growth habits in the unimproved species have poor horticultural interest (Fig. 3). Plants of C*. angustifolia* (Fig. 4) and *C. micropetala* are particularly robust but are short day flowering species with little summer interest. The growth habit of *C. glutinosa*

is ideal for hanging baskets but the bloom size is small (Fig. 5) without the flowering intensity of *C. hyssopifola.* The color range of flowers is most diverse in *C. procumbens* with a range of pastel shades, reds and purples. A recently introduced species, *C. splendida* var. *viridiflava* (Fig. 6), appears to have great potential as an ornamental because of compact growth habit and abundant display of nice white flowers throughout the growing season.

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Fig. 1. *Cuphea* 'David Verity' is presumed to be a hybrid of *C. ignea* and *C. micropetala* because it displays morphological traits of the two species.

Fig. 2. Cultivars resulting from crosses between *C. llavea* and *C. procumbens* have been introduced from Australia which combine the horticultural attributes of the two species.

Fig. 3. Most wild-type species of *Cuphea* have growth habits and flower traits with limited horticultural interest.

Fig. 4. Plants of C*. angustifolia* are robust but are short day flowering with little summer interest.

Fig. 5. The growth habit of *C. glutinosa* is ideal for hanging baskets but the bloom quality and flowering intensity needs improvement.

Fig. 6. *Cuphea. splendida* var. *viridiflava* has great potential as an ornamental because of compact growth habit and abundant white flowers throughout the growing season.

Relationships between *Hydrangea indochinensis*, *H. macrophylla*, *H. scandens*, and *Dichroa febrifuga* based on SSR markers

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Index Words: *bigleaf hydrangea*

Significance to Industry: Numerous *Hydrangea macrophylla* cultivars exist that demonstrate a wide array of desirable ornamental traits but breeders continue to search for new genetic diversity. Interspecific hybrids between *Hydrangea* species closely related to *H. macrophylla*, such as *H. scandens*, are a potential source of new traits. Recent evidence suggests that *Dichroa febrifuga* is also closely related to *H. macrophylla* and freely interbreeds. Here we report on the potential for breeding between *H. macrophylla* and *H. indochinensis*.

Nature of Work: *Hydrangea macrophylla,* or big leaf hydrangea, is the most popular hydrangea due to showy inflorescence with a long blooming season, broad green foliage, and moderate cold hardiness. Many closely related species have desirable characteristics that could be incorporated into a hydrangea breeding program (10). For example, some *H. scandens* species are known to produce flowers earlier than *H. macrophylla* and are less susceptible to powdery mildew (5). Species of *H. scandens* also exhibit burgundy foliage on purplish stems in full sun situations (3). *Hydrangea scandens* subsp*. chinensis* has white sterile flowers and yellow fertile flowers giving the flower head an over-all yellow appearance. *Dichroa febrifuga* is an evergreen shrub producing persistent, fleshy, iridescent-blue berries. Blue inflorescences begin to appear in spring, even in the absence of available aluminum in the soil, and continues through the summer. *H. indochinensis* is a small to medium evergreen shrub with ornamental value, particularly the dark purple color on the abaxial, or underside, of the leaves.

McClintock (7) considered *H. indochinensis* a synonym for *H. macrophylla* subsp. *stylosa,* while Hinkley (3) identified it as *H. scandens* subsp. *indochinensis.* Dirr (2) and Church (1) have chosen to name it an individual species of *Hydrangea*, but Dirr states "the validity of this species is taxonomically tenuous". McClintock established her findings based solely on herbarium specimens, whereas Hinkley and Dirr explore plants in nature with an eye for breeding. The data included in this report were based on genetic markers called simple sequence repeats, or SSR. SSR data have indicated close relationships between *H. macrophylla* and *Dichroa febrifuga* and *H. scandens* (10). These relationships were confirmed by the production of fertile *D. febrifuga* × *H. macrophylla* (4, 5, 9) and *H. macrophylla* × *H. scandens ssp. angustipetala* hybrids (5). The objective of this study is to use SSRs to determine if *H. indochinesis* also exhibits a close genetic relationship to *H. macrophylla*. If so, it is highly possible that it can be used as a source of new germplasm for hydrangea breeding.

