Plant Breeding and Evaluation

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Induced Tetraploidy in Japanese Cedar

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Significance to the Industry: Currently, the industry standards of Japanese cedar exhibit an off color during winter that is undesirable, reducing their market potential. Tetraploid Japanese cedars may provide a non-winter-browning form that producers would be able to market as a superior alternative to current cultivars of Japanese cedar or Leyland cypress. Evergreen plants that may be used for screening in the southeastern U.S. are few and a non-winter-browning form of Japanese cedar may represent an economically important alternative for growers.

Nature of Work: Cryptomerias, also commonly called Japanese cedars or sugi, offer an alternative to Leyland cypress due to their limited pest problems (13; 14), ability to perform well under the hot, humid conditions of the southeastern U.S., and tolerance of heavy soils during both wet and dry conditions (13). However, in full sun they exhibit a browning in winter that can be undesirable (personal observation). Winter browning in Japanese cedar occurs in sun-exposed leaves during periods of low temperature indicating that photoinhibition plays a role (6; 8). Ida (8) reported that chloroplasts in sun-exposed leaves were transformed into rhodoxanthin-containing chromoplasts during winter, resulting in the brown-red color.

In Japanese forestry nurseries, tetraploid forms of Japanese cedar have been identified based on their leaves which are thickened and twisted and remain green during winter (2). Tetraploids were shown to be more resistant to oxidative damage from UV light and air pollution than diploids due to increased antioxidant levels (12). Negative selection has been imposed based on the lack of utility of tetraploids in a silvicultural setting but tetraploid Japanese cedars may have great potential for use in the nursery and landscape industries in the U.S. by providing non-winter-browning forms that consumers find more attractive. Therefore, the current research was conducted to develop tetraploid forms of Japanese cedar.

Seed of *Cryptomeria japonica* were received from Lawyer Nursery, Inc., Plains, MT. Approximately 1,000 seeds were sown in germination trays containing 8 pine bark : 1 sand (v/v) amended with 0.91 $kg \cdot m^{-3}$ (2.0 $lb \cdot yd^{-3}$) dolomitic lime and 0.45 $kg \cdot m^{-3}$ (1.0 $lb \cdot yd^{-3}$) Micromax (The Scotts Co., Marysville, OH) and allowed to germinate in a glasshouse with day/night set temperatures of 27/20 °C (81/68 °F) . Approximately 600

seedlings germinated and were moved to the laboratory and grown under constant light (28 µmol·m^{-2}·s^{-1}) supplied by cool-white fluorescent lamps at 25 °C (77 °F). Seedlings were sprayed to run-off daily for 30 d with an aqueous solution containing 150 □M oryzalin (supplied as Surflan® AS, United Phosphorus Inc., Trenton, NJ) + 0.1% SilEnergy™ (Brewer International, Vero Beach, FL), an organosilicate surfactant, using a standard spray bottle. After 30 days, approximately 500 surviving seedlings were moved to a glasshouse and transplanted into six trays containing the pine bark substrate described above. Seedlings were allowed to grow for four months and then transplanted into small containers filled with 1 Pro-Mix BX with Biofungicide (Premier Horticulture, Quakertown, PA) : 1 pine bark/sand mixture described above (v/v), and fertilized weekly at a rate of 100-ppm N using TotalGro 20N-4.4P-17.6K water soluble fertilizer (SDT Industries, Winnsboro, LA). Morphology indicative of polyploidy in Japanese cedar (2; Fig. 1) was used to select 237 seedlings for transplantation. After one month of growth [180 days after treatment (DAT)], the ploidy level of the seedlings were determined using flow cytometry.

Flow cytometry, as described in Contreras et al. (4) with modifications, was conducted on the 237 selected seedlings with altered leaf morphology. Five plants were evaluated during each analysis by bulking leaves. For bulked samples, a comparable amount of mature leaf tissue from five plants was collected and analyzed. In bulked samples where a single peak was observed, all plants were recorded as being composed solely of tissue at the ploidy level corresponding to the fluorescence channel. For bulked analyses in which there were multiple peaks, plants were analyzed individually to determine ploidy of individual plants. Samples that were ambiguous were analyzed individually using an internal standard [(*Pisum sativum* L. 'Ctirad'; 2C = 8.76 pg) (5)]. The internal standard was used to calculate the 1Cx DNA content of the diploid [1Cx DNA (pg) = (MRF Sample/MRF Standard) × 8.76 pg)/2)]. DNA content of the unknown samples was calculated and ploidy determined based on the 1Cx DNA content of the diploid. A random subset of 20 individuals identified as tetraploid 180 DAT were re-analyzed using flow cytometry 270 DAT to determine if they remained stable tetraploids.

Results and Discussion: Treating *C. japonica* seedlings for 30 consecutive days with 150 □M oryzalin + 0.1% SilEnergy™ successfully induced tetraploidy. Numerous preliminary experiments were unsuccessful in developing tetraploids, including soaking seeds in various rates of colchicine, germinating seeds in various rates of colchicine, and treatment of shoot tips of seedlings and stem cuttings (3) with various rates of oryzalin. We hypothesized that previous applications of oryzalin were unsuccessful because they were too short (one to five days) and did not contain a surfactant to penetrate the cuticle. A total of 237 seedlings were analyzed using flow cytometry and of these, 219 (92.4%) contained tetraploid cells (Table 1). Flow cytometric analysis of seedlings revealed 197 (83.1%) tetraploids, 22 (9.3%) cytochimeras, and 18 (7.6%) diploids (Table 1). Morphology of induced tetraploids (Fig. 1), primarily thickened and twisted leaves, was similar to that previously described (2) and provided a phenotypic marker for selection

during transplantation that was over 92% accurate. Tetraploids had thicker leaves than diploids (Fig. 1) but leaf length and growth rate displayed variation typical of a seedling population. Other populations grown from the same seed source had no individuals that exhibited leaf morphology indicative of polyploidy (personal observation), which provides strong indication that the tetraploids recovered were the result of the treatment and not chance seedlings. Also, Chiba (2) reported that recovery of polyploids as chance seedlings was several orders of magnitude lower (0.0005%) than observed in the current report.

Compared to the number of studies on induced polyploidy in angiosperms, there have been few in gymnosperms and no reports of induced polyploidy in Japanese cedar (1). This is the first study reporting the use of oryzalin as a mitotic inhibitor to induce polyploidy in a gymnosperm. Previous studies on gymnosperms used colchicine to treat seed (7; 9; 10; 11), shoot-tips of seedlings (7), or injection into vascular tissue (9).

Plants were initially evaluated using flow cytometry 180 DAT and then a random subset of 20 tetraploid plants were reassessed 270 DAT which were all found to contain only tetraploid cells. However, continued evaluation of the induced tetaploids in the current study will be required to determine long term stability. Furthermore, since shoot tips were treated, the roots of individuals with tetraploid leaves are presumed to be diploid. Therefore, to obtain plants with only tetraploid cells, stem cuttings will be used to propagate tetraploids when sufficient material is available. Long-term evaluation will be conducted at multiple locations to determine if the induced tetraploids will be superior with regard to winter character.

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Table 1. Results of treating *Cryptomeria japonica* seedling shoot tips with 150 µM oryzalin + 0.1% SilEnergy™ for 30 consecutive days to induce tetraploids.

Ploidy	No. seedlings	Percent of total
2*x* (Diploid)	18	7.6
2*x* + 4*x* (Mixoploid)	22	9.3
4*x* (Tetraploid)	197	83.1
Total[z]	237	100

[z]237 seedlings selected based on foliar morphology from ≈500 surviving seedlings after beginning 30 d treatment on ≈600 seedlings.

Figure 1. Two leaves from an induced tetraploid of *Cryptomeria japonica* (left) exhibiting thickened and twisted morphology and two leaves of diploid (right) with wild-type phenotype.

Optimizing *in vitro* Growth Conditions for *Magnolia* 'Ann'

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Significance to Industry: *Magnolia* (*M. liliiflora* 'Nigra'× *M*. *stellata* 'Rosea') 'Ann' (NA 28344; PI 326570) is a member of the 'Little Girl' series of magnolias that have become widely popular. *Magnolia* 'Ann' is characterized by a desirable combination of traits including prolific and remontant flowering and a shrubby form. Due to difficulties in propagating magnolias from cuttings or grafting, development and optimization of *in vitro* propagation methods would be desirable. *Magnolia* 'Ann' is also a triploid, interspecific hybrid that is reportedly sterile (22). *In vitro* chromosome doubling may be an approach to develop allopolyploids with restored fertility (4) and provide an opportunity to use this cultivar in future breeding programs. To enhance the ornamental qualities of 'Ann', micropropagation protocols were developed as a platform for propagation and future ploidy level manipulation. Murashige and Skoog basal medium (MS)(17) supplemented with 2 µM benzylamino purine (BAP) provided high shoot proliferation, while Lloyd and McCown Woody Plant Medium (WPM)(8) containing charcoal may be used to produce elongated plantlets more suitable for rooting and *ex vitro* establishment.

