Plant Breeding and Evaluation

Tom Ranney
Section Editor and Moderator

New *Callicarpa* Species with Breeding Potential

Ryan N. Contreras and John M. Ruter
University of Georgia, Dept. of Horticulture, Tifton, GA 31793

rncontre@uga.edu

Index Words: beautyberry, species evaluation, ornamental plant breeding

Significance to Industry: There is a great deal of available *Callicarpa* L. germplasm that has yet to be utilized by the nursery industry in the U.S. Taxa currently being evaluated are likely to have potential as breeding material or direct commercial marketability. With new breeding material and selections for introduction the number of beautyberry cultivars for use in southeastern gardens has the potential to expand greatly.

Nature of Work: *Callicarpa* L. is a genus of ~150 species of shrubs and trees distributed throughout the world including warm-temperate and tropical America, SE Asia, Malaysia, Pacific Islands, and Australia (5) with the greatest concentration of species found in SE Asia, specifically the Philippine Islands (1). Of the New World species the highest concentration occurs in Cuba, with ~20 native species (1). There are currently four species commonly found in cultivation in the U.S.: *C. americana* L., *C. bodinieri* Lév., *C. dichotoma* (Lour.)K.Koch, and *C. japonica* Thunb. with a limited number of varieties or cultivars of each to choose from (3). Beautyberries, desired primarily for their handsome berries produced in fall, have been selected for white-fruiting varieties, finer textured varieties, increased berry production, and variegated foliage. These varieties are desirable but do not possess especially novel characters. Further evaluation of the available germplasm has the potential to increase the number of cultivars available in the trade that exhibit novel traits and are well adapted to southern gardens.

Results and Discussion: The following list contains a number of *Callicarpa* species that are not widely distributed or are not found in the trade in the U.S. that may have potential either as introductions or as breeding material in the beautyberry improvement project currently being conducted at The University of Georgia. Evaluations are currently being conducted at the Center for Applied Nursery Research in Dearing, Ga. and the Coastal Plain Experiment Station in Tifton, Ga.

***C. acuminata* H. B. K.** – Throughout Central America south into Colombia. 1.5 – 6 m (10 m) shrub or tree (6), remaining much smaller in southeastern gardens. Corollas pale yellowish-white to white (6). Wine-red fruit distinctly different from other species. Has performed well at the JC Raulston Arboretum (JCRA) in Raleigh, NC; vigorous growth with extremely heavy fruit bearing; flowers over an extended period such that there are flowers and fruit on the plant at the same time.

***C. cathayana* Chang** – Mixed mountain slopes or valleys of China. Shrub to 3 m tall with slender branchlets that are tomentose when young. Good fall color, large clusters of fruit.

***C. formosana* Rolfe** – Southeastern to southern China, the Philippines, Taiwan, and Japan (2). Shrub to 4 m high; leaves elliptic to oblong-lanceolate; flowers purple to pinkish or rarely white. Marginally cold hardy in USDA zone 8.

***C. kwangtungensis* Chun** – North River region of Guangdong (formerly Kwangtung) on the southern coast of China. Upright growth habit with attractive and unique purple tint to the leaves. A specimen at the JCRA has been reluctant to flower and has set little fruit possibly because of the lack of proper pollinators or pollenizers. Flowers reported to have mild fragrance (7) that has not been observed in any received material.

***C. longissima* (Hemsl.) Merr.** – China, Japan, Vietnam, and Taiwan (2). Shrubs or trees 1-3 m (7 m) tall with long lanceolate leaves 17-23 cm (2) that resemble peach leaves. Purple flowers, more ornamental than other species; fruit are larger than *C. americana*. Has performed very well at the Coastal Plain Experiment Station in Tifton, Ga.; vigorous growth with good form and very heavy fruit production; evergreen; appears to be self-fertile.

***C. pedunculata* R. Br.** – New South Wales and Queensland, Australia. Shrubs to 3 m with branches covered in loose hairs; leaves with velvety pubescence, 7-12 cm long with acuminate point (4). Good form in a container; appears to be self-fertile. Sprouts back from the ground in Tifton, Ga.

***C. rubella* Lindley** – Southeast Asia from China south to Malaysia. Deciduous shrub to 2 m tall with loose habit; obovate leaves are distinct, heavily toothed; self-pollination in the greenhouse yielded no fruit indicating that it may be self-incompatible therefore would require companion plants to produce fruit.

Literature Cited:

1. Atkins, S. 1999. *Callicarpa japonica*: *Labiatae*. Curtis Bot. Mag. 16(2):79-83.
2. Chen, Tien-Tsai, Shu-Miaw Chaw, and Yuen-Po Yang. 1998. A revision of *Callicarpa* (Verbenaceae) of Taiwan. Taiwania 43(4):330-345.
3. Dirr, M.A. 1988. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. Stipes, Champaign, Ill.
4. Elliot, W.R. and D.L. Jones. 1982. Encyclopedia of Australian Plants Suitable for Cultivation. Vol. 2. Lothian Publishing Company, Sydney, Australia.
5. Harden, G.J. (ed.). 1992. Flora of New South Wales. Vol. 3. New South Wales University Press, Kensington NSW, Australia.

6. Moldenke, H.N. 1981. Additional materials toward a monograph of the genus *Callicarpa*. XXVIII. Phytologia 49(5):470-482.
7. Moldenke, H.N. 1982. Additional materials toward a monograph of the genus Callicarpa. XXIX. Phytologia 50(7):470-500.

Trichostema Hybridization

Bruce L. Dunn* and Jon T. Lindstrom
University of Arkansas, 316 Plant Sciences Building, Fayetteville, AR 72701

bldunn@uark.edu

Index Words: Ornamental Plant Breeding, Blue Curls, Interspecific Hybrid, Intersectional Hybrid, Hybrid Sterility

Significance to Industry: Plant releases add new excitement and choices for a consumer and industry as a whole. One such new release from a *Trichostema* breeding program at the University of Arkansas is the hybrid *Trichostema* 'Blue Myth'. This release has the desirable qualities of no seed set, ease of propagation, long flowering time, and compact growth. It will make an excellent patio perennial due to its long bloom time and ease of growth. It has potential for use in areas where water is limited, and may be a suitable substitute for locations where *T. lanatum* can not be successfully grown due to its cultivation difficulties.

Nature of Work: *Trichostema* L. is a North American genus in Lamiaceae. Species in the genus can be found across the continental United States and extending north into Canada and south into Mexico (4). The genus consists of approximately 18 species with a center of diversity likely occurring in California, where 10 species grow (9).

Originally, Lewis had proposed five sections (*Paniculatum* F.H. Lewis, *Rhodanthum* F.H. Lewis, *Chromocephalum* F.H. Lewis, *Trichostema* Benth., and *Orthopodium* Benth.) within the genus with *T*. *purpusii* Brandegee. placed in sect. *Rhodanthum* by itself (5). Lewis (6) later reported that *T*. *purpusii* and *T*. *arizonicum* A. Gray were more closely related to each other than any other species in the genus; therefore, he included *T*. *purpusii* in with *T*. *arizonicum* in sect. *Paniculatum*. Sections *Paniculatum* and *Chromocephalum* both have the same chromosome numbers (n=10).

Of the species, *T*. *lanatum* Benth. sect. *Chromocephalum*, is probably the most recognizable species as it occurs in limited cultivation in California (1, 3, 10). The flowers have a pleasant aromatic fragrance, and are a favorite of hummingbirds and butterflies (1, 2). *Trichostema lanatum* grows vigorously during the summer and early autumn dry season making it an excellent plant for xeriscaping (2). In cultivation, some *Trichostema* species, such as *T*. *purpusii*, are easier to grow than others. *Trichostema lanatum* has been reported as being somewhat difficult to maintain in cultivation unless supplied with perfect drainage (8).

The main objective was to make the first artificial crosses within the genus by hybridizing *T*. *lanatum* with *T*. *purpusii* and *T*. *arizonicum* in hopes of generating a commercially accepted hybrid with greater landscape appeal and culturing ease.

Controlled reciprocal pollinations between *T. arizonicum* and *T. lanatum* were made on 24 Aug. 2004. Controlled reciprocal pollinations between *T. purpusii* and *T. lanatum* were made on 12 Sept. 2006. On greenhouse-grown plants, flowers were emasculated prior to anthesis, and pollination occurred once a day for 3 days after emasculation. Mature seeds from the *T. arizonicum* × *T. lanatum* cross were collected on 5 Sept. 2004, and subsequently sown on 20 Sept. 2004. Mature seeds from the *T. purpusii* × *T. lanatum* cross were collected on 10 Nov. 2006, and subsequently sown on 12 Nov. 2006. All seeds were planted in pots using a 1:1 ratio of Fafard #2 (Portland, Ore.) mix and perlite, and placed in a Park Seed Biodome™ (Greenville, S.C.).

Results and Discussion: Five viable seedlings were obtained from the *T. arizonicum* (female) and *T. lanatum* (male) cross, of which two later died. One viable seedling was obtained from the *T. purpusii* (female) and *T. lanatum* (male) cross. Crosses where *T*. *lantum* was the female did not result in progeny.

The *T. purpusii* × *T. lanatum* hybrid**,** which flowered for the first time on 14 May 2007, unfortunately, did not retain the unique pink corolla lobe color. The plant showed hybrid vigor since the flower size was greater than either parent. This hybrid is currently being evaluated in the greenhouse, and was field planted spring 2007.

One of the *T. arizonicum* × *T. lanatum* hybrids, was selected in 2006 and named ‘Blue Myth’ based on its unique flower color. ‘Blue Myth’ was superior to the other two progeny and either parent in growth habit and flower production. It has a number of similar characteristics matching the parents, while also having characteristics intermediate of either parent. On the hybrid ‘Blue Myth’, the stamen, lip, non-exserted corolla-tubes, and growth habit resembled *T*. *arizonicum*, while the pistil and corolla lobes resembled *T*. *lanatum* (Table 1). Leaf morphology and the slight presence of pubescence on the stem, sepals, and corolla lobes were intermediate between the parents (Table 1).

The greenhouse-grown plant of ‘Blue Myth’ began flowering in Apr. 2005, seven months after sowing, and has continued uninterrupted, unlike either parent, showing outstanding and consistent blooming qualities since that time. Peak flowering occurs in spring. In the greenhouse, ‘Blue Myth’ has not produced seed for two consecutive summers despite attempts to self and sib-cross the hybrid.

Propagation of 'Blue Myth' was not difficult as softwood cuttings rooted at high percentages within 2-3 weeks after being dipped in 1,000 ppm K-IBA (data not shown). We were also successful in propagating 'Blue Myth' by plant tissue culture. Cuttings rooted from 'Blue Myth' were field-planted at the University of Arkansas Research Farm in the spring of 2006. 'Blue Myth' has been successfully grown in ground with full sun in Arkansas. Neither the parents nor 'Blue Myth' have survived a winter in Fayetteville, Ark. (USDA Zone 6b/7a).

Literature Cited:

1. Crane, K. 2007. Yerba Buena Nursery (*Trichostema lanatum*). Viewed on 13 May 2007. http://www.yerbabuenanursery.com/online_album/0810.htm

2. Dalby, R. 2004. Blue Curls. Am. Bee J. 144:127-128.

3. Evans, M., and J. Bohn. 2001. *Trichostema lanatum*, Woolly Bluecurls. Viewed on 13 May 2007. http://www.treeoflifenursery.com/main/PDFs/Plant-Profiles/Trich_lana.pdf

4. Lewis, H. 1945. A revision of the genus *Trichostema*. Brittonia. 5: 276-303.

5. Lewis, H. 1960. Chromosome numbers and phylogeny of *Trichostema*. Brittonia. 12:93-97.

6. Lewis, H., and Rzedowski, J. 1978. The genus *Trichostema* (Labiatae) in Mexico. Madroño. 25:151-154.

7. Royal Horticultural Society. 1966. Royal Horticultural Society Colour Chart. Royal Hort. Soc., London.

8. Schmidt, M.G. 1970. Woolly blue curls-*Trichostema lanatum*. Amer. Hort. Mag. 49:83-84.

9. USDA, NRCS. 2007. The PLANTS Database (*Trichostema*). Viewed on 10 May 2007. http://plants.usda.gov/java/profile?symbol=TRICH9.

10. Wilson, B. 2007. *Trichostema lanatum* Woolly Blue Curls. Viewed on 1 May 2007. http://www.laspilitas.com/plants/680.htm.

Table 1: Morphological measurement comparison for *Trichostema lanatum*, the interspecific hybrid 'Blue Myth', and *T. arizonicum*.

Characteristics	*T. lanatum*	'Blue Myth'	*T. arizonicum*
Growth habit			
Branching	shrub	shrub	suffructescent
Leaves			
Length/width ratio	11.7-24.1	4.6-5.5	1.7-2.3
Shape	linear lanceolate	lanceolate	ovate
Margin	entire	entire	undulate
Flowers			
Top posterior corolla lobe length/width ratio	1.7-2.6	1.7-2.3	1.8-2.5
Bottom posterior corolla lobe length/width ratio	1.3-1.7	1.4-2.2	(1.6) 2.2-3.2
Anterior corolla lobe (lip) length/width ratio	1.7-2.4	1.1-1.6	1-1.8
Petal color[z]	93B	86B	155B
	purplish-blue	purple	white
Lip color[z]	89B	89B	88A/155B
	royal purple	royal purple	violet w/ white stripes
Pubescence	dense	sparse	sparse
Stamen			
Length (mm)	25-29	9-15	16-20
Anther color[z]	101B	1C	155A
	blue	greenish-yellow	off-white
Filament color[z]	77B	76A	155D
	wine	light purple	off-white
Pistil			
Length (mm)	33	27	23
Style tip color[z]	83A	83A	76D
	dark purple	dark purple	light lavender
Style color[z]	83D	76A	76D
	purple	light purple	light lavender

[z] Color ratings based on the Royal Horticultural Society Colour Chart (7).

Genetic Diversity of Flowering Dogwood (*Cornus florida* L.) in Tennessee

Denita Hadziabdic[1], Xin wang Wang[1], Robert N. Trigiano[1], Benjamin M. Fitzpatrick[2], Bonnie H. Ownley[1], Mark T. Windham[1], Timothy A. Rinehart[3], and Qiuyun Xiang[4].

[1] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996
[2] Department of Ecology & Evolutionary Biology, University of Tennessee, Knoxville, TN 37996
[3] USDA-ARS, Southern Horticultural Laboratory, Poplarville, MS 39470
[4] North Carolina State University, Raleigh, NC 27695

rtrigian@utk.edu

Index Words: *Cornus florida*, Flowering Dogwood, SSR, Microsatellite, Genetic Diversity

Significance to Industry: Indigenous to the eastern United States, flowering dogwood (*Cornus florida* L.) is commonly found in forests and urban landscapes. Native dogwood populations are important not only for their ornamental attributes, but also as a food source for wildlife. Over the past two decades, dogwood trees have been severely affected by dogwood anthracnose caused by *Discula destructiva* Redlin (Redlin, 1991), resulting in a mortality range from 48-98% in the northeast US and Appalachian highlands (1;2;3;6;7).

Nature of Work: Twenty polymorphic Simple Sequence Repeat (SSR) or microsatellites primer pairs were selected to examine native dogwood populations in Tennessee. These molecular markers are highly reproducible and used for studying genetic diversity and creating linkage maps, since they occur in abundance in the genomes of most eukaryotes (4). Five samples were taken at each of eight locations in Tennessee (Figure 1) and analyzed using PCR and the HDA-GT12™ Genetic Analyzer. The data was entered as a binary matrix, where a 0 and 1 coded for absence and presence of a locus, respectively. The NTSYS (Exeter Software, Setauket, New York) program served as a tool for computation of putative alleles. The objective of this project was to asses the genetic diversity of flowering dogwood in Tennessee using microsatellite loci. This project is a subset of a larger program designed to assess genetic diversity of flowering dogwood throughout the southeastern United States.

For the purpose of this publication, data from five highly polymorphic markers was compiled for all 36 Tennessee samples (Table 1) and analyzed for shared allele frequencies, which consisted of allele size variation of 81 SSR loci.

Genetic distances were calculated for all TN samples and then used to construct a dendrogram showing clustering of individuals from the different sample populations. Identical samples were shown on a single vertical line, whereas ones showing genetic differences were shown on separate (horizontal) branches of the dendogram (Figure 2).

Results and Discussion: Preliminary results from five SSR primer pairs of *C. florida* individuals collected throughout the state of Tennessee demonstrated that considerable genetic diversity exists in dogwood populations. The distribution of frequency of many alleles was significantly different between sample populations, suggesting that sharing of common alleles between physically distant populations. The dendrogram generated from the cluster analysis verified that samples collected in Chattanooga, Jackson/Nashville Mile 170 (along I-40 interstate, west of Nashville) and Newport area, respectively (TN 1-1, TN 2-3 and TN 41-5) are identical for all alleles revealed by the five primer pairs (Figure 2). University of Tennessee Plateau Experiment Station and Jackson/Nashville Mile 170 (TN 2-2 and TN 3-3, respectively) samples also shared the same alleles, suggesting that randomization of allele distribution is more common then previously expected. This preliminary study indicated that maintaining a relatively high level of diversity is favored by obligate outcrossing species such as flowering dogwood. Further research and additional data analysis is necessary to give us a better understanding of population distribution and genetic diversity of this important tree for the nursery industry.