Data from 31 SSR loci were compiled for 19 samples and analyzed for shared allele frequencies to better understand the genetic relationships between *H. macrophylla*, *H. scandens*, *Dichroa* and *H. indochinensis*. Genetic variation was calculated for each taxa by comparing effective numbers of alleles to differences in allele frequencies between taxa (6). Allele sharing statistics were used independently of ploidy differences and all alleles were represented as diploid (8).

Results and Discussion: Genetic similarity between individual samples is shown in Figure 1 which is rooted with *H. paniculata* 'Limelight'. All samples clustered by species except for *H. indochinensis*, which appears to be more closely related to *Dichroa febrifuga* than the other *Hydrangea* species. The native geographic range of these two species may account for this relationship. *Hydrangea macrophylla* and *H. scandens* are native to China and Japan while *H. indochinensis* and *Dichroa* are native to Nepal and Vietnam. Multiple *H. indochinensis* samples are needed to complete the analysis, particularly wild-collected samples from China and Vietnam. Regardless, our analysis suggests that *H. indochinensis* is within the genetic group of species capable of interbreeding with *H. macrophylla*.

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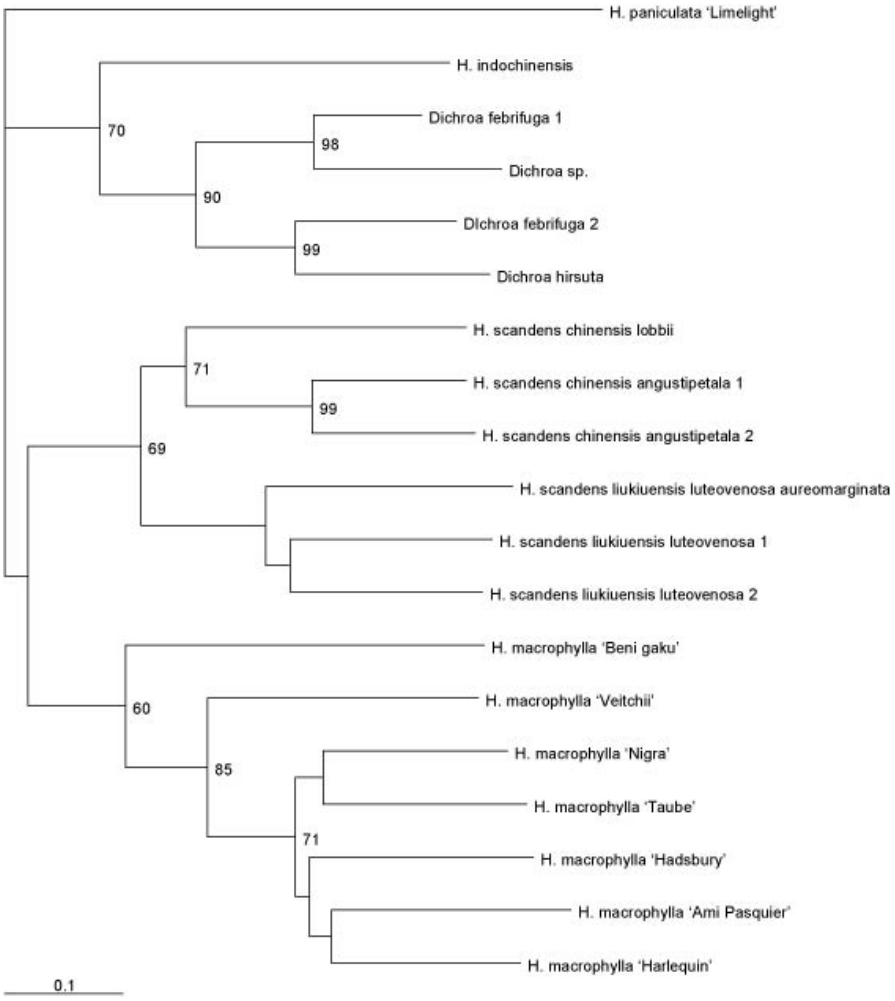

Figure 1. This neighbor-joining tree is rooted with *H. paniculata* 'Limelight'. Numbers correspond to 100 bootstrap replicates where higher numbers indicate more statistical support. Bootstrap values less than 50 are not shown. All of the species form separate groups except for *H. indochinensis*, which appears more closely to *Dichroa* than to the other *Hydrangea* species.

