Plant Breeding and Evaluation

Tim Rinehart

Section Editor

Ploidy Levels and Interploid Hybridization in Panicle Hydrangea (*Hydrangea paniculata*)

Winston T. Beck and Thomas G. Ranney
Department of Horticultural Science, North Carolina State University
Mountain Horticultural Crops Research and Extension Center
455 Research Dr., Mills River, NC 28759

tom_ranney@ncsu.edu

Index Words: cytotype, DNA content, genome size, plant breeding, polyploidy, reproductive biology

Significance to Industry: *Hydrangea paniculata* is a medium to large shrub noted for its excellent floral displays throughout summer, followed by notable winter interest as the flowers dry and persist through the seasons. Although this species is native to Japan, Taiwan, and parts of China, it is grown in landscapes around the world (3, 9).Breeding efforts have been recently been directed towards smaller stature, quality architecture, panicle size, and coverage of sterile (showy) florets, among other attributes. In nature, *H. paniculata* occurs as diploid, tetraploid, and hexaploid cytotypes, where 1*x* = 18. There is evidence that most plants in cultivation are tetraploids (10). However, little is known about the ploidy levels of specific cultivars or potential for interploid hybridization and fertility of anisoploid progeny. To better understand the reproductive biology and to further breeding efforts within *H. paniculata* the objectives of this study were to: 1) determine the ploidy levels of diverse clones and cultivars, and 2) determine fertility and cytotypes of interploid and anisoploid hybrids. Although most cultivated plants were tetraploids, pentaploids and hexaploids were also found. Information on ploidy levels of specific cultivars will facilitate the development of more strategic and efficient breeding programs. The potential for interploid hybridization and maintenance of fertility of pentaploids may also allow for the introgression of traits between these cytotypes and the development of improved cultivars.

Nature of Work: Samples of cultivated *H. paniculata* were collected from public gardens and arboreta for ploidy determination. Interploid crosses were completed between unnamed tetraploid *H. paniculata* and hexaploid *H. paniculata* 'Dharuma', a 1989 introduction to the market (9) to create pentaploids. Pentaploids (H2008-081-010 and H2008-149-032) were open-pollinated to create the H2012-191, H2012-189, and H2012-185 populations. 'Jane' Little Lime® (4*x*) x H2008-076-030 (5*x*) generated the H2013-133 population. The reciprocal cross, H2008-076-030 x 'Jane' Little Lime® generated the H2013-132 population. H2008-081-010 (5*x*) x 'Phantom' (4*x*) generated the H2012-127 population. Due to self-incompatibility in *H. paniculata*, interploid crosses were completed by isolating parents in pollination cages.

Flow Cytometry. Relative genome sizes (DNA content) were determined using flow cytometry (4). Tissue samples (~1 cm^2) were collected from expanding leaves, placed in a plastic petri dish with ~0.1g of young floral bud tissue from *Magnolia virginiana* 'Jim

Wilson' serving as an internal standard with a known genome size of 2C = 3.92 pg (5). Samples were finely chopped with a razor blade in 0.4 mL of nuclei extraction buffer (Partec CyStain UV Precise P Nuclei Extraction Buffer; Partec GMBH, Munster, Germany) and filtered through a 50 µm nylon mesh filter. Nuclei were stained with1.6 mL 4',6-diamidino-2-phenylindole (DAPI) immediately prior to analysis with a flow cytometer (Partec PA II, Munster, Germany). Samples were run until 5000 nuclei were counted with a CV of less than 5%. Two subsamples were analyzed for each accession.

Cytology. 'Jane' Little Lime®, H2009-149-046, and 'Dharuma' were used to confirm and calibrate ploidy levels with genome sizes for 4X, 5X, and 6X plants, respectively. Actively growing root tips were excised from containerized plants in late July and placed into 3 mL of 2mM 8-hydroxyquinoline/0.248mM cycloheximide. Roots were incubated in hydroxyquinoline/cycloheximide solution for 2 HR at 23°C, and moved to 40°F for an additional 3 HR. Roots were then fixed in 3mL of 3 parts 95% Ethanol : 1 part propionic acid fixative solution at 70°F for 18 HR prior to examination.

Results and Discussion: Ploidy levels and chromosome numbers for 'Jane' Little Lime®, H2009-149-046, and 'Dharuma' were confirmed to be tetraploid ($2n = 4x = 72$), pentaploid ($2n = 5x = 90$), and hexaploid ($2n = 6x = 108$), respectively. Ploidy levels were calibrated with genome sizes based on these representative cytotypes. Relative genome sizes were determined for 46 cultivars, 12 wild-collected accessions, and 61 interploid/anisoploid crosses of *H. paniculata* (Table 1). Mean genome sizes were found to be 7.4, 8.5, and 10.5 pg for 4*x*, 5*x*, and 6*x* plants respectively, clearly differentiating among ploidy levels. Overall mean1C_x genome size was1.80pg, slightly higher than values reported by Zonneveld (10) when using propidium iodide stain and *Agave americana* as an internal standard.

The vast majority of cultivated *H. paniculata* were found to be tetraploids (or near tetraploids) with 2C genome sizes ranging from 6.4 to 7.96 pg. 'Dharuma' and 'Praecox' were the only hexaploid cultivars found, though several wild-collected hexaploids from Japan were also identified. These two cultivars are noted for early blooming, although the panicles on 'Dharuma' have a flattened, less attractive appearance than other cultivars (2, 7, 8). The bloom-time differences and floral morphologies of 'Dharuma' and 'Praecox' have been noted before. However, these differences were thought to be a result of sub-specific variation, rather than the effect of ploidy (9). Three commercial cultivars were found to be pentaploid including 'Bulk' Quick Fire®, 'SMHPLQF' Little Quick Fire™, and 'Wim's Red' Fire and Ice. These most likely resulted from interploid hybridization, potentially with 'Dharuma' as a parent (9).

Interploid crosses between tetraploids and hexaploids produced pentaploid progeny with genome sizes ranging from 8.1 to 8.8 pg. Unlike many anisoploids, pentaploid *H. arborescens* maintained some fertility as both males and females. Crosses between pentaploids and tetraploids tended to produce progeny with genome sizes within or near the tetraploid range, suggestion a bias towards euploidy/isoploidy. Open pollenated pentaploids produced progeny with genome sizes ranging from 7.3 pg (~4X) to 9.0 pg

(~5X), as well as one plant with 12.8 pg (~7.5X), most likely resulting from an unreduced gamete from one parent. Reed (6) showed that *H. macrophylla* can produce unreduced gametes with some regularity. Triploids reported in by Funamoto (3) in *H. paniculata* could have been the result of unreduced gametes from diploid parents or potentially from interploid hybridization.