Literature Cited:

1. Hiers, J. K. and J. P. Evans. 1997. Effects on anthracnose on dogwood mortality and forest composition of the Cumberland Plateau (U.S.A.). Conservational Biol. 11:1430-1435.
2. Jenkins, M. A. and P. S. White. 2002. *Cornus florida* L. mortality and understory composition changes in western Great Smoky Mountains National Park. J. Torrey Bot. Soc. 129:194-206.
3. McEwan, R. W., R.N. Mueller, M. A. Arthur and H. H. Housman. 2000. Temporal and ecological patterns of flowering dogwood mortality in the mixed mesophytic forest of eastern Kentucky. J. Torrey Bot. Soc. 127:221-229.
4. Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingley S. and Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2: 225–238.
5. Redlin, S.C. 1991. *Discula destructiva* sp. nov., cause of dogwood anthracnose. Mycologia 83:633-642.
6. Sherald, J. L., T. M. Stidham, J. M. Hadidian and J. E. Hoeldtke. 1996. Progression of the dogwood anthracnose epidemic and the status of flowering dogwood in Catoctin Mountain Park. Plant Dis. 80:310-312.

7. Williams, C. E and W. J. Moriarity. 1999. Occurrence of flowering dogwood (*Cornus florida* L.), and mortality by dogwood anthracnose (*Discula destructive* Redlin), on the Northern Allegheny Plateau. J. Torrey Bot. Soc. 126:313-319.

Acknowledgement: The authors thank the USDA (Agreement number 58-6404-2-0057) for financial support.

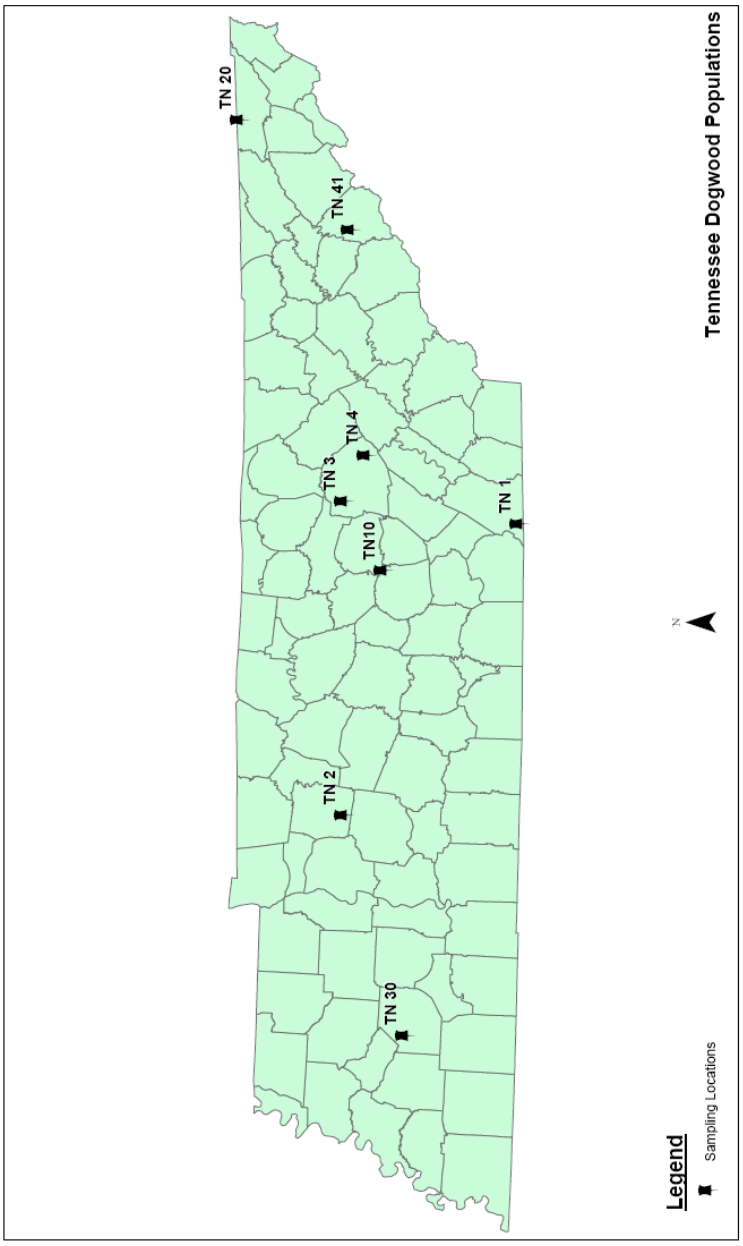


Figure 1. Native dogwood sampled populations in the state of Tennessee. Data was generated using Arc View program.

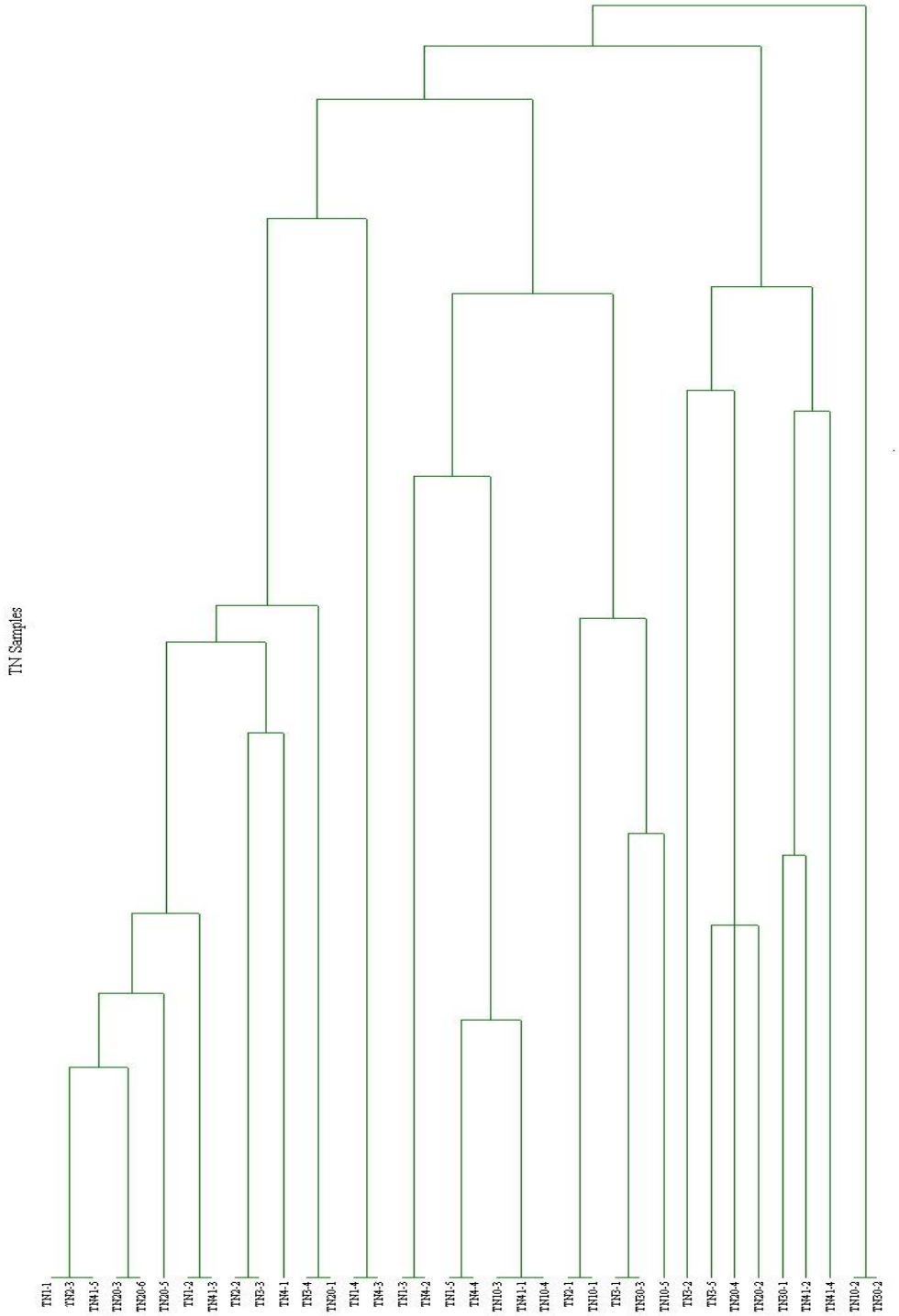


Figure 2. Dendrogram showing clustering of samples in the state of Tennessee. First number indicates sample site or population and second number is subsample within the local population.

Table 1. Tennessee flowering dogwood locations with GPS coordinates.

Sample	Subsample	Latitude	Longitude	General Location
TN 1	(1-5)	35° 00′ 28.0	85° 18′ 49.7	Chattanooga, TN
TN 2	(1-3)	36° 00′ 64.4	87° 22′ 75.5	Jackson/Nashville Mile 170
TN 3	(1-5)	36° 00′ 87.4	85° 07′ 99.2	Plateau Exp. Stat/Rose PT
TN 4	(1-4)	35° 52′ 88.8	84° 48′ 61.3	Ozone, TN
TN10	(1-5)	35° 47′ 57.0	85° 38′ 23.9	Rock Island State Park
TN 20	(1-6)	36° 34′ 33.5	82° 23′ 30.1	Kingsport/Bristol Hwy 11 W
TN 30	(1-3)	35° 37′ 58.7	88° 56′ 57.1	I-40 Exit 74/Memphis/Nashville
TN 41	(1-5)	35° 56′ 64.7	83° 12′ 14.6	I-40 near Newport Mile 435

In vitro Screening for Salt Tolerance in *Hibiscus* Species

H. F. Sakhanokho[1], C. Pounders[1], and R.Y. Kelley[2]
[1]USDA-ARS, Southern Horticultural Laboratory, Poplarville, MS 39470
[2]USDA-ARS, Corn Host Plant Resistance Research Unit, Mississippi State, MS 39762

hsakhanokho@msa-stoneville.ars.usda.gov

Index Words: *Hibiscus acetosella*, *Hibiscus dasycalyx*, *Hibiscus aculeatus*, *in vitro* salt selection

Significance to Industry: Salinity constitutes one of the major stress factors affecting plant growth (14, 16). This is a concern in many areas of the world, including the U.S. Gulf Coast where extreme weather events such as hurricanes and coastal flooding can periodically increase soil salinity. Evaluating for salt tolerance using conventional breeding methods can be labor intensive because it is a complex trait controlled by several genes. Tissue culture techniques, which allow for the processing of large numbers of samples in a short period of time, have been used to screen and/or produce salt-tolerant lines and plants in several species, including wheat, rice, barley, potato, sunflower, and sugarcane (1, 2, 3, 6, 8, 12, 17), but very little research has been undertaken to assess salinity tolerance of ornamental plants (17).

Nature of Work: The species used in this study included red and green variants of *Hibiscus acetosella*, *Hibiscus dasycalyx*, and *Hibiscus aculeatus*. *H. acetosella* is native to tropical west Africa (10) and grown as an ornamental for the attractiveness of its deep burgundy red, maple-like leaves. *H*. *dasycalyx*, also known as the Neches River rose mallow, is a federally listed candidate endangered species that is native to Texas (5). *H. aculeatus* (rosemallow, comfortroot, or Pineland hibiscus) is a hardy species with light yellow flowers and red eye. It is native to the lower coastal plains of southeastern North America (15). The objective of the study was to use *in vitro* meristem culture to evaluate and select hibiscus plants for salt (NaCl) tolerance. Five salt concentration levels, 0.1%, 0.2%, 0.4%, 0.8%, and 1% (w/v), were used in addition to the control treatment that contained no salt. Meristems were cultured for 30 days using a published protocol (11). The number of plants that rooted at day 10, 20, and 30 in each treatment was recorded as well as plant height after 30 days of *in vitro* culture.

Results and Discussion: Both root formation and shoot growth were adversely affected by increasing salinity in all three species (Table 1). In the control treatment, shoot growth was significantly higher in both variants of *H. acetosella* than in either *H. aculeatus* or *H. dasycalyx*. *H. dasycalyx* performed as well as both *H*. *acetosella* variants for shoot height at 0.2% NaCl concentration, but at

0.4% NaC concentration, *H. dasycalyx* performed better than any of the two other species for the same trait (Table 1). At 0.8 and 1% NaCl concentrations, there were no differences among the species for shoot height. For root development, *H. dasycalyx* performed better than any other species under saline conditions after 30 days (Table 1). In addition, there was very little callus formation even at higher salt concentrations in *H. dasycalyx* as compared to the other species (Fig. 1D). Reports on *in vitro* salt tolerance studies suggest that rooting and root growth are highly affected by salt and also positively correlated with salt tolerance at the whole plant level (4, 9). Based on the results obtained here, *H. dasycalyx* appears to be more salt tolerant than the other species tested. This species, which can be found only in three wetlands in eastern Texas as it is threatened by interspecific hybridization with *H. laevis* and *H. moscheutos* as well as loss of preferred wetland habitat along the Neches River and its tributaries (5, 7, 13), must have developed salt tolerance under its harsh wetland environments.

Literature Cited:

1. Alvarez, I., L. M. Tomaro, and P. M. Bernvides. 2003. Changes in polyamines, proline and ethylene in sunflower calluses treated with NaCl. Plant Cell Tiss. Org. Cult. 74:51-59.
2. Barakat, M. N. and T. H. Abdel-Latif. 1996. *In vitro* selection of wheat callus tolerant to high levels of salt and plant regeneration. Euphytica 91:127-140.
3. Benavides, P. M., L. P. Marconi, M. S. Gallego, E. M. Comba, and L. M. Tomaro. 2000. Relationship between antioxidant defense systems and salt-tolerance in *Solanum tuberosum*. Aust. J. Plant Physiol. 27:273-278.
4. Cano, E.A., F. Perez-Alfocea,V. Moreno, M. Caro, and M. C. Bolarin. 1998. Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. Plant Cell Tiss. Org. Cult. 53:19-26.
5. Creech, D., D. Parish, and B. Clark. 1999. Saving the Neches River rose mallow, *Hibiscus dasycalyx*. Native Plant Soc.of Texas News 17:1-3.
6. Gandonou, C. B., T. Errabii, J. Abrini, M. Idaomar, and N. S. Senhaji. 2006. Selection of callus cultures of sugarcane (*Saccharum* sp.) tolerant to NaCl and their response to salt stress. Plant Cell Tiss. Org. Cult. 87:9-16.
7. Klips, R.A. 1995. Genetic affinity of the rare eastern Texas endemic *Hibiscus dasycalyx* (Malvaceae). Amer. J. Bot. 82:1463-1472.
8. Lutts, S., J. Bouharmont, and J. M. Kinet. 1999. Physiological characterization of salt-resistant rice (*Oryza sativa*) somaclones. Aust. J. Bot. 47:835-849.
9. Martinez, C. A., M. Maestri, and E. G. Lani. 1996. *In vitro* salt tolerance and proline accumulation in Andean potato (*Solanum spp*.) differing in frost resistance. Plant Sci. 116:177-184.
10. Menzel, M. Y. and F. D.Wilson. 1961. Chromosome and crossing behavior of *Hibiscus cannabinus*, *H. acetosella*, and *H. radiatus*. Amer. J. Bot. 48:651-657.

11. Sakhanokho, H. F. 2007. *In vitro* multiple shoot induction and plant regeneration from shoot apex of *Hibiscus acetosella* Welw. ex. Hiern. J. Crop Improv. (in press).
12. Sibi, M. L. and M. Fakiri. 2000. Androgenèse et gynogenèse, sources de vitrovariation et de tolérance à la salinité chez l'orge *Hordeum vulgare*? Sécheresse 11:125-132.
13. Smith, E. and D. Creech. 1995. A propagation and reintroduction strategy for the Neches River rose mallow, *Hibiscus dasycalyx*. HortScience 30:805.
14. Tester, M. and R. Davenport. 2003. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91:503-507.
15. Wilson, F. D. 1994. The genome biogeography of *Hibiscus* L. section Furcaria DC. Genetic Resources and Crop Evolution 41:13-25.
16. Yang, A., L. Zhu, S. Zhao, and J. Zhang. 2004 Induction of multiple bud clumps from inflorescence tips and regeneration of salt-tolerant plantlets in *Beta vulgaris*. Plant Cell Tiss. Org. Cult. 77:29-34.
17. Zollinger, N., R. Koenig, T. Cerny-Koenig, and R. Kjelgren. 2007. Relative salinity tolerance on intermountain western United States native herbaceous perennials. HortScience 42:529-534.

Table 1. Effect of different NaCl concentrations on *in vitro* root development and height of hibiscus plants.

		Rooting (%)				
NaCl (%)	**Species**	**Day 10**	**Day 20**	**Day 30**	**Total**	***Shoot height (cm) after 30 days in culture.**
0	*H. acetosella* (green)	59.1ab	4.5ab	6.8ab	70.4ab	8.7a
0	*H. acetosella* (red)	78a	6a	6ab	90a	8.3a
0	*H. aculeatus*	68.6ab	2.9c	5.7ab	77.4ab	5.3b
0	*H. dasycalyx*	59.4ab	2.9c	10.1a	72.4ab	5.2b
0.1	*H. acetosella* (green)	42.6ab	42.6ab	6.4ab	91.6b	5.5bc
0.1	*H. acetosella* (red)	33.3ab	48.9a	8.9ab	91.1b	5.2bc
0.1	*H. aculeatus*	36.1ab	33.3ab	11.1a	80.5bc	8.4a
0.1	*H. dasycalyx*	**67.8a**	32.2ab	0c	**100a**	6.8ab
0.2	*H. acetosella* (green)	40.4b	32c	8.5	80.9b	7.4a
0.2	*H. acetosella* (red)	19.6c	62.7a	3.9a	86.2ab	7.3a
0.2	*H. aculeatus*	20c	55.6b	2.2b	77.8bc	5.5b
0.2	*H. dasycalyx*	**80.4a**	9.8d	2b	**92.2a**	**7.5a**
0.4	*H. acetosella* (green)	2.1d	22.9c	10.4a	35.4bc	4.0b
0.4	*H. acetosella* (red)	6.3c	31.3a	9.4a	47b	3.8b
0.4	*H. aculeatus*	41.5b	26.4ab	9.4a	77.3a	4.1b
0.4	*H. dasycalyx*	**69.8a**	4.7d	4.7b	**79.2a**	**7.3a**
0.8	*H. acetosella* (green)	0b	0b	0b	0b	1.9a
0.8	*H. acetosella* (red)	0b	0b	0b	0b	2.1a
0.8	*H. aculeatus*	0b	0b	0b	0b	1.7a
0.8	*H. dasycalyx*	**6.6a**	**14.8a**	**4.9a**	**26.3a**	1.8a
1	*H. acetosella* (green)	8a	4a	0b	12b	1.8a
1	*H. acetosella* (red)	0c	0b	0b	0c	1.7a
1	*H. aculeatus*	8.3a	0b	0b	8.3b	1.4a
1	*H. dasycalyx*	**3.3b**	**3.3a**	**16.4a**	**23a**	1.7a

*Means followed by the same letter within the same column and NaCl concentration are not significantly different (P = 0.05) according to Tukey's HSD test. Means represent the averages of 50 explants (2 replications of 25 per treatment).

