Plant Breeding and Evaluation

Timothy Rinehart

Section Editor
Genetic Variation of Magnolia sieboldii K. Koch ‘Colossus’ and Magnolia grandiflora L. ‘Kay Parris’ F1 Seedlings Using ISSR Markers

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Significance to Industry: Magnolia species, cultivars, and hybrids have been revered ornamental plants since cultivation began in the 1700’s. In particular, Magnolia grandiflora cultivars are prevalent in the southeastern nursery industry with over 100 cultivars readily documented in popular reference literature. Recent taxonomic revisions based upon morphological observation and molecular phylogeny derived from cpDNA analysis have clarified relationships within Magnoliaceae. This has provided breeders with new inspiration to attempt crosses once assumed futile. While an understanding of taxonomic relationships, verification of ploidy levels, and genome size are clues to a breeder about reproductive compatibility, more information is needed to better predict reproductive fecundity and compatibility beyond the F1 generation. Inter Simple Sequence Repeat (ISSR) markers can provide us with a more detailed view of genetic content to verify parentage, measure variation among siblings of controlled crosses, and potentially facilitate development of novel and desirable cultivars.

Nature of Work: Magnolia sieboldii K. Koch ‘Colossus’ is a diploid (2n=2x=38) cultivar (9) derived from this deciduous species native to China, Japan, and Korea (2, 10). Specimens of this cultivar produce slightly remontant, nodding white flowers with a greater tepal count than the typical species. Red stamens provide an intriguing bullseye appearance. M. sieboldii ‘Colossus’ is a multi-stem shrub or small tree adaptable to USDA Zones 4-7. Magnolia grandiflora L. ‘Kay Parris’ is a cultivar selection of this hexaploid species (2n=6x=114) (9), which is native from Virginia to Florida, and east to Texas. It is a densely pyramidal evergreen tree, producing remontant white flowers from an early age, and has demonstrated excellent hardiness north of the natural range of the species. Each species has great breeding potential, evidenced by their incorporation in a variety of interspecific, intersectional, and interploid hybrids (5, 9). Prolific magnolia breeder Dennis Ledvina of Green Bay, Wisconsin, who passed away in May 2015, was the first to select a cultivar worthy of commercial introduction (M. x ‘Exotic Star’) from a cross of M. sieboldii with M. grandiflora. Inspired by the success of Mr. Ledvina, several
controlled crosses were performed in May 2013 between a specimen of *M. sieboldii* ‘Colossus’ and *M. grandiflora* ‘Kay Parris’ located in the Spartanburg Community College Arboretum to generate a controlled F1 population for evaluation. A total of 25 seeds were harvested from 2 fruits in August 2013 and subjected to a float test to determine viability. Following 5 months cold stratification, 16 seeds germinated. Among them, 12 seedlings survived during early production and were then transplanted into 1-gallon containers. Leaf tissue of 9 individuals growing with adequate vigor had been harvested for this study. Previously sampled accessions of this interspecific hybrid have been verified as tetraploid (2n=4x=76) by flow cytometry (9). When the cross is performed in this direction, verification of hybridity is clear as all seedlings possess evergreen foliage favoring the hexaploid pollen parent, but some variation in vigor, leaf size, shape, and color is evident. Due to the young age of this F1 population, growth habit and floral characters are still unknown. Therefore DNA analysis is necessary to reveal genotypic variations that may not yet be phenotypically apparent.

Molecular techniques, such as cpDNA analysis (1), had been applied for Magnoliaceae for its phylogenetic studies. ISSR markers is a useful new method in plant genetic research (4, 11). They have been widely used in the study of plant genetic structure (7), genetic diversity and genetic relationships (6). In this study, our goals are to analyze the genetic variation between the parents and F1 seedlings, assess the genetic diversity of these F1 siblings, and prescribe a series of crosses to generate a recombinant F2 population for further study with enhanced ornamental potential.

One fresh leaf per plant was selected from each of the 9 seedlings of *Magnolia sieboldii* K. Koch ‘Colossus’ × *Magnolia grandiflora* L. ‘Kay Parris’, and their parents. To prepare for DNA extraction, sample tissue was ground under liquid nitrogen using a mortar and pestle. Tissue not immediately subjected to extraction was stored at -80°C. Genomic DNA was extracted using the QIAGEN DNeasy Plant Mini Kit. The concentration and purity of DNA were determined using a spectrophotometer (ThermoScientific NanoDrop Lite) at A260 and A280 and stored at -20°C until use (8). Nuclear DNA was then PCR-amplified in an Eppendorf Mastercycler Pro with ISSR primers. Following an initial screen of 100 primers, 10 were selected for further analysis (Table 1). The PCR reaction mixture in each tube was 20µl containing 10 µl Ampli Taq Gold 360 Master Mix, 3 µl DNA, 1 µl primer and 6 µl ddH2O. The PCR program was initial denaturation for 5 min at 94°C, followed by 40 cycles of 94°C for 30 sec, annealing at 50°C-60°C for 45 sec (temperature dependent on recommendation per primer), extension at 72°C for 1.5 min and a final extension at 72°C for 7 min. Tubes were held at 4°C until removal. PCR products were electrophoresed on 1.2% agarose gel in 0.5X Tris Borate Edta (TBE) buffer with 2 drops of ethidium bromide per 120 ml solution at 120 V for 80 min. A 100 bp DNA ladder was used as a standard molecular weight for comparison to bands produced from the parental and sibling DNA. Gels were photographed under UV light in a UVP BioDoc-ItTM Imaging System.

Bands were scored manually. ISSR scores were recorded as either present (1) or absent (0) to generate a binary matrix set. Bands with the same size were considered to
be allelic according to the weight of the 100bp DNA ladder. Weak or ambiguous bands were excluded from the analysis (3). Gene differentiation between parental and F1 samples were estimated by the coefficient of gene differentiation using PAUP version 4.0.

**Results and Discussion:** Ten ISSR primers revealed 96 bands in sizes ranging from 200 to 1500bp in the 11 individuals of the two generations, among which 85 bands (88.5%) were polymorphic. The number of bands varied from 7 to 15, with average of 9.6 bands per primer. The ISSR primer UBC 817 amplified the highest percentage of polymorphic bands at 100.0%, while the primer UBC 807 produced the least number of polymorphic bands among the 10 primers at 57.1% (Table 1). For each taxon, number of bands was from 45 (XX) to 58 (SG and SI).

Pairwise distance between taxa varied from 0.167 to 0.604. Since *M. sieboldii* ‘Colossus’ (XX) had bigger deciduous foliage without red brown pubescences on the back and *M. grandiflora* ‘Kay Parris’ (XY) had smaller evergreen foliage (Fig. 2), ISSR markers revealed that they had significant genetic difference at 0.604. Among the siblings, the smallest distance was between seedling H (SH) and seedling I (SI) at 0.167 (Fig. 3) and the largest between seedling A (SA) and seedling G (SG) at 0.396 (Fig. 4). The average distance from the seed parent (XX) was 0.279, while to pollen parent (XY) at 0.494. Obviously, the hexaploid pollen parent contributed much more genetic diversity than that of diploid seed parent. All these 9 seedlings were verified interspecific hybrids not only by polidy levels (9), but also by the ISSR markers.

The dendrogram based on ISSR data indicated that the UPGMA tree was composed of two groups. The first one contained only the seed parent and the second one clustered the pollen parent and all F1 siblings. The 2nd group was formed by four subgroups (Fig. 1). The pollen parent (XY) and seedling C (SC) was joined by genetic distance of 0.219. Morphologically, SC had the most similar foliage shape and size to XY. SH and SI both had small leaves, low vigor, and similar growth pattern (habit). It was understandable that they were the closest siblings genetically. The above four taxa formed the first subgroup (Fig. 2, 3 and 4) and with closer genetic makeup. The second subgroup contained SA, the most vigorous plant, and the cluster SB and SF. They definitely closer genetically, but no distinguished different at the current growing stage. The third subgroup formed by SD and SE with almost identical appearance. The fourth subgroup was seedling G (SG), the only sibling with distinctly lanceolate foliage (Fig. 3 and 4).

Unique bands and band patterns were important for further genetic studies (12). In this ISSR evaluation, 17 unique bands were generated and 8 of them were from seed parent and only one from the pollen parent. The recombination of DNA from this controlled cross hybridization yielded four siblings with unique bands, 3 each from SE and SG and one each from SC and SD. Since seedling G with distinguished lanceolate foliage and 3 unique bands, it is possible that the gene(s) that controlled lanceolate leaf shape might be from these unique band regions. Further studies should be conducted to verify this hypothesis. It is too early to evaluate the band patterns in associated with...
plant growth and development at this early stage and the data will be utilized for future studies.

The analytical results of ISSR showed seedling C has the closet relationship with the pollen parent and all the seedlings are similar to the pollen parent validating the known 3:1 ratio of genetic contribution from *M. grandiflora* and *M. sieboldii*. Further evidence was that all siblings shared 11 bands with pollen parent and only 3 from seed parent. Plants will continue to be evaluated until reaching maturity for anticipated variation of growth habit and floral characteristics. These results should be applied for our future controlled *Magnolia* breeding.

**Acknowledgement**

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**Literature Cited**


Table 1: Sequences of 10 primers successfully used in the ISSR analysis and number of amplified bands per primer and their polymorphism.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Ta(^a) (°C)</th>
<th>TB(^b)</th>
<th>PB(^c)</th>
<th>PPB(^d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC807</td>
<td>(AG)8T</td>
<td>52</td>
<td>7</td>
<td>4</td>
<td>57.1</td>
</tr>
<tr>
<td>UBC811</td>
<td>(GA)8C</td>
<td>52</td>
<td>10</td>
<td>9</td>
<td>90.0</td>
</tr>
<tr>
<td>UBC 812</td>
<td>(GA)8A</td>
<td>52</td>
<td>10</td>
<td>9</td>
<td>90.0</td>
</tr>
<tr>
<td>UBC 814</td>
<td>(CT)8A</td>
<td>52</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>UBC 815</td>
<td>(CT)8G</td>
<td>54</td>
<td>15</td>
<td>14</td>
<td>93.3</td>
</tr>
<tr>
<td>UBC 817</td>
<td>(CA)8A</td>
<td>54</td>
<td>8</td>
<td>8</td>
<td>100.0</td>
</tr>
<tr>
<td>UBC 827</td>
<td>(AC)8G</td>
<td>52</td>
<td>9</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>UBC 828</td>
<td>(TG)8A</td>
<td>52</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>UBC 834</td>
<td>(AG)8YT</td>
<td>56</td>
<td>14</td>
<td>13</td>
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</tr>
<tr>
<td>UBC 835</td>
<td>(AG)8YC</td>
<td>56</td>
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<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>9.6</td>
<td>8.5</td>
<td></td>
<td>88.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Annealing temperature.  
\(^{b}\) Total number of bands.  
\(^{c}\) Polymorphic bands.  
\(^{d}\) Percentage of polymorphic bands.

Figure 1. Dendrogram of Magnolia parents and F1 siblings based on ISSR data.
Figure 2. Comparative image of parental foliage and intermediate foliage of hybrid (all back sides).

Figure 3. Comparative image of F1 population after transplant to one gallon containers.
Figure 4. Varying foliage characteristic representative siblings from each cluster of the dendrogram during Spring 2015 flush.
Inheritance of Floral and Plant Size Traits in *Hydrangea macrophylla*

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**Index words:** hydrangea, plant breeding, heritability, transgressive segregation

**Significance to industry:** Here we establish transgressive segregation as a favorable method of producing novel and extreme phenotypes in *Hydrangea macrophylla*. All inflorescence and plant size traits showed some level of transgressive segregation with one family having 46% of full-siblings with phenotypes that were extreme relative to either parent. These results will guide parental choice in experimental crosses with *Hydrangea* and provide germplasm for continued species improvement.

**Nature of Work:** Five species of *Hydrangea* (*H. macrophylla*, *H. paniculata*, *H. quercifolia*, *H. arborescens* and *H. anomala* subsp. *petiolaris*) are widely cultivated in the U.S. as landscape plants (1). *Hydrangea* is the second most valuable woody ornamental genus after *Rosa* with an estimated sales of over $73 million in 2007 (2). Hydrangeas - prized for their large showy flowers and lush green foliage - are sold for landscape use, patio and container use, and as cut flowers in the floral industry. By far the most popular member of the genus is *H. macrophylla*, with over 1,200 cultivars named world-wide (3). Although this species has a breeding history stretching hundreds of years, room for improvement exists for a number of environmental and ornamental traits.