Another point of interest is the lack of diploid plants in commerce and from the wild-collected material. While Funamoto and Ogawa (3) suggested that there were no morphological differences between cytotypes of *H. paniculata*, it appears that tetraploids and to a lesser degree hexaploids have been preferentially selected for horticultural use.

These results document genome sizes and ploidy levels of diverse cultivars, hybrids, and wild-collected accessions of *H. paniculata*. Furthermore, it was found that interploid hybrids can be easily produced and that pentaploid hybrids maintain fertility and can produce offspring with a wide range of cytotypes including aneuploids, (near) euploids, and higher ploidy levels resulting from unreduced gametes.

Acknowledgements: We would like to thank those at the institutions that have donated material for research purposes: Chicago Botanic Gardens, Morton Arboretum, J.C. Raulston Arboretum, Smithgall Woodland Gardens, and Juniper Level Botanic Gardens, as well as the staff of the Mountain Crop Improvement Lab for their support on this project.

Literature Cited

1. Bulk, R. (2006). Hydrangea plant named 'Bulk'. United States Plant Patent PP16812. Canada Food Inspection Agency. 2012. Applications Accepted for Filing. Plant Varieties Journal, 85. 16 August, 2013. http://www.inspection.gc.ca/english/plaveg/pbrpov/journal/appsaccept85e.shtml#hydr
2. Dirr, M. A. (2009). Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. 6th edition. Stipes Publishing L.L.C., Champaign, IL.
3. Funamoto, T. and Ogawa, M. (2002). A Cytogeographical Study in Hydrangea paniculata Sieb. (Saxifragaceae s.l.) in Japan. Chromosome Science, 6, 73-82.
4. Kao, K.N. 1975. A Nuclear Staining Method for Plant Protoplasts p. 60-62. In: O.L. Gamborg and L.R. Wetter (eds.). Plant Tissue Culture Methods. National Research Council of Canada Prairie Regional Laboratory, Saskatoon, Saskatchewan
5. Parris, K. J., Ranney, T. G., Knap, H. T., Baird, W. V. 2010. Ploidy Levels, Relative Genome Sizes, and Base Pair Composition in *Magnolia*. J. Amer. Soc. Hort. Sci. 135(6): 533-547.
6. Reed, S. M., T. A. Rinehart. 2007. Analysis of Ploidy Level and Its Effects on Guard Cell Length, Pollen Diameter, and Fertility in *H. macrophylla*. HortScience 42(3): 483-488.
7. Reed, S.M., T. A. Rinehart. 2009. Simple-Sequence Repeat Marker Analysis of Genetic Relationships Within *Hydrangea paniculata*. Hort Science 44 (1): 27-31.

8. Sanders, C. 2009. The RHS *Hydrangea paniculata* Trial. The Plantsman 8 (3): 168-175.
9. Van Gelderen, J. and Van Gelderen, D. M. (2004). Encyclopedia of Hydrangeas. Portland, OR: TimberPress.
10. Zonneveld, B. J. M. (2004). Genome Size in Hydrangea. Chapter in: Van Gelderen, J. and Van Gelderen, D. M. (2004). Encyclopedia of Hydrangeas. Portland, OR: Timber Press. 245-251.

Table 1. Relative genome sizes and ploidy levels determined via flow cytometry for cultivars and hybrids of *H. paniculata.*

Source[z]	Taxa	Relative 2C genome size [mean ± SE (pg)]	Ploidy level (*x*)
SWG	2012SM12118 Taiwan	7.71 ± 0.02	4
MOR	417-99 ‘White Tiara’	7.51 ± 0.31	4
MOR	533-57 Kyoto U. Exp. Station, Japan	7.26 ± 0.07	4
MOR	101-99 Götenburg B.G., Sweden	7.67 ± 0.22	4
MOR	149-2011 ‘Paszam'	7.30 ± 0.17	4
CBG	‘Angustipetala’	7.04 ± 0.15	4
CBG	‘Barbara’ The Swan™	7.96 ± 0.04	4
CBG	‘Big Ben’	7.34 ± 0.11	4
PDN	BL 17-01	6.87 ± 0.08	4
CBG	‘Bokrathirteen’ Sweet Summer	7.30 ± 0.05	4
JCR	‘Bokratorch’ Magical™Flame	7.88 ± 0.53	4
CBG	‘Bombshell’	7.29 ± 0.07	4
CBG	‘Boskoop’	6.87 ± 0.05	4
CBG	‘Brussel’s Lace’	7.53 ± 0.13	4
CBG	‘Burgundy Lace’	7.36 ± 0.17	4
CBG	‘Chantilly Lace’	7.28 ± 0.02	4
CBG	‘Dolly’	7.31 ± 0.03	4
CBG	‘Dvppinky’ Pinky Winky®	7.28 ± 0.13	4
CBG	‘Floribunda’	7.35 ± 0.03	4
CBG	‘Greenspire’	7.20 ± 0.07	4
CBG	‘Hypmad I’ White Diamonds®	7.06 ± 0.03	4
CBG	‘Hypmad II’ Tickled Pink®	7.31 ± 0.01	4
CBG	‘ILVOBO’ Bobo®	7.29 ±0.13	4
CBG	‘Jane’ Little Lime®	7.17 ±0.02	4
CBG	‘Kyushu’	7.67 ± 0.17	4
CBG	‘Le Vasterival’ Great Star®	7.43 ± 0.08	4