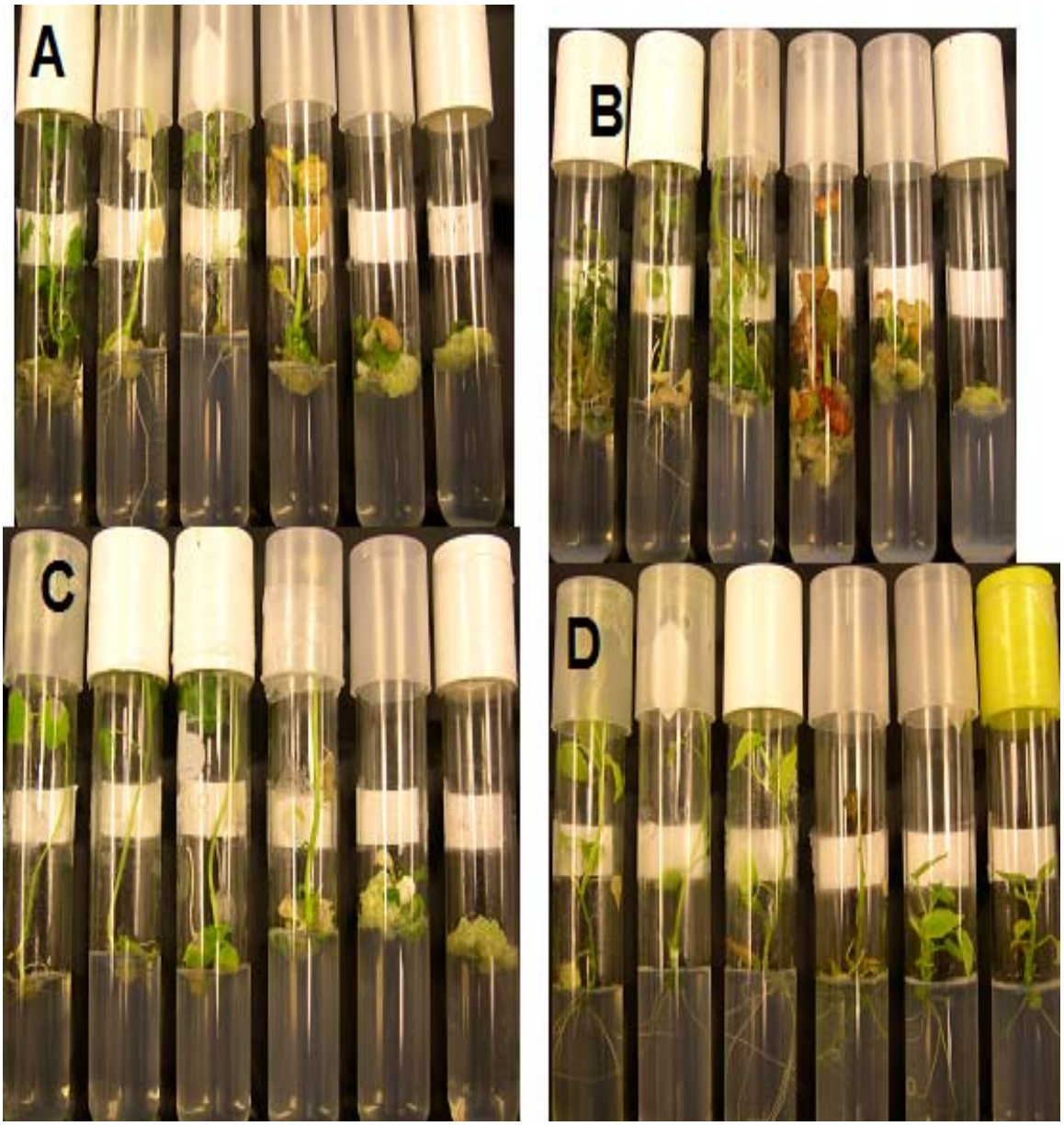


Fig. 1. *In vitro* salt tolerance evaluation in three *Hibiscus* species: **(A)** *H. acetosella* (green variant), **(B)** *H. acetosella* (red variant), **(C)** *H. aculeatus*, and **(D)** *H. dasycalyx*. The effect of increasing salt concentration (left to right: 0, 0.1, 0.2, 0.4, 0.8, and 1%) on each genotype is indicated for the four genotypes. Higher salt concentrations generally resulted in substantial callus formation, except for *H. dasycalyx* (**D**, last two test tubes).

Ploidy Levels and Genome Sizes of Diverse Species, Hybrids, and Cultivars of Rhododendron L.

Jeff R. Jones[1], Thomas G. Ranney[1], Nathan P. Lynch[1], and Stephen L. Krebs[2]

[1] N.C. State University, Dept. of Horticultural Science, 455 Research Dr. Fletcher NC, 28732

[2] David G. Leach Research Station, The Holden Arboretum, 9500 Sperry Road, Kirtland, OH 44094

jeff_jones@ncsu.edu

Index Words: Cytology, Flow Cytometry, Genome Size, Polyploidy

Significance to Industry: Polyploidy has been a central pathway in the evolution of plants and is an important consideration in plant breeding as it can influence fertility, crossability, plant vigor, and gene expression. In some cases, polyploid plants can also have desirable characteristics including thicker leaves and petals, enhanced vigor, and larger flowers that persist longer. This research provides an extensive survey of polyploidy in the genus *Rhododendron* L. and provides further insights into the genetics, evolution, and reproductive biology of rhododendron as well as serving as a valuable database for breeders.

Nature of Work: Many of the more than 800 *Rhododendron* species have been reported to be diploid with 2*n* = 2x = 26 (1). However, polyploidy occurs naturally in some rhododendron species, particularly within the *Pentanthera* and *Rhododendron* subgenera. Although some information has been published on polyploidy of *Rhododendron* species, there has been limited sampling and there is little data for specific clones or cultivars. The chromosomes in rhododendron are small and can be difficult to view and count. Determination of chromosome numbers by light microscopy is therefore not a practical method for establishing ploidy levels of large numbers of individual cultivars and clones. However, flow cytometry can provide a fast and accurate determination of nuclear DNA content (genome size) that is related directly to ploidy level among closely related taxa. Flow cytometry is also effective for detecting mixaploidy or cytochimeras. The objectives of this project were to determine the ploidy level and genome size of a diverse collection of species, hybrids, and cultivars of rhododendron using a combination of flow cytometry and traditional cytology. Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry. Two hundred diverse species and cultivars were acquired from various sources that included taxa from the *Hymenanthes* (Blume) K. Koch., *Rhododendron* L., *Tsutsusi* (Sweet) Pojarkova, and *Pentanthera* G. Don. subgenera. Stained nuclei from newly expanded leaf or petal tissue was analyzed using a flow cytometer (PA-I, Partec, Münster, Germany) to determine relative DNA content. Genome

sizes were determined by comparing mean relative fluorescence of each sample with an internal standard, *Pisum sativum* L. 'Ctirad', with a known genome size of 9.09 pg and calculated as: 2C DNA content of sample = 9.09 pg × (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 (3). Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 13) 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. In situations where cytometric results were not consistent with published research, chromosomes were counted using standard cytology techniques (2).

Results and Discussion: Flow cytometry was an effective method for determining genome size and ploidy levels of rhododendron. Analysis of variance demonstrated significant effects of both subgenus and ploidy level on 2C genome size ($P<0.05$). Genome sizes (2C) within ploidy level for a given subgenus had a narrow range providing clear distinction among ploidy levels (Table 1). Mean 1Cx monoploid genome size was conserved across ploidy levels within a subgenus (Table 1). As expected from past reports, all of the sampled species within the *Hymenanthes* were diploid. However, many interspecific hybrids were polyploids. Hybridity has been shown to increase formation of unreduced gametes even when the parental species might not exhibit the same characteristic (5). Tetraploids arising from interspecific hybrids included 'Horizon Monarch', 'Lem's Monarch', 'Point Defiance', and 'Gentle Giant'. 'Vulcan' tetraploid was found to be a 2x + 4x mixaploid that apparently arose from an asexual mitotic doubling event within a single histogenic layer. Several chemically induced tetraploids were found including 'Everlasting Tetra', R. *fortunei* Lindl. (NCSU 2005-175), 'Super Nova', and the mixaploid 'Briggs Red Star'. Concordant with previous findings, polyploidy was common among species and their hybrid derivatives from subgenus *Rhododendron*. *Rhododendron augustinii* Hemsl. and its hybrids were found to be tetraploids, while *R*. *maddenii* Hook. f. clones were found to be hexaploids and octoploids. 'Bubblegum' and 'Northern Starburst' were both tetraploids developed from *in-vitro* colchicine treatments. Polyploidy was not common among the evergreen azaleas (subgenus *Tsutsusi*) with the exception of two chemically induced tetraploids. The majority of deciduous azaleas (subgenus *Pentanthera*) were found to be diploids as has been reported previously and *R*. *calendulaceum* (Michx.) Torr. was confirmed as a tetraploid. However, our results indicated that natural polyploidy is more prevalent among deciduous azalea species than previously thought. All of the *R. atlanticum* (Ashe) Rehder and *R. austrinum* (Small) Rehder accessions tested in this study were polyploids (mostly tetraploid and a few triploid), as were some of the *R. flammeum* (Michx.) Sarg. This is notable because in all earlier reports, only one instance of polyploidy (triploid) in these three North American species has been reported (4). Cytometric results in

the present study were confirmed by chromosome counts on somatic cells from fifteen accessions of both *R. atlanticum* and *R. austrinum*, which showed that they were tetraploids, 2*n* = 4x = 52 (Figs. 1 and 2). Both diploid and tetraploid accessions were observed for *R. flammeum* representing a natural polyploid series. Many deciduous azalea cultivars were found to be polyploids including the tetraploids 'Admiral Semmes', 'Gibralter', 'Gold Dust', 'Lemon Lights', 'MaryDel', 'My Mary', 'Klondyke', 'Snowbird', and the octoploid 'Fragrant Star'.

Literature Cited:

1. Ammal, E.K.J., I.C. Enoch, and M. Bridgwater. 1950. Chromosome numbers in species of rhododendron. Rhododendron Year Book 5:78-91.
2. Contreras, R.N., T.G. Ranney, and S.P. Tallury. 2007. Reproductive behavior of diploid and allotetraploid *Rhododendron* L. 'Fragrant Affinity'. HortScience 42(1):31-34.
3. De Schepper, S., L. Leus, M. Mertens, E. Van Bockstaele, and M. De Loose. 2001. Flow cytometric analysis of ploidy in Rhododendron (subgenus Tsutsusi). HortScience. 36(1):125-127.
4. Li, H. 1957. Chromosome studies in the azaleas of eastern North America. Amer. J. Bot. 44(1):8-14.
5. Ramsey, J. and D.W. Schemske. 1998. Pathways, mechanisms, and rates of polyploidy formation in flowering plants. Annu. Rev. Ecol. Syst. 29: 467-501.

Table 1. Summary of means and ranges for 2C, holoploid genome size (ρg) and 1Cx monoploid genome size (ρg) by sub-genus and ploidy level.

	Ploidy level				
Sub-genus	Diploid (2x)	Triploid (3x)	Tetraploid (4x)	Hexaploid (6x)	Octoploid (8x)
Hymenanthes	2C = 1.50 ± 0.01[1] A (1.41-1.64) 1Cx = 0.75 ± 0.01 A (0.71-0.82)	2C = 2.17 ± 0.05 B (2.06-2.22) 1Cx = 0.72 ± 0.02 A (0.69-0.74)	2C = 3.01 ± 0.04 C (2.89-3.37) 1Cx = 0.75 ± 0.01 A (0.72-0.84)	NA	NA
Rhododendron	2C = 1.65 ± 0.05 A (1.32-1.86) 1Cx = 0.83 ± 0.02 A (0.66-0.93)	2C = 2.01 ± -- B (NA) 1Cx = 0.67 ± -- B (NA)	2C = 3.06 ± 0.05 C (2.78-3.25) 1Cx =0.77 ± 0.01 AB (0.70-0.81)	2C = 4.48 ± 0.04 D (4.39-4.61) 1Cx = 0.75 ± 0.01 AB (0.73-0.77)	2C = 5.70 ± 0.28 E (5.42-5.97) 1 Cx = 0.72 ± 0.03 AB (0.68-0.75)
Pentanthera	2C = 1.63 ± 0.01 A (1.51-1.74) 1Cx = 0.81 ± 0.01 A (0.76-0.87)	2C = 2.48 ± 0.06 B (2.30-2.60) 1Cx = 0.83 ± 0.02 A (0.77-0.87)	2C = 3.24 ± 0.02 C (3.00-3.88) 1Cx = 0.81 ± 0.00 A (0.75-0.97)	NA	2C = 6.40 ± .03 D (6.32-6.46) 1Cx =0.80 ± 0.00 A (0.79-0.81)
Tsutsusi	2C = 1.26 ± 0.01 A (1.22-1.30) 1Cx = 0.63 ± 0.01 A (0.61-0.65)	2C = 1.93 ± 0.03 B (1.88-1.98) 1Cx = 0.65 ± 0.01 AB (0.63-0.66)	2C = 2.68 ± 0.08 C (2.60-2.75) 1Cx = 0.67 ± 0.02 B (0.65-0.68)	NA	NA

[1]Values represent means ± SEM followed by (ranges) derived from the entire data set. Means followed by different letter, within a row, are significantly different, P<0.05.

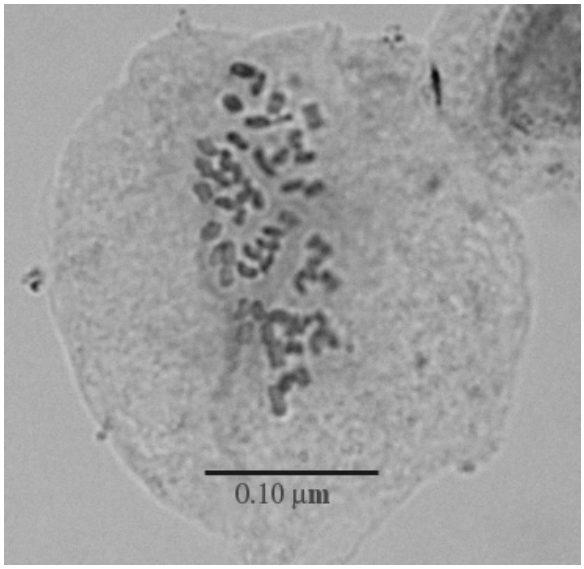


Figure 1. Photomicrograph of root tip cell of *R. atlanticum* (H2004-054-002) in prophase with 2*n* = 4x = 52 somatic chromosomes.

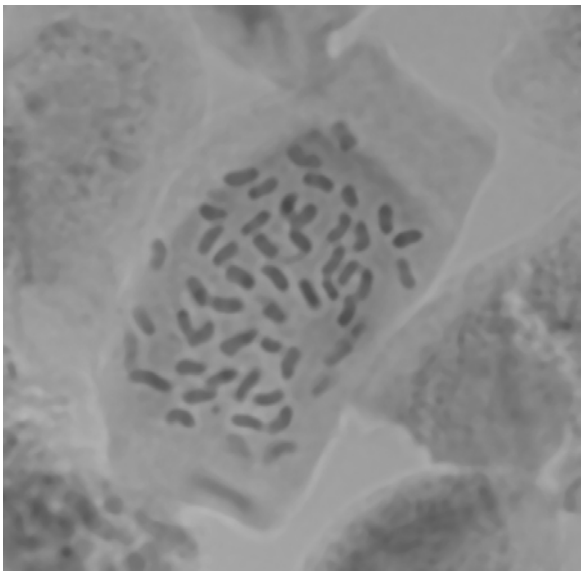


Figure 2. Photomicrograph of root tip cell of *R. austrinum* (2006-223) in prophase with 2*n* = 4x = 52 somatic chromosomes.

Inheritance of leaf variegations in *Miscanthus*

Darren Touchell, Jeremy Smith and Thomas Ranney

NC State University, Dept. of Hort. Science, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Fletcher, NC 28732-9244

darren_touchell@ncsu.edu

Key Words: Miscanthus, leaf variegation, breeding, genetics, invasive

Significance to Industry: Miscanthus is a grass native to Asia and has become a popular ornamental plant in the United States. It is a valuable nursery crop and also has significant potential as a bio-fuel crop. In some areas of the United States, and particularly in Western North Carolina, *Miscanthus* has naturalized along roads side verges and disturbed areas raising environmental concerns.

Miscanthus contains 14 species of which *M. sinensis* is most common in the nursery industry. Currently, there are over 100 named varieties of *M. sinesis* (1), which are noted for their variegations, different foliage characteristics, compactness and inflorescence color. Vertical and horizontal leaf variegations are the most valued characteristics. Understanding the genetics and heritability of these desirable characteristics will help in developing strategies to simultaneously breed for ornamental traits and non-invasiveness.