Transgressive segregation occurs when trait values for offspring in experimental crosses fall outside (either above or below) the range of values recorded for the parents. Transgressive segregation is important to plant breeders as a source of novel or extreme traits and is ubiquitous in plant hybrids, where an estimated 58% of traits show transgression (4). While widespread, it is difficult to predict which traits or parental combinations may be the most suitable for producing transgressive segregants. *The objective of this research is to evaluate the inheritance of inflorescence characteristics and plant size in related full-sibling families, determine the level of transgressive segregation present, and select full-sibling families for further species improvement.* Results will be compared to flow cytometry data to determine the effect of ploidy on phenotype, be used inform future breeding and in vitro ploidy manipulation experiments, and provide germplasm for further species improvement.
Controlled pollinations in 2010 produced a series of three *Hydrangea macrophylla* full-sibling families with similar genetic backgrounds, where each family had at least one parent in common. The *Hydrangea macrophylla* varieties ‘Princess Juliana’, ‘Trophee’, and ‘Zaunkoenig’ were used in the following crosses: ‘Princess Juliana’ x ‘Trophee’, ‘Zaunkoenig’ x ‘Princess Juliana’, and ‘Trophee’ x ‘Zaunkoenig’. Controlled crosses and seed germination were carried out in 2010 and 2011, respectively, following the method of Reed (5). Plants were grown in 26.5 L containers under 60% shade and micro-irrigated using spray stakes. Growing media consisted of pine bark amended with 6.6 kg·m⁻¹ 19N-2.1P-7.4K Osmocote Pro fertilizer (Scotts-Sierra Horticultural Products Co., Maryville, Ohio), 0.6 kg·m⁻³ Micromax (Scotts-Sierra Horticultural Products Co.), 0.6 kg·m⁻³ iron sulfate, and 0.2 kg·m⁻³ Epsom salts.

The following variables were used to describe plant inflorescence and size: Number of inflorescences, number of inflorescences with at least one fully expanded sepal, inflorescence width (mean of three on each plant), sepal width (mean of three on each plant), height, and stem width. Additional floral data included flower color (white, pink, purple, or blue), sepal color (1 = white, 2 = light pink, 3 = pink, and 4 = dark pink), and sepal margin wave (0 = no wave, 1 = little wave, 2 = moderate wave, 3 = very wavy). Number of inflorescences and number of inflorescences with at least one fully expanded sepal were measured on 5/22/2015; all variables were measured on 6/8/2015. All data analysis was performed using the SAS 9.4® software system (6). The general linear model procedure (PROC GLM) was used to partition variance in inflorescence and plant size means into sources attributable to family and environment (error) and to estimate variance components for calculation of narrow-sense heritability. For flower color data, a chi-square test for association between family and flower color was performed using PROC FREQ. The CORR procedure was used to produce correlations between variables. Percent transgressive segregants was determined by counting the number of siblings in each family with trait values above or below the range of parental values.

**Results and Discussion:** Mean values for flower and plant size variables are shown in Table 1. A total of 81 plants in three *Hydrangea macrophylla* full-sibling families were measured; 54 of these flowered during the data collection period. Variance in inflorescence number was extremely large due to several 0 values in a single family, ‘Princess Juliana’ x ‘Trophee’. On 5/22/2015, ‘Princess Juliana’ x ‘Trophee’ had significantly fewer flowers open than the other crosses; by 6/8/2015 the percentage of flowers open was not significantly different between families (Figure 1). Between 5/22 and 6/8, full-sib families added an average of 1 inflorescence per plant with no difference between families in the number of new inflorescences.

Full-sibling families differed significantly in sepal width, plant height, and stem width (Table 2). ‘Princess Juliana’ x ‘Trophee’ was the tallest family and had the thickest stems. Bi-modal pollen grain distribution was identified in the cultivar ‘Trophee’ (7), indicating the possibility of unreduced gametes pairing with normal, haploid gametes to produce triploid individuals. Work is ongoing to determine ploidy levels of full-sibling families; the existence of triploids in the full-sibling family with ‘Trophee’ as a male would establish a link between ploidy and phenotype.
The majority of plants had white or pink flowers (Table 2). Chi-square analysis showed no association between family and flower color ($\chi^2 = 8.6, p = 0.19$) though 13% of ‘Zaunkoenig’ x ‘Princess Juliana’ siblings had either blue or purple flowers. ‘Zaunkoenig’ x ‘Princess Juliana’ also had the largest inflorescences and the widest sepals (Table 2). Because of this large inflorescence size and variability in flower color, the most favorable individuals from the ‘Zaunkoenig’ x ‘Princess Juliana’ full-siblings will be used as parents in further Hydrangea macrophylla improvement. ‘Trophee’ x ‘Zaunkoenig’ full-siblings had the darkest colored sepals and the waviest sepal margins due to two unique individuals. These plants will be used to study inheritance of these traits and to introduce them into superior germplasm.

The lacecap flower type appears dominant to the mophead flower type as demonstrated by the lack of mophead flowers in the F₁ families with ‘Zaunkoenig’ as a parent (Table 2). Heritability for floral and plant size traits ranged between 0.08 for margin wave and 0.62 for height. Narrow-sense heritability represents the portion of phenotypic variation controlled by additive gene action and is crucial to predicting outcomes of plant breeding. The higher the narrow sense heritability, the greater the response to selection. The traits with the two highest heritabilities, plant height and sepal width, are both desirable traits for improvement. As these traits are not significantly correlated (Table 3), it should be possible to produce compact (or large) plants with large sepals from this population.

Transgressive segregation is a major mechanism by which novel or extreme phenotypes arise. In this study, the percentage of offspring with values more extreme than their parents ranged between 7% for height and 42% for stem width (Table 4). (In other words, 42% of all offspring had stem width values either above or below the range of stem width values of their parents.) An analysis of variance was performed to partition variation in transgressive segregation to sources attributable to trait and full-sibling family. While trait was not a significant source of variation ($F = 1.3, p = 0.35$), family did explain a significant portion of the variation in transgressive segregation ($F = 3.5, p = 0.051$). These results indicate that predicting transgressive segregation for specific traits is impractical; any trait may display transgressive segregation and the level of transgressive segregation is unrelated to heritability. Instead, certain crosses may be identified that increase the probability of seeing extreme traits in the offspring. Specifically, the more similar the phenotypes of the parents, the greater the likelihood that transgressive segregation will be observed (4). In this study, the full-sib family ‘Trophee’ x ‘Zaunkoenig’ had 46% of offspring with trait values more extreme than their parents, significantly more than the other two families where 19% and 20% of offspring showed transgressive segregation (Table 4). ‘Trophee’ and ‘Zaunkoenig’ are both compact varieties with serrate sepal margins, and dark pink sepals. Recommendations for observing extreme phenotypic values include using parents of the same species (intraspecific crosses), using domesticated, self-compatible species, and using parents with fixed genetic differences (4). While the genetic make-up of Hydrangea macrophylla varieties is largely unknown, results herein indicate that transgressive segregation should be a major source of novel and extreme traits for this species.
Literature Cited
6. SAS Software System v. 9.4. Cary, NC, USA.

Table 1. Overall range, mean (± standard error), and variance for seven inflorescence and plant size variables for three 5-year old full-sibling Hydrangea macrophylla families.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Range</th>
<th>Mean±SE</th>
<th>Variance</th>
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</thead>
<tbody>
<tr>
<td>Inflorescences (no.)</td>
<td>81</td>
<td>0 - 37</td>
<td>7.4±1.1</td>
<td>88.3</td>
</tr>
<tr>
<td>Inflorescence width (cm)</td>
<td>54</td>
<td>8.5 - 22.4</td>
<td>13.7±0.4</td>
<td>8.16</td>
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<tr>
<td>Sepal width (cm)</td>
<td>54</td>
<td>2.0 - 5.6</td>
<td>4.1±0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>81</td>
<td>38 - 89</td>
<td>64.3±1.2</td>
<td>113.2</td>
</tr>
<tr>
<td>Stem width (mm)</td>
<td>81</td>
<td>3.3 - 13.0</td>
<td>7.1±0.2</td>
<td>2.85</td>
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<tr>
<td>Sepal color*</td>
<td>54</td>
<td>1 - 4</td>
<td>1.9±0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Margin**</td>
<td>54</td>
<td>0 - 3</td>
<td>0.7±0.1</td>
<td>0.8</td>
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* Sepal color was based on a qualitative scale where 1 = white, 2 = light pink, 3 = pink, and 4 = dark pink.
** Margin wave was based on a qualitative scale where 0 = no wave, 1 = little wave, 2 = moderate wave, 3 = very wavy.
Table 2. Mean (± standard error) inflorescence and plant size for three 5-year old full-sibling *Hydrangea macrophylla* families.

<table>
<thead>
<tr>
<th>Flower color</th>
<th>Inflor. width, cm</th>
<th>Sepal width, cm</th>
<th>Sepal color*</th>
<th>Margin wave**</th>
<th>Height (cm)</th>
<th>Stem Width (mm)</th>
<th>Flower type (M or L)†</th>
</tr>
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<tbody>
<tr>
<td>parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Princess Juliana</td>
<td>12.0±0.8  3.3±0.4  1 0</td>
<td>90.0±3.0  7.5±0.4</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trophee</td>
<td>12.5±1.1  3.5±0.2  3 1</td>
<td>57.0±12.4  5.9±0.2</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Zaunkoenig</td>
<td>11.6  3.9  3 3</td>
<td>34 4.7</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>full-sib families</th>
<th>No.</th>
<th>% sibs with</th>
<th>Inflor. width, cm</th>
<th>Sepal width, cm</th>
<th>Sepal color*</th>
<th>Margin wave**</th>
<th>Height (cm)</th>
<th>Stem Width (mm)</th>
<th>Flower type (M or L)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Princess Juliana</td>
<td>19</td>
<td>67</td>
<td>13.8±0.8 a 3.3±0.2 b  1.7±0.2 a  0.5±0.3 a 71.4±2.2 a 7.9±0.4 a</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Trophee</td>
<td>45</td>
<td>45</td>
<td>14.3±0.5 a 4.3±0.1 a  1.9±0.1 a  0.6±0.2 a 64.1±1.4 b 7.0±0.2 ab</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Zaunkoenig</td>
<td>17</td>
<td>10</td>
<td>12.1±0.8 a 4.3±0.2 a  2.3±0.2 a  1.1±0.3 a 54.2±2.7 c 6.3±0.5 b</td>
<td>L</td>
<td></td>
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<thead>
<tr>
<th>Trait</th>
<th>h²</th>
<th>0.16</th>
<th>0.58</th>
<th>0.12</th>
<th>0.08</th>
<th>0.62</th>
<th>0.20</th>
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</table>

* Sepal color was based on a qualitative scale where 1 = white, 2 = light pink, 3 = pink, and 4 = dark pink.
** Margin wave was based on a qualitative scale where 0 = no wave, 1 = little wave, 2 = moderate wave, 3 = very wavy.
† M = Mophead, L = Lacecap

Tukey’s mean separation is shown in lower-case letters; for each variable, means with the same letter are not significantly different at the α=0.05 level.

Table 3. Correlations between inflorescence and plant size variables measured on three 5-year old full-sibling *Hydrangea macrophylla* families. Values are Pearson correlation coefficients; p values are reported below. (n = 54 plants).

<table>
<thead>
<tr>
<th>Inflor. width</th>
<th>Sepal width</th>
<th>Height</th>
<th>Stem width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflor. width</td>
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<td>0.5223</td>
<td>&lt;.0001</td>
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<tr>
<td>Sepal width</td>
<td>0.5223</td>
<td>1</td>
<td>-0.0721</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>0.48884</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 4. Percentage of transgressive segregants in three 5-year old full-sibling *Hydrangea macrophylla* families.

<table>
<thead>
<tr>
<th>Full-sib family</th>
<th>Percent transgressive segregants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inflor. Width</td>
</tr>
<tr>
<td>Princess Juliana</td>
<td>0.25</td>
</tr>
<tr>
<td>× Trophee</td>
<td>0.47</td>
</tr>
<tr>
<td>Zaunkoenig</td>
<td>0.45</td>
</tr>
<tr>
<td>× Princess Juliana</td>
<td></td>
</tr>
<tr>
<td>Trophee</td>
<td>0.39 a</td>
</tr>
<tr>
<td>× Zaunkoenig</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Tukey’s mean separation is shown in lower-case letters; for each family and variable, means with the same letter are not significantly different at the $\alpha=0.05$ level.

* Sepal color was based on a qualitative scale where 1 = white, 2 = light pink, 3 = pink, and 4 = dark pink.