CBG	'Little Lamb'	7.28 ± 0.02	4
CBG	'Mega Pearl'	7.30 ± 0.19	4
CBG	'Interhydia' Pink Diamond	7.02 ± 0.16	4
PDN	PD 01-01	6.57 ± 0.03	4
PDN	PD 14-01	6.74 ± 0.06	4
PDN	PD 14-01	6.41 ± 0.02	4
CBG	'Pink Lady'	7.45 ± 0.08	4
CBG	'Renhy' Vanilla Strawberry™	7.29 ± 0.01	4
CBG	'Revival' Honeycomb™	7.48 ± 0.02	4
CBG	'Ruby' Angel's Blush™	7.21 ± 0.12	4
SWG	'Shadow'	6.92 ± 0.09	4
JCR	'Shikoku Flash'	7.54 ± 0.10	4
CBG	'Silver Dollar'	7.33 ± 0.04	4
CBG	'Skylight'	7.29 ± 0.07	4
CBG	'SMHPFL' Fire Light®	7.27 ± 0.13	4
PDN	TCM 12-666	7.40 ± 0.02	4
CBG	'Unique'	7.52 ± 0.13	4
CBG	'Webb's Grandiflora'	7.22 ± 0.05	4
CBG	'White Lace'	6.93 ± 0.00	4
CBG	'White Lady'	7.42 ± 0.02	4
CBG	'White Moth'	7.00 ±0.19	4
CBG	'White Tiara'	7.27 ± 0.04	4
CBG	'WRHPBB2' Polar Bear™	7.54 ± 0.11	4
CBG	'Zwijnenburg' = 'Limelight'	7.15 ± 0.06	4
CBG	'Bulk' Quick Fire® (1)	8.76 ± 0.21	5
CBG	'SMHPLQF' Little Quick Fire™	8.47 ± 0.14	5
CBG	'Wim's Red' Fire and Ice	8.79 ± 0.04	5
CBG	'Dharuma'	10.48 ± 0.01	6
MOR	30-98 – Mt. Iwo, Hokkaido, Japan.	10.56 ± 0.03	6
MOR	374-82 'Praecox'	10.52 ± 0.16	6
MOR	135-99 Kuta, Kyoto, Japan.	10.73 ± 0.05	6
MOR	140-2009 'Dharuma'	10.49 ± 0.17	6
MOR	180-2001 Chiba U. Japan.	10.24 ± 0.04	6
MOR	186-2000 Tohaka U. Sendai, Japan.	10.41 ± 0.04	6

	4*x* × 6*x* Crosses		
MCI	H2008-076-030	8.53 ± 0.13	5
MCI	H2008-081-010	8.77 ± 0.01	5
MCI	H2008-081-018	8.12 ± 0.15	5
MCI	H2008-081-024	8.37 ± 0.10	5
MCI	H2008-082-011	8.53 ± 0.10	5
MCI	H2008-082-027	8.56 ± 0.03	5
MCI	H2008-095-002	8.45 ± 0.14	5
MCI	H2008-095-021	8.42 ± 0.08	5
MCI	H2008-095-027	8.67 ± 0.08	5
MCI	H2009-146-046	8.69 ± 0.04	5
MCI	H2009-149-032	8.28 ± 0.07	5
	5*x* × 4*x* Crosses		
MCI	H2013-127-001	7.52 ± 0.05	~4.2
MCI	H2013-127-002	7.52 ± 0.08	~4.2
MCI	H2013-127-003	7.48 ± 0.03	~4.2
MCI	H2013-127-004	7.57 ± 0.15	~4.2
MCI	H2013-127-005	7.51 ± 0.02	~4.2
MCI	H2013-127-009	7.62 ± 0.08	~4.2
MCI	H2013-127-010	7.66 ± 0.05	~4.3
MCI	H2013-127-015	7.58 ± 0.02	~4.2
MCI	H2013-127-016	7.62 ± 0.12	~4.2
MCI	H2013-127-025	7.63 ± 0.02	~4.2
MCI	H2013-127-028	7.51 ± 0.05	~4.2
MCI	H2013-132-001	7.61 ± 0.04	~4.2
MCI	H2013-132-002	8.19 ± 0.78	~4.6
MCI	H2013-132-003	7.61 ± 0.06	~4.2
MCI	H2013-132-004	8.17 ± 0.11	~4.5
MCI	H2013-132-005	7.47 ± 0.01	~4.2
MCI	H2013-132-006	7.68 ± 0.15	~4.3
MCI	H2013-132-007	9.71 ± 0.10	~5.4
MCI	H2013-132-008	8.10 ± 0.20	~4.5
MCI	H2013-132-009	7.64 ± 0.01	~4.3
MCI	H2013-132-010	8.85 ± 0.07	~4.9
	4*x* × 5*x* Crosses		
MCI	H2013-133-001	7.41 ± 0.02	~4.1
MCI	H2013-133-002	7.23 ± 0.01	~4.0
MCI	H2013-133-003	7.21 ± 0.07	~4.0
MCI	H2013-133-004	7.45 ± 0.01	~4.1

		5*x* Open Pollinated	
MCI	H2012-185-004	7.58 ± 0.21	~4.2
MCI	H2012-185-005	8.93 ± 0.33	~5.0
MCI	H2012-185-006	8.95 ± 0.01	~5.0
MCI	H2012-185-008	8.44 ± 0.19	~4.7
MCI	H2012-185-010	7.96 ± 0.23	~4.4
MCI	H2012-185-011	7.50 ± 0.31	~4.2
MCI	H2012-185-013	7.82 ± 0.09	~4.4
MCI	H2012-185-015	7.74 ± 0.04	~4.3
MCI	H2012-185-022	8.04 ± 0.04	~4.5
MCI	H2012-185-023	8.07 ± 0.03	~4.5
MCI	H2012-185-024	8.17 ± 0.30	~4.5
MCI	H2012-185-028	8.97 ± 0.13	~5.0
MCI	H2012-189-002	8.46 ± 0.02	~4.7
MCI	H2012-189-003	12.78 ± 0.04	~7.1
MCI	H2012-189-004	8.05 ± 0.03	~4.5
MCI	H2012-191-001	8.86 ± 0.21	~4.9
MCI	H2012-191-002	7.62 ± 0.02	~4.2
MCI	H2012-191-003	8.85 ± 0.00	~4.9
MCI	H2012-191-004	8.60 ± 0.06	~4.8
MCI	H2012-191-005	8.72 ± 0.15	~4.9
MCI	H2012-191-006	7.32 ± 0.02	~4.1
MCI	H2012-191-007	8.00 ± 0.15	~4.5
MCI	H2012-191-008	8.00 ± 0.15	~4.5
MCI	H2012-191-009	8.22 ± 0.06	~4.6
MCI	H2012-191-010	7.25 ± 0.05	~4.0

[z] Source Codes: CBG; Chicago Botanic Gardens. JCRA; JC Raulston Arboretum, Raleigh, NC. MCI; Mountain Crop Improvement Lab, Mills River, NC. MOR; Morton Arboretum. PDN; Plant Delights Nursery, Raleigh, NC. SWG; Smithgall Woodland Garden, Gainesville, GA.
[x] Also known by the name “Early Sensation” in Europe.