Nature of Work: Leaf variegations arise from mutations that make chloroplast formation unstable. While these mutations are most often found in the chloroplast genome they can also occur in the nuclear genome (3). Mutations in the nuclear genome can be distinguished from those in the chloroplast genome by the inheritance pattern. Specifically, chloroplast mutations are typically inherited maternally, while mutations in the nuclear genome show Mendelian inheritance (2). In the present study we report on the mode of inheritance for horizontal and vertical leaf variegations in *Miscanthus sinensis*.

M. sinensis 'Strictus' (ST) and *M. sinensis* 'Variegatus' (VAR) were selected as two cultivars representing horizontal and vertical leaf variegations, respectively. Reciprocal di-hybrid F_1 and F_2 populations were produced to study inheritance of the two variegations. Backcrosses (BC) population to each parent were also produced. Further backcrosses with *M. sinensis* 'Zebrinus'(ZEB), *M. sinensis* 'Little Zebra' (LZ) and *M. sinensis* 'Superstripe' (SS) were conducted to determine if horizontal variegations in these cultivars was attributed to the same gene. Similarly, backcrosses with *M. sinensis* 'Silberfeil' (SILB) were conducted to determine if vertical variegations could be attributed to the same gene. All crosses were conducted during the summer of 2006. Hybridizations were

performed by placing the two flower heads in a paper bag and shaking for 30 s. Seed was collected after 30 d and germinated on moist filter paper. Germinated seed was transferred to pinebark substrate supplemented with 0.75 kg/m^3 micronutrients (Micromax) and 1.5 kg/m^3 lime. Phenotypes were scored during the spring/summer of 2007. It was hypothesized that horizontal and vertical variegations were independent traits and inherited in a Mendelian recessive manner. Chi-square analysis was used to analyze departures from expected rations on segregating families.

Results and Discussion: Reciprocal F_1 crosses yielded progeny that were all non-variegated and green, with the exception of 3 horizontally variegated plants and 1 vertically variegated plant, which were attributed to infrequent self-pollination (Table 1). This suggests that both horizontal and vertical variegations were not maternally inherited characteristics arising from mutations in the chloroplast genome.

In the F_2 progeny, a lower than expected number of horizontally variegated plants (19 observed vs. 31.3 expected) and combined horizontally and vertically variegated plants (3 observed vs. 10.4 expected) were observed, therefore having a poor fit to the expected 9:3:3:1 ratio. The chi-square test for independence of linkage ($\chi^2 = 1.24$, $P = 0.2$) suggested no evidence of linkage between the horizontal and vertical variegations. However, segregation ratios for BC populations strongly fit the expected 1:1 ratio for variegated:non-variegated phenotypes for both horizontal and vertical variegations (Table 1). Further, phenotypic expression was erratic and seemed to be influenced by seasonal and/or other factors not considered in this study. The lack of uniformity in expression may have influenced data collection and it is possible that further variegations will be expressed as plants mature. The combination of these data and observations suggest that simple recessive inheritance is currently the most likely model for both horizontal and vertical leaf variegations in *M. sinensis*.

Progeny from crosses with other horizontally and vertically variegated cultivars provide good fits to a 1 variegated: 1 green ratio, strongly suggesting that the genes responsible for variegations remain the same across cultivars. However, the intensity of variegations often varied within each cross. Therefore it is likely there are other genes that interact with the phenotypic expression of variegations.

Horizontal and vertical variegations in *Miscanthus sinensis* are likely to be inherited in a simple recessive Mendelian manner. However, the expression of variegations (e.g., phenology, intensity, etc.) appears to by modified by other factors. Further studies are currently being conducted to develop variegated, non-invasive ornamental cultivars.

Literature Cited:

1. Darke, R. 2007. The encyclopedia of grasses for living landscapes. Timber Press.Portland.
2. Fawole, I. 2001. Maternal inheritance of plant variegation in cowpea, *Vigna unguiculata* (L.) Walp. Theoretical and Applied Genetics. 102: 459-462.
3. Sakamoto, W. 2003. Leaf-variegated mutations and their responsible genes in *Arabidopsis thaliana*. Genes and Genetic Systems. 78: 1-9.

Table 1. Segregation analysis for variegated foliage traits in *Miscanthus sinensis*

Cross[Z]	Population	Progeny (No. of plants)				Expected Ratio	χ^2	*P*
		Green	Horizontal	Vertical	H+V			
ST x VAR	F_1	75	3	0	0	1:0:0:0	_	_
VAR x ST	F_1	70	0	1	0	1:0:0:0	_	_
F_1 x F_1	F_2	111	19	33	3	9:3:3:1	14.57	.05
ST x F_1	BC_H	16	14	0	0	1:1:0:0	0.17	0.5
VAR x F_1	BC_V	33	0	39	0	1:0:1:0	0.51	0.5
ZEB x F_1	BC_{ZEB}	21	20	0	0	1:1:0:0	0.05	0.5
LZ x F_1	BC_{LZ}	12	15	0	0	1:1:0:0	0.37	0.5
SS x F_1	BC_{SS}	30	26	0	0	1:1:0:0	0.3	0.5
SILB x F_1	BC_{SILB}	32	0	34	0	1:0:1:0	0.08	0.5

[Z]ST = 'Strictus', VAR = 'Varigatus', ZEV = 'Zebrinus', LZ = 'Little Zebra', SS = 'Superstripe', SILB = 'Silberfiel'

Assessing Fertility among Cultivars of Winged Euonymus

Thomas G. Ranney, Thomas A. Eaker, and Joel A. Mowrey

NC State University, Dept. of Hort. Science, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Fletcher, NC, 28732-9244

tom_ranney@ncsu.edu

Index Words: Alien Species, Adventive Species, Fertility, Invasive Species, Invasiveness, Naturalized Exotic Species, Non-indigenous Species, Seedless, Sterility, Weed Free

Significance to Industry: Invasive species are an important issue for the nursery industry. A limited number of non-native species that are grown for landscape use can be weedy to the point of being invasive, i.e., their introduction causes or is likely to cause economic or environmental harm or harm to human health that outweighs any beneficial effects (http://www.invasivespeciesinfo.gov/docs/council/isacdef.pdf). Winged euonymus (*Euonymus alatus*), for example, has been found to naturalize over broad areas and habitats (http://www.natureserve.org/explorer/). Concern over potential environmental impacts has recently led numerous states to ban or limit sales of winged euonymus. However, winged euonymus continues to be an economically, aesthetically, and environmentally important crop. As such, the selection/development of seedless/noninvasive cultivars would be an ideal solution whereby these valuable plants can be utilized without detriment. The objective of this study was to evaluate and compare fertility of *E. alatus* 'Compactus' with *E. alatus* 'Odom' Little Moses™ (USPP# 13168), a cultivar that has been observed to have low fertility in field and production settings.

Nature of Work: Container-grown plants of *E. alatus* 'Compactus' and 'Odom' were grown in full sun, with drip irrigation, and standard production practices at the Mountain Horticultural Crops Research Station in Fletcher, N.C. in 2004. Six plants of each cultivar were arranged in a completely randomized design. Plants were similar in size and ranged from 3 to 4 feet in height and 2 to 3 feet in width. In addition to natural pollination, 3 branches on each plant were marked and all receptive flowers on those branches were hand pollinated daily with a mixture of fresh pollen collected each day from both 'Compactus' and 'Odom'. Up to 100 fruit were randomly collected from each plant. Seeds were extracted and sowed in an equal mixture of peatmoss and perlite. Seeds were then subjected to cold, moist stratified at 40 °F for 90 days, followed warm, moist stratification (65-75 °F) for 90 days, followed by as second period of cold, moist stratification at 40 °F for 90 days, then germinated in a glass greenhouse at 65-75 °F. Pollen germination tests were performed in 5 mL Petri dishes containing Brewbaker-Kwack media

supplemented with 15% sucrose and solidified with 2% agarose (2). Pollen grains with pollen tubes greater than one-half the diameter of the pollen grain after 6 h were scored as germinated. Each replicate included100 pollen grains. Data were collected for the total number of fruit formed on each plant, number of flowers and fruit produced on branches receiving supplemental hand pollination, and percent pollen germination.

Results and Discussion: Pollen germination under our experimental conditions averaged 16% and was not significantly different between the two cultivars indicating similar levels of male fertility (Table 1). Natural pollinators including a variety of Diptera and Hymenoptera insects were prevalent and actively visiting flowers throughout the bloom period. Total fruit set for open pollinated plants averaged 270 for 'Compactus' and was significantly more than the average of 7 for 'Odom', despite all plants being similar in size with a similar number of flowers. However, fruit set per flower, on branches that received supplemental hand pollination, averaged 0.06 and was not significantly different between cultivars. Seed germination was 51% for 'Compactus' and significantly greater than the 11% germination for 'Odom'.

Overall fruit production for 'Odom' was only 3% of that compared to similar sized plants of 'Compactus', yet fruit set was similar on branches that received supplemental pollination. These results suggest that the lower fruit production found for 'Odom', may have resulted from lower natural pollination. The different cultivars appeared to have similar numbers of flowers, but the foliage and branching of 'Odom' was much denser than 'Compactus', which might have deterred pollinators. In addition to producing fewer fruit, seed germination of 'Odom' was only 22% that of 'Compactus', demonstrating reduced post-fertilization fertility.

The frequency, intensity, and distribution of new propagules (e.g., seeds) is a principal factor in predicting invasiveness (1, 3, 4). Reduced fertility, including reduced fruit production and reduced seed germination, would limit propagule pressure and invasive potential. In a study of scotch broom (*Cytisus scoparius*), Sheppard et al. (5) estimated that a 62% reduction in seed set was needed to reverse dominance of this species in native grasslands. Under our conditions, the cultivar 'Odom' had 2.6% of the fruit set and 22% of the germination rate of 'Compactus', corresponding to 0.6% of the total reproductive potential (a 99.4% reduction). The reduced fruit set and reduced seed germination of 'Odom' should reduce the potential for this cultivar to naturalize. However, because some of the reduced fruit set of 'Odom' appears to be from reduced pollination efficacy, it would be prudent to evaluate fertility in other environments with other pollinators. Additional research is warranted to better understand how a substantial reduction in fertility impacts invasive potential.

Literature Cited:

1. Crawley, M.J. and S.L. Brown. 1995. Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proc. R. Soc. Lond. B Biol. Sci. 259:49-54.
2. Marquard, R.D. 1992. Pollen tube growth in *Carya* and temporal influence of pollen deposition on fertilization success in pecan. J. Amer. Soc. Hort. Sci. 117: 328-331.
3. Martínez-Ghersa, M.A. and C.M. Ghersa. 2006. The relationship of propagule pressure to invasion potential in plants. Euphytica. 148:87-96.
4. Sakai, A.K., F.W. Allendorf, J.S. Holt, D.M. Lodge, J. Molofsky, K.A. With, S. Baughman, R.J. Cabin, J.E. Cohen, N.C. Ellstrand, D.E. McCauley, P. O'Neil, I.M. Parker, J.N. Thompson, and S.G. Weller. 2001. The population biology of invasive species. Ann. Rev. Ecol. Syst. 32:305-332.
5. Sheppard, A.W., P. Hodge, Q. Paynter, and M. Rees. 2002. Factors affecting invasions and persistence of broom *Cytisus scoparius* in Australia. J. Appl. Ecol. 39: 721-734.

Acknowledgements: Thanks to Odom Nursery for providing support for this project.

Table 1. Pollen germination, fruit set, and seed germination for two cultivars of *Euonymus alatus*.

Taxa	Pollen Germination (%)	Fruit Set per Plant	Fruit Set per Flower (%)[z]	Seed Germination (%)
'Compactus'	18 a	270 a	7.4 a	51 a
'Odom'	14 a	7 b	5.4 a	11 b

[z]Received supplemental hand pollinations with a mixture of pollen.

Exploring Crossability Among *Rudbeckia* L. Species

Irene E. Palmer, Thomas G. Ranney, Nathan P. Lynch, and Richard E. Bir

North Carolina State University, Dept. of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive Fletcher, NC 28732

irene.palmer@centre.edu

Index Words: Apomixis, Coneflower, Cytology, Flow Cytometry, Plant Breeding, Ploidy, Pseudogamy

Significance to Industry: Rudbeckia are valuable nursery crops that offer broad adaptability and exceptional ornamental merit. Interspecific hybridization between durable, perennial species and showy, annual species could lead to the development of valuable new cultivars.

Nature of Work: The genus *Rudbeckia* L. is widespread throughout North America and contains approximately 30 species of annuals, biennials, and perennials known for their colorful ray corollas and prominent disk-shaped receptacles. The annual species *R. hirta* L. includes cultivars with a diverse range of flower colors and forms; however, this species is short lived and susceptible to diseases including rhizoctonia rot (*Rhizoctonia* sp.) and cercospora leaf spot (*Cercospora* sp.) (3,4). Other rudbeckia species, including *R. fulgida* Ait., *R. missouriensis* Engelm. ex C.L. Boynton & Beadle, and *R. subtomentosa* Pursh., are reliable perennials with superior disease resistance (1).

There has been little published on the genetics and breeding of rudbeckia. Urbatsch (6) reported that the *Rudbeckia* subgenus *Rudbeckia* (including *R. hirta, R. fulgida*, *R. missouriensis,* and *R. subtomentosa*) has a base chromosome number of x=19. Polyploids have been reported to exist in *R. hirta* and *R. fulgida* var. *speciosa* (5). However, information on interspecific crossability and ploidy levels of specific cultivars is lacking. The objectives of this study were to determine the ploidy levels and DNA contents of selected species and cultivars, and to evaluate self-compatibility and crossability among species to facilitate the future development of new hybrids.

Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement regardless of ploidy level) were determined via flow cytometry for all the parental taxa and progeny. Approximately 0.25 in^2 (1.6 cm^2) of leaf or petal tissue was chopped with a razor blade in a petri dish containing 400 µL of extraction buffer (CyStain UV Precise P, Partec, Münster, Germany). The suspension was filtered through 50-µm nylon mesh and nuclei were stained

using 1.6 mL staining buffer containing 4', 6-diamidino-2-phenylindole (DAPI) (CyStain UV Precise P, Partec). The suspension was analyzed using a flow cytometer with fluorescence excitation provided by a mercury arc lamp (PA-I Ploidy Analyzer, Partec). The mean fluorescence of each sample was compared with an internal standard of known genome size [*Pisum sativum* L. 'Ctirad', 2C = 9.09 ρg; (2)]. Chromosome counts were performed on *R. hirta* 'Toto Gold', *R. hirta* 'Indian Summer', *R. missouriensis*, *R. subtomentosa*, and *R. fulgida* var. *sullivantii* 'Goldsturm' to calibrate DNA content with ploidy level for each species. Root tips were collected and placed in 2 mM 8-hydroxyquioline for 3-5 h at 54F (12C). Roots were then rinsed with cold (39F, 4C) distilled water and placed in 6:3:1 solution of 95% ethanol: chloroform: glacial acetic acid fixative for 24 h at room temperature. Samples were rinsed with a 70% ethanol solution, placed in 70% ethanol solution, and stored at 39F (4C). The following week, samples were removed from storage and transferred to 30% aqueous ethanol solution for 12 min, followed by two 15 min rinses in distilled water. Roots were then hydrolyzed for 1 h at room temperature in 1 N HCl, followed by a quick rinse in distilled water, were placed in Feulgen stain for 2 h at room temperature. Root tips were excised and placed on a glass microscope slide with a drop of 1% aceto-carmine stain, squashed with a coverslip, and viewed at 1,500 ×. Base 1Cx monoploid DNA content (i.e., DNA content of the non-replicated base set of chromosomes with x = 19) was calculated for each species as 2C DNA content / ploidy level. DNA content data were subjected to analysis of variance and means separation using the Tukey HSD procedure.

Taxa selected for breeding included ten *R. hirta* cultivars, three *R. fulgida* varieties, *R. missouriensis*, and *R. subtomentosa* (Table 1). Forty-three interspecific crosses were completed in a greenhouse with at least eight pollinated inflorescences per cross. Pollinations included reciprocal crosses between *R. hirta* cultivars and the five remaining taxa. Inflorescences were pollinated daily until all ray flowers passed anthesis. Self-pollinations were also performed on separate inflorescences of the same taxa. Achenes were collected after flower senescence and sown.

Results and Discussion: Chromosome counts documented that *R. hirta* 'Toto Gold' was a diploid (2*n* = 2x = 38) and *R. hirta* 'Indian Summer' was a tetraploid (2*n* = 4x = 76) with an average 1Cx value of 3.91 ρg (Table 1). Our seedlings of *R. missouriensis* and *R. subtomentosa* were diploids with 1Cx values of 4.6 ρg and 5.7 ρg, respectively. *Rudbeckia fulgida* var. *sullivantii* 'Goldsturm' was a tetraploid with a 1Cx value of 9.28 ρg. Based on these standards, ploidy levels of the remaining cultivars were then estimated for each species (Table 1). Mean 1Cx DNA content (genome size) was similar among cultivars of *R. hirta*, regardless of ploidy level (i.e., there was no apparent genome downsizing at higher ploidy levels). However, 1Cx DNA content varied close to 300% among species, emphasizing the need to calibrate DNA content with ploidy level separately for each species within this subgenus.