Hydrangea macrophylla Sun Tolerance for the Deep South

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Index Words: bigleaf hydrangea, cercospora leaf spot

Significance to Industry: Although thoughts of hydrangea in full bloom are associated with hot summer days in the south, there is a lack of published information regarding hydrangea performance in the deep-south which includes USDA cold hardiness zones 8 and 9. Landscapers, retailers, and consumers rely on information gathered from vastly different sources including promotional literature. Replicated studies on *Hydrangea macrophylla* (Thunb.) Ser. are only associated with disease tolerance in the deep south, not necessarily sun or heat tolerance. Results from this study will help professionals and consumers in the selection of more sun tolerant varieties of *Hydrangea macrophylla* for warmer climates.

Nature of Work: Tree loss during hurricane Katrina in 2005 dramatically reduced the amount of shade in Southern Mississippi landscapes, particularly near houses. Trees removed during or after the hurricane are not expected to be replaced due to safety concerns. In this study we used full sun conditions that are commonly found in post-hurricane landscapes and evaluated the performance of 26 *Hydrangea macrophylla* cultivars that were listed as tolerant to full sun, at least according to anecdotal evidence (1, 2). None of the literature suggests that tolerance to sun enhanced tolerance to drought. It is widely accepted that even cultivars planted in sufficient shade require afternoon watering to keep foliage from wilting. However, increased sun exposure has been associated with increased cercospora leaf spot incidence (Yonghou Li, personal communication).

In 2006, we initiated a sun tolerance trial of 26 *H. macrophylla* cultivars to determine if cultivars described as “tolerating full sun” would survive the extreme heat and humidity of the deep south. Our hypothesis was that most anecdotal evidence for sun tolerance applies to more temperate zones and no cultivar would tolerate full sun exposure in USDA Hardiness Zone 8b, or American Horticultural Society Heat Zone 9. One-year-old plants were purchased from Amethyst Hill Nursery in Aurora, OR and transplanted into 3-gallon pots in May, 2006. Plants were grown for one season under 50% shade with drip irrigation in pine bark media amended with Osmocote 18-6-12, Micromax and dolomatic limestone. In December 2007, plants were transplanted into a raised landscape bed topped with pine bark mulch located in front of the Thad Cochran Southern Horticulture Laboratory in Poplarville, MS. Four replicates were planted using a completely random block design. Plants are approximately 3 feet apart. The bed is oriented east of the building, receiving full sun from daybreak until 4:00 pm during the

summer. Irrigation was supplied via drip lines for 30 minutes daily in the afternoon to prevent wilting. Plants were not pruned and no pesticides, herbicides, or fungicides were used in proximity to the study plot to minimize leaf damage that might be mistaken for environmental responses.

Overall plant quality was evaluated monthly from April 2007 until October 2007 using a visual rating and nominal scale of 1 to 5, where 1 indicates plant death and 5 indicates a plant with no visible damage. At the conclusion of first year, quality ratings were averaged to produce an overall mean per plant and per cultivar, with error determined from 4 replicates. In addition to overall plant quality, we recorded number of flowers, height, and sun scorch ratings but these data are not presented in this preliminary report. Replicate plots were planted at Crystal Springs, MS, Baton Rouge, LA, and McMinnville, TN but these data have not been analyzed.