Inheritance of Powdery Mildew Resistance in Flowering Dogwoods (*Cornus florida* L.)

Lipi Parikh, S. Kodati, M. T. Mmbaga and Gurong Zhang

Department of Agricultural and Environmental Sciences
College of Agriculture, Human and Natural Sciences
Tennessee State University
3500 John A Merritt Blvd, Nashville, TN 37209

lparikh@my.tnstate.edu

Index Words: *Cornus florida*, powdery mildew, disease management, *Erisyphe pulchra*

Significance of Work: Powdery mildew of *Cornus florida* is one of the most destructive diseases in nursery production throughout the Southeastern U.S. Planting resistant cultivars is the most successful long term solution to powdery mildew control problem in dogwoods. Host plant resistance is a cornerstone of IPM and a key strategy for powdery mildew disease management. Efforts to breed for resistance have been undertaken, but only a few cultivars show powdery mildew resistance. The mode of inheritance for powdery mildew resistance in flowering dogwoods is not understood; such information will facilitate the breeding efforts and allow plant breeders to develop new cultivars with novel phenotypes and disease resistance.

Nature of work: *Cornus* is a large genus of trees, and shrubs with approximately 65 species collectively known as dogwoods. Several species of dogwoods are cultivated for their showy bracts, attractive fall color of foliages, twigs and fruit color (5). Flowering dogwoods (C*. florida*) and Kousa dogwood (C. *kousa*) are the two major species grown in the nursery production industry (4). Flowering dogwood is an economically important ornamental tree with 30 million dollars in total sales in U.S. and 23.2% of the total US dogwood supplies are from Tennessee (9, 10). In early 90's powdery mildew caused by *Erisyphe pulchra* emerged as one of the most destructive diseases of dogwood that limits nursery production of flowering dogwood. In addition to reducing the aesthetic appeal and commercial value of infected plants, it reduces the plant growth, thereby increasing the time needed for infected plants to reach its prime size for sale (5, 11).

Effective fungicides for powdery mildew control have been identified, but frequent application is required season long, that has increased production costs and forced small growers out of business. Host resistance to powdery mildew would be the best method to manage the powdery mildew disease problem in flowering dogwoods; it is friendly to the environment and less expensive for growers. Host resistance has become a cornerstone of IPM and a key strategy in plant disease management. While efforts to breed for powdery mildew resistance are underway, only a few cultivars show powdery mildew resistance in different locations. Understanding the pattern of inheritance of powdery mildew resistance from parents to progeny is important to facilitate breeding strategies.

This study was carried out at Tennessee State University, main campus, Nashville, TN and Otis L. Floyd Nursery Research Center, McMinnville, TN. The aim of this study was to understand the mode of inheritance of resistance/susceptibility to powdery mildew in flowering dogwoods.

Controlled hand pollinations were conducted between susceptible cultivar of *C. florida* 'Cherokee Princess' (CP) and resistant selections R14, MI9 and 'Cherokee Brave' (CB). On an average, 50 sets of 8 inflorescences were selected from each parent and the inflorescence was covered with breathable plastic bags before flowers opened so as to eliminate pollen contaminations. Since dogwood is self-incompatible (7), flower emasculation was not needed. Crosses were conducted by controlled hand pollination by rubbing the anthers on stigma of female parents in 2004, 2011 and 2012. Breathable bags were maintained until seeds from the crosses were harvested. Normal procedure in depulping the seed was done manually and seeds were air-dried at room temperature, vernalized at 4°C for 3 months to overcome the dormancy. Following seed germination at 4°C, seeds were sown and seedlings grown in 4 inch pots in greenhouse environment. The progeny seedlings were exposed to powdery mildew pathogen using air-borne spores from previously infected plants and evaluated for disease severity in the year after controlled crosses were conducted. Disease severity was monitored and rated using a 0-5 scale where, 0= no disease, 1 = 1-10%, 2 = 11- 25%, 3 = 26- 50% ,4 = 51- 75% and 5 = 76- 100% of foliage area with powdery mildew symptoms (5) (Fig. 1). Disease rating was scored 3 times starting in end of June, July and August. The highest disease reading scores were used for subsequent data analysis. Data obtained from disease severity measurements was analyzed using Microsoft Excel. The distribution frequency for the progeny disease severity readings was plotted to determine whether the distribution was continuous or formed discrete classes. Narrow sense heritability (h^2) was calculated by using the regression between mid-parent value and progeny mean. The slope of regression line will be the value for h^2 that would suggest effect of additive gene for the disease resistance trait (8).

Results and Discussion: Controlled crosses between the parents CP (susceptible), CB (moderately resistant), R14 and MI9 (resistant selections) *C. florida* produced 'pseudo' F2 progeny plants with a wide range of susceptibility to powdery mildew disease that ranged from highly susceptible to resistant reactions (Figure 1). Some of the progeny susceptibility levels exceeded the parental susceptibility. Powdery mildew disease severity for the parents in the three years' study is shown in Table 1. The lowest disease severity was from selections R14 followed by MI-9, Cherokee Brave exhibited moderate resistance (Table 1).

The distribution frequency of powdery mildew disease readings for pseudo F_2 progeny of controlled crosses of 2005, 2012 and 2013 is shown in the Figures 1, 2 and 3. The distribution showed a continuous phenotypic variation (Fig 2, 3, 4). No discrete classes of powdery mildew reactions were observed; amongst progeny population from each cross, the transgressive segregations fit a normal curve with slight skew. This type of continuous distribution of powdery mildew disease severity amongst pseudo F_2 progeny indicates that the resistance to powdery mildew in *C. florida* has a quantitative inheritance. These results are similar to a report on bacterial leaf spot disease resistance in pseudo F2 population of Mulbery (8). In 2006 and 2009, Han et al. (1)and Bokmeyer et al. (3) reported similar continuous distributions of grey leaf spot and brown patch disease severity on perennial rye grass and tall fescue respectively and suggested that the inheritance was quantitative (1,3).