Nature of Work: Tissue culture is a useful tool for propagation and plant breeding. *In vitro* protocols provide a foundation for ploidy level manipulation and allow for the rapid propagation of valuable cultivars. Previous *in vitro* propagation studies on *Magnolia* have focused on endemic species for conservation, including *M. acuminata* var. *cordata* (15,16), *M. dealbata* (12), *M. denudata* (1), *M. fraseri* (15,16), *M. macrophylla* (13,16), *M. obovata* (7), *M. officinalis* (21), *M. pyramidata* (14,16), *M. sieboldii* (9), *M. sinicum* (5), and *M. virginiana* (15,16). However, little work has been done on micropropagation of ornamental *Magnolia* taxa with the exception of *M.* × *soulangiana M.* (6,11), *M. grandiflora* (18,19) and *M. delavayi* (10). These studies have indicated that media composition and plant growth regulators are important factors influencing the *in vitro* propagation of Magnolia. Shoot proliferation in Magnolia during micropropagation has been reported to be difficult due to the high content of phenolic substances (5, 18). Therefore, the objectives of this study were to evaluate a range of basal media

compositions, phenolic binding agents, and cytokinins in a series of experiments to optimize *in vitro* growth conditions for 'Ann'.

'Ann' stock cultures were maintained on MS basal salts and vitamins, 2 µM BAP, 30 g/l sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar at the N.C. State Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, N.C. Cultures were maintained at 23 °C (73°F) under a 16h photoperiod.

The effect of basal media composition was tested with five basal salt compositions and vitamins (MS, ½ MS, WPM, Blaydes Modified Basal Medium (Blaydes)(2), and Driver and Kuniyuki basal salt mixture (DKW)(3) in factorial combination with phenolic binding agents (none, 1 g/l polyvinylpyrrolidone (PVP)(12), or 1 g/l charcoal). All media were supplemented with 30 g/l sucrose, 2 µM BAP, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate arranged in a completely randomized design. After eight weeks, data were collected on shoot number, shoot length, root number, fresh weight, and dry weight.

In a separate experiment, the effect on plant growth of three cytokinins; BAP, meta-topolin (mT), and 6-(γ,γ-dimethylallylamino) purine (2iP)(11) at three concentrations (2, 4, and 8 µM) with or without 1 g/l charcoal was evaluated in a completely randomized design with a factorial arrangement of treatments. Based on the results of the first experiment, basal media consisted of MS basal salts and vitamins, 30 g/l sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate, arranged in a completely randomized design. After eight weeks, data were collected on shoot number, shoot length, root number, root length, fresh weight, and dry weight. Data for both studies were subjected to analysis of variance (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC). Means separations were based on LSD.

Results and Discussion: In the first experiment, the influence of media composition and phenolic binding agents on plant growth were examined. Media composition, phenolic binding agents and their interaction had a significant effect on shoot number, root number, fresh weight and dry weight (Table 1). In general the number of shoots produced per explant was lower on both Blaydes media and media supplemented with activated charcoal. Fresh weight was significantly lower on Blaydes and WPM medium as well as media supplemented with activated charcoal, while phenolic binding agents (PVP and charcoal), as well as WPM and DKW reduced dry weight. Rooting increased on WPM media and media containing charcoal. There was no interaction between media composition and phenolic binding agents on shoot length. Even though shoot length was less on Blaydes media overall, shoots were significantly longer on all media containing charcoal (Table 1). Reduced shoot proliferation and increased shoot elongation and

rooting in response to charcoal have been found for *Acacia mearnsii* and *Anacardium occidentale* (cashew) (20).

In the second experiment, the influence of cytokinins, cytokinin concentration and charcoal was examined. There was a significant interaction between cytokinin and charcoal that influenced shoot number, shoot length and fresh weight, while a complex interaction between cytokinin, cytokinin concentration and charcoal affected dry weight (Table 2). In general, shoot number was higher on media containing BAP, regardless of concentration, and lower on media containing charcoal. Interestingly, mT and 2iP did not promote shoot proliferation. Meta-topolin has been reported to produce longer, greener and less hyperhydrated shoots and may be an alternative cytokinin to BAP (23). Unexpectedly, cytokinin concentration did not have a significant effect on shoot number. Similar to the first experiment, charcoal significantly promoted root formation.
This study demonstrated that there are several different media components that interact to influence in vitro growth of 'Ann'. Based on the results, MS media supplemented with BAP provides high shoot proliferation, while WPM containing charcoal may be used to produce elongated plantlets more suitable for rooting and *ex vitro* establishment. Further studies are required to optimize rooting media. Protocols developed in this study will be used in future experiments focused on the development of allopolyploids to restore fertility.

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Table 1. Summary of means for growth responses to different in vitro culture media and phenolic binding agents.

Media	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
MS	none	3.2±0.2^{A}	17.2±1.8^{C}	0.2±0.07^{DE}	5.6±0.60^{B}	0.56±0.04^{AB}
	PVP	2.8±0.3^{AB}	20.0±2.8^{BC}	0.1±0.10^{DE}	4.8±0.45^{B}	0.58±0.04^{AB}
	Charcoal	1.1±0.1^{C}	24.2±1.7^{AB}	0.6±0.03^{BC}	2.8±0.22^{CD}	0.42±0.03^{BC}
½ MS	none	2.8±0.2^{AB}	24.4±2.6^{AB}	0.3±0.14^{CDE}	8.1±1.21^{A}	0.69±0.08^{A}
	PVP	2.6±0.2^{AB}	19.9±1.1^{BC}	0.1±0.04^{DE}	4.6±0.35^{BC}	0.53±0.04^{B}
	Charcoal	1.1±0.1^{C}	22.2±1.7^{B}	0.3±0.08^{CDE}	2.1±0.24^{D}	0.35±0.03^{C}
WPM	none	2.3±0.2^{AB}	19.9±1.9^{BC}	0.7±0.20^{B}	2.4±0.38^{D}	0.39±0.06^{BC}
	PVP	2.6±0.3^{AB}	16.5±1.6^{C}	0.4±0.19^{BCD}	1.9±0.57^{D}	0.33±0.05^{D}
	Charcoal	1.2±0.1^{C}	22.4±1.3^{B}	1.8±0.16^{A}	2.4±0.16^{D}	0.42±0.03^{BC}
DKW	none	2.6±0.4^{AB}	22.7±3.0^{B}	0.1±0.04^{DE}	4.9±1.07^{B}	0.50±0.04^{B}
	PVP	2.9±0.4^{A}	19.0±1.0^{BC}	0.0±0.00^{E}	4.2±0.79^{BC}	0.49±0.08^{BC}
	Charcoal	1.1±0.1^{C}	30.1±4.1^{A}	0.7±0.20^{BC}	3.0±0.53^{C}	0.29±0.04^{D}
Blaydes	none	1.2±0.2^{C}	8.2±2.1^{D}	0.3±0.12^{CDE}	2.2±0.24^{D}	0.62±0.09^{AB}
	PVP	1.5±0.2^{C}	13.6±3.2^{CD}	0.1±0.11^{DE}	2.2±0.44^{D}	0.45±0.06^{BC}
	Charcoal	0.9±0.1^{C}	14.2±1.3^{CD}	0.6±0.10^{BC}	0.9±0.16^{D}	0.47±0.04^{BC}
Analysis of Variance[2]						
Media		**	**	**	**	**
PBA		**	**	**	**	**
Media x PBA		*	NS	**	**	*

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.

[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. PBA=Phenolic Binding Agent.

Table 2. Summary of means for *in vitro* growth responses to different concentrations of cytokinins and phenolic binding agents.