Results and Discussion: Average plant quality ratings are shown in Figure 1. 'Ayesha', 'Blue Wave', 'Grayswood', and 'La France' all produced average quality ratings of 3.3, which was the highest for any cultivar. Statistically, there is very little separation between quality ratings (data not shown) but this is expected given the variation shown as error bars in Figure 1. Significant differences may be observed as we combine data from multiple years and locations. Surprisingly, only three plants died during the summer. The average quality ratings differ by only one point (2.3 to 3.3) indicating that no cultivar performed well under these conditions but no cultivar performed exceptionally poorly. The highest single quality score for any plant was 3.8 for one of the 'Ayesha' replicates.

We expect to continue rating cultivars for three years because popular publications suggest that fully established, acclimated hydrangeas in full sun landscape conditions perform significantly better than do new plantings. Our study also includes a few *H. macrophylla* subspecies *serrata* cultivars, or mountain hydrangeas, which have been described as having better sun tolerance than *H. macrophylla* subspecies *macrophylla*, or bigleaf hydrangeas.

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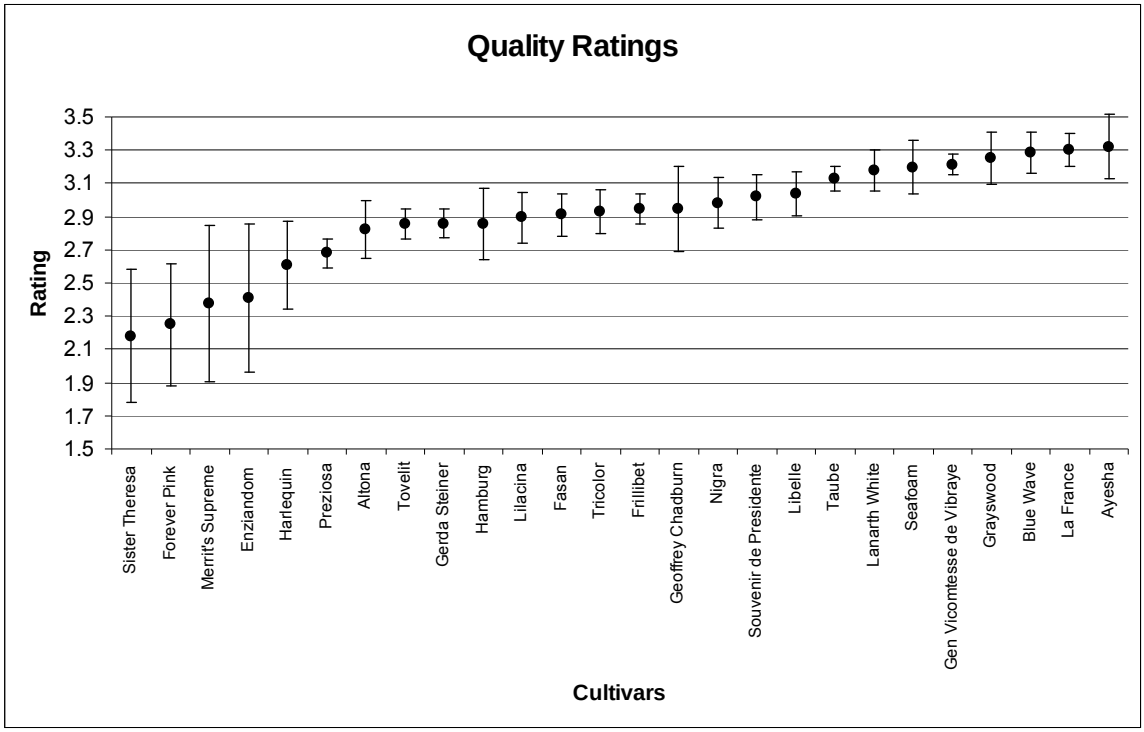

Figure 1. Average plant quality ratings for 26 *H. macrophylla* cultivars grown in full sun.