Narrow sense heritability estimates based on the mid-parental value and progeny mean regression analysis were 0.69, 0.84 and 0.86 for the progeny of the controlled crosses of 2005, 2012 and 2013 respectively (Figure 5, 6, 7). The R squared values for the 2005 progeny was 0.54 and 2012 and 2013 had the R squared values of 1.0 and 0.69. High narrow sense heritability suggested the presence of additive gene effects. Bovi et al. in 2004 also reported high narrow sense heritability values for sooty mold resistance in *Archonotophoenix* palm (2).

The continuous distribution, high narrow sense heritability estimates (0.69, 0.84, and 0.86) suggested that powdery mildew disease resistance may be controlled by additive gene effects. Powdery mildew severity is highly affected by environmental conditions especially temperature and moisture. It is likely that environmental variations from year to another somewhat affected disease severity and contributed to variations in disease severity from year to year. However, the narrow-sense heritability, h2 = VA/VP, for all crosses in the three years show the proportion of genetic variation that is due to additive genetic values (VA) that is most important in plant selections. Further analysis of the data using broad sense heritability estimates using analysis of variance have not been done, but the numerous crosses done all suggest quantitative resistance of additive genes. Thus, we expect that the determination of mode of inheritance for powdery mildew resistance from this study will facilitate plant breeding to develop a new generation of powdery mildew resistant cultivars.

Literature Cited

1. Bokmeyer, J. M., Bonos, S. A., and Meyer. W. A. (2009). Inheritence charateristics of brown patch resistance in tall fescue. *Crop Science, 49,* 2302-2308.
2. Bovi, M.L. A., Deresende, N. D. V., Saes L. A., and Uzzo, R. P. (2004). Genetic analysis for sooty mold resistance and heart of palm yield in *Archontophoenix*. *Science Agriculture (Piracicaba, Braz), 61,*178-184
3. Han, Y., Bonos, S. A., Clarke, B. B., and Meyer, W. A. (2006). Inheritence of resistance to grey leaf spot disease in perenial rye grass. *Crop Science, 46,* 143-1148
4. Hillier Nurseries, Hiller, A., and Coombes, J.J. 2007. Hillier's manual of trees and Shrubs. Davis and Charles, Newton Abbot, United Kingdom.
5. Mmbaga, M. T., & Sauvé, R. J. (2004). Multiple disease resistance in dogwood for three foliar pathogens. J Arboriculture: 30(2) : 101- 10.
6. Ranney TG, Grand LF, Knighten JL (1995). Susceptibility of *cornus kousa* cultivars and hybrids to dogwood anthracnose and powdery mildew. *J. of Arboriculture*, 21:11-16.
7. Reed, S.M. 1999. Development of a labor - efficient hand pollination procedure for flowering dogwood. J. Environ. Hortic. 17: 92 94.

8. Rita Banerjee, N. K. Das, S. G. Doss, A. K. Saha, A. K. Bajpai, and B. B. Bindroo. (2012). Narrow sense heritability estimates of bacterial leaf spot resistance in pseudo F2 (F1) population of mulberry (Morus spp.). *European Journal of Plant Pathology*. doi:10.1007/s10658-011-9894-z
9. U.S. Department of agriculture.1998. Census of horticultural specialties, USDA, Natl.Agr. Stat. Serv., Wash., D.C.
10. US Department of Agriculture. 2009. 2007 census of agriculture, Washington DC
11. Windham, M. T., Trigiano, R. N., & Windham, A. S. (1962). Susceptibility of Cornus Species to Two Genera of powdery mildew. J. Environ. Hortic. 23: 190 - 192.

Table 1: Powdery mildew disease severity of parents in greenhouse conditions at the location of the progeny

Parents	Year evaluated	Location (greenhouse)	Disease severity
Cherokee Princess	2005	Tennessee State University Otis Floyd Nursery Research Center, McMinnville	4.5
Cherokee Brave			2.5
MI9			1.5
Cherokee Princess	2012	Tennessee State University Otis Floyd Nursery Research Center, McMinnville	4.5
R14			1.0
MI9			1.5
Cherokee Princess	2013	Tennessee State University Otis Floyd Nursery Research Center, McMinnville	3.0
R14			0.5
MI9			1.0
Cherokee Brave	2013	Tennessee State University Main campus (Nashville)	3.5
R14			1.0
MI9			2.0

Figure 1: Disease severity rated on flowering dogwoods in greenhouse at Tennessee State University, Otis L. Floyd Nursery research center, McMinnville, TN: (a) 0 (b) 1 (c) 2 (d) 3 (e) 4 (f) 5

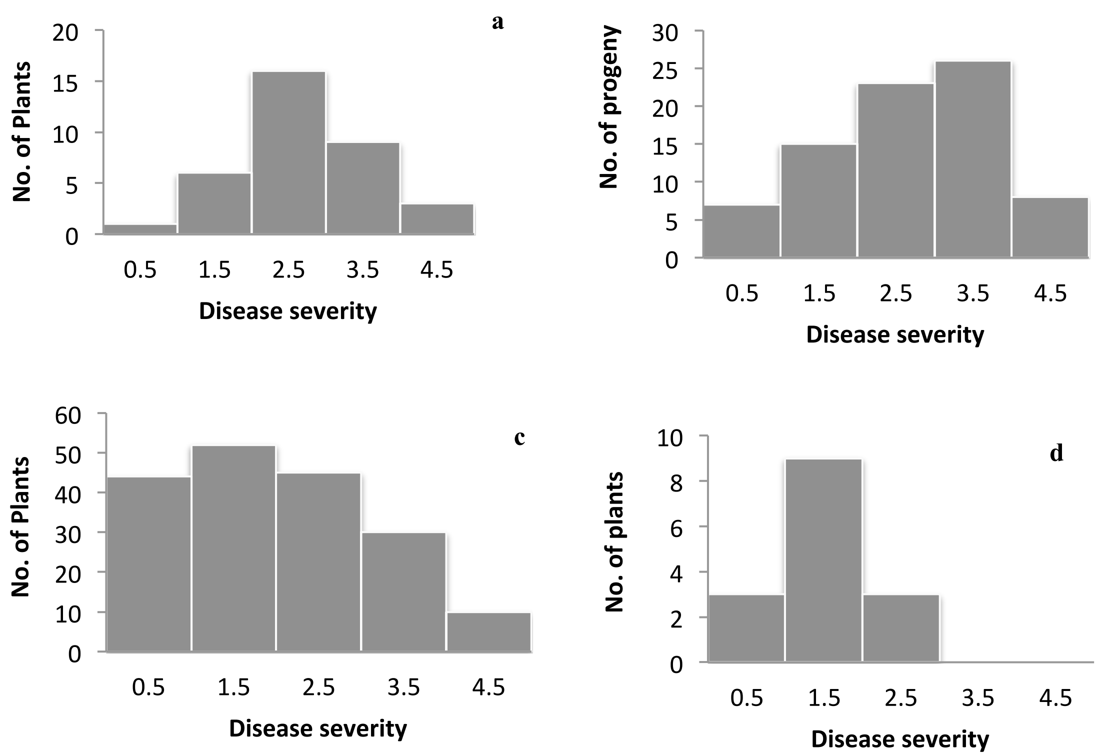

Figure 2. (Controlled crosses evaluated in 2005): (a) MI9 X Cherokee Princess. (b) Cherokee Princess X MI9. (c) MI9 X Cherokee Brave (d) Cherokee Brave X MI9.