** Margin wave was based on a qualitative scale where 0 = no wave, 1 = little wave, 2 = moderate wave, 3 = very wavy.

Figure 1. Percent inflorescences open on three 5-year old full-sibling *Hydrangea macrophylla* families on two dates. Mean number of inflorescences for each cross was 13.7, 13.3 and 12.0 on 5/22/2015 and 14.6, 14.3, and 13.0 on 6/8/2015. Error bars represent standard deviation.
**In Vitro Polyploid Induction of Ophiopogon planiscapus**

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**Index Words:** chromosome doubling, flow cytometry, oryzalin, polyploidy, somatic embryogenesis, tissue culture, whole genome duplication

**Significance to Industry:** Mondo grass (*Ophiopogon* spp.) are versatile and valuable landscape plants with a variety of ornamental uses (2). The diversity of species and traits in this genus provide opportunities for the breeding and development of new cultivars. However, variations in ploidy levels complicate breeding systems. Embryogenic callus derived from embryos of the diploid cultivar *Ophiopogon planiscapus* ‘Nigrescens’ was subjected to *in vitro* ploidy manipulation to produce tetraploids that will be evaluated for ornamental character, size and vigor, and for use as breeding lines to hybridize with other naturally occurring tetraploid species.

**Nature of Work:** The genus *Ophiopogon* contains approximately 65 species, many of which are popular as ornamental landscape plants due to their versatility and adaptability (3). Of particular interest is the cultivar *Ophiopogon planiscapus* ‘Nigrescens’ which is characterized by dark, almost black, grass-like foliage and upright flowers. Although ‘Nigrescens’ is highly valued for its unique foliage color its propagation and production is typically hindered by its slow growth. Development of tetraploid ‘Nigrescens’ may enhance vigor and facilitate hybridization with existing polyploid species. Induced polyploids often show an increase in size and growth rate with larger flowers and longer bloom periods (5, 8). These induced tetraploids may also provide new opportunities for interspecific hybridization with naturally occurring tetraploids of *Ophiopogon japonicus* and may be useful in intergeneric crosses with the tetraploid *Liriope* species *L. gigantea*, *L. muscari*, and *L. platyphylla* (3, 8). The dinitroanaline herbicide oryzalin (Surflan®) has been effectively used as a mitotic inhibitor to induce chromosome doubling (6); however, the concentrations and exposure times can vary according to species (1). The objectives of this study were to produce tetraploid *Ophiopogon planiscapus* clones for future breeding work and to determine the optimal concentration and duration of exposure to oryzalin for chromosome doubling.

**Somatic embryogenesis:** Embryogenic callus was induced from mature embryos excised from open pollinated seeds of *Ophiopogon planiscapus* ‘Nigrescens’ collected in September 2012. Callus induction and proliferation medium consisted of Murashige and Skoog’s (MS) basal salts and vitamins (7) supplemented with 2% sucrose, 5 µM
benzylamino purine (BAP), 5 µM naphthalene acetic acid (NAA), and 0.08 g/L adenine hemisulfate. Medium was adjusted to a pH of 5.75 ± 0.03 and solidified with agar at 0.65%. Callus was subcultured onto fresh medium every 6 to 8 weeks and incubated in the dark at 23 ºC (73.4 ºF) to allow callus proliferation.

Oryzalin Treatment: A liquid medium consisting of MS basal salts and vitamins and 2% sucrose was prepared for chromosome doubling. Medium was adjusted to a pH of 5.75 ± 0.03. A 3 mM stock solution of oryzalin was prepared in 95% ethanol. This stock solution was added to flasks containing cooled autoclaved media to obtain the concentrations of 7.5 µM, 15 µM, and 30 µM oryzalin. The control solution (0 µM oryzalin) received the addition of 5 ml of 95% ethanol. Fifteen jars per solution concentration were prepared.

The experimental design consisted of a completely randomized, four × three factorial, with four oryzalin concentrations (0, 7.5, 15 and 30 µM) and three exposure durations (3, 6 or 9 days). Each treatment combination consisted of five replications with each replication containing five calli (subsamples).

On day zero of the experiment, five callus pieces were placed in each jar. Jars were sealed with parafilm and placed on a rotary shaker in the dark. Five jars of each concentration (0, 7.5, 15 and 30 µM) were removed from the shaker after 3, 6, or 9 days. Upon removal, calli were transferred to a liquid MS medium and replaced on the rotary shaker for 24 hours to remove residual oryzalin. Calli were then transferred onto embryogenic maintenance MS medium and incubated in the dark. After 8 weeks, data was collected on the number of surviving calli.

Flow Cytometry: Calli were allowed to recover in the dark for a period of at least 28 days and thereafter resulting shoots that arose from the callus were analyzed using flow cytometry. Samples were prepared by placing leaf tissue in a petri dish with 400 µL of nuclei extraction buffer and chopping finely using a razor blade. The resulting solution was filtered and 1600 µL of a nucleotide staining buffer solution (CyStain UV Precise P Staining Buffer, Partec, Munster, Germany), containing 4', 6-diamidino-2-phenylindole (DAPI), was added to the solution. Stained nuclei were analyzed using a flow cytometer (Partec, PA-II).

The mean relative fluorescence for each sample was compared with that of a confirmed diploid Ophiopogon (3, 4) to determine if ploidy levels had been affected by treatment. All tetraploid and any strongly mixoploid (>50% tetraploid nuclei) shoots were subcultured into jars containing shoot maintenance medium consisting of MS basal salts and vitamins supplemented with 2% sucrose and 10 µM BAP. Medium was adjusted to a pH of 5.75 ± 0.03 and solidified with 0.65% agar. Cultures were placed under cool white fluorescent lights (60 µmol m⁻²s⁻¹) with a 16 h photoperiod at 23 ºC (73.4 ºF). After a period of regrowth these shoots were retested to confirm their ploidy. Diploid shoots were discarded and any strongly mixoploid or tetraploid shoots were subcultured. A population of diploid O. planiscapus was also maintained in culture on a solidified MS medium containing 10 µM BAP for use as a control for flow cytometry.
Results and Discussion: Regression analysis showed there was no influence of treatment duration or interaction between duration and concentration of oryzalin on callus survival or shoot ploidy (Proc GLM, SAS version 9.4; SAS Institute, Cary, NC). However, callus survival did decrease with increasing oryzalin concentration in a linear fashion \( y = -1.0108x + 90.933 \), \( R^2 = 0.9882 \), \( p < 0.0002 \) (Fig. 1). This reduction in survival illustrates that the oryzalin was affecting the callus at these concentrations.

Oryzalin concentration also influenced the number of diploid and mixoploid shoots recovered (Fig. 2). The mean number of diploid shoots recovered after treatment followed a quadratic model \( y = 0.2306x^2 - 8.5134x + 98.373 \), \( R^2 = 0.9894 \), \( p < 0.0001 \) where the percentage of diploid shoots decreased and then began to rise again as concentration increased (Fig. 2). This trend was seen in reverse in the mean number of mixoploid shoots recovered \( y = -0.2002x^2 + 7.7026x -0.6843 \), \( R^2 = 0.9979 \), \( p < 0.0001 \). As the concentration of oryzalin increased the mean number of mixoploids increased until it began to fall again at higher concentrations (Fig. 2). Although there was no significant effect of concentration on the number of tetraploid shoots recovered, a limited number of tetraploids were successfully recovered from treated callus (7.5 and 15 µM) evidencing that oryzalin is an effective agent for polyploid induction in *Ophiopogon planiscapus* (Fig. 2). A number of mixoploid shoots that were kept in tissue culture eventually stabilized as tetraploids at a later date. Due to variations in the rate of cell division and cell fitness, lineages of different cell cytotypes may eventually outcompete one another through this type of endocytotypic selection.

Clones of all tetraploids are currently being multiplied and will subsequently be rooted on a half-strength medium consisting of Murashige and Skoog’s (MS) basal salts and vitamins supplemented with 2% sucrose and 5 µM (NAA). Clones will be removed as they root and placed in a greenhouse under intermittent mist to acclimate for use in future breeding projects.

Acknowledgements: This work was funded, in part, by the North Carolina Agricultural Research Service, Raleigh; the North Carolina Biotechnology Center, Research Triangle Park, NC; and the Kenan Institute, Raleigh, NC. Thanks are also expressed to Tom Eaker, Nathan Lynch, Joel Mowrey, Andra Nus and the staff at the Mountain Horticultural Crops Research and Extension Center for their technical assistance.

Literature Cited:


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**Figure 1**: Percentage of callus surviving as a function of oryzalin concentration.

(y = -1.0108x + 90.933, R² = 0.9882, p < 0.0002).
Figure 2: Percentage of diploid, mixoploid and tetraploid shoots recovered as a function of oryzalin concentration. 
(Diploid: $y = 0.2306x^2 - 8.5134x + 98.373$, $R^2 = 0.9894$, $p < 0.0001$). 
(Mixoploid: $y = -0.2002x^2 + 7.7026x - 0.6843$, $R^2 = 0.9979$, $p < 0.0001$).
Fire Blight Resistance Among Interspecific and Interploidy F₁ Hybrids of Cotoneaster

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Index words: *Erwinia amylovora*, disease resistance, Maloideae

Significance to the Industry: Cotoneaster is a common shrub or groundcover that once was extremely popular in the Southeastern U.S., but has largely gone out of favor. The decline in popularity of Cotoneaster is due, in part, to severe susceptibility of popular cultivars to fire blight, as well as few releases of new cultivars from breeding programs. With such diversity in this genus and its widespread adaptability to drought and poor soil conditions, we believe that targeted breeding to develop new cultivars with fire blight resistance could be a boon to the nursery and landscape industries.

Nature of Work: Five parents (two diploids and three tetraploids) were used in controlled crosses in a glasshouse (Table 1). Crossing schemes were designed based on phenotype, ploidy level, and disease resistance from the work of Rothleutner et al. (2, 3). We used flow cytometry and morphology to confirm our hybrids, which was feasible because all crosses, except *C. × suecicus* ‘Coral Beauty’ x *C. thymifolius*, were interploidy crosses with the sexual diploid ‘Coral Beauty’ as the seed parent. We performed flow cytometry analysis of DAPI-stained nuclei using a CyFlow PA (Partec, Münster, Germany) and *Pisum sativum* ‘Ctirad’ (2C = 8.76 pg) as an internal standard. We speculate that progeny of three of our controlled crosses in the glasshouse were the result of self-pollination; however, we had imperfect exclusion of potential pollinators, therefore we refer to these progeny as resulting from open-pollination.

We screened 15 genotypes for disease resistance in a randomized complete block design (RCBD) according to Rothleutner et al. (3). Briefly, selections (7 F₁, 3 OP, 5 parents) were asexually propagated by stem cuttings and grown in a glasshouse. With the exceptions of *C. simsonii* (1 clone/block), *C. splendens* (3 clones/block), and *C. thymifolius* (4 clones/block), there were three blocks with five clones per block. The youngest two leaves on actively growing shoots were bisected with scissors dipped in \(10^5\) CFU/mL of a virulent strain (Ea153) of *Erwinia amylovora*, the causal agent of fire blight. Shoot lesions were measured weekly and we present the mean percent shoot infection ± SEM eight weeks after inoculation as:

\[
\% \text{Shoot infection} = \left(\frac{\text{Lesion length}}{\text{Total shoot length}}\right) \times 100
\]

Results and Discussion: Flow cytometry was effective to determine which of our attempted interploidy interspecific crosses were successful and which likely resulted in self-pollination of our diploid parent (Table 1). In the case of the homoploid cross of ‘Coral Beauty’ x *C. thymifolius*, morphology was used to confirm that the progeny was a
successful cross. This seedling had very small and narrower leaves similar to C. thymifolius compared to its seed parent, ‘Coral Beauty’, and also compared to other progeny we identified as OP seedlings from ‘Coral Beauty’ using flow cytometry.

Disease severity was variable among parents, interspecific progeny, and open-pollinated seedlings (Fig. 1). Even though plants of a single accession were clonal, we observed greater than expected variation in susceptibility; particularly notable in this respect was C. divaricatus. However, this large variance was due to two plants in block 3 that had 100% shoot infection. Of the 15 clones of this species inoculated, seven were asymptomatic with no visible signs of infection. Progeny of interspecific crosses exhibited transgressive segregation. In some cases the progeny were more resistant than their parents (‘Coral Beauty’ x C. divaricatus and ‘Coral Beauty’ x C. thymifolius) and in other cases the progeny were more susceptible than either parent (‘Coral Beauty’ x C. splendens H2011-01-003, ‘Coral Beauty’ x C. simsonii).