A Microaray Study of Aluminum Toxicity in Tomato Root Culture

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Index words: Transcriptional factors, protein kinases, hormones, biogenic amines, tomato, microarray, Al toxicity

Significance to Industry: Aluminum (Al^{3+}) is one of the most abundant elements in the earth's crust. This element can be deadly to nursery plants in acidic soil (pH <5.0) where it hampers root elongation and absorption of essential elements such as calcium and phosphorus. Because of the many problems associated with soluble aluminum, field-grown nursery stock should be grown at higher pH levels where it is not soluble. Acid-loving plants such as azaleas and hydrangeas are more tolerant to aluminum than other nursery crops and grow well at low soil pH levels. Sensitive plants can be grown safely at low pH levels in soil-less potting media because they contain little or no aluminum. This research was conducted to identify genes that are induced or suppressed in tomato, as the experimental plant, under toxic soluble aluminum levels. Once DNA sequences of genes of interest are known, they could be used to clone homologous genes in nursery crop species and used to improve their ability to grow in acidic/high Al soils.

Nature of Work:
Germinated tomato 'Money Maker' seeds were maintained for 12 days in a liquid culture solution containing aluminum sulphate (20uM); the culture solution was replaced every 4 days. Total RNA was extracted from root tissue and amplified using the Amino Allyl MessageAmp II aRNA Amplification Kit (Ambion, USA). The cyanine-dye labeled aRNA was hybridized with probes on a cDNA microarry (Tom1) (http://bti.cornell.edu/CGEP/). Hybridization was conducted at 43^{o}C in the dark for 16 h. After washing, following the protocols found on the microarray supplier's website (http://bti.cornell.edu/CGEP), images were obtained on a GenePix 4000B slide scanner and the data acquisition was using Genepix 6.0 software (Molecular Devices). The raw data was loaded into GeneSpring (v.7) (Agilent Technologies, Inc., USA) using the GenePix-format defaults and normalized with the Lowess curve. Fold differences between treated and control were subjected to a t-test with a multiple testing correction using 5% Benjamini-Hochberg false discovery rate. Genes that had over a 1.4 fold change were considered as Al-affected and are presented in this paper.

Results and Discussion:
Genes in tomato that underwent significant changes in activity (P<0.05, FDR) under Al stress are classified into five groups: gene transcription, signal transduction, abiotic and biotic stress-induced, biogenic amines, and hormone synthesis (Table 1). Changes in activity are expressed in fold between the control and gene.

Group1. The transcriptional regulation group contains genes that function as transcriptional regulators or activators. Suppressed genes include the DEAD/DEATH box helicase and transcriptional activators FHA1. Helicase genes are involved in unwinding nucleic acid. Expression of FHA1 (Forkhead-associated domains) is associated with controlling gene replication and subsequently cell cycle progression (2, 4). The suppression of these genes is related to inhibition of cell division due to aluminum toxicity. The six genes that encode for MADs-box protein, bZIP transcriptional factors, zinc finger protein and GT-related trihelix DNA-binding protein were induced by Al^{3+}.

Group 2. The signal transduction gene group includes the suppressed LysM domain-containing receptor-like kinase 3 (8), three up-regulated genes that encode for kinase proteins (serine/threonine kinase and calcium-dependent protein kinase 2) and a 14-3-3 protein. These genes function in the signal transduction pathway of a wide range of fundamental regulatory processes such as response to pathogenic attack, apoptotic cell death, and cell cycle control (1).

Group 3. The stress-inducible gene group contains nine genes that were induced 1.5 to 2 fold following Al treatments. These genes are also induced by other biotic and abiotic stresses. The salt and dehydration-induced proteins and the calcineurin-like phosphoesterase (salt-induced) (7) had higher transcript levels under Al treatments. Genes for defense to plant pathogenic attacks include those that respond to fungi (eli3 gene) (5) and bacterial infection as well as disease resistance (NP24 protein and CC-NBS-LRR). Lipoxygenase enzymes are involved in growth and development, pest resistance, senescence, and wounding responses (6). Results of this study indicate that some of the molecular regulation underlying Al stress could be in common with other stress factors.