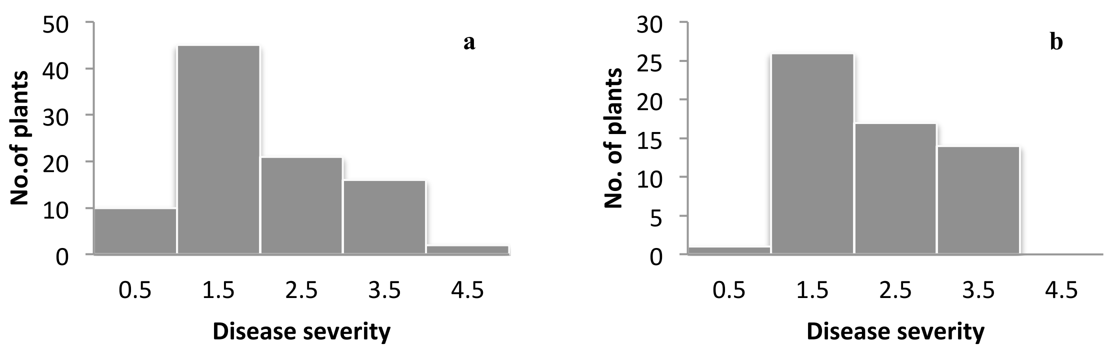

Figure 3. (Controlled crosses evaluated in 2012): (a) R14 X Cherokee Princess. (b) MI9 X Cherokee Princess.

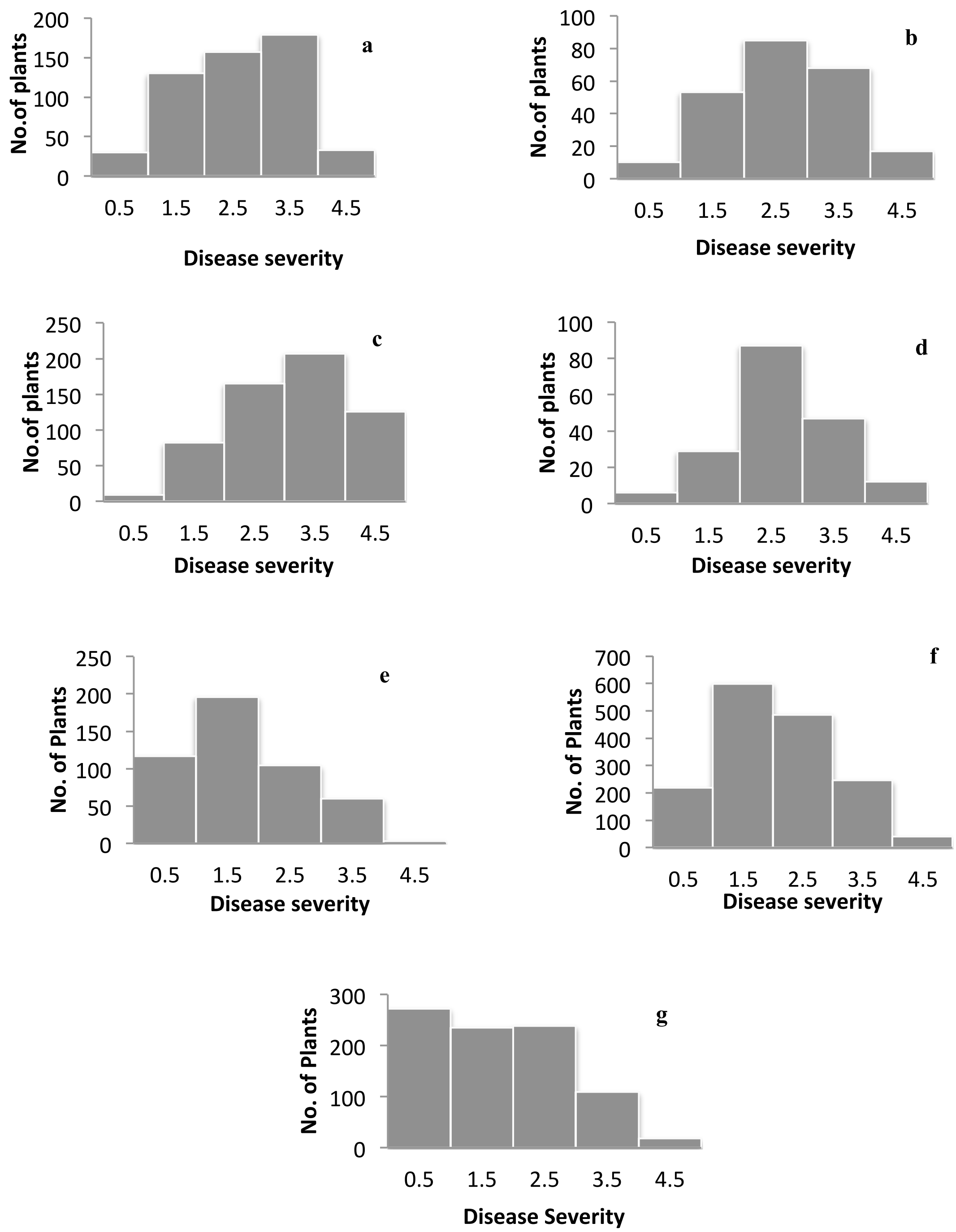

Figure 4 (Controlled crosses evaluated in 2013): (a) R14 X Cherokee Brave (b) Cherokee Brave X R14 (c) MI9 X Cherokee Brave (d) Cherokee Brave X MI9 (e) R14 X Cherokee Princess (f) Cherokee Princess X R14 (g) Cherokee Princess X MI9.