To visualize trends among progeny we graphed percent shoot necrosis of full-sib progeny and open-pollinated seedlings of ‘Coral Beauty’. There were two genotypes of ‘Coral Beauty’ x C. splendens, three genotypes of open-pollinated seedlings from ‘Coral Beauty’, and three genotypes of ‘Coral Beauty’ x C. divaricatus (Fig. 2). The mean of the two selections of ‘Coral Beauty’ x C. splendens (8.5%) was intermediate of its parents, which were 11.1% (‘Coral Beauty’) and 0% (C. splendens), respectively. The mean of the open-pollinated seedlings of ‘Coral Beauty’ was substantially lower (2.2%) than ‘Coral Beauty’. It is unclear why these progeny showed nearly 10% lower shoot necrosis than ‘Coral Beauty’. Inadvertent pollination with a resistant parent is very unlikely because the leaf morphology and genome size is consistent with ‘Coral Beauty’. The progeny that were most distinct from their parents were derived from ‘Coral Beauty’ x C. divaricatus. The mean shoot necrosis of the three genotypes from this cross was 1.2%, which was substantially lower than either parent (11.1% and 20.8%).

The unpredictable heritability of fire blight resistance is not uncommon and has been observed in the progeny of several Asian pear species crossed with ‘Doyenné du Comice’ (1). In their study, they recovered transgressive segregants in both directions, similar to the results in our study with Cotoneaster. In crosses with various pear species, they observed that resistance was inherited as dominant and monogenic, polygenic, or contributed by a major gene (1). Due to similar variability of resistance among different interspecific crosses of Cotoneaster and the limited number of progeny, it is difficult to identify the mode of inheritance at this point and we continue to rely on phenotypic screening of all progeny. However, with increased progeny number, we hope to identify the mode of inheritance among various sources of resistance to fire blight in Cotoneaster.
Literature Cited

Table 1. Relative holoploid (2C) genome size of Cotoneaster F1 interspecific hybrids and seedlings resulting from open-pollination (OP); C. * × suecicus* ‘Coral Beauty’ was the seed parent of all seedlings.

<table>
<thead>
<tr>
<th>Taxon/Cross</th>
<th>Accession</th>
<th>2C (pg)</th>
<th>Ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. * × suecicus* ‘Coral Beauty’</td>
<td>10-0166</td>
<td>1.53</td>
<td>2x</td>
</tr>
<tr>
<td>C. divaricatus</td>
<td>10-0089</td>
<td>3.05</td>
<td>4x</td>
</tr>
<tr>
<td>C. simsonii</td>
<td>09-0032</td>
<td>3.18</td>
<td>4x</td>
</tr>
<tr>
<td>C. splendens</td>
<td>09-0024</td>
<td>3.03</td>
<td>4x</td>
</tr>
<tr>
<td>C. thymifolius</td>
<td>10-0122</td>
<td>1.55</td>
<td>2x</td>
</tr>
<tr>
<td>‘Coral Beauty’ x C. splendens</td>
<td>H01-002</td>
<td>2.42</td>
<td>3x</td>
</tr>
<tr>
<td>‘Coral Beauty’ x C. splendens</td>
<td>H01-003</td>
<td>2.61</td>
<td>3x</td>
</tr>
<tr>
<td>‘Coral Beauty’ OP(^z)</td>
<td>H01-004</td>
<td>1.66</td>
<td>2x</td>
</tr>
<tr>
<td>‘Coral Beauty’ OP(^y)</td>
<td>H02-001</td>
<td>1.84</td>
<td>2x</td>
</tr>
<tr>
<td>‘Coral Beauty’ x C. divaricatus</td>
<td>H02-002</td>
<td>2.35</td>
<td>3x</td>
</tr>
<tr>
<td>‘Coral Beauty’ x C. divaricatus</td>
<td>H02-003</td>
<td>2.42</td>
<td>3x</td>
</tr>
<tr>
<td>‘Coral Beauty’ x C. divaricatus</td>
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</tr>
<tr>
<td>‘Coral Beauty’ OP(^y)</td>
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<tr>
<td>‘Coral Beauty’ x C. simsonii</td>
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<tr>
<td>‘Coral Beauty’ x C. thymifolius</td>
<td>H04-001</td>
<td>1.68</td>
<td>2x</td>
</tr>
</tbody>
</table>

\(^z\) Attempted cross between ‘Coral Beauty’ x C. splendens that likely resulted in accidental self-pollination.
\(^y\) Attempted cross between ‘Coral Beauty’ x C. divaricatus that likely resulted in accidental self-pollination.
Figure 1. Percent shoot necrosis eight weeks after inoculating five parents and 10 F₁ or OP progeny selections with *Erwinia amylovora* strain Ea153 at 10⁹ CFU/mL. Plants were grown from rooted stem cuttings in a glasshouse immediately before and during disease screening.
Figure 2. Percent shoot necrosis eight weeks after inoculating three parents, F1, and OP progeny selections with *Erwinia amylovora* strain Ea153 at $10^9$ CFU/mL. Plants were grown from rooted stem cuttings in a glasshouse immediately before and during disease screening. There were two selections of ‘Coral Beauty’ x *C. splendens* and three selections of ‘Coral Beauty’ OP and ‘Coral Beauty’ x *C. divaricatus* pooled with means and SEM presented.
Genome Sizes and Ploidy Levels of Maples: Sample Handling and Preliminary Estimates

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Index words: flow cytometry, Acer, polyploidy

Significance to the Industry: Maples are important landscape trees that make up a significant portion of the urban canopy. The genus is rich with diverse forms and there are species broadly distributed—primarily in the temperate zone of the northern hemisphere. In 2009, wholesale production of maples was valued at more than $190 million in the United States (11). Even though this genus is an important nursery crop and significant breeding and selection has occurred, there is relatively little information available on genome sizes; and there are many gaps in the literature on chromosome numbers. Many plant breeders are inducing polyploidy in various species of maples to develop sterile, or semi-fertile, triploids. However, there are known examples of natural polyploids in the genus that could be used for breeding odd-ploidy selections that may have reduced fertility. Using naturally occurring polyploids would reduce breeding time by many years, and may provide growers with sterile selections not subject to regulation or banning imposed on some species (e.g. Acer platanoides, A. ginnala syn. A. tataricum ssp. ginnala). Due to the large number of species, cultivars, and hybrids in the genus, it may take longer to conduct a genus-wide survey of genome sizes than material will remain viable in cold storage. Therefore, in addition to making preliminary genome size estimates we had a secondary goal to evaluate the use of dried plant tissue that would enable long-term storage until sampling is possible.

Nature of Work: Genome sizes can be used to determine ploidy levels once calibrated by chromosome counts. Genome size data is also a useful tool for identifying hybrids in cases where genome size differences exist between parents. For example, plant breeders use interploidy crosses to develop odd ploidy (e.g. triploid, pentaploid) cultivars with reduced fecundity; therefore, resulting in selections with a reduced chance of escaping cultivation. Today, ploidy analysis is primarily done using flow cytometry (FCM) due to its reliability, convenience, and speed. Studies on best practices of flow cytometry generally recommend measuring cellular DNA content using fresh plant tissue (6,8). Fresh material has a limited shelf life; thus, completing large-scale surveys of genome size can be problematic. This can also be an issue when sampling in remote locations where there is limited cold storage (1). Use of dried plant tissue to determine ploidy levels was first applied fairly recently (6, 10). Currently, there are few studies using desiccated material and among these, both approaches and conclusions have varied. Furthermore, there are no studies demonstrating this technique in maples.
Plant material. Material from a total of 53 individuals (Table 1) was collected from two Oregon nurseries (Heritage Seedlings, Stayton; J. Frank Schmidt and Sons, Boring) including 27 species and 23 cultivars. We selected a subset of 10 taxa that included 2x, 3x, 4x, 5x, and 6x individuals to further test combinations of sample vs. standard handling (Fig. 2). Fresh material, including newly expanded leaf tissue, vegetative buds, and floral buds, was refrigerated for up to two weeks before sampling. Stem cuttings were rooted and received an accession number (Table 1). These plants are being maintained in containers. Samples from each individual were dried by placing material in individual nylon mesh bags, which were placed in an airtight container containing Drierite (W.A. Hammond DRIERITE Co. LTD, Xenia, Ohio) desiccating anhydrous calcium sulfate. A preliminary study was done comparing different drying methods (silica vs. oven) of Acer, and we determined that relative genome sizes were not different between the different drying methods. We chose anhydrous calcium sulfate because it is the simpler and more practical choice for material collected in the field for long-term storage.

Flow cytometry. Flow cytometry was conducted according to Contreras et al. (3) with the modification that when using dried tissue, 3 to 5 times more leaf or bud tissue was used than with fresh. Three subsamples were measured for each taxon. At least 3000 cells were analyzed per sample and had a CV that was less than 10.0 with few exceptions (<1% of samples). Data were subjected to mixed model analysis (PROC MIXED; SAS Institute Inc., Cary, NC) and means were separated based on Tukey’s HSD (α = 0.05). Data for holoploid genome size are presented as means ± SEM.

Cytology. Cytology was conducted according to Contreras et al. (2) and chromosomes were viewed with a light microscope (AxioCam by Zeiss; Jena, Germany) using ×1000 oil immersion.

Results and Discussion: Our preliminary analysis of the 53 individuals sampled indicated there were 40 diploids (2n = 2x = 26), four triploids (2n = 3x = 39), six tetraploids (2n = 4x = 52), one pentaploid (2n = 5x = 65), and two hexaploids (2n = 6x = 78). Monoploid (1Cx) genome size ranged from 0.69 to 1.27 pg with a mean of 0.93 pg (Table 1). We attempted to calibrate genome size and ploidy level by performing chromosome counts and were successful with a diploid and triploid accession (Fig. 1); but higher ploidy level counts were ambiguous and will require additional work. However, we confirmed that the comparatively large monoploid genome size of A. pseudoplatanus (1.14 to 1.27 pg) is not due to incorrect interpretation of data. Figure 1B shows that A. pseudoplatanus 14-139 is a triploid, which confirms the monoploid genome size presented. Also of note in this species is the cultivar ‘Puget Pink’. This cultivar has been reported to be apomictic due to homogeneity of seedling populations (E. Hammond, personal communication); however, we tested two seedlings (‘Puget Pink’ – 1 and ‘Puget Pink’ – 3) and identified that each were a different cytotype. Therefore, we confirmed that ‘Puget Pink’ produces sexually derived seed. Our small sample size is not conclusive, and more work is necessary to confirm our report of triploidy, which differs from most reports of A. pseudoplatanus as a tetraploid (9).
Another particularly interesting group is Series Rubra, which contains A. rubrum, A. saccharinum, and their hybrid A. ×freemanii. Acer rubrum has most commonly been reported as a diploid (9), hexaploid, and octoploid (2n = 8x = 104) (4); while A. saccharinum has been reported only as a tetraploid (4, 9). However, Freeman (7) reported in the original manuscript describing hybrids of red and silver maple that red maple varies between 36, 54, or 72 somatic chromosomes while silver maple has 26. It is not clear why his report differs from other literature, and he provided no photomicrographs. Dirr (5) describes both ‘Autumn Blaze’ and ‘Celebration’ as having an affinity with A. ×freemanii, and we identified both cultivars as tetraploids. This supports that silver maple is diploid, and the red maple parent was hexaploid; however, this is no more likely than if both parents were tetraploid. It is worth noting that ‘Brandywine’, which Dirr describes as a “true” red maple was identified as a hexaploid. Further work is needed to resolve this group.

Overall, dry tissue provided acceptable results to provide genome size estimates and ploidy analysis of maples. Our results are preliminary and there was instrument error greater than generally acceptable. We expect to have coefficient of variation (CV%) less than 7%, in this study some samples exceeded 10%. We are validating our findings, but we were able to draw some conclusions. When all taxa were pooled together there was a significant difference (p<0.001) between mean values of fresh vs. dry material, with dry treatment resulting in higher genome size. However, when we performed t-tests on individual taxa, only 9 of the 53 dry samples were significantly different than the fresh (Table 1). On the other hand, some taxa could not be analyzed when dry material was used, while fresh leaves or buds of all taxa produced clear results except for A. miyabei ssp. miaotaiense, which was recalcitrant regardless of material. However, more recently this taxon was run with no issue, so previous difficulty may have been due to the age of material collected.

Other studies that have investigated using desiccated material appear to have used fresh internal standard material, or fail to mention which type of standard material was used. The subset of 10 taxa we tested using different combinations of sample and standard handling included the entire ploidy range in the study, and we observed greater variation among treatments of higher ploidy selections than in diploids (Fig. 2). Sample treatment was not significant but we found that standard treatment was significant and using dry standard resulted in a slightly larger genome size. This finding is significant because it demonstrates that studies using desiccated sample material with fresh internal standards may not be providing the most accurate estimate and should be interpreted cautiously.