The self-pollinated inflorescences produced no viable seed indicating a high level of self-incompatibility (data not shown). Crosses between species yielded 0 to 38 seedlings per inflorescence with a total of 844 seedlings. However, 2C DNA contents of all but one of these seedlings were similar to the maternal parent, suggesting that these plants arose through pseudogamy: a process whereby pollination stimulates apomixis. Apomixis has been recorded previously in *R. triloba* L. (3x) and *R. fulgida* (4x) (5). Overall, interspecific crossability among these species was found to be extremely low, with the production of only one successful hybrid: *R. subtomentosa* (2C = 22.8 ρg) × *R. hirta* 'Toto Gold', (2C = 7.4 ρg) which yielded a seedling with an intermediate 2C DNA content of 15.2 ρg, confirming hybridity. Cytology also documented two sets of 19 chromosomes of disparate size, consistent with both parents (Fig. 1).

Results from the cytology and cytometry component of this project documented ploidy levels and DNA contents of selected species and cultivars. Breeding and crossability components determined a high level of self-incompatibility among these taxa, but found that pseudogamy appears to be prevalent following interspecific pollination. Although successful interspecific hybridization was rare and difficult to achieve, it is possible and future efforts may lead to the development of improved cultivars.

Literature cited:

1. Armitage, A.M. 1997. Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. 2nd ed. Stipes, Champaign, Ill.
2. Doležel, J., J. Greilhuber, S. Lucretti, A. Meister, M.A. Lysák, L. Nardi, and R. Obermayer. 1998. Plant genome size estimation by flow cytometry: inter-laboratory comparison. Ann. Bot. 82:17–26.
3. Fulcher, A., W.C. Dunwell, and D. Wolfe. 2003. *Rudbeckia* taxa evaluation. Proc. SNA Res. Conf., 48th Annu. Rpt. p. 510-512.
4. Harkess R.L, and R.E. Lyons. 1994. *Rudbeckia hirta* L.: A versatile North American wildflower. HortScience 29(3):134, 227.
5. McCrea, K.D. 1981. Ultraviolet floral patterning, reproductive isolation and character displacement in the genus *Rudbeckia* (Compositae). M.S. thesis. Purdue University. West Lafayette, Ind.
6. Urbatsch, L.E., B.G. Baldwin, and M.J. Donoghue, 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction site data. Syst. Bot. 25(3):539-565.

Acknowledgements:

Thanks are expressed to Bluebird Nursery, Clarkson, Nebr. and North Creek Nurseries, Landenberg, Penn. for providing plants for this study.

Table 1. DNA content, ploidy level, and chromosome number of selected *Rudbeckia* taxa.

Taxa	2C DNA Content (ρg)	1Cx DNA Content (ρg)	Ploidy Level	2*n* Chromosome Number
R. hirta 'Goldilocks'	7.1[Z]± 0.10 a	3.5[Z]± 0.05 a	2x	38
R. hirta 'Marmalade'	7.3 ± 0.03 a	3.6 ± 0.02 ab	2x	38
R. hirta 'Toto Gold'	7.4 ± 0.07 a	3.7 ± 0.04 ab	2x[Y]	38[Y]
R. hirta 'Toto Rustic'	7.4 ± 0.15 a	3.7 ± 0.07 ab	2x	38
R. hirta 'Sonera'	14.2 ± 0.34 b	3.5 ± 0.08 a	4x	76
R. hirta 'Cherokee Sunset'	14.6 ± 0.17 bc	3.6 ± 0.04 ab	4x	76
R. hirta 'Autumn Colors'	15.4 ± 0.26 bc	3.8 ± 0.07 ab	4x	76
R. hirta 'Prairie Sun'	16.0 ± 0.23 bcd	4.0 ± 0.06 abc	4x	76
R. hirta 'Indian Summer'	16.4 ± 0.32 bcd	4.1 ± 0.08 abc	4x[Y]	76[Y]
R. hirta 'Tetraploid'	16.7 ± 0.44 cd	4.2 ± 0.11 bc	4x	76
R. missouriensis	18.3 ± 0.02 d	4.6 ± 0.01 cd	2x[Y]	38[Y]
R. subtomentosa	22.8 ± 0.18 e	5.7 ± 0.04 e	2x[Y]	38[Y]
R. fulgida var. *fulgida*	35.2 ± 1.10 f	8.8 ± 0.27 f	4x	76
R. fulgida var. *speciosa*	36.8 ± 0.15 f	9.2 ± 0.39 f	4x	76
R. fulgida var. *sullivantii* 'Goldsturm'	37.1 ± 0.56 f	9.3 ± 0.14 f	4x[Y]	76[Y]

[Z]Values are means, n = 2 to 5 ± SEM. Values followed by a common letter, within a column, are not significantly different, P ≤ 0.05.
[Y]Confirmed through cytology.

Temperature Regulation of Photorespiration Genes in *Pachysandra terminalis*

Suping Zhou

Institute of Agricultural and Environmental Research, Tennessee State University, 3500 John A Merritt Blvd, Nashville, TN 37209

zsuping@tnstate.edu

Index words: Temperature Stress, Evergreens, Real-time PCR, Gene Expression

Significance to Industry: Genes encoding for two key photorespiration enzymes, glycolate oxidase (*PtGOX*) and NADPH-dependent hydroxypyruvate reductase (*PtHPR*), were found differentially regulated by chilling and heat stresses. Findings from this study will contribute to the understanding of molecular control in cold tolerance and heat-sensitivity in some evergreen species.

Nature of Work: Temperature is one of the most important environmental factors affecting plant development and crop productivity (1). *P. terminalis* is a cold-hardy evergreen plant, the plants normally have very little or no new growth when the temperature is above 28-30^{o}C. The objective of this study is to find the controlling point for cold tolerance through evaluation of gene transcript accumulation when young seedlings were subjected to two extremes of temperature stresses. The treatments were conducted on cuttings with four leaves, two dark green (mature) and two light green (fresh leaves) (Yoder Brothers, Inc., PA, USA). Upon arrival, plants were kept in an incubator programmed at 25^{o}C, and a 12/12 h (light/dark) photoperiod with light intensity of 100 $\mu molm^{-2}s^{-1}$ photosynthetically active radiation (PAR) provided with fluorescent light tubes for 2 d. Temperature treatments were performed in the continuous darkness at 5 and 38^{o}C. Seedlings incubated at 25^{o}C were the control. The two fresh tissues were collected after 1d, 2d, 3d and 7d. Total RNA was extracted from the collected leaf tissues and the genomic DNA was removed with DNase I digestion using reagents supplied by the Genhunter company (TN, USA).

We have previously isolated the cDNA sequences of the glycolate oxidase (*PtGOX,* accession DQ442286*)* and NADPH-dependent hydroxypyruvate reductase (*PtHPR,* accession DQ442287) (4). Gene-specific primers were designed using OligoPerfect Designer at Invitrogen website and these primers were purchased from the same company. Real-time qRT PCR assay was performed following the instruction in the SYBR-green PCR mix/RT kit (Applied

Biosystems, CA, USA). The 18S ribosomal RNA (rRNA) was selected as the house keeping gene to normalize the real-time PCR result. Its primers were designed using the GenBank sequence (accession: NC003071) with an amplicon size of 110bp.The forward and reverse primers were 5'-CATCAGCTCGCGTTGACTAC-3'/5'-CACTTCACCGGATCATTCAA -3', respectively. The PCR reaction mixture contained cDNA (100pg total RNA), primer mix (250nM each) and 2X PCR master mix supplied in the SYBR-green PCR mix/RT kit. The PCR amplification was preformed using a program of 40 cycles of 94^{o}C, 30s, and 60^{o}C, 1 min on a 7000 Real Time PCR System (Applied Biosystems). At the end of the PCR cycles, the data were analyzed with the ABI Prism 7000 SDS software and presented as Ct values.

Each measurement had 3 replications for every cDNA sample and the experiments were repeated three times independently. The results were averaged and mean was considered as the Ct value for each RNA sample. The Ct value of each gene was normalized with the 18S rRNA to obtain the value of ΔCt. Values of ΔΔCt relative to 25^{o}C for each treatment was used to calculate relative gene expression following the procedure described in the Relative Quantification Using the Comparative Ct Method in the User Bulletin #2, ABI Prism 7700 Sequence Detection System (Applied Biosystems).

Results and Discussion: Chilling stress increased the relative mRNA abundance of *PtGOX* and *PtHPR* (Table 1). After 7 d incubation at 5^{o}C, relative transcription levels of *PtGOX* and *PtHPR* showed 1.80, and 12.97 fold increases, respectively. At 3 d of chilling stress, mRNA transcript levels of *PtHP*R (3.97 fold up) were higher in cold treated leaf tissues while *PtGOX* had no obvious difference. During the first day of chilling stress, *PtGOX* and *PtHPR* transcription levels were all higher than corresponding values at 25^{o}C, but only the *PtGOX* reached a significant level. The *PtHPR* was 1.48 fold higher than the control, but the changes in the values were below the background values in both comparisons (0.84-2.63/0.64-1.56), thus the change cannot be considered significant. Two d of cold treatment induced a 2 and 1.5 fold reduction for *PtHPR*. When exposed to 38^{o}C, the plants had more copies of *PtHPR* transcripts, less copies of *PtGOX*. After 3 d at 38^{o}C, *PtHPR* transcript level was 2.37 fold higher, however, the *PtGOX* transcription level declined by 2 fold (Table 2).

Stability and accumulation of gene transcripts and tolerance/sensitivity to temperature stress: In this study, we compared the genes that are involved in the photorespiration pathway to see if there is a correlated regulation in their expression. The results indicate that under chilling stress the changing pattern of the *PtGOX* and *PtHPR* are very similar. Compared to chilling stress, relative gene expression at high temperature (38^{o}C) was different for the two photorespiratory genes. While *PtHPR* gene transcripts accumulation was enhanced by heat stress, relative expression of *PtGOX* was reduced

significantly. This result may be related to the heat sensitivity of the plant species. The optimum growth temperature for *P. terminals* plants is 22-25^{o}C. The dark green mature leaves can survive summer at the State of Tennessee, USA where the highest temperature can temporarily reach 35-38^{o}C on hot days. But, plants have little new growth during hot seasons in summer. In our laboratory, we have also observed those fresh or light green leaves formed at 25^{o}C cannot normally develop into mature dark green leaves when transferred to high temperature (>35^{o}C) environment. In the current study, the q-RT-PCR was performed with light green fresh leaves. The mRNA transcript level of *PtGOX* was significantly reduced after incubation at 38^{o}C for 2 d. This reduction can from suppression of gene transcription, or enhanced degradation of the gene transcripts. Combining the physiological response of the fresh leaves to high temperature and changes in the expression of *PtGOX*, it is suggested that reduced expression of *PtGOX* might be associated with the heat-sensitivity and the absence of new growth in hot summer. Stability and enhanced accumulation of mRNA of glycolate oxidase has been observed in heat tolerant species (2). Studies on transgenic tobacco shows sufficient activities of glycolate oxidase are necessary for maintaining normal photosystem under heat stress (3**).** All these evidences show that temperature regulation of *GOX* is directly correlated to the genetic characteristics of different plant species.

The high stability and increased abundance of *PtHPR* mRNA transcripts indicates that this gene may be regulated by a different mechanism. In the photorespiration pathway, the substrate of HPR is hydroxypyruvate which is derived from serine. The product of HPR is glycerate, which leaves peroxisome, and enters the chloroplast to participate Calvin cycle. Serine that feeds into peroxisome can be from other resources in addition to photorespiration, or from previous reserves. In addition to gene transcription, enzyme activity of hydroxypyruvate reductase has shown to be very stable at high temperature (3).

Acknowledgements: Financial support was from USDA–CSREES Capacity Building Program.

Literature cited:

1. Crosatti, **C.,** P. P. de Laureto.**,** R. Bassi**,** and L. Cattivelli**.** 1999. The Interaction between Cold and Light Controls the Expression of the Cold-Regulated Barley Gene *cor14b* and the Accumulation of the Corresponding Protein. Plant Physiol. 119: 671-680.
2. Rizhsky, L., H. Liang, and R. Mittler. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol*.* 130: 1143–1151.
3. Yamaguchi, K., and M. Nishimura. 2000. Reduction to below Threshold Levels of Glycolate Oxidase Activities in Transgenic Tobacco Enhances Photoinhibition during Irradiation. Plant and Cell Physiol. 41: 1397-1406.
4. Zhou, S. P., R. Sauve, and A. Abudullah. 2005. Identification of genes regulated by low temperature in *Pachysandra terminalis* Sieb.et Zucc using cDNA differential display. HortScience 40:1995-1997.

Table 1 Relative abundance of gene transcripts in leaves of *P. terminalis* under chilling stress

Genes	1d		2d		3d		7d	
Folds	25°C	5°C	25°C	5°C	25°C	5°C	25°C	5°C
PtGOX	1.00[a] (0.76-1.31) [b]	**1.97 (1.33-2.91)**	1.00 (0.84-1.19)	1.19 (1.05-1.35)	1.00 (0.85-1.18)	1.01 (0.78-1.32)	1.00 (0.94-1.06)	**1.80 (1.48-2.19)**
PtHPR	1.00 (0.64-1.56)	1.48 (0.84-2.63)	1.00 (0.59-1.70)	0.65 (0.38-1.13)	1.00 (0.76-1.32)	**3.97 (3.18-4.95)**	1.00 (0.91-1.10)	**12.97 (11.00 - 15.29)**

Bolded values indicate that the treatment transcript level is significantly higher than the control. [a]: Fold change which is equal to $2^{-\Delta\Delta CT}$; b: range of the fold change which is $2^{-\Delta\Delta Ct + s}$ and $2^{\Delta\Delta Ct - s}$; $\Delta\Delta C_t = \Delta C_t (5^oC) - \Delta C_t (25^oC)$, s is the standard deviation.

Table 2 Relative abundance of gene transcripts in leaves of *P. terminalis* under heat stress

Genes	1d		2d		3d	
Folds	25°C	38°C	25°C	38°C	25°C	38°C
PtGOX	1.00 [a] (0.76-1.31)[b]	1.08 (1.04-1.12)	1.00 (0.84-1.19)	***0.72 (0.60-0.86)***	1.00 (0.85-1.18)	0.47 (0.42-0.53)
PtHPR	1.00 (0.64-1.56)	1.49 (1.12-1.99)	1.00 (0.59-1.70)	0.82 (0.55-1.22)	1.00 (0.76-1.32)	**2.75 (1.82-4.17)**

Bolded and italicized indicates that the treatment transcript level is significantly lower than the control. Bolded indicates that the treatment transcript level is significantly higher than the control. [a]: Fold change which is equal to $2^{-\Delta\Delta CT}$; b: range of the fold change which is $2^{-\Delta\Delta Ct + s}$ and $2^{\Delta\Delta Ct - s}$; $\Delta\Delta C_t = \Delta C_t (38^oC) - \Delta C_t (25^oC)$, s is the standard deviation.

Genotyping of Japanese Flowering Cherries Accessions via AFLP Markers

Maya A. Holcombe, A. Naseer Aziz and Roger J. Sauve

Institute of Agricultural and Environmental Research, Tennessee State University
3500 John A. Merritt Blvd., Nashville, TN 37209-1561

mholcombe@mytsu.tnstate.edu

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

Significance to Industry: Flowering group of cherries comprised of some 200 species in the genus *Prunus*. As popular ornamentals, Japanese flowering cherries have pink or white flowers with delicate sweet scent. There is a great confusion over their naming and identification due to the abundance of species. Amplified fragment length polymorphism (AFLP) markers can be used for true-to-type identification of these important nursery plants. AFLP marker's profiling can benefit the nursery industry by reducing costs of genetic characterization and increasing the efficiency of developing new types. This research illustrates the uses of these genetic markers and standardization of AFLP amplification for flowering cherry accessions.

Nature of Work: Among the *Prunus* species, beauty can be found in inflorescence, foliage and bark of the flowering cherries making them garden ornamentals for all seasons (2). Their uses are increasing as residential, recreational, public, and industrial plants. Therefore, development of genetic identification methods for reliable selection of commercially suitable cherries is imperative. The AFLP technique is based on detection of genomic restriction fragments by PCR amplification without prior sequence knowledge (3). In this study AFLP marker's were used for the genetic characterization of selected flowering cherry accessions.

Following the manufacturer's protocols, DNA samples from all accessions were isolated utilizing DNeasy Plant Mini Kit (Qiagen, Santa Clara, CA) and grinding matrix along with Bio Fast Prep System (Q. Biogene, Irvine, CA). To verify DNA isolation 1% gel electrophoresis (1) was used. For gel-imaging the AlphaImager 2000 system (Alpha Innotech, San Leandro, CA) was used and the DNA concentrations from extracted samples were quantified using a spectrophotometer (Eppendorf, Hamburg, Germany). AFLP System Analysis Kit (Invitrogen™ Life Technologies, Carlsbad, CA) was used for subsequent assays. AFLP analyses require restriction digest, ligation of adaptors, pre-amplification, and selective amplification of the plant genomic DNA samples (3), Agarose (1%) gel analyses were conducted to check restriction digestion, pre-amplification and selective amplification of the plant genomic DNA samples.