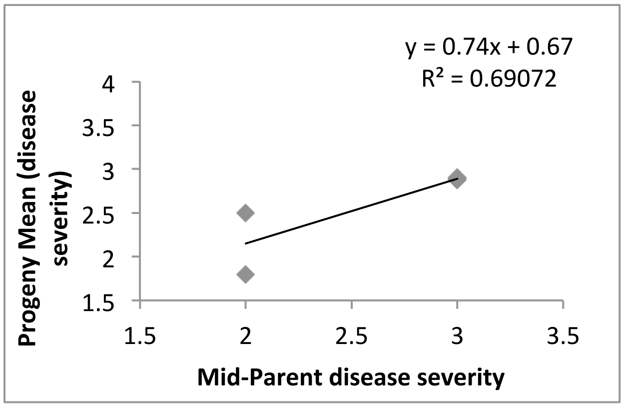

Figure 5: Mid-parent progeny regression of pseudo F2 population means on the mid-parent values from 4 crosses from 3 *C. florida* evaluated in 2005.

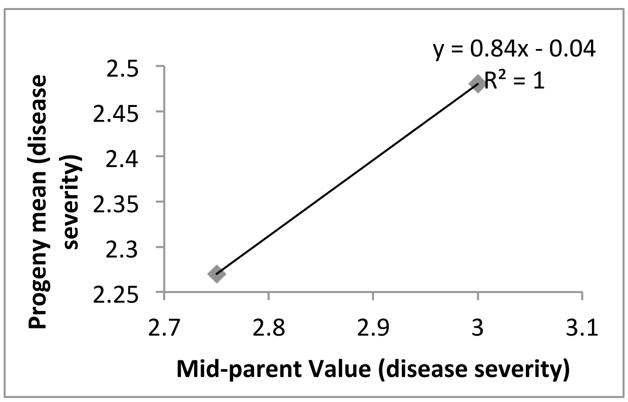

Figure 6: Mid-parent progeny regression of pseudo F2 population means on the mid-parent values of 2 crosses from 3 *C. florida* parents evaluated in 2012.

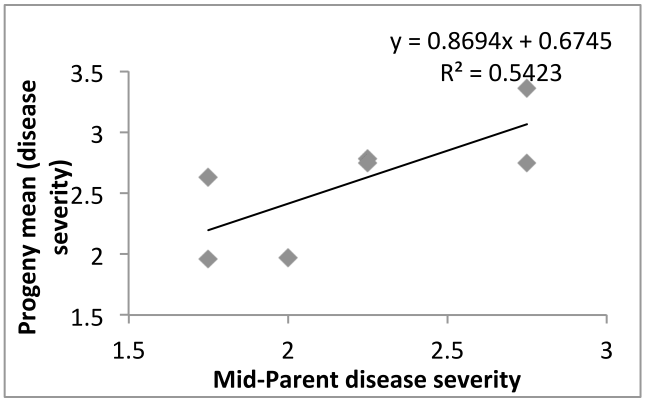

Figure 7: Mid-parent progeny regression of Pseudo F2 population means on the mid-parent values of 7 crosses from 4 *C. florida* parents evaluated in 2013.

Acer saccharum ssp. *skutchii*, the Mexican Mountain Sugar Maple

David Creech

Stephen F. Austin State University, SFA Gardens, 1924 Wilson Drive
Nacogdoches, TX 75962-3000

dcreech@sfasu.edu

Index words: *Acer saccharum* ssp. *skutchii*, skutch maple, drought and alkalinity tolerance, landscape evaluation, propagation

Significance to Industry: While sugar maple enjoys a sizeable market in the U.S and many cultivars are available, the Mexico mountain sugar maple or skutch maple, *Acer saccharum* ssp. *skutchii*, remains relatively unknown. This disjunct wing of sugar maple has endured over 9000 years of divergent evolution apart from relatives in eastern North America (4). The tree is similar in many respects to *Acer saccharum*, sugar maple, and *Acer saccharum* var. *floridanum (*syn. *A. barbatum),* the Florida maple, but features larger leaves, and perhaps the biggest samaras found in *Aceraceae*. The skutch maple offers opportunities for enhanced growth rate, interesting fall and spring foliage color, alkalinity tolerance, drought tolerance and good adaptation to well drained sunny locations in landscapes of the southern USA, east to west. Dirr lists 47 varieties of sugar maple in the industry (1). None include the diverse genetics of this western relative, the skutch maple of Mexico.

Nature of Work: SFA Gardens comprises 128 acres (52 ha) of on-campus property at Stephen F. Austin State University (SFA), Nacogdoches, Texas. Initiated in 1985, SFA Gardens focuses on diversity, collections, rare and unusual species and cultivar collections, and promotes plants that perform well in East Texas and Louisiana landscapes. SFA Gardens has an extensive collection in the *Aceraceae*, with western genotypes well represented. The skutch maple is a genetic resource for breeders interested in improving growth rate, and drought, heat, and alkalinity tolerance.

Background: In 1993, Simpson and Hipps assigned nine, possibly ten, maples to the southwestern USA (6). They noted that certain provenances of *A. grandidentatum*, Bigtooth maple, and the Caddo maple, a unique genotype of *A. saccharum* from southwestern Oklahoma*, w*ere particularly good performers in higher pH soils. Skutch maple, or the cloud forest sugar maple, *Acer saccharum* ssp. *skutchii*, is the least known in the USA and is the southern and western most sugar maple. It occurs naturally as six disjunct populations, with five in Mexico and one in Guatemala (7). First described in Guatemala in 1936 (5), the skutch maple is a rare relic from the Miocene era, probably separated from those in North America since the end of the Pleistocene over 9000 years ago (4). Lara-Gomez characterized variation in isolated skutch maple populations in Mexico (2). Yalma Luisa Vargas-Rodriguez, who graduated from Louisiana State University in 1995, hypothesized that the "six forest sites in Jalisco, Tamaulipas, Veracruz, and Hidalgo (Mexico) contain a unique and ancient flora, were connected and shared species before the Pleistocene, and currently function as tree refuges of that ancient flora" (8). Because of the small number of populations and low numbers in each population, Vargas-Rodriguez proposed to include Skutch maple in the IUCN Red List Catalog and as Endangered in the Guatemalan Species Red List.