Cytokinin	Conc. (µM)	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
BAP	2	None	2.64±0.4 ^{A}	18.6±1.9 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.23±0.03^{A}
		Charcoal	1.00±0.0 ^{C}	18.2±1.0 ^{ABC}	1.40±0.5^{A}	1.33±0.1 ^{D}	0.18±0.01^{AB}
	4	None	2.40±0.1 ^{A}	19.2±0.8 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.2±0.02^{A}
		Charcoal	1.08±0.1 ^{C}	17.0±0.7 ^{BCD}	0.60±0.2^{AB}	1.34±0.2 ^{D}	0.17±0.02^{B}
	8	None	2.40±0.2 ^{A}	22.5±0.7 ^{A}	0.00^{B}	1.00±0.0 ^{D}	0.19±0.02^{A}
		Charcoal	1.15±0.1 ^{BC}	18.9±1.7 ^{ABC}	1.00±0.6^{A}	1.37±0.2 ^{CD}	0.14±0.01^{BC}
mT	2	None	1.40±0.2 ^{BC}	13.6±1.7 ^{D}	0.00^{B}	2.00±0.0 ^{BC}	0.11±0.01^{C}
		Charcoal	1.25±0.2 ^{BC}	19.7±2.2 ^{AB}	0.75±0.5^{AB}	1.99±0.3 ^{BC}	0.20±0.02^{A}
	4	None	1.33±0.2 ^{BC}	15.4±1.8 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.16±0.03^{BC}
		Charcoal	1.06±0.1 ^{C}	16.7±3.0 ^{BCD}	0.33±0.3^{B}	1.65±0.4 ^{BC}	0.13±0.01^{BC}
	8	None	1.55±0.3 ^{B}	14.9±1.4 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.14±0.03^{BC}
		Charcoal	1.13±0.1 ^{BC}	15.5±0.1 ^{CD}	0.00^{B}	2.33±0.3 ^{AB}	0.13±0.01^{BC}
2iP	2	None	1.06±0.1 ^{C}	19.6±2.5 ^{ABC}	0.00^{B}	3.00±0.0 ^{A}	0.21±0.04^{B}
		Charcoal	1.10±0.1 ^{BC}	17.9±2.8 ^{ABCD}	1.00±0.4^{A}	1.84±0.4 ^{BC}	0.18±0.02^{A}
	4	None	1.12±0.1 ^{BC}	13.9±1.7 ^{D}	0.00^{B}	3.00±0.0 ^{A}	0.13±0.01^{BC}
		Charcoal	1.20±0.1 ^{BC}	19.0±0.7 ^{ABC}	0.83±0.2^{AB}	2.01±0.2 ^{B}	0.22±0.02^{A}
	8	None	1.06±0.1 ^{C}	20.7±1.8 ^{AB}	0.67±0.7^{AB}	2.37±0.6 ^{AB}	0.13±0.02^{BC}
		Charcoal	1.00±0.0 ^{C}	17.9±1.2 ^{ABCD}	0.00^{B}	3.00±0.0 ^{A}	0.23±0.02^{A}
Analysis of Variance[2]							
Cytokinin			**	*	NS	**	**
Conc.			NS	NS	NS	NS	NS
PBA			**	NS	**	NS	NS
Cytokinin x Conc.			NS	NS	NS	NS	NS
Cytokinin x PBA			**	*	NS	**	**
Conc. x PBA			NS	NS	NS	*	NS
Cytokinin x Conc. x PBA			NS	NS	NS	NS	**

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.

[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. Conc. = Concentration; PBA=Phenolic Binding Agent.

Production of Triploids in *Hydrangea macrophylla*

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Index Words*:* *bigleaf hydrangea*, triploidy, unreduced gametes

Significance to Industry: Bigleaf hydrangea cultivars with the triploid number of chromosomes typically have highly desirable traits such as dark foliage, strong stems and large, deeply colored inflorescences. While it would be desirable to produce new triploid cultivars, the origin of the existing triploids is unknown. This study identified one diploid cultivar ('Trophee') that produced triploid progeny when used as the pollen parent in hybridizations. This study is the first step towards developing a system by which triploids can routinely be produced in bigleaf hydrangea breeding programs.

Nature of Work: Polyploid forms of ornamental plants are considered desirable because they often exhibit shorter stature, darker foliage, larger inflorescences and extended flowering period. While most bigleaf hydrangea [*H. macrophylla* (Thunb) Ser.)] cultivars are diploids, 24 triploid cultivars have been reported (4, 8). These cultivars, along with three other cultivars that we have recently observed to be triploids using flow cytometric analysis, are listed in Table 1. In general, triploid cultivars of *H. macrophylla* have dark foliage, strong stems and large, deeply colored inflorescences (2, 7) – all traits that are highly desirable in this popular ornamental shrub.

No information as to the origin of *H. macrophylla* triploids is available. Autotriploids are produced experimentally from hybridization of tetraploid and diploid individuals, but in nature they usually arise from the union of unreduced (2n) and haploid (n) gametes. Most of the triploid cultivars of *H. macrophylla* were introduced by European breeders in the first half of the 20th century. It is not known whether these breeders had access to tetraploid forms of *H. macrophylla* that are not available in the trade today or if they were using parental stocks that produced unreduced gametes.

The most direct method of screening for unreduced pollen grains is the examination of the range of pollen sizes produced by an individual (1). The presence of "giant" pollen has been associated with 2n status and has been used as an indicator of unreduced gamete formation. The frequency distribution of pollen grain size among 19 diploid cultivars of *H. macrophylla* was examined for evidence of unreduced gamete production (4). All but one cultivar examined exhibited a range of pollen grain sizes that peaked at

or near the mean value, but most produced a few grains considerably larger than the mean size. The only cultivar that deviated substantially from this pattern was 'Trophee', which displayed a bimodal distribution. The objective of this study was to determine if triploids of *H. macrophylla* could be produced by using 'Trophee' as the pollen parent in hybridizations to other diploid cultivars.

Controlled pollinations were made in summer 2008, using previously published hybridization techniques (5). 'Alpengluhen', 'Early Sensation' (Forever and Ever®), 'Harlequin', 'Otaska' and 'Princess Juliana', all of which are verified diploids with no evidence of unreduced gamete production (4), were used as seed parents and 'Trophee' was used as the pollen parent. Seed were collected in fall 2008 and sown in winter 2009. Previously published flow cytometric techniques (6) were used for determining ploidy of progeny obtained from these crosses. Genome sizes were calculated as nuclear DNA content for unreduced tissue (2C) as: 2C DNA content of tissue = (mean fluorescence value of sample ÷ mean fluorescence value of standard) × 2C DNA content of standard. *Pisum sativum* L. 'Ctirad', with a 2C content of 9.09 pg (3), was used as the internal standard.

Results and Discussion: No seed were produced from 'Early Sensation' × 'Trophee' or 'Otaska' × 'Trophee' hybridizations (Table 1). Seed obtained from 'Alpengluhen' × 'Trophee' and 'Harlequin' × 'Trophee' hybridizations did not germinate, but 20 'Princess Juliana' × 'Trophee' seedlings were obtained.

A previous study using DAPI-stained nuclei of *H. macrophylla* cultivars found genome sizes ranging from 4.5 to 5.0 pg DNA for diploids and 6.9 to 7.3 pg DNA for triploids; ploidy was verified by chromosome counts of three diploid and three triploid culitvars (4). Flow cytometric analysis of the parental cultivars used in this study revealed a genome size of 4.63 pg DNA for 'Princess Juliana' and 4.86 pg DNA for 'Trophee', verifying diploidy in both cultivars. One 'Princess Juliana' × 'Trophee' seedling had 4.86 pg DNA, indicating that it is a diploid. The other 19 seedlings appear to be triploids with genome sizes ranging from 7.11 to 7.33 pg DNA.

'Trophee' had previously been identified as a cultivar that potentially produces a large quantity of unreduced pollen grains (4). The results of this study indicate that 'Trophee' can be used as the pollen parent in hybridizations to produce triploid progeny. Unfortunately, no progeny were obtained from hybridizations of 'Trophee' with four of the five maternal cultivars tested. Further work is needed to examine the role of the maternal parent in hybridizations to 'Trophee' and to identify other *H. macrophylla* cultivars with a high frequency of unreduced pollen grain production. This work represents the first step towards developing a method for the production of desirable polyploidy progeny in *H. macrophylla*.

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Table 1. Verified triploid cultivars of *H. macrophylla*. Numbers in parentheses indicate source of information from Literature Cited; Reed = unpublished data.