Group 4. Two genes in the biogenic amine synthesis pathway were affected. The arginine decarboxylase 1 in the putrescine biosynthesis pathway was suppressed (Al/control ratio: 0.67) and the histidine decarboxylase which converts histidine into histamine was induced. Putrescine is the diamine precursor of polyamines such as spermidine and spermine. Enhancement of the arginine decarboxylase pathway, as measured by the accumulation of putrescine in apple callus culture, plays an important role in the stress response (3). Acidic (pH< 5.0) conditions can also induce arginine decarboxylase activity and higher putrescine titer in leaf segments of *Avena sativa* L. 'Victory' (9). In this study, the tomato root culture medium was acidic (pH<5.0), but the gene that encodes for arginine decarboxylase was down-regulated. Further work needs to be done to clarify the temporal change in enzyme activity as it correlates with the accumulation of putrescine in tomato under Al stress.

Group 5. Genes for hormone metabolism are mostly associated with ethylene and Jasmonic acid (JA) synthesis. The Al induced genes encode for S-adenosylmethionine synthetase (AdoMetS) and 12-oxophytodienoate reductase 3. AdoMetS catalyzes the formation of S-AdoMet which is a precursor for ethylene biosynthesis. 12-

oxophytodienoate reductase 3 is a key enzyme for JA biosynthesis. Both ethylene and JA are known to activate responses to stress conditions in other plant species. Ethylene affects root architecture in plants grown under nutrient deficiency conditions. High levels of AdoMetS protein were found in Al treated tomato roots (unpublished data). Under Al stress, root growth was very stunted, with few very short lateral root branches. These observations indicate that ethylene production regulated by SAM may be one of the causal mechanisms for controlling root development under Al stress.

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Table 1. Genes in tomato roots that underwent significant changes in activity (P<0.05) under aluminum stress expressed in fold changes.

5'-SGN unigene best hit	* Fold
Group 1 Gene transcription	
DEAD/DEAH box helicase, putative (*Arabidopsis thaliana*)	0.70
Transcriptional activator FHA1 (*Nicotiana tabacum*)	0.67
MADS-box protein 15 (*Petunia x hybrid*)	1.41
bZIP transcription factor ATB2 (*Glycine max*)	1.42
bZIP transcription factor (*N. tabacum*)	1.59
AP2 domain transcription factor, putative (*A. thaliana*)	1.85
Zinc finger (C3HC4-type RING finger) protein family (*A. thaliana*)	1.56
GT-related trihelix DNA-binding protein (*A. thaliana*)	2.49
Group 2 Signal transduction	
LysM domain-containing receptor-like kinase 3 (*Medicago truncatula)*	0.54
Serine/threonine Kinase (*Persea americana*)	1.57
Ccalcium-dependent protein kinase 2 (*N. tabacum*)	1.73
14-3-3 protein 7	1.42
Group 3 Stress-induced genes	
Hypersensitive-induced response protein (*A. thaliana*)	2.04
Disease resistance protein (CC-NBS-LRR class), putative (*A. thaliana*)	2.15
NP24 protein precursor (Pathogenesis-related protein PR P23) (Salt-induced protein)	1.47
Dehydration-induced protein family (*A. thaliana*)	1.47
Erwinia induced protein 1 (*S.tuberosum*)	1.47
Disease resistance protein (CC-NBS-LRR class), putative (*A. thaliana*)	1.47
Lipoxygenase (EC 1.13.11.12) - tomato	1.56
Calcineurin-like phosphoesterase family (*A. thaliana*)	1.50
ELI3 [*Lycopersicon esculentum*]	1.67
Group 4 Biogenic amines	
Arginine decarboxylase 1 (*D. stramonium*)	0.67
Histidine decarboxylase (HDC) (TOM92)	2.24
Group 5 Hormone biosynthesis	
S-adenosylmethionine synthetase 3	3.14
Serine carboxypeptidase -related (*A.thaliana*)	1.80
12-oxophytodienoate reductase 3 (*L. esculentum*)	1.90

*A fold value above 1 means that the gene expression is up regulated while less than 1 means that it is down regulated. A fold of 1 indicates no change in activity.