Literature Cited


Table 1. Calculated relative genome sizes and ploidy levels of 53 individuals from 10 sections, 14 series, and 27 species of genus *Acer*. Genome sizes were estimated using flow cytometry analysis of DAPI-stained nuclei and *Pisum sativum* 'Ctirad' (2C = 8.76 pg) as an internal standard. Ploidy levels were inferred from genome size calibrated by chromosome counts performed using light microscopy of root tip squashes.

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<thead>
<tr>
<th>Taxon</th>
<th>Source</th>
<th>Acc.</th>
<th>1C (pg)</th>
<th>2C (pg)</th>
<th>Ploidy</th>
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<td></td>
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<td><strong>Series Acer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>A. pseudoplatanus</em> - (green-leaved)</td>
<td>H.S.</td>
<td>14-139</td>
<td>1.15</td>
<td>3.44±0.09a</td>
<td>3x^v</td>
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<tr>
<td><em>A. pseudoplatanus</em> - (purple-leaved)</td>
<td>H.S.</td>
<td>14-138</td>
<td>1.14</td>
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<td><em>A. pseudoplatanus</em> 'Puget Pink' - 1</td>
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### Series Negundo

*A. negundo* 'Sensation'  
J.F.S.  0.69  0.73  1.38±0.03a  1.46±0.04a  2x

### Section Palmata

#### Series Palmata

*A. circinatum* 'Pacific Sprite'  
H.S.  14-0165  0.88  -  1.75±0.03  -  2x
*A. circinatum*  
H.S.  14-0154  0.84  0.95  1.68±0.05a  1.90±0.02b  2x
*A. japonicum* 'Ed Wood #2'  
J.F.S.  0.97  1.08  1.95±0.02a  2.17±0.02b  2x
*A. japonicum* 'Green Cascade'  
H.S.  14-0171  0.93  1.04  1.85±0.02a  2.07a  2x
*A. palmatum* 'Ara Kawa'  
H.S.  14-0179  0.90  -  1.79±0.02  -  2x
*A. palmatum* 'Butterfly'  
H.S.  14-168  0.94  0.99  1.87±0.02a  1.99±0.12a  2x
*A. palmatum* 'Emperor 1'  
H.S.  14-166  0.97  -  1.94±0.03  -  2x
*A. palmatum* 'Fireglow'  
H.S.  14-0180  0.94  1.07  1.88±0.07a  2.15±0.09a  2x
*A. palmatum* 'Karasu gawa'  
H.S.  0.95  0.99  1.90±0.07a  1.98a  2x
*A. palmatum* 'Kiyo hime'  
H.S.  0.97  1.02  1.94±0.07a  2.05±0.06a  2x
*A. palmatum* 'Mikawa Yatsubusa'  
H.S.  14-0167  0.95  1.07  1.91±0.05a  2.15a  2x
*A. palmatum* 'Ukigumo'  
H.S.  14-0170  0.91  -  1.82±0.01  -  2x
*A. pseudosieboldianum*  
H.S.  14-0156  0.89  0.96  1.79±0.04a  1.92a  2x
*A. shirasawanum* 'Aureum'  
H.S.  14-0169  0.92  1.05  1.85±0.02a  2.10±0.04b  2x

#### Series Sinensis

*A. campbellii*  
H.S.  14-0140  0.96  -  1.92±0.01  -  2x
*A. campbellii*  
H.S.  14-142  1.02  1.08  2.03±0.08a  2.16±0.02a  2x

### Section Pentaphylla

#### Series Trifida

*A. buergerianum*  
H.S.  14-0157  0.95  1.02  1.91±0.08a  2.03±0.06a  2x

### Section Platanoida

#### Series Platanoida

*A. miyabei* ssp. *mioitaense*-A  
H.S.  0.93  0.90  1.87±0.02a  1.81±0.01a  2x
*A. miyabei* ssp. *mioitaense*-B  
H.S.  0.86  0.94  1.73±0.02a  1.88±0.00b  2x
*A. platanooides* 'Columnare'  
J.F.S.  0.92  0.99  1.83±0.03a  1.98±0.07a  2x
*A. platanooides* 'Crimson King'  
J.F.S.  0.89  0.95  1.77±0.02a  1.89±0.13b  2x
*A. platanooides* 'Princeton Gold'  
J.F.S.  0.90  0.94  1.80±0.04a  1.87±0.15a  2x
*A. truncatum*  
H.S.  14-0159  0.87  0.89  1.74±0.09a  1.78±0.04a  2x

#### Section Rubra

#### Series Rubra

*A. ×freemanii* 'Autumn Blaze'  
J.F.S.  14-0181  0.96  0.96  3.85±0.03a  3.82a  4x
*A. ×freemanii* 'Autumn Blaze'  
J.F.S.  14-0182  0.98  1.01  3.91±0.05a  4.04±0.06a  4x
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### Section Trifoliata

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#### Series Mandshurica

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*H.S. = Heritage Seedlings, Stayton, OR; J.F.S. = J. Frank Schmidt and Sons, Boring, OR.

*Accession number from the Ornamental Breeding Program, Oregon State University, Corvallis, OR. Those with accession numbers were maintained as rooted cuttings or container plants.

*Monoploid genome size.

*Holoploid genome size presented as mean ± SEM; there is no SEM value for taxa that only had one successful replicate. Means followed by the same letters within row are not significantly different.

*Indicates ploidy was confirmed with cytology.

*Putative hybrid accession received from Keith Warren of J. Frank Schmidt and Sons Nursery.
Fig. 1. Photomicrographs of chromosome spreads prepared using meristematic root tips of (A) *Acer truncatum* 14-0159 (2n = 2x = 26) and (B) *A. pseudoplatanus* 14-0139 (2n = 3x = 39) used to calibrate monoploid genome size.
Fig. 2. Genome size estimates of 10 taxa calculated using flow cytometry analysis of DAPI-stained nuclei using *Pisum sativum* ‘Ctirad’ as an internal standard. Samples and standards were prepared as follows: dry sample/dry standard, dry sample/fresh standard, fresh sample/dry standard, or fresh sample/fresh standard.
‘Pam’s Mountain Bouquet’, ‘Empire’ and ‘Red Steeple’:
Three *Cornus kousa* Introductions by the University of Tennessee

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**Index Words:** Chinese dogwood, columnar form, disease tolerance, exfoliating bark, fused bracts, selection

**Significance to Industry:** *Cornus kousa* or Chinese dogwood is a large-bracted dogwood native to Asia and a closely related sister species of *C. florida* (flowering dogwood). Cultivars of this species generally flower about one month after flowering dogwood, although some accessions may delay flowering for even longer periods. Many *C. kousa* cultivars and lines exhibit better tolerance to diseases, such as powdery mildew and dogwood anthracnose, than most *C. florida* cultivars, and are not plagued with boring insect problems. Here, we introduce three new cultivars of *C. kousa*, which have unique horticultural characteristics as well as exceptional tolerance to dogwood anthracnose and powdery mildew.

**Nature of Work:** We aimed to select accessions of *C. kousa* with superior horticultural characteristics from a collection of related seedlings. Three accessions were selected and released by the University of Tennessee.

**Results and Discussion:** The remainder of the report is a condensed and abbreviated version of Wadl et al. 2014 published in HortScience 49(9):1230-1233 (1) and used with permission from the American Society for Horticultural Science. For additional details including color and shape of leaves, color of bark, etc., please consult the HortScience publication listed above.

We received a collection of about 400 open-pollinated seeds from Ms. Polly Hill, Barnard’s Inn Farm, MA in 1989. Seeds were stratified, germinated and grown in containers and eventually planted at the Tennessee Forest Resources AgResearch and Education Center, Oak Ridge, TN in 1994. The plants were rated for dogwood anthracnose and powdery mildew susceptibility/tolerance over the next five years and plants that exhibited signs and symptoms of the two diseases were rogued from the plot. The remaining plants were observed for horticultural characteristics over the next 15 years. Three plants were selected to be released by Tennessee AgResearch and named ‘Pam’s Mountain Bouquet’, ‘Empire’ and ‘Red Steeple’. We have included a brief cultivar description for each of the three cultivars.

‘Pam’s Mountain Bouquet’ (PP 25,575) has a spreading form that prolifically flowers, and prominently features fused bracts (Figure 1A; Table 1). Eighty-three percent...
of the bracts of individual inflorescences were fused along one or more sides (Table 1). The ovate bracts are pure white with an inflorescence diagonal mean length 7.4 cm (2.9 inches); a mean width of 5.3 cm (2.1 inches) and a mean peduncle length of 6.8 cm (2.7 inches). The mean number of flowers per inflorescence was 34. The completely fused bracts (Figures 1B and C) remain encircling the peduncle after senescing. Dogwood anthracnose and powdery mildew were not observed on this cultivar.

‘Empire’ (Figure 2A) is a very narrow columnar form growing to 10.0 m (about 32 feet) tall by 1.2 m (about 4 feet) wide after 20 years. It possesses brilliant, non-fused, ovate white-bracts (Figure 2B), which are slightly overlapping. Outside mean bract length is 3.2 cm (1.3 inches) and outside bract mean width (widest point) is 3.1 cm (1.2 inches). Inside bract mean length is 3.2 cm (1.3 inches) with the inside bract mean width (widest point) of 3.1 cm (about 1.2 inches). The peduncle is stiff with a mean length of 3.7 cm (1.5 inches) and there are about 25 flowers per inflorescence. Another notable feature of the cultivar is the exfoliating bark (Figure 2C), which adds year-around interest. Dogwood anthracnose and powdery mildew were not observed on this tree.

‘Red Steeple’ has a columnar-shaped canopy and grows to about 13 meters (about 15 feet) after 20 years (Figure 3A) with red foliage (Figure 3B) that fades to green with warmer temperatures. The white bracts have a red tint along the margins in cool temperatures, but fades with heat. The bracts slightly overlap (about 30% at widest point; Figure 3C). Outside bract mean length is 3.4 cm (1.3 inches) and a mean width of 2.0 cm (about 0.8 inches); whereas inside bracts have mean length of 3.3 cm (about 1.2 inches) and a mean width of 1.9 cm (about 0.75 inches). The mean number of flowers per inflorescence is 26. The peduncle is stiff with a mean length of 4.5 cm (1.8 inches). Dogwood anthracnose and powdery mildew were not observed on this tree.

These three cultivars are well-suited to the warmer temperatures found in the southern United States (US) and should be easily adaptable to the cooler northern US. A limited amount of budwood has been distributed to wholesale nurseries in Tennessee and Japan and trees are now available.

Acknowledgement: The authors gratefully acknowledge the financial support of the project through USDA/ARS grant number: 5864041637 and AgResearch.

**Literature Cited**

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Figure 1. *Cornus kousa* ‘Pam’s Mountain Bouquet’. A. Original tree after 20 years. (B-C) Close-up of bract display.
Figure 2. *Cornus kousa* ‘Empire’. (A) Original tree after 20 years of growth. (B) Brilliant white bracts that slightly overlap. (C) Exfoliated bark.
Figure 3. *Cornus kousa* 'Red Steeple'. (A) Original tree after 20 years of growth. (B) Red foliage that fades with high temperatures. (C) Bract display.
Developing Non-Invasive Callery Pears: Fertility and Reproductive Biology of Triploid Cytotypes


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Index Words: aneuploidy, flow cytometry, genome size, invasive, plant breeding, Pyrus spp., seedless, unreduced gametes

Significance to Industry: Pyrus calleryana has become a popular landscape tree in the United States and is grown in USDA hardiness zones 5 to 8(9). It is valued for its abundance of white flowers, fall color, broad adaptability, and pest resistance. As a species, P. calleryana is not without problems and can be susceptible to fireblight, splitting and breakage of trunks and branches in older trees, and most recently the concern of invasiveness (1, 3). Birds readily eat the fruits and spread the seeds of the species into areas near where it is planted. In many areas, P. calleryana has naturalized in old fields and along highways. Pyrus calleryana is a diploid species (2n = 2x = 34) (4). Development of triploid plants could result in seedless, low fertility cultivars of P. calleryana and related hybrids that would be desirable as an alternative for those in the current landscape. Triploids typically have low fertility due to unbalanced chromosome segregation in meiosis. However, triploids can have limited fertility resulting from formation of apomictic embryos, unreduced gametes and the union of aneuploid gametes (3). The objective of this study was to evaluate fertility and reproductive pathways in selected triploids of Pyrus calleryana.