Results and Discussion: For this project, AFLP amplification data was obtained from six Prunus accessions, namely *P. incisia* 'February Pink 104 ER', *P.* 'Snow Fountain', *P. incisia*, *P. serulata*, *P. maximowicizii* '62 ER', and *P.* 'Fudan Zakara 1 ER'. DNA was isolated and purified from all these six plant samples. These DNA preparations were evaluated using 1% agarose gel electrophoresis and were found to be of good quality for the restriction digestion in subsequent analysis. Using AFLP separation and analysis kit (Invitrogen^{TM}, Carlsbad, CA) restriction digest, ligation of adaptors, pre-amplification, and selective amplification was performed on each flowering cherry sample. For selective AFLP amplifications there were16 primers that annealed to AFLP adaptors ligated to plant sequences. Eight primers are designed for *EcoR* I adaptors (E-AAC, E-AAG, E-ACA, E-ACC, E-ACG, E-ACT, E-AGC, and E-AGG) and while other eight primers were designed for *Mse* I adaptors (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT). All 64 primer pair combinations consisting of eight *EcoR* I and *Mse* I primers each were used for selective AFLP amplification. After each successful AFLP step, gel (1%) electrophoresis was used to discern that procedures were properly conducted. The electro-grams also identified and evaluated the primer pairs that provided most amplification for each accession. From the preliminary results obtained, we were able to confirm that out of total 64 combinations, the number of AFLP primer pairs that were suitable for ample polymorphism in these flowering accessions were as follows; 51 for *P.* sp. 'Snow Fountain', 43 primer combinations for *P*. sp 'Fudan Zakara 1 ER', 41 primer combinations for *P.* sp 'Fudan Zakara 1 ER', 37 for *P. incisia* 'February Pink 104 ER'; 36 for *P. incise*; and 34 for *P. maximowicizii* '62 ER' (Table 1). There were 16 AFLP EcoR I and Mse I primer pair combinations that depicted amplification in all six Japanese flowering cherry samples (Table 2). The uses of AFLP primers have not been previously reported on these plants. These primer pairs are being used for comparative AFLP profiling of all accessions under study.

Literature Cited:

1. Aziz, A.N. 1998. Genetic analysis of the anther-derived progeny and isolated pollen grains of a culture-responsive Solanum clone. Ph.D. Thesis. Department of Biology, University of New Brunswick, Fredericton, Canada. p 100.
2. Clark, R.B. 1963. Cherries for mist of white and pink, p. 59-72. In: R. B. Clark (Ed.).
3. Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23(21):4407–4414.

Acknowledgement: The authors gratefully acknowledge Dr. M. Pooler of the USDA National Arboretum for supplying the Japanese flowering cherry accessions. The graduate assistantship was provided by USDA-CREES Capacity Building Grant. Tennessee State University's Institute of Agricultural and Environmental Research (IAgER) supplied the research facilities.

Table 1: Identification of AFLP[1] primer pairs that provided selective amplification of molecular marker's from six flowering cherry accessions.

	e1*	e2*	e3*	e4*	e5*	e6*	e7*	e8*
m1*	1,3,5†	2,3,4 5,6†	1,2,3 4,5,6†	1,2,3 4,5,6†	1,2,5†	6†	1,2,3 4,5,6†	2,3,4 5,6†
m2*	1,4,6†	3,4†	1,2,3 4,5,6†	1,2,3 4,5,6†	1,2,3 4†	2,4,5 6†	1,2,3 4,5,6†	3†
m3*	1,2,3 4,5,6†	2†	2†	1,2,4†	1†	1,2,3 4,5,6†	1,2,3 4,5,6†	2,4,5 6†
m4*	2,5,6†	1,2,3 4,5,6†	1,2,3 4,5,6†	2,3,6†	2,4†	2,6†	2,3,4 5,6†	2,6†
m5*	1,2,4†	1,6†	1,2,3 4,6†	1,2,3 4,5,6†	1,2,3 4†	1,2†	1,2,4 5,6†	1,2,3 6†
m6*	1,3,5 6†	4,5†	6†	1,2,3 5,6†	4,5 6†	1,2,3 4,5,6†	1,2,3 4,5†	1,2†
m7*	2,4,6†	2,4†	2,3,4,5†	1,2,3 4,5,6†	1,2,3 4,5,6†	2†	1,2,3 4,5†	2,3,4†
m8*	2,5,6†	1,2,3 4,6†	1,2,3 4,5,6†	1,2,4 5,6†	2,3,4 6†	2,6†	1,2,3 4,6†	2,4†

[1]Amplified Fragment Length Polymorphism (Invitrogen™ Life Technologies, Carlsbad, CA).
*e1= E-AAC, e2= E-AAG, e3= E-ACA, e4= E-ACC, e5= E-ACG, e6= E-ACT, e7= E-AGC, e8= E-AGG and *Mse* I adaptors: m1= M-CAA, m2= M-CAC, m3= M-CAG**,** m4**=** M-CAT, m5= M-CTA, m6= M-CTC, m7= M-CTG, m8= M-CTT.
† 1= *P. incisia* 'February Pink 104 ER', 2= *P.* 'Snow Fountain', 3= *P. incisia*, 4= *P. serulata,* 5= *P. maximowicizii* '62 ER', and 6= *P.* 'Fudan Zakara 1 ER'.

Table 2: The 16 AFLP primer pairs* that depicted selective amplification of the DNA maker's in *P. incisia* 'February Pink 104 ER', *P.* 'Snow Fountain', *P. incisia* , *P. serulata*, *P. maximowicizii* '62 ER,' and *P.* 'Fudan Zakara 1 ER'.

E-AAC/M-CAG, E-AAG/M-CAT, E-ACA/M-CAA, E-ACA/M-CAC, E-ACA/M-CAT, E-ACC/M-CAA, E-ACC/M-CAC, E-ACC/M-CTA, E-ACC/M-CTG, E-ACC/M-CTT, E-ACG/M-CTG, E-ACT/M-CAG, E-ACT/M-CTC, E-AGC/M-CAA, E-AGS/M-CAC, A-AGC/M-CAG

*Each pair consists of primers designed for both *Eco*R I (E) and *Mse* I (M) adaptors. The three selective nucleotides of each primer are shown.

Changes in RNA Levels in Daylilies during Flower Maturation and Senescence

A. Abraham [1], R. Sauve[1], A. Aziz[1] and J. Carter[2]

[1]Institute of Agricultural and Environmental Research, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209
[2] College of Agriculture, Home Economics and Allied Programs, 1005 State University Drive, Fort Valley State University, Fort Valley, GA 31030

Index Words: *Hemerocallis spp.***,** RNA, programmed cell death

Significance to Industry: The understanding of the process that occur during flower maturation and senescence will result in the development of protocols to slow down the rate of senescence in daylily and possibly add another flowering perennial to the cut flower industry.

Nature of Work: Daylilies (*Hemerocallis spp*.) are popular perennial plants which are widely grown for their attractive and showy flowers. They exhibit a high resistance to pests and diseases, and are adaptable to a wide range of soils and climatic conditions. Because of the above characteristics, they are used extensively in both home and commercial landscapes world-wide. However, the daylily is unsuitable for use as a cut flower due to its short flower-life. Their botanical name, *Hemerocallis* is derived from the Greek terms *Hemero* (meaning for a day) and *callis* (meaning beauty), i.e. beauty for a day referring to the fact that each flower lasts only one day. The daylily belongs to the category of flowers whose cell death is not triggered by ethylene (1). Thus, the daylily provides a model system to study floral senescence and events related to cell death. The petals exhibit a highly predictable program of senescence that occurs over a 24 hours period after the flower opens (2).

This study was performed to identify genes that express during flower maturation and senescence. Flower buds at the following seven stages of development were assayed: at ¾" in length, one inch, 1 ½", at opening, halfway opened, fully opened and when they began to wilt. RNA was extracted from all samples using RNApure^{TM} Reagent (GeneHunter Co., Nashville, TN). Ten 150µL of chloroform per ml of lysate was added and centrifuged at 4^0C for 10 minutes. Following removal of the upper phase, equal volume of isopropanol was added and soaked on ice for 10 minutes followed by centrifugation for 10 minutes at 4°C. The supernatant was removed and the RNA pellet was washed with 1 ml of cold 70% Ethanol at 4^0C. Following the removal of Ethanol, the residual liquid was removed and air dried. The RNA was resuspended in 50 µL of DEPC-water. Subsequently the concentration of RNA extracts were measured by diluting 1 µL of the RNA in 1ml of water. The RNA yeilds are summarized in Table 1. The trace amounts of DNA contamination was removed by Dnase I treatment with message clean kit (GenHunter Nashville, TN).

Results and Discussion: Results from this study show variation in transcription activity during flower maturation as depicted by the total RNA levels in flower buds. The concentration of RNA in the flower buds rapidly began to reduce as the bud began to open. The RNA level was at its lowest level when the flowers were almost completely open and began to increase again when they were fully open and continued to increase until the beginning of senescence (Table-1). These results indicate that anabolic and catabolic activity occurs during flower bud formation and during flower senescence.

Acknowledgement: This project was partially funded by a USDA Capacity Building Grant awarded to Dr. J. Carter at Fort Valley State University.

Literature Cited:

1. Gulzar, S., Tahir, I., Amin, I., Farooq, S. and Sultan, S.M. 2005. Effect of cytokinins on the senescence and longevity of isolated flowers of daylily (*Hemerocallis fulva*) cv Royal Crown sprayed with cyclohesimide. Acta Hort. 669:395-404

2. Rubinstein, B. 2000. Regulation of cell death in flower petals. Plant Molecular Biology 44:303-318.

Table 1. Amount of total RNA at different stages of daylily.

Treatment	Total RNA (ng/ul)	Total RNA (ng/50ul)
bulb size less than one inch	2955.0	147750
bulb size about one inch	4103.4	205170
bulb size greater than one inch	4828.8	241440
bulb about to open	2248.8	112440
bulb not fully opened	1721.2	86060
fully opened flower	1998.4	99920
when the flower wilts	2598.6	129930

One Hundred Miles of Natural *Rhododendron* Communities

Donglin Zhang[1,2], Xun Chen[2,3], Fang Geng[2], Zhihui Li[2] and Riqing Zhang[2]

[1]Department of Plant, Soil, and Environmental Science
University of Maine, Orono, ME 04469, USA
[2]College of Resource and Environment, Central South Forestry University
Changsha, Hunan 410004, China
[3]Guizhou Academy of Sciences, Guiyang, Guizhou 550001, China

donglin@maine.edu

Index Words: Baili, Breeding, Community, Exploration, Guizhou, Hundred Li, Hybrid, Introduction, Ornamental, Plant, Rhododendron

Significance to Industry: Natural plant resources are important to potential ornamental plant exploration, which ultimately leads to breeding and introduction of better ornamental plants for the nursery and greenhouse industries. One hundred miles of natural *Rhododendron* communities (Baili Rhododendron communities) are located in western Guizhou Province of China. The communities consist of broad-leaf evergreen *Rhododendron*. A total of 19 species and one natural, double-flower form have been identified. Among them, 11 species have been introduced to US and European countries while 8 species are new to our US gardens. Detailed exploration with focus on potential ornamental *Rhododendron* species and new hybrids should be conducted in Baili Rhododendron areas. Future collaboration on plant exploration, introduction, breeding, and better management of this ornamental treasure is desperately needed.

Nature of Work: *Rhododendron* are popular ornamental plants in today's market. The genus *Rhododendron* has 854 to 1,000 natural taxa [1, 6, 7]. With the ability of the species to freely hybridize, more than 5,000 cultivars have been introduced to the market [5]. Dirr [3] recorded 1558 taxa and cultivars in his manual. The genus is widely distributed through Asia, Europe, and North America. Two species are recorded in Australia, but none are native in South America or Africa. China is a center of diversity with a total of 571 species. Among them, 409 species are endemic in China [6].

Guizhou is one of the provinces in China with rich *Rhododendron* species. A total of 86 species, 3 varieties, and 1 form were collected in Guizhou [2, 4]. Hundred miles of Rhododendron communities are in western Guizhou with latitude $27^0$17' to $27^0$20'N, longitude $105^0$50' to $106^0$00'E, and elevation from 1,500 to 1,800 meters [4]. After thousands of years of natural selections, Baili Rhododendron communities reached today's pure stands. The climate is a subtropical type with

dry and chilly spring and wet and warm summer. The annual mean temperature is 11.8^{0}C (53.3^{0}F). The highest average temperature is 20.7^{0}C (69.3^{0}F) in July and the lowest month 1.6^{0}C (33.6^{0}F) in January. The annual precipitation is 1180.8 mm (46.5"). The raining days reach 221 days per year and the raining months are from May to October. The annual relative humidity is 84%. The full sunny days are only 21.4 days per year. Others are raining and cloudy days. Several types of soils have been described in Hundred Miles of Rhododendron areas. Generally, these soil types are acidic, with pH range of 4.5-6.3. Obviously, this chilly and lower light environment with acid soil is the best for some of Rhododendron species.

In the past four years, we have investigated Baili Rhododendron communities. The purpose of this paper is to share the preliminary results of their natural species and communities. Further studies should focus on their species with better ornamental potentials and natural hybrids.

Results and Discussion: Hundred Miles of Rhododendron communities are bush vegetation. They are not typical Rhododendron communities as that in high mountains (more than 3,000 m (11,800 ft) above the sea level). Also, they are not zonal climax vegetations. The zonal vegetations for Baili Rhododendron areas are evergreen broadleaf forests, especially under the elevation of 2,000 m (7,870 ft). Baili Rhododendron plants are dominant species under tree layer of the communities. With human intervention in the last century, all trees in the communities were removed for various commercial purposes. The understory plants (*Rhododendro*n spp.) became dominant species.

Three distinguished communities in Baili Rhododendron are *Rhoodendron irroratum* Community, *Rhododendron simsii* community, and *Quercus-Castanea-Rhododendron* community. The dominant species for the first two communities are *R. irroratum* and *R. simsii*, while the dominant *Rhododendron* species for third one are *R. arboretum* and *R. delavayi*. Other *Rhododendron* species are mixed with these four species or deciduous oak species. Preliminary investigation concluded that both *R. irroratum* and *R. simsii* communities have some pure stands. Although *Quercus-Castanea-Rhododendron* community has some mixed species, the number of *R. delavayi* is stable with very strong natural succession. Some populations had 59% of *R. delavayi* with basal diameters more than 14 cm (5 in) (aged plants). Others showed that number of plants decreased as the plant basal diameters increased and 60% or more plants had less than 8cm (3 in) basal diameter. In a 15 m^2 (160 ft^2) plot, 319 natural seedlings from age 3-5 years were counted. With proper protection and management, *Quercus-Castanea-Rhododendron* community will be in succession to *Rhododendron delavayi* communities.

It is a magnificent experience to visit One Hundred Miles of Rhododendron (Baili Rhododendron). After a moment of thrill, authors had been able to collect and identify 19 taxa (18 species and one variety). Although the following species

have been mentioned in Hortus Third [1] and The Royal Horticultural Society Dictionary of Gardening [7], the phenotypes of some species and hybrids may have great potential for *Rhododendron* breeding.

Rhododendron ambiguum Hemsley, not in RHS.
Rhododendron arboreum Smith
Rhododendron argyrophyllum Franchet
Rhododendron calophytum Franchet
Rhododendron decorum Franchet
Rhododendron delavayi Franchet (*Rh. arboretum* ssp. *delavayi* in RHS)
Rhododendron irroratum Franchet
Rhododendron × *pulchrum* Sweet = *Rh. indicum* × *Rh. mucronatum* in RHS.
Rhododendron siderophyllum Franchet
Rhododendron simsii Planchon
Rhododendron wiltonii Hemsley & E. H. Wilson

Variations in *Rhododendron* habit, foliage, flower color and quality, and fruit are phenomenal. The preliminary survey indicated that the following species were new to our ornamental market. Although it takes time and a lot research to determine their performance in our gardens, these natural taxa will enhance our breeding work and enrich the new plant list for our nursery industry.

Rhododendron agastum I. B. Balfour & W. W. Smith
Rhododendron anthosphaerum Diels
Rhododendron atrovirens Franchet
Rhododendron bachii H. Léveillé (= *Rh. ovatum* in RHS)
Rhododendron cavaleriei H. Léveillé
Rhododendron fuchsiiflorum H. Léveillé
Rhododendron peramoenum I. B. Balfour & Forrest
Rhododendron simsii var. *bailii* (a new variety will be published)

Generally, Baili Rhododendrons bloomed from late march to early June, with peak season from end of March to middle May. The diversity of flower colors, sizes, and floral structures are phenomena. It is possible that natural hybridizations among the species produced some new plants. Further plant exploration and collection should be conducted.

Acknowledgement: Financial support from Guizhou Academy of Science, Guizhou Provincial Government, University of Maine, and Central South University of Forestry and Technology is appreciated. Great thanks should go to the field technicians and graduate students of Guizhou Academy of Science for their helps.

Supporting Online Material:
http://www.umaine.edu/maineplants/PubDZ/SNA07Rhodo.pdf
Slide presentation for the 52nd SNA Research Conference.

Literature Cited:

1. Bailey, Liberty H. and Ethel Z. Bailey. 1976. Hortus third. MacMillan, New York, NY, USA.
2. Chen, Xun and Huamei Wu. 2003. Rhododendron species from Guizhou, P.R.China. Guizhou Science and Technology Press, Guiyang, Guizhou, China.
3. Dirr, Michael A. 1998. Manual of woody landscape plants, their identification, ornamental characteristics, culture, propagation and uses. Stipes Publishing L.L.C., Champaign, IL. pp 221-224.
4. Li, Yongkang. 1986. Flora Guizhouensis (Tomus 3). Guizhou People's Publishing House, Guiyang, Guizhou, China.
5. Liu, Zhenye. 1985. Scientific survey of the hundred li Rhododendron Forest Area in Guizhou. Guizhou Society for Science and Technology, Guiyang, Guizhou, China.
6. Raven, Peter and Zhenyi Wu. 2005. Flora of China (Ericaceae – *Rhododendron*) 14:260-455.
7. Royal Horticultural Society (RHS). 1999. The royal horticultural society dictionary of gardening. MacMillan Reference Limited, London, UK.

Breeding Intra- and Inter-specific *Cornus* hybrids

Phillip A. Wadl[1], John A. Skinner[1], Xinwang Wang[1], Timothy A. Rinehart[2], Sandra M. Reed[3], Vincent R. Pantalone[4], Mark T. Windham[1], and Robert N. Trigiano[1]

[1]Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37996, [2]USDA-ARS Southern Horticultural Laboratory, 810 Highway 26 West, Poplarville, MS 39470, [3]USDA-ARS Floral and Nursery Plants Research Unit, US National Arboretum, Tennessee State University Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110, [4]Dept. of Plant Sciences, The University of Tennessee, Knoxville, TN 37996

Index Words: Flowering Dogwood, Kousa Dogwood, Hybridization, Pollination

Significance to Industry: Flowering and kousa dogwood were used in controlled crosses to develop intra- and inter-specific hybrids. Results demonstrate that honeybees are effective in performing controlled pollinations and that honeybee-mediated pollinations provide an alternative to time-consuming hand-pollinations for *Cornus*.