Cultivar	Cultivar (cont.)
Admiration (8)	Kardinal (4)
Altona (4)	Marechal Foch (4)
Blaumeise (4, 8)	Masja (4)
Domotoi (4)	Merritt's Supreme (4)
Eisvogel (4, 8)	Miss Hepburn (4)
Enziandom (4)	Mousmee (Reed)
Europa (4, 8)	Mowe (8)
Geoffrey Chadbund (Reed)	Nachtigall (4, 8)
Gerta Steiniger (8)	Oregon Pride (4)
Gertrude Glahn (4)	R.F. Felton (8)
Goliath (Reed)	Sybilla (8)
Hamburg (4)	Taube (4)
Heinrich Seidel (4)	Todi (4)
Holstein (4)	

Table 2. Results of hybridization of *Hydrangea macrophylla* 'Trophee' as the pollen parent with five *H. macrophylla* cultivars

Hybridization	No. flowers pollinated	No. seed obtained	No. seed germinated
Alpengluhen × Trophee	10	11	0
Early Sensation × Trophee	18	0	- - -
Harlequin × Trophee	93	33	0
Otaska × Trophee	7	0	- - -
Princess Juliana × Trophee	83	521	20

DNA Fingerprinting Eastern Redbud Cultivars (*Cercis canadensis*) using SSR Markers

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Index Words: ornamental tree, microsatellite, genetic diversity

Significance to Industry: Eastern redbuds are popular ornamental ts because of their small size, showy spring bloom, heart-shaped glossy leaves, and adaptability to diverse environmental conditions (1). At least twelve cultivars of redbud have been introduced in the past decade with variations in foliage, flower color, or plant habit. We developed SSR markers for *Cercis canadensis* and used them to analyze 25 eastern redbud cultivars. Markers successfully differentiated among all the cultivars tested except where plants were derived from sport mutations. We are currently investigating the genetic relationship between *C. canadensis* cultivars, breeding germplasm, and wild-collected populations.

Nature of Work: In this study, we present data for a subset of SSR loci, 76 out of the 130 high-quality loci, which were selected out of hundreds of SSR loci identified from a SSR-enriched library. SSR markers are abundant in eukaryotic genomes and are highly reproducible (3). Previously, we have used SSR markers to estimate genetic diversity, identify cultivars, and verify parentage of woody ornamental plants. The 25 samples in this study do not include all commercially available germplasm but represent a broad cross-section of material in the trade including recently released cultivars. Our objective is to show reproducible DNA fingerprints for cultivars, establish unambiguous identification where possible, and verify limitations. This research is part of a larger program designed to assess genetic diversity of redbud throughout the southeastern U.S., accelerate redbud breeding, and provide genetic tools for cultivar identification.

Data from polymorphic markers were compiled for all samples (Table 1) and analyzed for shared allele frequencies. Nei's minimum genetic distances were calculated for all samples (2). Dendograms were generated to show clustering of botanical varieties and related taxa (Figure 1). Identical genotypes, typically from identically-named samples

acquired from different sources, are shown on a single vertical line. Genetic distances were also analyzed by Principal Coordinates Analysis (PCoA) and depicted in a scatter-plot (Figure 2). Plots were generated using NTSys software (4).

Results and Discussion: The tree in Figure 1 shows a clear distinction between *C. canadensis* and the other two botantical varities, *C. canadenssis* var *mexicana* and *texensis*. *Mexicana* is only represented by one sample so the separation and bootstrap support between *mexicana* and *texensis* cannot be verified. Interestingly, *C. canadensis* is split into two groups with weak bootstrap support from 1000 replicates. The genetic basis for these two clusters will be explored in further studies and may be due to hybridization between subspecies. Previous work has shown fertility in crosses between *canadensis* and *texensis* and *mexicana* suggesting that genetic backgrounds of cultivated forms may include taxonomic combinations (5). Additional wild-collected germplasm is needed. Because our samples are all cultivars, the two clusters might also correlate with ornamental traits that have been repeatedly selected for during breeding efforts.

Duplicate samples acquired from different nurseries are included in Figure 1 and show 100% identical DNA fingerprints. Cultivars such as ‘Solar Eclipse’ and ‘Rising Sun’ have 100% identical SSR data because ‘Solar Eclipse’ is a sport mutation of ‘Rising Sun’. This represents a limitation for SSR markers since we cannot genetically distinguish between clonally-derived materials, although morphologically they are easily identifiable. ‘Covey’ is sold under the trademark Lavender Twist and our results confirm this information. Other cultivars with close genetics associations include ‘Forest Pansy’ and ‘Greswan’, ‘Ace of Hearts’ and ‘Little Woody’, and ‘Tennessee Pink’ and ‘Crosswicks Red’. Some of these associations are likely due to pedigree. For example, ‘Forest Pansy’ and ‘Greswan’ both have intense purple leaves that remain during the summer. Such results are consistent with previous work, which documented that important ornamental traits are heritable (5). Future pedigree analyses will explore improved breeding strategies using both cultivated material and wild-collected trees.

In an effort to more closely examine the two groups within *canadensis*, we used principal coordinates to plot the samples in Figure 2. There are no clear associations between tree size, form, flower color and genetic group. However, weeping forms are only found in one group of *canadensis*. This may be due to pedigree or inheritance but there is probably not a single genetic source for weeping since this trait is also found in ‘Traveler’, a cultivar from *texensis*. Eastern redbud cultivars display a collection of novel ornamental traits including growth habit, leaf color, variegation, flower morphology, and flower color. Many of these traits are transmitted by simple inheritance. Thus, the SSR markers described here are an ideal starting point for accelerated breeding of new cultivars and trait identification using genetic mapping strategies.

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Table 1. Samples used in this study include commercially available cultivars representing three botanical varieties of *C. canadensis*. *Cercis chinensis* 'Don Egolf' was included as the outgroup to root the tree shown in Figure 1

Cultivar	Species	Height (feet)	Width (feet)	Form and foliage	Flower color
Rising Sun	*C. canadensis* var. *canadensis*	8-12	8-12	upright, new growth is orange maturing to lime green	lavender
Solar Eclipse	*C. canadensis* var. *canadensis*	8-12	8-12		lavender
Forest Pansy	*C. canadensis* var. *canadensis*	20-30	20-30	upright, dark purple foliage fades to green	lavender pink
Greswan	*C. canadensis* var. *canadensis*	20-25	20-25	Round to slightly oval, burgundy leaves fade to green	burgundy
Appalachian Red	*C. canadensis* var. *canadensis*	20-30	20-30	upright	bright pink
Hearts of Gold	*C. canadensis* var. *canadensis*	20-30	20-30	vase to rounded, yellow leaves mature to green	lavender pink
Ace of Hearts	*C. canadensis* var. *canadensis*	12	15	dense crown, dome-shaped canopy	light violet
Little Woody	*C. canadensis* var. *canadensis*	10	8	vase-shaped with an ascending-spreading crown	lavender pink
Pauline Lilly	*C. canadensis* var. *canadensis*	12-15	12-15		pink
Silver Cloud	*C. canadensis* var. *canadensis*	15-20	15-20	upright, white speckled dark green foliage	lavender pink
Tennessee Pink	*C. canadensis* var. *canadensis*	20	20	round shape	rosy pink
Crosswicks Red	*C. canadensis* var. *canadensis*				
Covey	*C. canadensis* var. *canadensis*	5	5	weeping, large zigzagging mound	lavender
Lavender Twist	*C. canadensis* var. *canadensis*	6-7	5	weeping, large zigzagging mound	lavender
Royal White	*C. canadensis* var. *canadensis*	15-25	15-25		white
Floating Clouds	*C. canadensis* var. *canadensis*	8-12	8-12	variegated foliage with irregular markings	lavender
Morton	*C. canadensis* var. *canadensis*	30	30	round form, purple seed pods	dark lavender pink
Pink Heartbreak	*C. canadensis* var. *canadensis*	10-15	8-10	weeping	lavender pink
Rubye Atkinson	*C. canadensis* var. *canadensis*	15-20	15		small, pink
Cascading Hearts	*C. canadensis* var. *canadensis*	3	3.5	round, weeping canopy	purplish-mauve
Flame	*C. canadensis* var. *canadensis*	20-25	15	flat topped vase or globe	double pink
Oklahoma	*C. canadensis* var. *texensis*	20-25	15-20	glossy foliage	deep red wine
Texas White	*C. canadensis* var. *texensis*	20	15-20	glossy foliage	white
mexicana	*C. canadensis* var. *mexicana*				
Traveller	*C. canadensis* var. *texensis*	5	5	weeping mound	purple
Don Egolf	*C. chinensis*	9	9-10	compact multibranched	bright pink