Nature of Work: A population of triploid Pyrus calleryana was established at the Mountain Horticulture Crops Research and Extension Center (MHCREC) in Mills River, NC. Fourteen triploids were selected from the population based on desirable traits including precociousness, heavy flowering, desirable forms, resistance to fire blight, and limited fruit set. Female fertility was determined based on fruit set (%), seeds per fruit, germination (%), and overall fertility expressed as the number of seedlings per flower from open pollinated trees. At flowering, three branches were selected on each tree and number of flowers were counted. Fruit was collected and seed were extracted and stratified for 90 days at 40°F (4°C) then moved to a greenhouse at 65-75°F (18-21°C) for 90 days to determine the number of viable seedlings. The experiment was completely randomized with three diploid and 14 triploid clones (Table 1).

Flow cytometry was used to determine the 2C genome sizes of parents and seedlings. Approximately 0.5 cm² of plant material was placed in a 55 mm plastic Petri dish, along with 0.5 cm² Pisum sativum L. ‘Citrad’, an internal standard with a known genome size of 2C = 8.75 pg. Four hundred µl of extraction buffer (CyStain UV Precise P Nuclei...
Extraction Buffer, Partec, Munster, Germany) was added to the Petri dish. Tissue was chopped with a razor blade for 30 to 60 seconds and incubated for approximately 30 seconds (no more than five minutes). The suspension was then filtered through Partec 50 µm CellTrics disposable filter into a sample tube. Nuclei were stained with 1.6 mL 4',6-diamindino-2-phenylindole (DAPI). Nuclei were analyzed using a flow cytometer (PARTEC PAII, Partec) to determine the mean sample nuclei florescence relative to that of the internal standard. Approximately 5,000 nuclei were measured per sample. Genome sizes of samples were calculated as: mean florescence value of sample x nuclear DNA content of standard / mean fluorescence value of standard.

**Results and Discussion:** Female Fertility. Fruit set, seeds per fruit, germination, seedlings per flower, and relative fertility all varied considerably among individual triploid clones (Table 1). Relative female fertility represents the number of seedling germinated per flower relative to the most fertile diploid control and ranged from 0.00 to 73.00% among the triploids. Of the 14 triploids used in this research, five accessions had a relative fertility of <2%. Three accessions, H2008-047-008, H2008-048-010, and H2008-049-015, had no measurable female fertility (Table 1). These results demonstrate that the impact of triploidy on female fertility in Callery pear varies on a case-by-case basis and needs to be evaluated for individual clones.

Reproductive Pathways. The mean 2C genome size of the three diploid cytotypes was 1.25 ± 0.05 (SEM) pg, whereas the mean for the fourteen triploid cytotypes was 1.88 ± 0.12 (SEM) pg (Table 1), thereby confirming their ploidy levels. The progeny from triploid maternal parents varied in genome size ranging from 1.35 to 3.11 pg with a mean of 1.70 pg (Fig. 1). The majority of the progeny from the maternal triploids had 2C genome sizes near a triploid level or between diploid and triploid levels which indicates that these parents are producing predominantly aneuploid gametes. Aneuploids typically have reduced fitness and fertility and can suffer from abnormal development (2). However, a few progeny from maternal triploids had genome sizes near diploid levels indicating that a generational reversion to a diploid cytotype may be possible in limited instances. Also, a limited number of progeny also had genome sizes greater than triploid, including near tetraploid and above, indicating fertilization from unreduced gametes from one or both of the parents (2). This study documented that some triploid Callery pears display substantial reductions in fertility (as much as 100%) and that most of the seedlings derived from triploid maternal parents were abnormal aneuploids with the infrequent production of some apparent isopoloids (2x and 4x). Selections of highly-infertile triploid cultivars should be a viable approach to reduce or eliminate self-sowing of Callery pears in the landscape.

**Acknowledgements:** This work was funded, in part, by the North Carolina Agricultural Research Service, Raleigh; the J. Frank Schmidt Family Foundation, Boring OR; the North Carolina Biotechnology Center, Research Triangle Park, NC; and the Kenan Institute, Raleigh, NC. Thanks are also expressed to Nathan Lynch, Joel Mowrey, Andra Nus and the staff at the Mountain Horticultural Crops Research and Extension Center for their technical assistance.
**Literature Cited**


**Table 1.** Ploidy level, 2C genome size, fruit set, number of seeds per fruit, germination, number of seedlings per flower, and relative fertility based on diploid controls of selected *Pyrus calleryana* cytotypes.

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<th>Ploidy (x)</th>
<th>2C Genome Size</th>
<th>Fruit Set (%)</th>
<th>Seeds/Fruit</th>
<th>Germination (%)</th>
<th>Seedlings/Flower</th>
<th>Relative Fertility* %</th>
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<td>3</td>
<td>1.75 ± 0.00</td>
<td>0.30 ± 0.01</td>
<td>1.33 ± 4.53</td>
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<td>H2008-048-010</td>
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<td>4.84 ± 0.14</td>
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<tr>
<td>H2008-048-019</td>
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<td>32.90 ± 0.10</td>
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<td>12.32 ± 0.14</td>
<td>0.10 ± 0.12</td>
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<td>25.45 ± 0.49</td>
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<td>16.32</td>
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<td>Diploid 3</td>
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<td>46.76 ± 0.22</td>
<td>3.16 ± 0.72</td>
<td>52.83 ± 0.15</td>
<td>0.80 ± 0.62</td>
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*2C DNA values represent the mean value of four subsamples conducted for each taxon.

*Values are means ± 1 SEM.

*Calculated as (seedlings/flower)/(0.80), where 0.80 is the number of seedlings per flower of the most fertile diploid control.
Figure 1. Frequency distribution of 2C genome sizes of seedlings derived from open pollinated diploid and triploid *Pyrus calleryana* cytotypes. Gate width for columns was based on a 95% prediction interval for diploid, triploid, and tetraploid parents calculated as the mean ± 1.96 (SEM).
Chemical Mutagenesis of *Galtonia candicans* Decne. Results in Altered Morphology and Reduced Fertility

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**Index words:** ethyl methane sulfonate, EMS, cape hyacinth, summer hyacinth

**Significance to Industry:** *Galtonia candicans*, cape hyacinth, is a cold hardy bulb species native to South Africa. While it is a relatively low input summer flowering bulb that produces flowers from early summer through frost, there has been little cultivar development. This species is also of interest to gardeners because its flowers attract pollinators throughout the summer season; however, once the racemes of blooms become laden with open flowers, the plant lodges. Additionally, this species produces very large quantities of seed and may have the potential to become a nuisance in the garden or perhaps escape cultivation. In this study, we sought to induce variation in the species through the application of ethyl methane sulfonate (EMS) in order to produce improved forms through reduced height that would decrease lodging and reduced fertility that would alleviate concerns over weeediness. A cape hyacinth cultivar with reduced height and fertility will provide a nursery crop that is potentially drought tolerant and can fit on shipping carts in wholesale nurseries and be attractive to consumers in garden centers. Additionally, results from this study will provide further insight into the effects of EMS on the morphology and fertility of related species in Asparagaceae, which could further aid in cultivar development.

**Nature of Work** *Galtonia candicans* is a self-pollinating crop with little variation. The species has a Royal Horticulture Society Award of Garden Merit due to the ease with which it performs in the garden. It requires little watering and is relatively pest and disease resistant. When planted in the Willamette Valley of Oregon, *G. candicans* flowers from June-July until frost providing season long interest. As observed by the authors, pollinators are drawn to *G. candicans* throughout the blooming period. Season-long blooming, attraction of pollinators, drought tolerance, and disease and pest resistance are all desirable traits in an herbaceous perennial, yet there has been little targeted breeding to improve form. The only reported cultivar found as of this writing is the double flowering form *G. candicans* ’Moonbeam’ (4) found in New Zealand. As reported by Armitage (2), the species grows to 4’ tall (personal observation in field was up to 5’ tall). Due to both height and weight of the flowers, the plant is prone to lodging unless planted in mass.

EMS is a chemical mutagen used by breeders to induce variation in relatively homogeneous populations. Through the application of EMS to seeds during the imbibition period, the chemical can be taken up by the embryo resulting in genome wide
point mutations. Resulting mutations include reduced height and fertility and changes in floral morphology (1,3,5).

**Mutagenesis** Seeds of *Galtonia candicans* were collected from a single plant at the Oregon State University (OSU) North Willamette Research and Extension Center during September, 2011. In early winter of that year, a factorial arrangement of treatments was applied to the seeds. The first factor of the treatment was a 24-hour imbibition treatment prior to EMS application (pre-soak or no pre-soak). The second factor was EMS concentration (0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%). This factorial arrangement of treatments produced 12 total treatments. Each treatment was replicated three times on experimental units of seed lots containing 300 seeds. After EMS treatment, the seed lots were sown in separate 1.33 L mum pots for germination. Mum pots were kept in greenhouse in completely randomized design.

**Germination and Field Planting** Germinated seedlings were counted and transplanted to 0.6 L pots in February, 2012. Potted seedlings remained in containers until field planting in May, 2013 at the OSU Lewis-Brown Farm, Corvallis, OR (USDA Hardiness Zone 8b). Treatments were planted in rows (not randomized), and the replications for each treatment were kept separate (Fig. 1).

**Field Evaluation** Quantitative and qualitative phenotype data were collected from M<sub>1</sub> plants in summers 2013 – 2014. Quantitative data collected included first flower date, leaf length/width, peduncle height, scape height, overall plant height, number of flowers per inflorescence, and number of scapes per plant at first flower. Qualitative data collected included lodging, flower form, flower markings/color, foliage texture, and foliage yellowing. Fruits were collected from randomly selected open-pollinated control and M<sub>1</sub> plants for fertility assessment. Number of fruits and seeds were recorded.

**Preliminary M<sub>2</sub> Germination Study** Open-pollinated seed was used in a preliminary germination study to determine seed viability. The germination study was conducted in a controlled greenhouse maintained at ~24℃. Families of control and M<sub>2</sub> seeds were sown in 1.33 L mum pots in arranged in a randomized block design. Only seed from the 0%, 0.2% and 0.4% were used. Only one M<sub>2</sub> seed from the 0.4% treatment was recovered. There was no 0.6% M<sub>2</sub> seed available for this germination study.

**Results and Discussion:** Germination percent of M<sub>1</sub> seed is reported in Table 1. The pre-soak treatment had a detrimental effect on the germination of M<sub>1</sub> seed resulting in high fatality. Of the seeds that were pre-soaked, only seeds in the control treatment (0% EMS) and the 0.2% EMS treatment germinated. None of the germinated seedlings from the pre-soak + 0.2% EMS survived for field planting. An ANOVA for completely randomized design was performed for germination data of the no-soak group of treatments. These results indicated that there was a significant effect due to EMS treatment. Fisher’s LSD was applied for means separation. This effect on germination can also be observed in Figure 2. As the concentration of EMS was increased, the germination percent decreased. Due to the effects of the pre-soak treatment on germination, this method is not a viable approach for EMS induced mutation in G.
candidans using the rate and duration we applied. It should be noted that the germination of M₁ seed lots (300 seed per lot) may have been affected by the sowing method. These were sown one seed lot per 1.33 L mum pot which may have resulted in decreased germination of the control lots. When M₂ seed were sown, seed lots were no greater than 30 seed per 1.33 L mum pot. As seen in Table 2, germination percent of the control was much higher than found in the M₁ generation. An ANOVA of M₂ found no block effects, and did show a significant difference due to EMS treatment. A means separation using Fisher’s LSD found that all three treatments (0%, 0.2%, and 0.4%) were significantly different.

As noted in previous studies (3, 5), an effect on plant height can be expected after treatment with EMS. This study found that the application of EMS does affect the height of cape hyacinth (Fig. 3). Height data were analyzed using ANOVA, and means separation was conducted using Fisher’s LSD. We observed that with an increase in EMS concentration, there was a significant decrease in mean overall plant height. In addition to a decrease in height, we observed a decrease in fertility. As shown in Table 2, there was a significant decrease in the average number of seed produced per capsule.

Other morphological mutations noted in the field were changes in inflorescence structure. In some cases, additional branching of the inflorescence was noted. There was also the observation of green markings on petals that resembled markings found on Leucojum spp. There were few mutations that resulted in changes to reproductive organs. In one case, there was the separation of the three carpels that resulted in an increase in flower size; however, this character was not consistent over the whole plant. A phenotype that was observed on a number of plants was a change in the orientation of the corolla. Usually, the corolla is pendulous and points toward the ground, but our mutants had flowers that were oriented toward the apex of the plant.

It should also be noted that the field planted M₁ generation withstood freezing temperatures the first winter they were in the ground. The Willamette Valley experienced an extreme winter with temperatures much colder than usual. In December 2013, the temperature reached a low of -18 °F and freezing temperatures were maintained for at least a week. These temperatures were uncharacteristically early and severe for the Willamette Valley. This supports the zone designation (USDA Hardiness Zone 6 – 9) stated by Armitage (2).