Nature of Work: Dogwood are important to retail and wholesale nurseries in the southeast and especially Tennessee where sales account for 23.2 % of dogwood trees sold in the United States (6). Flowering dogwood (*Cornus florida* L.) and kousa dogwood (*C. kousa* Hance) are the most popular in the ornamental horticulture industry, although other species, such as *C. nuttallii*, *C. angustata*, *C. mas* and *C. sericea*, are often used. Flowering dogwood is renowned for its showy floral display, which occurs from April to May. The Asian equivalent of the flowering dogwood is the kousa dogwood, which typically blooms a month after the flowering dogwood. There are over 200 named cultivars of flowering and kousa dogwood and six inter-specific hybrids that were released as the Stellar series (3). Although pink or red-bracted cultivars exist, the overwhelming majority of the cultivars available are white-bracted.

Flowering dogwoods have been severely affected by two foliar fungal diseases, dogwood anthracnose and powdery mildew. Dogwood anthracnose has resulted in mortality of flowering dogwood in natural and landscape settings. The only flowering dogwood with known resistance to dogwood anthracnose is the white-bracted cultivar ‘Appalachian Spring’ (7). Powdery mildew causes economic losses due to the stunted growth of seedlings and lack of growth in older trees. Up to 100% of the foliage of liners and seedlings may be affected by powdery mildew, leading to possible mortality. The only flowering dogwood cultivar released prior to 2000 that demonstrates good resistance to powdery mildew is

'Cherokee Brave', which has deep red bracts. In 2000, three powdery mildew resistant white-bracted dogwood cultivars were released by the Tennessee Agricultural Experiment Station as the Appalachian series (8). Both dogwood anthracnose and powdery mildew have caused increased costs to dogwood growers and many small nurseries no longer grow dogwoods due to high production costs.

The popularity of kousa dogwood has increased in recent years due to its resistance to dogwood anthracnose and powdery mildew as compared to flowering dogwood. Hybrids between *C. kousa* and *C. florida* have shown both resistance and susceptibility to dogwood anthracnose and powdery mildew (2, 4). This range of resistance allows the development of new intra- and inter-specific cultivars with multiple disease resistance or a combination of disease resistance and specific ornamental traits.

Practically all dogwood cultivars currently available have been derived from either vegetative bud sports or from seedling selections and not from controlled crosses. Development of improved cultivars with desired combinations of specific traits requires controlled crosses. Often these crosses are done manually and the procedure is a time intensive process. The inflorescence of flowering and kousa dogwood consists of 20-30 flowers that are considered self-incompatible (1, 5). Self-incompatibility allows for breeding to be more efficient by not having to emasculate flowers. We have coupled the self-incompatibility of dogwood with the natural ability of honeybees (*Apis melifera* L.) to perform controlled pollinations of flowering and kousa dogwood to create intra- and inter-specific hybrids that have improved disease resistance and desirable ornamental qualities.

Container grown trees were placed into cages (8 ft x 8 ft x 8 ft) screened with fiberglass mesh for use in honey bee mediated intra- and inter-specific crosses. The cages are necessary to exclude unwanted pollinators. Crosses were conducted in spring 2006 at the University of Tennessee, Knoxville. A pheromone and sugar solution was applied to the base of the bracts to entice the honey bees to visit the flowers multiple times. Percent fruit set was calculated as the number of inflorescences with fruit / number of inflorescences and was used to determine the efficiency of the crosses. Mature seeds were extracted and cleaned in fall 2006 and stratified until germination occurred. Germinated seeds were planted into composted pine bark medium and the seedlings were grown under shade in a greenhouse.

Results and Discussion: In the case of the *C. florida* intra-specific crosses, fruit set was practically nonexistent (Table 1). We believed that the poor fruit set resulted from the extremely high average temperatures that occurred during flowering. The temperature during May 2006 in Knoxville, Tenn. was equal to the

30-yr average. Observed fruit set on the *C. kousa* intra-specific crosses was high (44%), fruit set was 49% when 'Blue Shadow' was the female parent and fruit set was 33% when 'Galilean' was the female parent (Table 1). For inter-specific crosses with either *C. florida* cultivar as the female parent and either *C. kousa* cultivar as the male parent no fruit set was observed (Table 1). The reciprocal of these crosses resulted in fruit set only when 'Galilean' was used as the female parent (Table 1).

These results demonstrate that a pheromone and sugar solution to attract honeybees inside a mesh enclosure can be used to advance a traditional breeding approach to create putative intra- and inter-specific hybrids of flowering and kousa dogwood.

Literature Cited:

1. Gunatilleke, C.V.S. and I.A.U.N. Gunatilleke. 1984. Some observations on the reproductive biology of three species of *Cornus* (Cornaceae). J. Arnold Arbor. 65:419-427.
2. Mmbaga, M.T. and R.J. Sauvé. 2004. Multiple disease resistance in dogwoods (*Cornus* spp.) to foliar pathogens. J. Arboric. 30:101-107.
3. Orton, E.R. Jr. 1985. Interspecific hybridization among *Cornus florida*, *C. kousa*, and *C. nutallii*. Proc. Inter. Plant Prop. Soc. 35:655-661.
4. Ranney, T.G., L.F. Grand, and J.L. Knighten. 1995. Susceptibility of *Cornus kousa* cultivars and hybrids to dogwood anthracnose and powdery mildew. J. Arboric. 21:11-16.
5. Reed, S.M. 2004. Self-incompatibility in *Cornus florida*. HortScience 39:335-338.
6. U.S. Dept. of Agriculture. 1998. Census of Horticultural Specialties. Washington, D.C.
7. Windham, M.T., E.T. Graham, W.T. Witte, J.L. Knighten and R.N. Trigiano. 1998. *Cornus florida* 'Applachain Spring': A white flowering dogwood resistant to dogwood anthracnose. HortScience 33:1265-1267.
8. Windham, M.T., W.T. Witte and R.N. Trigiano. 2003. Three white-bracted cultivars of Cornus florida that are resistant to powdery mildew. HortScience 38:1253-1255.

Table 1. Honey bee mediated intra-specific (*C. florida* × *C. florida* and *C. kousa* × *C. kousa*) and inter-specific (*C. kousa* × *C. florida* and *C. florida* × *C. kousa*) crosses in 2006.

Parents	No. of inflorescences	Percent fruit set	No. of seed	No. of seedlings
AS[1] × CB[2]	117	2.6	4	4
CB × AS	82	6	0	0
BS[3] × Gal[4]	439	49	125	115
Gal × BS	227	33	159	120
Gal × CB	73	2.7	2	2
CB × Gal	47	0	0	0

[1]*C. florida* 'Appalachian Spring'
[2]*C. florida* 'Cherokee Brave'
[3]*C. kousa* 'Blue Shadow'
[4]*C. kousa* 'Galilean'

Clarifying Taxonomy and Nomenclature of *Fothergilla* (Hamamelidaceae) Cultivars and Hybrids

Thomas G. Ranney[1], Nathan P. Lynch[1], Paul R. Fantz[1] and Paul Cappiello[2]
[1]NC State University, Dept. of Hort. Science, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Fletcher, NC, 28732-9244
[2]Yew Dell Gardens, P.O. Box 1334, 6220 Old LaGrange Rd, Crestwood, KY 40014

tom_ranney@ncsu.edu

Index Words: Breeding, Cultivars, *Fothergilla gardenii*, *Fothergilla major*, *Fothergilla ×intermedia,* Genome Size, Hybrid Fothergilla, Interspecific Hybridization, Polyploidy, Witch-alder, Flow Cytometry, Cytology

Significance to Industry: *Fothergilla* L. spp. (fothergilla or witch-alder) are exceptional garden plants that display showy, white, fragrant flowers in a terminal spike that resembles a bottlebrush. Summer foliage color can be dark green to blue-green with fall foliage ranging from and including multi-colored combinations of yellow, orange, maroon, and scarlet. *Fothergilla* have few pest problems, they tolerate a broad range of climates (USDA hardiness zones 4-9), soil types, and shade. As a result, *Fothergilla* have become valuable nursery and garden plants. However, clear differentiation among *F. gardenii* Murray, *F. major* Lodd., and potential hybrids can be difficult based solely on morphological characteristics. A combination of chromosome counts and DNA contents was used to clearly differentiate among *F. gardenii* (*2n* = 4x = 48), *F. major* (*2n* = 6x = 72), and hybrids (*2n* = 5x = 60). *Fothergilla ×intermedia* Ranney and Fantz (hybrid fothergilla) is proposed as the name for these hybrids. The correct classification and nomenclature for 17 different taxa are presented.

Nature of Work: There are two species of *Fothergilla, F. gardenii* and *F. major* and both are native to the Southeastern United States. *Fothergilla gardenii* is found in wet savannas and pocosins in the Coastal Plain of North Carolina, South Carolina, Georgia, Florida, and Alabama (Weaver, Jr., 1969). This species is generally smaller in stature (3-10 dm) than *F. major,* and is distinguished sometimes by smaller leaves that are generally toothed only on the upper half and symmetric at the base. Cytology determined a chromosome number of *2n* = 4x = 48 (Weaver, Jr., 1969). In contrast, *F. major* is found on upland sites in the piedmont and mountains of North Carolina, South Carolina, Georgia, Alabama, Tennessee, and Arkansas (Weaver, Jr., 1969). This species generally is larger in stature (7-65 dm) than *F. gardenii* and is sometimes distinguished by larger leaves that generally are toothed from below the middle and conspicuously asymmetric at the base. Cytology determined a chromosome number of *2n* = 6x = 72 (Weaver, Jr., 1969). The two species of *Fothergilla* are sometimes confused and attempts to properly identify them based on morphological characteristics is often inconclusive. There has also been speculation that the two species of *Fothergilla* hybridize (Dirr, 1998). Hybrids between these species should have a chromosome number of *2n* = 5x = 60.

Microscopic determination of chromosome numbers is not a practical approach for separating species and hybrids among large numbers of cultivars. However, flow cytometry can provide a fast and accurate determination of nuclear DNA content that is related directly to ploidy level (among closely related taxa) and can be used as a taxonomic tool (Doležel, et al., 1998).

The objectives of this research were to verify the existence of hybrids between *F. gardenii* and *F. major,* and to clarify the proper taxa designations for clones of *Fothergilla* commonly grown in the nursery industry. A comparison of morphological characteristics was made among diverse clones representing both species and potential hybrids from collections of *Fothergilla* at the N.C. State University, Mountain Horticultural Crops Research and Extension Center, Fletcher, NC (NCSU) and Yew Dell Gardens, Crestwood, KY (YDG). Morphological measurements were taken on lamina length, lamina width, leaf margin dentation location (strictly above the middle, to the middle, or extending to below the middle), symmetry of leaf base (symmetrical, variable, or asymmetrical), stipule length, stamen number, and hypanthium depth and width at anthesis. Twelve measurements were taken for each leaf morphology character and six measurements were taken for each flower morphology character for each clone.

Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement irrespective of ploidy level) were determined via flow cytometry (Greilhuber et al., 2005). Nuclei isolation and staining from approximately 12 stamen filaments followed protocols provided by Partec (Partec GmbH, Münster, Germany). The mean fluorescence of each sample was compared with an internal standard of known genome size [*Pisum sativum* L. 'Ctirad', 2C = 9.09 pg; (Doležel et al., 1998)]. A minimum of 4,500 nuclei were analyzed to calculate the ratio of sample peak to the internal standard for determining genome size [2C pg = (mean fluorescence of sample peak/ mean fluorescence of internal standard peak) $\times$ 9.09 pg]. Two to six subsamples were analyzed for each taxa. Chromosome counts were conducted on root tips collected in the morning from newly rooted stem cuttings of *Fothergilla* 'Mt. Airy'.

Results and Discussion: Cytological examination of 14 mitotic cells revealed that *Fothergilla* 'Mt. Airy' was a pentaploid with *2n* = 5x = 60 (Fig. 1), thereby confirming it is a hybrid between the tetraploid *F. gardenii* and hexaploid *F. major*. *Fothergilla* 'Mt. Airy', a confirmed pentaploid, was used as a reference to compare the approximate genome sizes (DNA content) for the different ploidy levels (Table 1). Genome sizes within species and hybrids had a narrow range providing clear distinction between the three taxonomic groups consistent with variations in ploidy levels (Table 1).

Separating hybrids from parental species was particularly challenging when based strictly on morphology. Most ranges for morphological measurements of

hybrids overlapped with one or the other parent (Table 1). One exception was that the lamina width of *F. gardenii* was consistently narrower than either *F. major* or the hybrids. In general, hybrids tended to resemble *F. major* more closely, likely resulting from higher ploidy level and gene dose that was contributed from *F. major.*

It was determined that the majority of cultivars represented in commerce were hybrids. To help clarify the taxonomy and nomenclature of *Fothergilla* spp., nothospecies *Fothergilla ×intermedia* Ranney and Fantz is proposed for the hybrid species name in accordance with Article H.3-5 (Greuter et al., 2000). Based on this study, we further identified the cultivars 'Appalachia', 'Bill's True Dwarf', 'Blue Mist', 'Harold Epstein', and 'Jane Platt' as *F. gardenii.* The cultivars 'Arkansas Beauty' and 'KLMG' Mystic Harbor™ were found to be *F. major*. The cultivars 'Blue Shadow', 'Eastern Form', 'KLMtwo' Beaver Creek®, one unnamed clone (YDG 2005-323-A), 'KLMfifteen' Red Monarch™, 'KLMsixteen' May Bouquet™, 'Mt. Airy', 'Red Licorice', 'Sea Spray', and 'Windy City' were hybrids, *Fothergilla ×intermedia.*

Acknowledgements: This research was funded, in part, by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643 and the North Carolina Association of Nurserymen, Raleigh, NC 27607-4904. The authors gratefully acknowledge the excellent technical assistance of Tom Eaker and Joel Mowrey at the Mountain Horticultural Crops Research and Extension Center, the staff at the Mountain Horticultural Crops Research Station, and Cassandra Finger and JoAnne Ficher at Yew Dell Gardens.

Literature Cited:

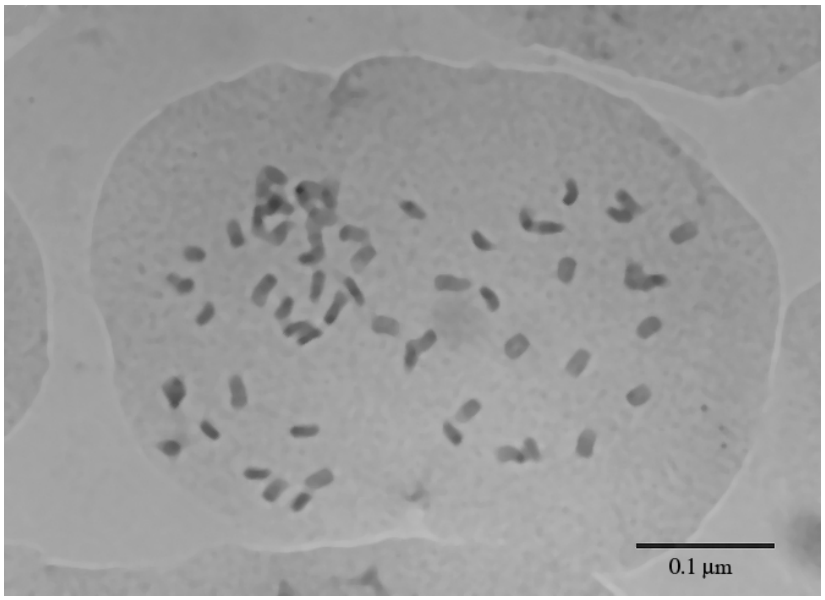
1. Dirr, M.A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publishing, Champaign, Ill.
2. Doležel, J., J. Greilhuber, S. Lucretti, A. Meister, M.A. Lysák, L. Nardi, and R. Obermayer. 1998. Plant genome size estimation by flow cytometry: inter-laboratory comparison. Ann. Bot. 82:17-26.
3. Greilhuber, J., J. Doležel, M.A. Lysák, and M.D. Bennett. 2005. The origin, evolution and proposed stabilization of the terms 'gemone size' and 'C-value' to describe nuclear DNA contents. Annal Bot. 95:255-260.
4. Greuter, W., J. McNeill, F.R. Barrie, H.M. Burdet, V. Demoulin, T.S. Filgueiras, D.H. Nicholson, P.C. Silva, J.E. Skog, P. Trehane, N.J. Turland, and D. L. Hawksworth. 2000. International Code of Botanical Nomenclature (Saint Louis Code). Koeltz Sci. Books, Köenigstein, Germany.
5. Weaver, Jr., R.E. 1969. Studies in the North American genus *Fothergilla* (Hamamelidaceae). J. Arnold Arboretum 50(4):599-619.

Table 1. Comparison of characteristics of *Fothergilla gardenii, F. ×intermedia*, and *F. major*.