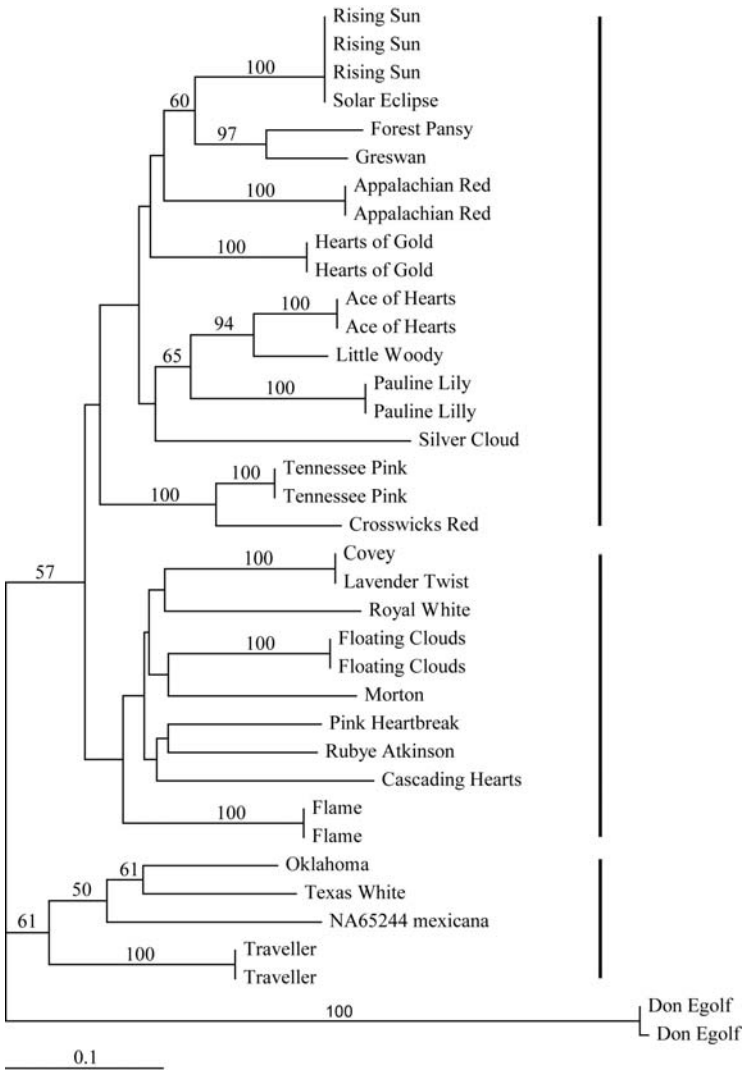

Figure 1. Neighbor-joining tree is based on Nei's minimum genetic distance. Numbers above branches indicate bootstrap support with 1000 replicates. Samples acquired from additional sources were included as duplicates to look for mislabeled cultivars. 'Covey' and Lavender Twist represent the same plant with two names. Sport mutations or clonally-derived material are not distinguished by our SSR markers as demonstrated by 'Solar Eclipse' and 'Rising Sun'. Vertical bars on the right indicate the three major groups and separate the two *C. Canadensis* var *canadensis* clusters from *mexicana* and *texensis*.

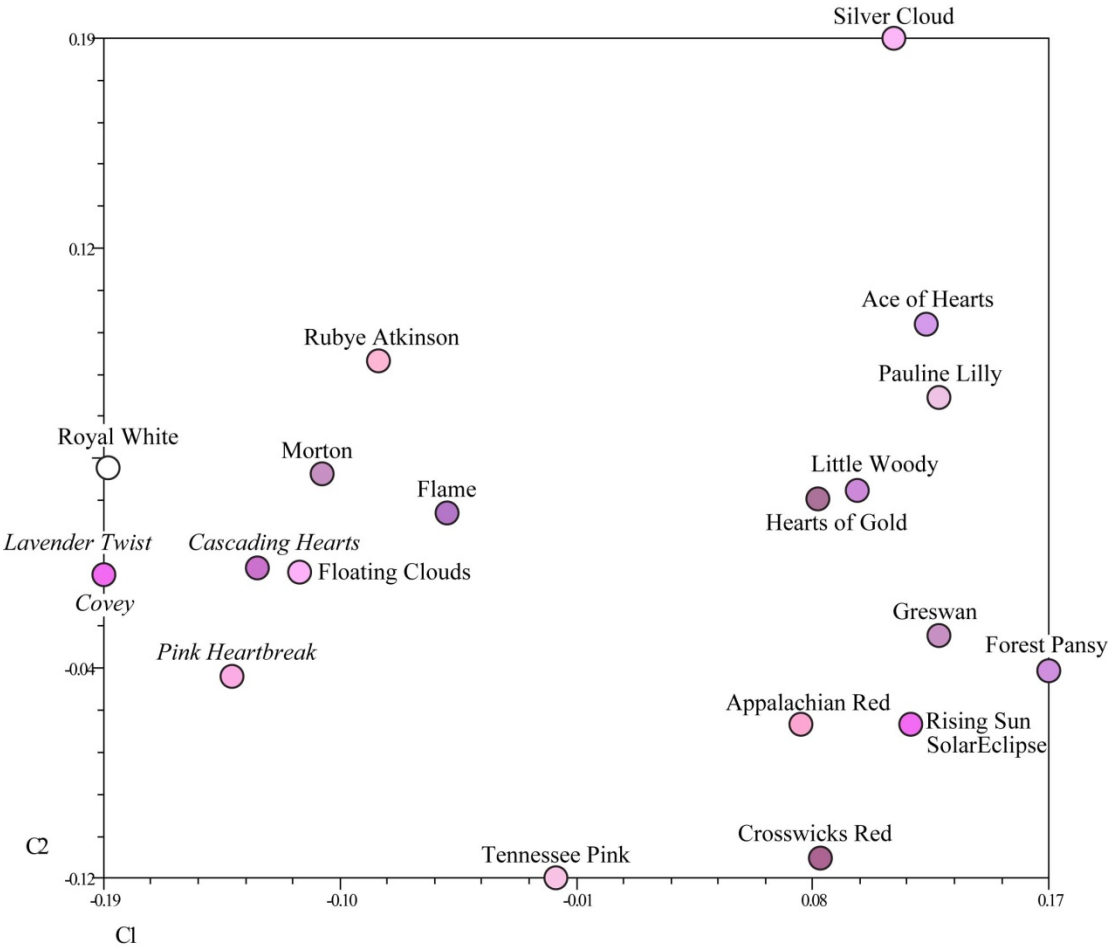

Figure 2. Principal coordinates analysis (PCoA) plot is limited to samples from the two *C. canadensis* var *canadensis* clusters and does not include *mexicana* or *texensis*. Circles indicate flower color. X and y-axis represent 36.38 and 11.76% of genetic variation, respectively. Ornamental traits do not cluster within a single group, except for weeping forms, which are only found on the left side of the plot for the samples we tested (cultivar names in italics).

Analysis of Ploidy Levels and Genome Sizes of *Berberis* L. and *Mahonia* Nutt. Species, Hybrids, and Cultivars

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Index Words: chromosome number, cytology, DNA content, flow cytometry, polyploidy

Significance to Industry: The sister genera, *Berberis* L. and *Mahonia* Nutt., represent the two largest groups within the family *Berberidaceae*, consisting of approximately 500 and 200 species respectively (6). This highly ornamental group of shrubs and small trees are valued for their evergreen or colored leaves, brilliant flowers, and often persistent fruit. Considering the tremendous diversity and the broad crossability found in these genera, the potential for breeding improved hybrids is considerable. However, a greater understanding of polyploidy in this genus would greatly enhance future breeding efforts. Polyploidy is an important factor in plant breeding as it can influence reproductive compatibility, fertility of progeny, morphology, and gene expression. Additionally, variation in genomic size can be used as an indicator of evolutionary history and taxonomic relationships (5, 10). This research provides an extensive survey of genome size and ploidy level of species, hybrids, and cultivars of *Berberis* and *Mahonia* that will serve as a valuable database for plant breeders, systematists, and evolutionary biologists.