The results presented here support the use of EMS treatment as a viable method to reduce plant height and fertility in Galtonia candidans. In future efforts to use this treatment on G. candidans, there should be no pre-soaking with water or the imbibition duration should be reduced along with a reduction in duration and rate of EMS. We will continue to explore the phenotypic effects of EMS on G. candidans, and the heritability of these mutations in later generations.
Literature Cited

Table 1. Mean seedling germination percent of the seed treatments of *Galtonia candicans* not receiving the 24-hour imbibition treatment.

<table>
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<th>EMS Concentration (%)</th>
<th>Mean germination (%)</th>
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<tr>
<td>0</td>
<td>39.56 ± 1.09 a¹</td>
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<tr>
<td>0.2</td>
<td>36.78 ± 1.25 b</td>
</tr>
<tr>
<td>0.4</td>
<td>36.56 ± 0.68 b</td>
</tr>
<tr>
<td>0.6</td>
<td>11.78 ± 3.90 c</td>
</tr>
<tr>
<td>0.8</td>
<td>0.89 ± 0.89 d</td>
</tr>
<tr>
<td>1.0</td>
<td>0.00 ± 0.00 d</td>
</tr>
</tbody>
</table>

¹Values followed by different letters within a column are significantly different, LSD, P ≤ 0.05.

Table 2. Mean number of seed per capsule collected from field planted *M₁ Galtonia candicans* and germination percent of *Galtonia candicans M₂ seed*.

<table>
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<th>EMS Concentration (%)</th>
<th>Average seed per capsule</th>
<th>Mean Germination (%)</th>
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<td>0</td>
<td>17.15 ± 0.68 a²</td>
<td>89.44 ± 2.42 a</td>
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<tr>
<td>0.2</td>
<td>3.06 ± 0.65 b</td>
<td>57.93 ± 4.56 b</td>
</tr>
<tr>
<td>0.4</td>
<td>0.00 ± 0.00 c</td>
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</table>

²Values followed by different letters within a column are significantly different, LSD, P ≤ 0.05. Data were analyzed using ANOVA, means separation using Fisher’s LSD.
**Figure 1.** Field layout of M₁ population of seedlings from EMS seed treatment of *Galtonia candidans*. Spacing between individual plants at planting was 0.3 m. Turf is planted in all areas outside of rows. All rows are on drip irrigation. Strip between rows ~0.9 m. Rows are planted in direction of arrow along bottom of figure.
Figure 2. Germination of *Galtonia candidans* seedlings 10 weeks after treating seed in 0, 0.2, 0.4, 0.6, 0.8, or 1.0% EMS for 24-h following a 24-h pretreatment in water (A) or no pretreatment (B).
Figure 3. Scape height of *Galtonia candicans* plants treated with varying concentrations of EMS measured at first flower. Increase in EMS concentration resulted in significant reduction in mean scape height. Data were analyzed using ANOVA, mean separation using Fisher’s LSD.
Thirty years of Plant Evaluation at SFA Gardens: A Retrospective

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Index words: Plant evaluation, woody ornamental trials, SFA Gardens, trees, shrubs.

Significance to Industry: SFA Gardens has prospered because of a mix of funding sources, an enthusiastic staff, and good luck. For nurserymen, landscapers and academics, SFA Gardens is an opportunity to absorb the breadth of landscape diversity we can enjoy in our region. It's an opportunity to acquaint oneself with many interesting and rarely encountered trees and shrubs, all planted in a landscaped venue. Visitors can make on-the-ground comparisons of similar varieties in side-by-side trials which will help to make informed decisions on what to grow. SFA Gardens is a dawn-to-dusk open public garden which means University students, the local population, and visitors from afar walk the collection. Building enthusiasm for gardening, farming, forestry and environmental science is one of our main responsibilities and SFA Gardens facilitates that.

Nature of Work: Since 1985 tree and shrub evaluation at SFA Gardens has been a key focus. Diversity for the sake of diversity is a major goal. In design, there's the benefit of repetition and massing to create a certain landscape effect. For the collector, this is space wasted. Plant evaluation at SFA Gardens can be separated into two general arenas. Trees, shrubs, and herbaceous perennials are planted into the landscape with some attention to plant exposure and drainage needs. The earliest plantings date back to the 1980s. The second strategy is a range of plantings that feature a “collection” based strategy, basically numerous varieties in side-by-side trials (crapemyrtles, azaleas, hydrangeas, Acer, Camellia, etc.) with the intention of collecting performance data and making rational observations (3, 4, 5, 6, 9). The goal of both strategies is the same: reaching a conclusion on what performs well and what doesn’t.

Location: Nacogdoches is in the Pineywoods vegetation region of East Texas, about two hours northeast of Houston, 3 hours southeast of Dallas and 50 miles east of the border with Louisiana. Nacogdoches is the oldest town in Texas, a small city of 30,000 citizens with a University student population of about 13,000. We are the Lumberjacks. Soils in East Texas are generally well drained, slightly acidic, and the native flora is dominated by pine, oak, river birch, sweetgum, sycamore, Florida maple, hornbeam, elm, hackberry, pecan and hickory.

SFA Gardens: SFA Gardens comprises 128 acres (58 ha) of on-campus property at Stephen F. Austin State University (SFA), Nacogdoches, Texas. SFA Gardens is the umbrella organization responsible for the activities, growth and development of five
theme gardens. Representing the oldest plantings, the 10-acre (4.5 ha) SFA Mast Arboretum was initiated in 1985 and includes the horticulture facility of the Agriculture Department. Second, the Ruby M. Mize Azalea garden is an 8-acre (3.2 ha) garden of primarily azaleas, camellias and Japanese maples that was dedicated in April, 2000. Third, the 42-acre (19 ha) Pineywoods Native Plant Center (PNPC) was dedicated by Lady Bird Johnson in April 2000. A relatively new land resource, SFA’s Recreational Trail and Gardens (SFARTG) was dedicated in March 2010 and comprises 68-acre (31 ha) acres of mostly undisturbed forest. As the result of a donor with a vision, a portion of the SFARTG has been set aside as the 8-acre (3.2 ha) Gayla Mize Garden, which is directly across University Drive from the Ruby M. Mize Azalea Garden. This new garden has allowed SFA Gardens to expand ornamental tree and shrub evaluation. SFA Gardens enjoys five full time employees and two half-time employees, all funded by a combination of state and external grant funding.

**Climate:** Nacogdoches is in Zone 8B of the Pineywoods region in East Texas with an average annual rainfall of 48 in (122 cm). June through August is characteristically hot and dry. 1 Sept 2000 was the record high, 112 F (44.4 C), and 23 Dec 1989 was the record low 0 F (-17.8 C). In 2010 and 2011, Nacogdoches experienced all-time record drought and heat. Total precipitation in 2010 was 22.3 in (56.6 cm) and 2011 was 35.4 in (89.9 cm), with one third of that coming late in the fall. Because much of SFA Gardens lies in the floodplain of LaNana creek, flooding is a reality. A June 17-18, 2015 flood because of Tropical Depression Bill forced the creek out of its banks into the garden. While the author can recount three flooding events of this severity in thirty years, we have reached the conclusion that flooding, while shocking, hasn’t been a long term tragedy.

**History of Plant Evaluation at SFA:** The beginning of the SFA Arboretum in 1985 as a student-driven project of the first Landscape Plant Materials class created a small garden on the south side of the Agriculture building. Generous donations from the nursery industry filled out a small .25 acre (.1 ha) plot. The first plant trials on the south side of the Agriculture building included a large petunia variety collection in raised beds. This was soon followed by a fascinating collection of fifty-two varieties of creeping Juniper, *Juniperus horizontalis* (2). ‘Blue Chip’ remains one of our favorites. In fact, the 1988 gift of a creeping juniper collection from J.C. Raulston, of the North Carolina State University Arboretum, Raleigh, North Carolina, may have stimulated, more than anything, our early interest in plant evaluation. As the garden grew, the focus remained on diversity. We envisioned the garden as a germplasm repository, as well as a long term ornamental evaluation program. Plants were accessioned, planted, and mapped. After thirty years, SFA Gardens is now home to many interesting and rare plants that have survived the test of time in a Pineywoods landscape.

**Trees and Shrub Evaluation:** SFA Gardens is home to a wide range of trees and shrubs scattered across gardens and landscapes. In most cases, plants have been placed in what is hoped to be an appropriate environment. For the most part, the azaleas, camellias and Japanese maple are under high pine canopy shade. Dry loving shrubs and small trees are placed in full sun locations, often on a mild berm to improve
drainage. Because much of the garden is bottomland, we pay particular attention to drainage. Not everything is perfect. In too many cases, shrubs brilliantly placed 20 years ago are now swamped by their neighbors. Many never reveal their full potential, something we deal with by pruning, moving the plants to a better location, or just letting them languish. The following represents a portion of our evaluation work. For any evaluation program, it would be imprudent to ignore the major market movers in the tree and shrub arena. SFA Gardens has done that. While the list of trees and shrubs in the collection numbers in the thousands, this paper focuses on those that have performed well, are well known in the market place, or have merit suggesting much greater use.

**Aceraceae:** Maples have long been a focus of SFA Gardens. The collection of Japanese and Full Moon maples exceeds three hundred varieties with the first planting dating back to 1988. SFA Gardens includes three evergreen Asian species that have performed well here for over 20 years. An interest in “western” genotypes led to an extensive planting of *Acer saccharum* ssp. *skutchii*, the Mexico mountain sugar maple. This species can be characterized as more alkaline and drought tolerant than the species (6). In December, 2010, 277 seedlings of the skutch maple were planted at the University’s Science Research Center near SFA. We are entering the first phases of selection, cutting propagation and distribution of superior forms.

**Cercis:** In the Deep South, redbuds are major trees in the small flowering tree category. Thirty years ago there were few varieties available. In 2015, the market place is seeing an increase in new introductions. Varieties in our trials include ‘Ace of Hearts’, ‘Ruby Falls’, ‘Merlot’, ‘Rising Sun’, ‘Alley Cat’, ‘Oklahoma’, ‘Solar Eclipse’, and ‘Hearts of Gold’. Two new recent releases from North Carolina State University are ‘Pink Pom Poms’ and ‘Carolina Sweetheart’, both breakout redbuds in an ever increasing market.

**Taxodium distichum**: SFA Gardens has a long history evaluating baldcypress. Baldcypress is in the cypress family, Cupressaceae, one of several ancient genera in the family commonly known as cypresses. Once three separate species under the genus *Taxodium*, we are currently accepting *Taxodium* as one species with three botanical varieties: 1) *Taxodium distichum* (L.) Rich.var. *distichum* (Baldcypress), 2) *Taxodium distichum* var. *imbricarium* (Nutt.) Croom (Pondcypress), and 3) *Taxodium distichum* var. *mexicana* Gordon (Montezuma cypress). SFA Gardens has collected Taxodium varieties and genotypes since the 1980s. The collection includes single representatives or multiples of over 92 varieties or selections representing the diversity in the three botanical varieties. In addition, the collection includes China-bred “hybrids” from the breeding and improvement program of the Nanjing Botanical Garden (NBG), Nanjing, China. The 2014 joint release with NBG of ‘LaNana’, tested as T-406, introduces a cross of baldcypress and Montezuma cypress with good leaf blight resistance, fine form, alkalinity tolerance, and fast growth rate (13). SFA Gardens Taxodium bulletins, planting plans, research reports and updates can be retrieved at the SFA Gardens Plants Webpage. A review article published in 2011 summarizes the breadth of Taxodium research at SFA Gardens (8).
**Quercus:** SFA Gardens has a wide collection of native and exotic oak species. However, a primary emphasis has been placed on “western” genotypes that harbor superior heat, drought, and alkalinity tolerance (7). Proven performers include *Quercus rysophylla*, *Q. canbyi*, *Q. polymorpha*, *Q. germanna*, *Q. glauoides*, *Q. tarahumara* and *Q. grisea*. The most recent introduction is *Quercus insignis*, a very rare Mexico species that features the largest acorns in the genus.