Characteristic	*F. gardenii*	*F. ×intermedia*	*F. major*
Chromosome no[z]	*2n* = 4x = 48	*2n* = 5x = 60	*2n* = 6x = 72
Genome size (2C)	4.2-4.5 ρg	5.2-5.5 ρg	6.2-6.4 ρg
Lamina length (cm)	3.4-5.4 (8)[y]	5.3-11.1	6.0-11.5
Lamina width (cm)	2.1-4.0	4.3-7.8 (9.5)	6.0-11.0
Leaf dentation location	Mostly toothed above the middle	Toothed above, interm., or below the middle	Toothed from below the middle
Leaf base	Symmetrical or variable	Asymmetrical or variable	Variable
Stipule length	3.9-8.8	3.8-10.9	6.0-10.0
Stamen no.	12-28	14-30	16-27
Hypanthium depth (mm)	0.7-2.3	0.9-2.6	1.0-2.7
Hypanthium width (mm)	1.0-2.2	1.0-3.4	1.4-3.0

[z]Chromosome numbers for *F. gardenii* and *F. major* were determined by Weaver, Jr. (1969).
[y]Numbers in parentheses indicate extreme ranges, but uncommon occurrences.

Figure 1. Photomicrograph of root tip cell of *Fothergilla ×intermedia* 'Mt. Airy' in prophase with 60 somatic chromosomes.



Four New Ornamental Plant Releases from the University of Arkansas

Jon T. Lindstrom, Bruce L. Dunn, Scott E. Renfro, Brent M. Burkett
Department of Horticulture, University of Arkansas, 316 Plant Sciences Building
Fayetteville, AR 72701

tranell@uark.edu

Index Words: Sterility, Blue Curls, Butterfly Bush, Bromeliad, Intergeneric, Gesneriad, Breeding

Significance to Industry: The University of Arkansas recently released four ornamental hybrid plants: *Trichostema* 'Blue Myth', *Buddleja* 'Asian Moon', ×*Sinvana* 'Mount Magazine' and *Puya* 'Posiedon's Trident'. These releases would be suitable for use as container or in-ground ornamentals across the southeastern and south-central United States. They were not patented.

Nature of work: ***Trichostema* 'Blue Myth':** *Trichostema* L. 'Blue Myth' is the result of crossing the white-flowered *T*. *arizonicum* Gray and the blue-flowered *T*. *lanatum* Benth. The genus *Trichostema*, in the family Lamiaceae, is comprised of 18 species and includes both annual and perennial types (2). The range of distribution for the genus includes central Mexico, southern Canada, and across the continental United States (2). Woolly Blue Curls, *T. lanatum*, is commercially available and is used in California landscapes (7). The suffrutescent *T. arizonicum* is occasionally recommended as a component for a xeric landscape (4). The native habit for this species includes western Texas, New Mexico, Arizona, and northern Mexico (2).

***Buddleja* 'Asian Moon':** Typical *B. davidii* Franch. cultivars are tetraploid and fertile. In certain areas of the world, butterfly bush is escaping from cultivation and could be considered an invasive plant (8). As part of a larger crossing study to assess *Buddleja* L. fertility, controlled pollinations were made on 10 May 2001 between the tetraploid *B*. *davidii* var. *nanhoensis* 'Moonshadow' (Chitt.) Rehd. (female parent), and the diploid *B*. *asiatica* Lour. (male parent) (1). One viable seedling was obtained from this cross, and it was planted in the field at the University of Arkansas Research Farm in 2002.

×*Sinvana* 'Mount Magazine':×*Sinvana* 'Mount Magazine' was the result of an intergeneric cross between the yellow-flowered *Sinningia conspicua* (Seem.) G. Nicholson and lavender-flowered *Paliavana tenuiflora* Mansf. Both plants are native to Brazil and are in the family Gesneriaceae. *Sinningia* Nees. is a large genus of primarily Brazilian gesneriads, many of which grow from a tuber and are seasonally dormant (3). The common florist's gloxinia is perhaps the best known member of this genus. *Paliavana* Vand. is closely related to *Sinningia* and is separated from the latter genus by its shrubby growth habit and lack of a tuberous root system (3).

***Puya* 'Poseidon's Trident:** *Puya* Molina 'Poseidon's Trident was the result of an interspecific cross between the blue-flowered *Puya tuberosa* Mez. and the chartreuse-flowered *Puya mirabilis* (Mez.) L.B. Sm. The genus *Puya* is native to South America and contains approximately 150 species. Members of the genus are commonly cultivated in areas with a Mediterranean climate. They are attractive due to their typical blue or green iridescent flowers as well as their dangerously sharp, spiny leaves. *Puya tuberosa* is a rare species thought to be endemic to Bolivia, while *Puya mirabilis* is widespread and common in both Argentina and Bolivia (6). *Puya mirabilis* is unusual in the genus because it flowers from seed in a relatively short period of time, 2-3 years after sowing seed.

Results and Discussion: *Trichostema* 'Blue Myth': The original cross was made on August 2004, and the progeny selected for release flowered for the first time in May 2005. The showy blue flowers were produced on a shrubby plant beginning in mid-summer and continuing through fall. The plant was sterile and flowered for an extended period of time. This was the first artificial cross between two species in the genus *Trichostema* L. The hybrid was propagated by soft-wood cuttings, which root in 2-3 weeks. It was also propagated via tissue culture. The plant would be suitable for use in patio, greenhouse or conservatory displays for its abundant summer flowers. It may also be useful as a landscape plant in frost-free areas.

***Buddleja* 'Asian Moon':** The whitish purple flowers on this hybrid were produced from late May until first frost. They were fragrant and attractive to butterflies. The foliage was typical for a *B. davidii* var. *nanhoensis* cultivar with a dark gray-green upper surface and whitish-green underside. Un-pruned plants reached 7 feet after three growing seasons, but height was easily controlled by pruning in very early spring. This selection was significant because it was the result of hybridization between a tetraploid and a diploid. The resulting hybrid was determined cytologically to be a triploid with seed set not being observed (5). Therefore, 'Asian Moon' might be suitable for cultivation in areas where butterfly-bush is known to escape from cultivation.

×*Sinvana* 'Mount Magazine': The original cross was made in July 2004 and the single resulting seedling flowered for the first time in July 2005. The fragrant white flowers had lavender lines in the throat and were produced on a shrubby plant beginning in mid-summer and continuing through fall. This was the first registered hybrid in the new hybrid genus ×*Sinvana.* The hybrid was propagated by cuttings taken in spring. Because this hybrid develops a tuberous stem, it is important to include part of the tuber when taking the cuttings. Such cuttings rooted in six weeks and began to flower the following summer. The plant would be suitable for use in greenhouse or conservatory displays for its abundant summer flowers. It may also be useful as a landscape plant in frost-free areas.

***Puya* 'Poseidon's Trident:** The original cross was made in July 2004 and 10 seeds were produced. All seeds germinated and the progeny selected for release flowered for the first time in June 2006. The upright, blue flowers were produced on a lightly branched inflorescence held stiffly above a rosette of spiny, silvery-gray leaves. Flowering occurred in early to mid-summer. This was the first registered hybrid using either *Puya* species, and only the second *Puya* hybrid registered. The hybrid was propagated by separating offsets (or pups) from the mother plant. It would be suitable for cultivation in areas where members of the genus *Puya* are currently grown, typically along the west coast of the United States, Florida, and areas near the Gulf Coast.

Literature Cited:

1. Burkett, B. M. 2001. Hybridization, production, and identification of *Buddleja davidii* (butterfly bush) and interspecific *Buddleja* hybrids. MS Thesis, Univ. of Ark., Fayetteville.
2. Lewis, H. 1945. A revision of the genus *Trichostema*. Brittonia:5(3)276-303.
3. Perret, M, A. Chautems, R. Spichiger, G. Kite, and V. Savolainen. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear *ncpGS.* Am. J. Bot. 90: 445-460.
4. Pima County demonstration garden xeriscape plant list. 29 May 2007 http://cals.arizona.edu/pima/gardening/xeri_plantlist.html.
5. Renfro, S. 2004. Artificial hybridization: Intersectional and interspecific breeding among Asiatic and Neotropical *Buddleja* species. MS Thesis, Univ. of Ark., Fayetteville.
6. Smith, L.B. and R.W. Downs. 1974. Pitcairnioideae (Bromeliaceae) Fl. Neotrop. Monogr. 14:1-658.
7. *Trichostema lanatum* woolly blue curls. 29 May 2007 http://www.laspilitas.com/plants/680.htm.
8. The University of Georgia, USDA APHIS PPQ and USDA Forest Service Forest Health Technology Enterprise Team. 2003. Invasive plants of the Eastern United States: Identification and control. 29 May 2007 http://www.invasive.org/eastern/ .

Evaluation of *Indigofera* Species for Ornamental Potential

David A. Knauft
University of Georgia, Dept. of Horticulture, Athens, GA 30602-7273

dknauft@uga.edu

Index Words: *Indigofera*, indigo, plant evaluation

Significance to Industry: Several species from the genus *Indigofera* are available in the nursery trade, although none are common. Most of the available species are likely perennial through zone 6, are drought and pest tolerant, have flower colors ranging from white through red, will flower for several months, and possess a range of plant types. This work is the first step in further evaluation of additional species and of assessing genetic variability available from which new cultivars may be selected.

Nature of Work: Over 750 accepted species of *Indigofera* have been described, making it the third largest genus within *Leguminosae*. (2, 5). Plants from the genus were likely the source of a deep-blue fabric dye used as early as Egyptian and Babylonian times. The species most frequently used as a source of deep-blue dye, *Indigofera tinctoria,* was grown in the southeastern United States during the mid-1700s. At one point during the American Revolution, it was valuable enough to be used as currency (1).

The genus has many species that possess ornamental traits. Flowers are characteristic of the legume family, and in many species are borne in large numbers, either singly or on racemes. Flower colors range from white to pink to red to purple, with many intermediate shades. Species generally have pinnately compound, attractive leaves and are often densely branched. Many species fix nitrogen and often have deep taproots, providing for efficient water and fertilizer use. Few disease or insect problems have been reported. While a vast majority of *Indigofera* species are tropical in origin, there are a number of cold-hardy species. Based on the aforementioned attributes, researchers have indicated the genus has significant ornamental promise (3). This work was conducted to evaluate ornamental potential of a range of *Indigofera* species and to examine genetic variability within promising *Indigofera* species.

Results and Discussion: Plants of available *Indigofera* species were obtained from the nursery trade. Given the large number of species and their relative rarity in the trade, there is some confusion about nomenclature. The International Legume Database and Information Service is an international database source that has documented accepted scientific names and synonyms for legumes. They are used as a source for nomenclature here (4). We obtained *I. amblyantha* (two sources)*, I. balfouriana* (one source, sold under pseudonym *I. dielsiana), I. decora* (two sources,

sometimes sold under common name Chinese indigo and/or pseudonym *I. incarnata*), *I. heterantha* (three sources, sometimes sold as Himalayan indigo, and also sold under several pseudonyms including *I. gerardiana, I. himalayensis, I. macrostachya* and *I. rubroviolacea*), *I. kirilowii* (five sources, sometimes sold as Kirilow indigo), and *I. pseudotinctoria* (three sources). There has been misapplication of *I. pseudotinctoria* to plants that are not actually of this taxon, as well as the use of the pseudonym *I. bungeana.* A single named *Indigofera* cultivar is commercially available, the low-growing 'Rose Carpet,' usually sold as a version of *I. pseudotinctoria. I. decora* is available in a standard pink-flowered form as well as a white-flowered type. In addition to plants obtained from commercial sources, seed of various species were obtained from the Plant Genetic Resources Conservation Unit of the USDA in Griffin, GA and Silverhill Seeds, Cape Town, South Africa.

A total of 58 taxa are being evaluated both in pots and in-ground at Athens and Watkinsville, GA. Species being evaluated are listed in Table 1. The five species available in the nursery trade all have been winter-hardy in our location, along with several unnamed species. Most of the seed-generated taxa were not reliably cold-hardy, which is to be expected given the primarily tropical origins of many *Indigofera* species. Flowering generally began in May or early June in Athens, GA although several species (*I. pungens* and *I. zollingeriana*) did not flower until late September. Several species did not flower (*I. arrecta, I. cytisoides, I .eriocarpa, I. filifolia, I. langbergesis*, and *I. zeyheri*) from seed the first year of this study and were not cold-hardy. Several species flowered for more than three months, including *I. amblyantha, I. balfouriana, I. decora, I. pseudotinctoria*. and two of the unnamed species. Flower colors varied from light pink to deeper pink/purple and salmon. Of the various annual species, an unnamed species from South Africa had very large flowers and may be a useful parent in crosses, although our initial artificial hybridization efforts were not successful.

I. amblyantha, I. heterantha, and *I. pseudotinctoria* produced sufficient open-pollinated seed in 2005 to allow evaluation of possible genetic variability within these species. Seed were germinated using a 15-second hot water treatment, and plants were evaluated for variability in 2006. Little variation in flower size, color, raceme length, or flowering duration was noted. Some variation in plant type was seen in *I. heterantha* and *I. pseudotinctoria*. These variations are being evaluated again in 2007. With the exception of the white- and pink-flowered forms of *I. decora* and the low-growing 'Rose Carpet' version of *I. pseudotinctoria*, there appears to be, at best, a small amount of genetic variability within *Indigofera* species available in the U.S. nursery trade. Because plants root easily from cuttings, it is possible that all plants trace back to a common, highly homozygous origin.

We feel that several of the species offer significant promise for the nursery industry. *I. amblyantha* produces a long-lived shrub that reaches at least six feet

in height and flowers for three months or more. *I. heterantha* produces a dramatic show with large numbers of deep pink flowers on a dark green shrub reaching three feet in height.

Several *I. pseudotinctoria* plants are vigorous, low-growing plants that also flower over an extended time. *I. hochstetteri* is an attractive plant that flowered over a long time period, and both *I. microcarpa* and *I. miniata* may be useful in hanging baskets. We have obtained seed of *I. amblyantha* and *I. heterantha* from additional sources and are evaluating these for possible variability. We also will evaluate seed irradiation as another potential source of genetic variation from which to select improved plant types.

Literature cited:

1. Balfour-Paul, J. 2000. Indigo. Fitzroy Dearborn Pub., Chicago. 264 pp.
2. Barker, N. P., B. D. Schrire, and J-H. Kim. 2000. Generic relationships in the tribe Indigofereae (Leguminosae: Papilionoideae) based on sequence data and morphology. In: P.S. Herendeen and A. Bruneau (editors). Advances in Legume Systematics (9):311-337. Royal Botanic Gardens, Kew.
3. Dirr, M. A. 1998. Manual of woody landscape plants. Stipes Publ. Co., Champagne, IL. 1187 pp.
4. ILDIS, 2007. International legume database and information service. (www.ildis.org, accessed May 23, 2007).
5. Schrire, B. D., 1995. Evolution of the tribe Indigoferae (Leguminosae-Paplionoideae). In: M. Crisp and J.J. Doyle (editors). Advances in Legume Systematics (7): Phylogeny, pp. 161-244. Royal Botanic Gardens, Kew.

Table 1. Observations of various *Indigofera* species grown in Athens, GA 2005 and 2006.

Scientific Name	Perennial	Plant type	Flowers
Indigofera amblyantha	Yes	Shrub to 6'	Profuse pink flowers on 3-4" racemes for most of growing season
Indigofera arrecta	No	Low-growing to 6"	None
Indigofera balfouriana	Yes	Shrub to 6'	Many pink flowers on 3-4" racemes for most of growing season
Indigofera capillaries	No	Low-growing to 8"	Profuse single salmon flowers through most of growing season
Indigofera cytisoides	No	Small shrub to 1.5'	None
Indigofera decora	Yes	Shrub 1-2', tends to sucker	Bright pink or white flowers on 4-8" racemes, more profuse early summer
Indigofera eriocarpa	No	Shrub to 1'	None
Indigofera filifolia	No	Shrub to 1'	None
Indigofera heterantha	Yes	Shrub to 2-2.5'	Many red-purple 4" racemes through much of growing season
Indigofera hilaris	No	Low-growing shrub to 6"	None
Indigofera hochstetteri	No	Compact light-green shrub to 2'	Large numbers of individual deep peach flowers through much of season
Indigofera kirilowii	Yes	Compact shrub 2-3'	Pink flowers on 5" racemes early summer
Indigofera langebergesis	No	Compact shrub to 2'	None
Indigofera microcarpa	No	Low-growing shrub to 6"	Pink racemes to 2"
Indigofera miniata	No	Low-growing shrub to 6"	Salmon-colored individual flowers
Indigofera pendula	Yes	Weak shrub to 5'	Long racemes of pink flowers reported, ours was weak
Indigofera pretoriana	No	Shrub to 3-4'	Peach-colored 2" inconspicuous racemes
Indigofera pseudotinctoria	Yes	Shrub to 3'	Upright, pink racemes to 4"
Indigofera pseudotinctoria 'Rose Carpet'	Yes	Low-growing shrub to 6", deterioriates late in season	Profuse, small pink racemes
Indigofera pungens	No	Small shrub to 1.5'	Small orange flowers
Indigofera schimperi	Yes	Shrub to 2-2.5'	Pink flowers on 5" racemes early summer
Indigofera sp. (Car. Nur.)	Yes	Shrub to 3-4'	Pink flowers on 3" racemes through much of season
Indigofera sp. E3DHC H97060 (Heronswood)	Yes	Did not survive	
Indigofera species #1 (Silverhill)	Yes	Shrub to 3'	Pink flowers on 3" racemes late in season
Indigofera species #2 (Silverhill)	No	Shrub to 2'	Large red individual flowers
Indigofera species #3 (Silverhill)	No	Shrub to 3'	Fuchsia flowers on 4" racemes
Indigofera suffruticosa	No	Shrub 3-4'	Small, inconspicuous 2" salmon-colored racemes near mainstem
Indigofera tinctoria	No	Shrub to 6-7'	Deep pink flowers on 6-8" racemes
Indigofera vicioides	No	Low-growing shrub to 1'	Many small, pink 2" racemes
Indigofera zeyheri	No	Low-growing shrub to 6"	Salmon-colored
Indigofera zollingeriana	No	Large, vigorous shrub to 6'	Deep pink 4-5" racemes late in season