Nature of Work: *Berberis* and *Mahonia* are each distributed into two well supported subgroups (6). Within *Berberis*, the Australes include all the species from Central and South America, the remaining species are placed in the Septentrionales, and occur entirely in the northern hemisphere except for two in East Africa and one in Java and Sumatra (1). Conversely, *Mahonia* are grouped longitudinally, with those of the eastern hemisphere in subgroup Orientales and all those of the western hemisphere (with the notable exception of *M. nervosa* (6)) in subgroup Occidentales. Although basic information on chromosome numbers, genome sizes and ploidy levels has been reported for some *Berberis* and *Mahonia*, sampling has been limited and little is known about ploidy levels of specific clones or cultivars. For *Mahonia,* most species have been reported to be diploid with $2n=2x=28$ (3,7), and in rare cases tetraploid with $2n=4x=56$ (8). Reports on *Berberis* species also favored $2n = 2x = 28$ diploids, though tetraploid species with $2n=4x=56$, were identified among both subfamilies (2,3). Flow cytometry is an efficient method for rapid determination of genome size in plants. For closely related taxa, where genome sizes are relatively conserved, flow cytometry can also be used for determination of ploidy level (4). The objectives of this study were to

determine genome sizes and ploidy levels of a diverse collection of species, hybrids, and cultivars of *Mahonia* and *Berberis* by using a combination of flow cytometry and traditional cytology.

A highly diverse collection of 52 *Berberis* taxa and 72 *Mahonia* taxa were obtained from various gardens and private collectors. Sampled taxa represented species from each of the four subgroups, many common cultivars, and a few purported artificial autopolyploids. Leaf tissue for each sample, as well as an internal standard (*Pisum sativum* 'Ctirad' 2C DNA = 8.76pg) was finely diced with a razor blade in a Petri dish containing 500 µL of nuclei extraction buffer. A solution containing 2 mL staining buffer, 12 µL propidium iodide (PI) stain, and 6µL RNAse was then added, and the samples were moved to a refrigerator at 4°C for one hour. A flow cytometer (Partec PA-II , Partec, Münster, Germany) was used to analyze the stained nuclei, with a minimum of 5,000 counts per sample, and two samples conducted for each taxon. Holoploid, 2C genome size was calculated as: 2C = DNA content of standard × (mean fluorescence value of sample/ mean fluorescence value of standard). The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers. Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 14) was calculated as (2C genome size / ploidy level) to assess variability in base genome size. Data were subjected to analysis of variance and means separation using the Waller procedure. Traditional cytology was conducted to verify previous work, and calibrate genome size with ploidy level. Actively growing root tips were immersed in a solution of (3: 1) 95% Ethanol : Propionic acid for 24 hours, then fixed in 70% Ethanol at 4°C for storage. Root tissue was removed from cold storage and hydrolyzed in a solution of (3:1) 95% Ethanol : 12N HCl for 5-10 seconds. Root tips were then placed into a drop of carbol fuschin stain on a glass microscope slide, and gently squashed with a coverslip. Chromosomes were counted using oil immersion at 1,500×.

Results and Discussion: The base, $1C_X$, genome size for *Mahonia* varied for each subgenus with a mean of 1.17 ρg for Occidentales and 1.27 ρg for Orientales (Table 1). There was no difference in base, $1C_X$ genome size, between the two subgenera of *Berberis*, but plants in the genus *Berberis* had a significantly higher mean (1.46 ρg) than either subgenus of *Mahonia*. There has been extensive debate among horticulturists and botanists as to whether *Mahonia* and *Berberis* are distinct genera, or should be collectively unified within *Berberis* (9). This data reveals a significant increase in genome size during the evolution of *Berberis*, apart from *Mahonia,* and provides support for maintaining these as separate genera.

Cytology performed on *Mahonia eurybracteata* 'Soft Caress' (2C genome size = 2.43 ρg) confirmed it to be $2n = 2x = 28$, allowing calibration of ploidy with genome size. Polyploid species were very infrequent in the taxa sampled for this project (Table 2). Within *Mahonia*, there were no tetraploid species found, nor were any accessions of *M*.

aquifolium and *M*. *nervosa* found to be tetraploid, as previously reported (8). The only polyploid *Mahonia* taxa found were *M*. *nervosa* MHCRS 2008-062 and *M*. *piperiana* x *nervosa* MHCRS 2006-136 which were estimated to be hexaploid (2*n*=6*x*=84), with respective 2C genome sizes of 7.45 ρg and 7.67 ρg. No natural polyploids were identified among the *Berberis* sampled in this study. However, artificially induced autopolyploids of *Berberis thunbergii* were confirmed with a mean 2C genome size of 5.9 ρg, and estimated to be tetraploid (2n=2x=56).

Overall, this study demonstrates that flow cytometry is an extremely useful tool for studying genome size and polyploidy in both *Berberis* and *Mahonia.* Data from this study provides valuable insights into evolutionary history, taxonomic treatment, and information on ploidy levels of specific taxa that will aid in the breeding and development of new hybrids.

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Table 1. Mean $1C_X$ Genome Size among the 4 subgenera of *Berberis* and *Mahonia*

Genus	Group	$1C_X$ Genome Size (ρg)	Taxa Sampled
Mahonia	Occidentales	1.17 ± 0.02 A[1]	24
	Orientales	1.27 ± 0.01 B	48
Berberis	Australes	1.45 ± 0.03 C	4
	Septentrionales	1.47 ± 0.02 C	48

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

Table 2. Mean 2C genome size and ploidy among key species of *Berberis* and *Mahonia*

Taxa	2C Genome Size (ρg)	Ploidy Level
Berberis thunbergii	2.93 ± 0.05	2x
Berberis thunbergii (oryzalin treated)	5.92 ± 0.07	4x
Mahonia eurybracteata 'Soft Caress'	2.43 ± 0.01	2x[1]
Mahonia nervosa	7.45 ± 0.04	6x
Mahonia piperiana x nervosa	7.67 ± 0.05	6x

[1]Chromosome number and ploidy level was confirmed via cytology.

Breeding *Rhododendron* species and cultivars for improved fragrance and cold hardiness

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Significance to Industry: *Rhododendron catawbiense* 'Vulcan's Flame' and a fragrant pink- flowered *R. decorum* variety were used in controlled crosses to develop inter-specific hybrids for improved fragrance and cold hardiness. Fragrance volatiles were collected using a headspace technique and identified using gas chromatography – mass spectrometry (GC-MS). Controlled pollinations were highly successful and with thousands of seeds produced, we expected to produce new Rhododendrons with improved fragrance and cold hardiness.

Nature of Work: The genus *Rhododendron* consists of over 900 species and infinite numbers of cultivars as a result of the species' ability to freely hybridize (1). Within the genus and among the hybrids is a wide variety of ornamental traits of value to rhododendrons including the following: size, ranging from only a few inches tall to giant trees reaching up to 80 feet or more; flower colors such as yellow, purple, blue, orange, red, and white; bloom time extending from midwinter to early fall; and leaves that are deciduous to evergreen. According to the 1998 Horticultural Specialties Census, U.S. nursery sales were in excess of $3 billion, with rhododendrons accounting for over $59 million in sales in the U.S.

Breeding of rhododendrons began less than 100 years ago in England, when Michael Waterer crossed two imported American species, *R. maximum* and *R. catawbiense* (2). Since this beginning, many amateur and professional breeders have taken advantage of wide variability in ornamental traits and the ability of the species to freely hybridize to develop treasured horticultural cultivars.

An evergreen species, *R. catawbiense* (Catawba rhododendron), is known to transmit to its offspring many valued traits such as cold hardiness from -20 to -30ºF, red, purple, or white flowers, ease of cultivation, resistance to heat, and tolerance of sunlight and exposure (2). In addition*, R. decorum* is known to transmit fragrance to its offspring (2,3).

There is little information available on the natural pollinators of *Rhododendron* species although most are pollinated by insects (4). Plants pollinated by insects typically have showy fragrant flowers and floral scent that adds to the horticultural value of rhododendron. There have been two studies that have documented floral volatiles. Godefroot et al. (5) characterized several cultivars of *R. simsii* and *R. scabrum* and found that *R. simsii* contained high volatile material, whereas *R. scabum* had only small amounts of volatiles. A cross of the two species resulted in a hybrid exhibiting good aroma. Tasdemir et al. (6) evaluated five species of *Rhododendron* and found that *R. luteum* contained the most diverse composition of volatiles, including aromatic compounds.

When the variability and the ability to freely hybridize within the genus *Rhododendron* is considered in combination with all of the previously mentioned characteristics of *R. catawbiense* and *R. decorum*, hybridization within the genus and between existing cultivars of these species becomes attractive for the development of new cultivars with improved floral scent and cold hardiness. The objective of this study is to assess whether rhododendron flowers of *R. catawbiense* and *R. decorum* emit volatile aromatic compounds and if so, to hybridize *R. decorum* to existing cultivars of *R. catawbiense*. Volatiles emitted from rhododendron floral tissues were collected in an open headspace sampling system. Charcoal-purified air entered the chamber at a flow rate of 0.8 L/min from the top through a Teflon hose. Volatiles were collected for 4 h by pumping air from the chamber through a SuperQ volatile collection trap. Plant volatiles were analyzed on a Shimadzu 17A gas chromatograph coupled to a Shimadzu QP5050A quadrupole mass selective detector.