**Rhodoendron:** Azaleas are major nursery and landscape shrubs in the South. Since the first plantings thirty years ago, the SFA Gardens collection has grown to include over 8000 azalea plants, which is comprised of more than 550 species and/or varieties. In the last decade, nothing has stirred the azalea industry more than the phenomenon of repeat blooming. While the major spring azalea bloom show at SFA Gardens is mid-March to mid-April, there have long been varieties that bloom at other times of the year. Since the late 1990s, reblooming azaleas have grown from only six to over 80 varieties that are part of various branding programs. First to impact the market and now with 29 varieties, Encore™ is the oldest brand, perhaps one of the best known brands in all of Horticulture. All begin with the word ‘Autumn’, which also imprints the plant into the buying public’s mind. More recent participants include 1) the Proven Winners® brand, the BLOOM-A-THON® group (six varieties), 2) Garden Debut’s® REBLOOM™ group (nine varieties), 3) the Gardener’s Confidence Collection, BLOOM-N-AGAIN®, which features 28 varieties, 4) HGTV’s Always Azaleas™ (five varieties), and, finally, 5) JBerry Nursery’s Dejavu Bloom™ series (five varieties) released in 2014. Deciduous azaleas are now a major focus at SFA Gardens and our goal is simple: to have the best collection of deciduous azaleas in the South. This is a more coherent group to work with and our collection now includes over 162 varieties or seedling selections on trial. They are characteristically truly fragrant, lose their leaves during the winter and feature blooms before the leaves emerge. We have long promoted deciduous azaleas as worthy of greater use. Once fully established after several years, we find them to be very drought and heat tolerant and rarely devastated by the impact of lacebugs, a common pest in southern USA landscapes.

evaluate only the new varieties that celebrate “reblooming”. This cooperative project is under way and it hasn’t taken long to realize that the market is packed with even more new varieties than our original estimate. At last count, there are over 95 new varieties of lacecap and mophead hydrangeas since 2006 that tout reblooming as a key attribute, and most fall under the umbrella of a major brand. Brands include Endless Summer® (Bailey), Forever & Ever™, Cityline™, Edgy™, Everlasting™ (Plants Nouveau), Mystical™, Hovaria® (Kaleidoscope®), Japanese Lady Series (Halo™, Frau™, and Angel™), Let’s Dance™ (Spring Meadow), Next Generation™ (Ball Ornaments), and Showstopper Hydrangeas™, a series promoted by HGTV which includes eight varieties. With brands, trademarks and patents making a stronger presence every year and nomenclature questions increasing, it’s easy to understand variety overload (1). While it’s hard to imagine improvements, future breeding projects might include better flower shedding, stronger stems, more reblooming, and burgundy foliage color (15).

*Vitex agnus-castus*, chaste tree, is a large shrub or small tree that has been much maligned across the southern USA for many years. The main feature is a summer bloom of bright blue, pink, or white blooms. The species is woody in the southern USA and an herbaceous perennial in more northern regions. We have six blue flowered varieties in our collection and find all of them to be good performers. ‘Montrose Purple’ and ‘LeCompte’ are most attractive. The first light pink-flowered variety, ‘Salinas Pink’, was introduced several years ago by Greg Grant, Research Associate at SFA Gardens. That variety was used as the foundation for finding the first true pink flowered form, ‘Flora Ann’, which is becoming more common in the trade. L.E. Cooke has introduced four new chaste tree varieties, and one, ‘Cooke’s Pink’™, features pink flowers. At this stage, the plant looks very similar to ‘Flora Ann’. Breeding goals would include dwarf forms, dense branching and more flowers (9, 10).

*Lagerstroemia*: Crapemyrtles are a major commodity in the southern USA with receipts exceeding 50 million dollars at the wholesale level. I remember when the Texas market only included red, pink and white, period. The “red” was usually a variety called ‘Watermelon Red’ which wasn’t quite red, but nearly so. Everything changed in the 1950s with the introduction of *Lagerstroemia faurei* by John Creech. Many of those early seedlings are still with us. One patriarch, now named ‘Bayou View’, rests on a Timberline Avenue in Shreveport and is a magnificent single trunk specimen of 100” circumference at breast height. This specimen is derived from the first seed distribution from the U.S. National Arboretum. ‘Townhouse’ and ‘Fantasy’ originated at the JCR Arboretum in Raleigh, NC. Many Texas horticulturists contend that Lynn Lowrey introduced the first hybrid, ‘Basham’s Party Pink’, which was found as a seedling near a *L. faurei*. This superseded the introductions by Donald Egolf of the U.S. National Arboretum’s breeding program. That program resulted in 25 excellent introductions, the Indian Tribe Series, with ‘Natchez’ and ‘Muskogee’ the first introductions, that are still fine trees in the landscape. Later, ‘Chickasaw’ and ‘Pocomoke’ were introduced as the first true genetic dwarfs. In Oklahoma, Dr. Carl Whitcomb, Stillwater, bred Dynamite® (true red) and Red Rocket® (true red), which are both 10’ to 15’ upright large shrubs, or small multi-stem trees. The last decade has seen a proliferation of varieties under the ever increasing presence of branding. The compact
Dazzle® series, a Filligree™ Series from Fleming’s, an Early Bird™ series, a Barnyard Collection™ introduced through McCorkle Nurseries and Plant Introductions, Inc., and Plant Introductions Inc. has introduced the Magic series, Coral, Plum, Purple, and Red. Purple Magic may be the best purple flower on the market. In 2012, Dirr provided a thorough and comprehensive treatment of the history of Lagerstroemia breeding to 2012 (9). Since 2012, nothing has excited the crapemyrtle industry more than dark burgundy foliage cultivars. Since the introduction of Lagerstroemia Delta Jazz™ ('Chocolate Mocha') (PP 21,540) there have been thirteen new varieties with burgundy foliage that lasts throughout the season. ‘Chocolate Mocha’ features small flower heads of bubblegum pink, an upright stature, and leaves best described as often cupped and less than attractive. In full sun, the variety is relatively free of disease, but in part shade conditions we have observed significant powdery mildew. The genes of this cultivar led to five Black Diamond™ varieties (JBerry Nursery) with flower colors ranging from red to white to blush. To confuse things a bit, Ebony Crapemyrtles and Black Diamond Crapemyrtles are the same clones under different names. Black Diamond ‘Pure White’ is 'Ebony & Ivory', ‘Best Red’ is 'Ebony Flame', ‘Blush’ is 'Ebony Glow', ‘Crimson Red’ is 'Ebony Fire', and ‘Red Hot’ is 'Ebony Embers'. That was followed by the release of four dark-foliaged Delta™ varieties by PDSI, Loxley, Alabama. Plant Introductions, Inc. has introduced two patented dark-foliaged varieties ‘Midnight Madness’ and ‘Moonlight Madness’. These are in the first year of trials at SFA Gardens and at LSU, Hammond, Louisiana. Future improvements might include dark foliage color on superior dwarf forms like ‘Cherry Dazzle’. The recent advent of Lagerstroemia scale, beginning in north central Texas a few years ago and now found elsewhere in the South, suggests a research focus on variety resistance and pest control. In November 2013, as part of a USDA scientific exchange team, we viewed the impact of Lagerstroemia scale in Beijing, Nanjing, and Kunming, China. In some cases, plants were reduced to pitiful specimens. Scientists at one location remarked that they had long had the scale, but the impact had gotten worse in the last two or three years. Some of my colleagues in China even speculated that this was a new form of scale brought in on USA hybrids cultivars.

*Chilopsis:* Desert willow, *Chilopsis linearis,* is a popular small landscape tree in central Texas and parts West. It’s drought hardy, alkaline tolerant and durable in the landscape. In more eastern USA locations, where it is rarely encountered, the tree demands very well drained soils and a full sun site. SFA Gardens is home to a wide range of varieties that have performed well over many years. ‘Bubba’, a Paul Cox, San Antonio, Texas, introduction remains a favorite. SFA Gardens is home to the world’s largest tree of this variety (9).

*Vaccinium:* Blueberries are a popular southern fruit and SFA has evaluated new selections and varieties since the early 1980s. We cooperate with USDA and other University breeding programs. Our primary University blueberry plot is located at the Pineywoods Native Plant Center and Mr. Louis Duffield, a local grower, serves as an on-farm cooperator. Over 100 selections and standard varieties are included. SFA introduced Earlibirdblue as a cooperative release with the USDA in 2013 (12).
Vitis rotundifolia: Muscadine grape evaluation is relatively new at SFA Gardens and our evaluation garden is located at the Pineywoods Native Plant Center. The collection includes most of the standard varieties, new releases, and, most recently, advanced selections from the breeding program of Stephen Stringer, USDA, Poplarville, Mississippi.

Actinidia: While many enthusiasts have successfully grown Actinidia chinensis vines in Texas, no one has successfully produced a good fruit crop until 2014. A trial planting of two varieties, ‘Golden Dragon’ and ‘Golden Dragon’ that are both Auburn University introductions, was established in 2011 at SFA Gardens. ‘Golden Dragon’ successfully fruited in 2014 and, at this writing, is poised for a second full crop in 2015. Kiwi trials are being expanded.

Single Plant Standouts: In most cases, it’s imprudent to base ornamental plant recommendations on a single plant in one garden setting. However, SFA Gardens is home to many one-of-a-kind species or varieties that have performed well here, been multiplied and performed well in other locales. They deserve further testing across a wider range of the South.

Magnolia species. The Asian magnolias deserve greater marketing. While seen in botanical gardens and the occasional home landscape, there are many well adapted Asian magnolias that fail to make a big presence in southern landscapes.

Cornus angustata. The Asian evergreen dogwood is an underutilized small tree or large shrub. It is durable, drought resistant, and pest free. Foliage is lustrous and the May blooms in our region are always appreciated. Edible fruit is another attribute. SFA Gardens has a good number of seedlings of ‘Empress of China’ under evaluation, now in their fourth year.

Celtis sinensis ‘Green Cascade’ is a strongly weeping form of Chinese hackberry that has made an impact at SFA Gardens. Introduced by Clifford Parks in the 1980s, SFA Gardens has one of the original distributions. Visitors and professionals always remark on its uncommon grace and form. It’s a durable and unique tree that improves with age.

Cornus florida ssp. urbiniana , Mexican dogwood, is a unique dogwood from the San Madre oriental mountain range in Mexico is relatively untested across the South. While individual specimens exist, there hasn’t been a market push. The white lantern-shaped blooms are stunning. With bracts that never separate, the anthers and stigma are somewhat protected from rain and wet forest humidity. Seed production on our trees has been disappointing. However, over the last few years we have made a good number of distributions to other gardens in the South.

Prunus X ‘Purple Pride’ is SFA Gardens sole patented plant is a chance seedling of a Chickasaw plum, Prunus angustifolia ‘Guthrie’, which was introduced by Charles Webb of Superior Trees in Florida. Key attributes of ‘Purple Pride’ include fast growth rate, nice natural form, burgundy foliage in full sun with red new growth, and resistance to early leaf drop in the autumn.
**Araucaria angustifolia**, Monkey puzzle tree or Parana Pine, is represented at SFA by a 1989-planted specimen over 30’ tall. The tree survived being replanted early in its life, a 0°F event in 1989 and the ravages of Hurricanes Rita and Ike in 2005 and 2008, respectively.

**Edgeworthia chrysantha**, paperbush plant, is rarely encountered in the Deep South and deserves greater use. This deciduous shrub typically reaches 8’ or taller and is usually slightly wider than tall. Large strappy leaves lend a tropical look to the plant. The key feature is winter bloom which occurs after the leaves have fallen. The foliage drops in mid December to reveal attractive bark and the large terminal flower buds. The flower buds open steadily from mid December to early March and produce a fragrant show of pendent white or yellow flowers. The orange-red form, *Edgeworthia* 'Akebono' (aka 'Red Dragon') on the market is less fragrant. Mike Dirr reports that a hybrid of the large tetraploid form and the shorter diploid form exist and should be entering the market in the future (14). One obscure fact associated with the plant is cultural. The author learned in several Chinese public gardens that the branches of paperbush are very pliable and young people can easily tie them into “love knots” which continue to grow unabated. This is evidently a favorite practice for young people in love and newlyweds.

**Conclusions**: After thirty years, SFA Gardens remains focused on finding, acquiring, growing, planting and enjoying as many new and different ornamental plants that we can plant, display and maintain. Whether that effort is sustainable is a matter of conjecture. I’ve concluded that side-by-side variety trials continue to be valuable to the nursery and landscape industry and popular with home horticulturists. However, I suspect the impact may be less than in years past. It’s apparent an ever-increasing flood of new woody ornamental introductions will continue to define the state of horticulture. By the time a final evaluation can be made, the list of varieties in the market will be a new one. Items will be dropped, new ones added and the cycle repeated. It’s a hyper competitive market. However, I remain convinced for those gardens with the space, enthusiasm, and will, there’s great opportunity to trial new varieties, advanced selections, rare plants, or interesting seedling populations, the latter holding great promise for the future of University woody ornamental introduction and promotion programs.

**Literature Cited**