Anthers and freshly dehised pollen were collected from a container-grown pink-flowered fragrant variety of *R. decorum* and the pollen was stored in the refrigerator until *R. catawbiense* 'Vulcan's Flame' bloomed. Pollen from *R. decorum* was applied to the emasculated flowers of the container-grown dark-red flowered 'Vulcan's Flame'. Mature seed capsules were collected in early fall 2009 and were prepared for germination following the methods of Rowe et al. (7). Mature seed capsules were stored in paper bags at 68 - 70°F for 30 days. Seeds were then removed from the capsules and stored in sealed glass bottles at 39°F. Seeds were germinated by planting into flats containing a 1:1 mixture of peat to sand. The flats were then placed at 77°F and subjected to a minimum daily photoperiod of ½ hour. Germinated seedlings were transplanted into pots containing composted pine bark and grown in a greenhouse.

Results and Discussion: 'Vulcan's Flame' has flowers that are dark red and are non-fragrant to the human nose, while *R. decorum* has flowers that are pink and are aromatic to the human nose. Gas chromatography-mass spectrometry (GC-MS) of fragrance volatiles collected from *R. catawbiense* 'Vulcan's Flame' and *R. decorum* flowers revealed similarities and differences.

Controlled pollinations were highly successful with 84% (26 / 31) of 'Vulcan's Flame' pollinated flowers setting seed capsules within three weeks after pollination. From mature seed capsules, thousands of seeds have been recovered and seed germination is in progress.

These results demonstrate that a traditional breeding approach can be used to create inter-specific hybrids of rhododendron that potentially have improved fragrance and cold hardiness.

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Identification of an unknown crape myrtle cultivar with SSR molecular markers

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Index words: *Lagerstroemia* L., simple sequence repeat, molecular identification

Significance to Industry: Most woody ornamental plants, including crape myrtle, are vegetatively propagated. Cultivars may have only minor phenotypic differences so that cuttings and plants of different cultivars may be very similar in appearance. Mislabeling of cultivars is a common occurrence throughout the nursery industry. There is a need for a method to identify crape myrtle cultivars based on genotype rather than phenotypic characteristics (6). Molecular markers can reliably identify clones, breeding lines, hybrids and cultivars, especially at an early stage with little time and cost (4, 5, 7, 9). The molecular markers used in this study could help identify mislabeled or unknown crape myrtle cultivars not only in breeding programs, but for producers across the nursery industry.

Nature of Work: Crape myrtle (*Lagerstroemia* L.) is a popular tree or shrub with an extended summer blooming period (up to ~120 d). It has various flower colors from white to red, a wide range of growth habits, and exfoliating trunk bark (3). *L*. *indica* L. crape myrtle was introduced to the United States in the late 1700's, and was first extensively planted in the eastern and southern United States. However, *L*. *indica* cultivars have limitations such as disease and insect susceptibility and lack of cold hardiness. Another *Lagerstroemia* genus, *L*. *fauriei* Kohene, was introduced from Japan in 1956 by Creech (3, 4), and Egolf integrated this new species into his *Lagerstroemia* breeding program, producing several widely adaptive and adopted cultivars in the last decades (2). Hybridization and vegetative propagation in various *Lagerstroemia* breeding programs has resulted in hundreds of crape myrtle cultivars for the nursery industry. Some of these cultivars are very similar in appearance, and as such mislabeling and/or misclassification are common occurrences in the trade.

Unknown or mislabeled plants often occur and a molecular method is needed to identify them instead of relying only on phenotypical or botanical classification methods. At the Texas A&M Research and Extension Urban Solution Center at Dallas, we encountered several mislabeled (unknown) crape myrtle plants with pink flowers that had been purchased from local suppliers. The objective of this study was to develop SSR (simple

sequence repeats or microsatellites) molecular markers that could be used to identify this unknown crape myrtle cultivar and for identification of future unknown cultivars.

Genomic DNA was isolated from young leaves of the crape myrtle cultivar 'Carolina Beauty'. Microsatellite isolation and identification methods were followed as previously published (8). Genomic DNA was isolated from the unknown plant (pink flowers) and 23 cultivars with pink or lavender flowers and a phenotype similar to that of the unknown plant. One SSR was used to screen the sample DNA of the cultivars and the unknown. The cultivars with the same PCR amplification profile on agarose gel as the unknown sample were further amplified with additional SSR markers until the unknown sample was distinguishable.

Results of Work: Ten SSR markers were obtained from a small DNA insert genetic library to test PCR amplification with genomic DNA of 'Carolina Beauty' and the unknown sample. All amplifications were successful. One of the 10 SSR markers, LID31, was selected to screen the 24 cultivars with pink flowers (including the unknown sample). Results showed that five cultivars ('Conestoga', 'Pink Velour', 'Pocomoke', 'Hopi' and 'Powhatan') had the same two bands on the PCR profile as did the unknown sample (Table 1, Figure 1A). The DNA of these 6 cultivars was further selected to be amplified with SSR primer LID38. Results indicated that 'Conestoga', 'Pink Velour', and 'Pocomoke' showed two bands, and 'Hopi', 'Powhatan' and the unknown sample showed one band (Figure 1B). To further distinguish if the unknown sample was 'Hopi' or 'Powhatan', another SSR primer LID09 was used to amplify the DNA of these 6 samples.

The results showed that 'Conestoga', 'Pink Velour', and 'Pocomoke' amplified only one band while 'Hopi', 'Powhatan', and the unknown sample amplified two bands (Figure 1C). Therefore, the unknown sample could be either the cultivar 'Hopi' or 'Powhatan'. Our next step was to carefully investigate the phenotypic appearance of these three plants that were growing both in the field and in the greenhouse. We determined that both 'Hopi' and the unknown plants were very close in phenotypic appearance with pink flowers, whereas, 'Powhatan' had lavender colored flowers (Figure 2). By the aforementioned sequence, we have determined that this unknown plant is the cultivar 'Hopi'.

In conclusion, three SSR markers were adequate to identify this unknown crape myrtle cultivar, followed by additional scrutiny of the phenotypic appearance of the whole plants in the field or greenhouse (Figure 2). These molecular markers (SSR) should have potential to identify other unknowns or mislabeled cultivars and to identify breeding lines in crape myrtle breeding programs.

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Table 1. Twenty-four crape myrtle cultivars with pink flower and SSR identification

Cultivar	Flower color	LID31*	LID38	LID09
Basham's Party Pink	lavender pink			
Caddo	bright pink			
Choctaw	clear, bright pink			
Comanchee	dark coral pink			
Conestoga	pink to lavender	2	2	1
Delta Blush	Pink			
Hopi	medium pink	2	1	2
Miami	dark pink			
Near East	soft pink			
Osage	clear pink			
Petite Pinkie Monkie	clear pink			
Pink Lace	bright pink			
Pink Velour	bright pink	2	2	1
Pocomoke	bright pink	2	2	1
Potomac	celar medium pink			
Powhatan	light lavender	2	1	2
Prarie Lace	medium pink			
Seminole	clear medium pink			
Sioux	dark pink			
Tuscarora	dark coral pink			
Tuskegee	dark pink to near red			
William Toovey	watermelon pink			
World's fair	watermelon red			
Unknown	pink	2	1	2

* Same PCR profile was showed by amplified band number, others not shown. See Figure 1.

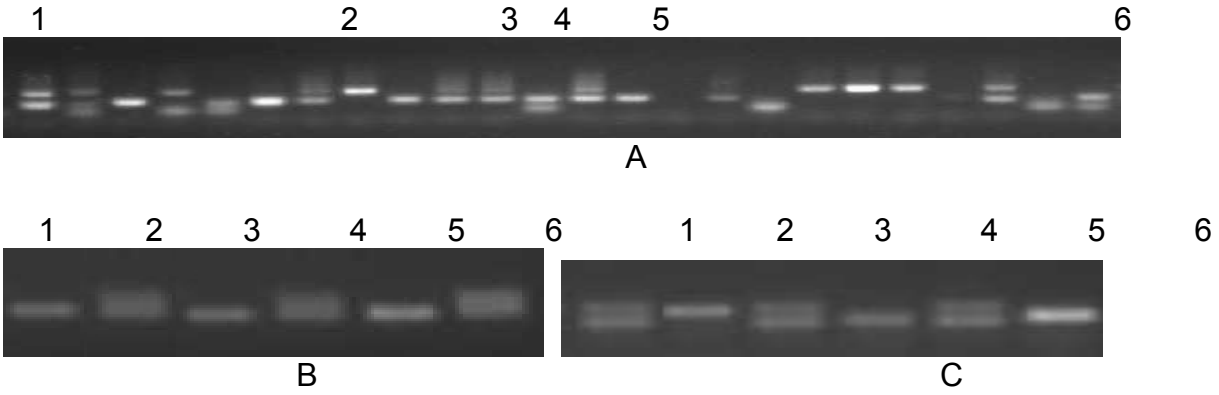

Figure 1. A. PCR profiles of SSR LID31 for the unknown sample and 23 crape myrtle cultivars with pink flowers. 1. Unknown tree; 2. 'Conestoga'; 3. 'Powhatan'; 4. 'Pink Velour'; 5. 'Hopi'; 6. 'Pocomoke'. B. Six samples showing the same profile with LID31 in A were amplified with LID38. C. Six samples showing the same profile with LID31 in A were amplified with LID09 to further identify the unknown sample.

Unknown plant Hopi Powhatan

Figure 2. Phenotypic appearance of the unknown plant, 'Hopi' and 'Powhatan' at the Dallas Center in 2009. These three cultivars showed the same PCR profiles with SSR markers LID 09, 31, and 38, but only 'Hopi' has a very similar phenotype with the unknown plant.

Inter- and Intra-Specific Hybrid Breeding in Crape Myrtle (*Lagerstroemia*)

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Significance to Industry: The woody ornamental genus *Lagerstroemia* L. is native to tropical regions of Southeastern Asia and Indo-Malaysia (4), and to date, these shrubs and trees are cultivated, and in some cases naturalized, in temperate and tropical regions worldwide (5). There are over 50 species in this genus (2, 4), which includes the popular cultivated "common" crape myrtle *L*. *indica* L. The disease-resistant ability of these *L*. *indica* cultivars have been significantly improved since another species, *L*. *fauriei* Kohene, was introduced from Japan by the U.S. National Arboretum (1). *L*. *fauriei* is resistant to powdery mildew and successfully improved and added to the ornamental traits of *L*. *indica* cultivars in interspecific breeding programs (3, 5, 6). To date, however, there are no *Lagerstroemia* cultivars possessing resistance to other diseases and insects, nor tolerance to abiotic stresses, nor other novel horticultural traits, like newer flower colors, or interesting growth habits, etc. Our inter- and intra-specific breeding programs are aimed at providing new breeding germplasm and cultivar(s) with broad adaptation and possessing the most desired horticultural traits for the nursery industry

Nature of Work: The long term goal of these breeding programs is to develop new crape myrtle cultivar(s) with broad adaptation to heat, drought, cold tolerance, disease and insect resistance, and unique/novel flower colors. The objectives of this long-term study are 1) to develop new germplasm with unique horticultural traits and 2) to create a mapping population to address the genetics of disease and insect resistance.

From the existing collections at the Texas AgriLife Research and Extension Center at Dallas and the USDA-ARS breeding program in Poplarville, MS (USDA-ARS SCA Agreement # 58-6404-9-395N), several different cultivars and breeding lines, which have either desirable flower color, powdery mildew and insect resistance, or environmental stress tolerance, were selected to make 17 cross combinations. All parent trees were grown in 5-gallon containers under greenhouse conditions. Controlled pollinations were made during summer 2009 following previously published procedures

(7). Pollen was collected from the male parent tree in the early morning before anthesis. A minimum of 60 flowers were emasculated before the flowers opened or anthers dehisced on each maternal tree. Previously collected pollen was applied to stigmas using a fine-tipped brush. After pollination, each maternal tree was covered with mesh until pod development (about 2 weeks). As a control, one panicle (50 or more flowers) was covered with mesh throughout pollination to exclude foreign pollen. Pods were harvested when they were dark brown or black and stored in a covered plastic box. These pods were dried under sunlight until all seeds were expelled. Seeds were counted and number of seeds per pod calculated.

Results of Work: Four species were incorporated into our breeding program through interspecific crosses in the summer 2009: *L. indica*, *L. subcostata* (NA40181), *L. fauriei* and *L. limii* (Table 1). A total of 16 cultivars or hybrids were selected as maternal or paternal parents. Approximately 3,403 flowers were pollinated and 1,809 fruits (pods) were set successfully (53.2%). Seeds per pod ranged from 4.1 to 14.4 with an average of 8.6, lower than observed in a previous study (6). The lower seed set may be attributable to the hot and dry 2009 summer in Dallas. Seed germination studies will begin between February and March 2010. All bagged self-pollinations had 0-5% pod set (data not shown).

The rate of pod setting varied with different crosses (Table 1), from the highest in the cross 'Cheyenne' × 'Velma's Royal Delight' (94.2%), to the lowest in the cross NA40181 (*L. subcostata*) × 'Carolina Beauty' (8.9%). Cross NA40181 (*L. subcostata*) × 'Velma's Royal Delight' also had a low pod set (19.3%). It seems that the gamete development of *L. subcostata* has some unknown problem when crossing with *L. indica*. However, seed set was higher for intra-specific crossing among *L. indica* cultivars, especially when 'Apalachee' and 'Catawba' were used as parents (Table 1). Whether used as paternal or maternal parent, 'Velma's Royal Delight' also showed higher combining ability in intra-specific breeding (data not shown). These three cultivars have beautiful lavender or dark purple flower color, so they could be good potential resources in breeding for deep purple crape myrtles. Consequently, these three cultivars served as parents in most of our crosses (Table 1) with the expectation of producing "true" (deep) purple germplasm in the segregating progenies.

Two complementary cultivars, 'Carolina Beauty' and 'Natchez', were used to create a mapping population. Both 'Carolina Beauty' and 'Natchez' are large crape myrtle trees which are widely planted in the eastern and southern U.S. 'Carolina Beauty' is red-flowered, aphid resistant and powdery mildew and flea beetle susceptible (greenhouse observation in Figure 1); 'Natchez' is white flowered, powdery mildew and flea beetle resistant and aphid susceptible and has superior exfoliating bark (3). An F1 population was created in order to map molecular markers and identify quantitative trait loci (QTLs) which are associated with disease and insect resistance.

In summary, ‘Apalachee’ and ‘Catawba’ have desired horticultural traits (flower color) and showed high combining ability with other crape myrtle species. ‘Carolina Beauty’ and ‘Natchez’ have complementary horticultural traits (flower color, powdery mildew and aphid resistance) and could be used to create a potential mapping population to address genetics and breeding issues for some quantitative traits, such as powdery mildew, flea beetle and aphid resistance etc. Genetic studies of flower color and powdery mildew and insect resistance will be performed based on F2 and/or BC1 populations.

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Table 1. Inter- and intra-specific hybridization in crape myrtle breeding program

Maternal	Paternal	No. pollinations	Pods	Pod setting(%)	Seeds	Seeds/pod
Cheyenne	Catawba	67	54	80.6	528	9.8
Cheyenne	Tonto	96	71	74.0	834	11.7
Cheyenne	Velma's R Delight	88	83	94.3	903	10.9
NA40181	Apalachee	295	228	77.3	1362	6.0
NA40181	Carolina Beauty	192	17	8.9	88	5.2
NA40181	Catawba	205	131	63.9	588	4.5
NA40181	New Orleans	136	59	43.4	239	4.1
NA40181	Velma's R Delight	450	87	19.3	404	4.6
Natchez*	Carolina Beauty	209	117	56.0	1382	11.8
Natchez	Velma's R Delight	456	213	46.7	1976	9.3
Ozark Spring	Peppermint Lace	86	16	18.6	100	6.3
Velma's Royalty Delight	Apalachee	108	83	76.9	1021	12.3
Velma's Royalty Delight	Peppermint Lace	206	165	80.1	1629	9.9
Velma's Royalty Delight	William Toovey	285	172	60.4	1573	9.1
William Toovey	Wichita	285	201	70.5	2895	14.4
Limii X Red Rocket	Tonto	113	55	48.7	439	8.0
Limii X Red Rocket	Velma's Royalty Delight	126	57	45.2	452	7.9

*To be used as genetic mapping population.

‘Carolina Beauty’ flower

‘Natchez’ flower

‘Carolina Beauty’ leaves
(showing powdery mildew symptoms)

‘Natchez’ leaves (showing aphids damage)

Figure 1. ‘Carolina Beauty’ and ‘Natchez’ flowers and leaves.