SFA Gardens Research: The oldest skutch maple at SFA Gardens was planted in 1994 and originated from seed collected in the Tamaulipas location (3) by John Fairey and Carl Schoenfeld of Yucca Do Nursery, Hempstead, Texas. Now twenty years old, the tree is 46 ft (14 m) tall with an 18 in (45.7 cm) diameter at breast height (dbh). It has grown well without irrigation, is oval shaped, exceptionally marcescent, and failed to develop showy fall color until after first flowering, an event that took 9 years to initiate. Fall color varies between butterscotch yellow to reds and oranges, depending on year.

Propagation: The samaras are large and showy. In our location, seed are often empty, with viable seed at less than 10%. In 2009 the tree produced a heavy seed crop and we allowed the samaras to fall and then covered the area with an inch of pine bark fines. The end result was over one thousand seedlings emerging early (January) that were not affected by frosts in February. Seedlings were potted, grown and distributed to interested nurseries and university research programs. In our trials, cutting propagation of young seedling trees is feasible because of their juvenile nature. Still, after numerous cutting batches, we have noted a poor <20% rooting/survival rate. Best results are two node June cuttings using 1500 PPM K-IBA as a ten second basal end dip.

Research Plots: In January 2011 SFA Gardens established 277 skutch maples at SFA's Science Research Center which is about five miles north and west of the campus (Figs. 1 and 2). The seedlings for this project were progeny of our sole flowering tree. The pollen source is unknown. However, there's some reason to suspect self-pollination. First, sugar maples are wind-blown self-pollinators. Second, the flowering time of the skutch maple is slightly earlier than nearby Florida maples. Third, the performance of seedling batches in central Texas indicates a degree of alkalinity tolerance not shared by Florida maples. However, there remains the possibility that the distributed trees are hybrids with our local Florida maples. The goal of this project was to improve on seed availability, improve seed set and viability, and to select superior clones. This is a cooperative project with SFA's College of Science and Mathematics through an informal agreement to use unutilized space at the Science Research Center for plant evaluation. The site is a full sun south facing gentle slope with a red clay loam (pH 6.7). SFA Gardens planted the seedlings spaced 15 ft (4.6 m) X 15 ft (4.6 m). Over the last three years, weed control has been backpack sprayer applications using glyphosate. The field drip irrigation system utilized ¾ inch black poly pipe with ½ gallon per hour emitters and a battery operated controller which delivered one to two gallons per plant per day during the irrigation season. The trees have not been pruned except to allow easier mowing and glyphosate application.

Climate: Nacogdoches is in Zone 8B of the Pineywoods region in East Texas with an average annual rainfall of 48 in (122 cm). June through August is characteristically hot and dry. 1 Sept 2000 was the record high, 112 F (44.4 C), and 23 Dec 1989 was the record low 0 F (-17.8 C). In 2010 and 2011, Nacogdoches experienced all-time record drought and heat. Total precipitation in 2010 was 22.3 in (56.6 cm) and 2011 produced 35.4 in (89.9 cm), with one third of that that coming late in the fall. Fortunately, 2012 and 2013 saw a return to normal summer temperatures and rainfall at 59.6 in (151 cm) and 43.4 in (110.2 cm), respectively.

Performance: Growth rate has been excellent. Trees were planted in January 2011. After three growing seasons, a 24 June 2014 random sample of 32 trees indicates an average tree height of 17.7 ft (5.4 m) $\pm$ 2.3 ft (.7 m). The tallest trees exceed 21 ft (6.4 m) in height and 4.5 in (11.4 cm) in diameter. Diameter at six inches averaged 2.9 in (7.4 cm) ± 0.49 in (1.2 cm). This random sampling did not include any replants or damaged trees. Deer have been a constraint at this site, not through browsing but through bucks rubbing their racks on young trees. Over the past three years we have replanted 23 trees due to extreme deer damage. In other cases the trees sprouted vigorously below the damage and a new leader was chosen to restore the tree.

Results: This seedling block includes significant diversity. For instance, we note an 11 day variation from earliest to latest leaf emergence in the spring. There is great variety in the color of new growth (green, yellow, red, salmon, pink). Fall foliage color has been brown with leaves held tight into January before falling. However, there is evidence this species does not characteristically develop good fall color until after flowering. Most trees are not of good habit with haphazard branches, a tendency to multi-leader, and bad crotch angles. However, in this seedling block, there's a variety of forms, including several fastigiate forms, one dwarf, and others with tighter habit and good shape. We have cutting propagated twelve selections and will be multiplying others in the next few years.

Genetic characterization. SFA Gardens is cooperating with Jason Grabosky, Rutgers University, New Brunswick, New Jersey in a maple characterization study. April Jackson, PhD candidate, is investigating variation within and among taxa included in the hard maple group. In particular, she is studying *Acer saccharum, Acer nigrum, Acer barbatum,* and *Acer leucoderme.* Populations of *Acer grandidentatum* as well as *Acer skutchii* were also represented in these studies. April is studying morphological, anatomical, and physiological traits that may enable species tolerance to water deprivation and increasing temperatures. She is genotyping germplasm collected over the last three years across natural ranges of the taxa to determine how genetically similar (or not) these taxa are. She will use phylogenetic analysis to address taxonomic discrepancies within this group of taxa and hopes to resolve classification and nomenclature listings. We are also cooperating with Richard Olsen of the National Arboretum, Beltsville, Maryland, in a similar project. In 2015, we hope to sample both our research plots and trees known to have originated from Mexico seed to determine parentage. Finally, our long term goal is to acquire seed of other provenances in Mexico, and, hopefully, Guatemala, to develop a documented ex situ collection of this species at SFA Gardens.

Cooperator observations

Brent Pemberton, Research Scientist, Texas Agrilife Research and Extension Center, Overton, TX: "We've had *Acer skutchii* in the ground for one full growing season. Looks good but still a small tree. It did survive all the cold weather this past year including 16^{o}F on Mar 3 after only being in the ground for one season."

Paul Cox, San Antonio Botanical Garden, San Antonio, TX (retired): "*Acer skutchii* at the SABG has put on the same height growth as *Taxodium mucronatum* during the first 10 years. It's fast. Leaves have never scorched but don't have the dramatic fall color reported from other areas."

James Spivey, nurseryman, Peerless Nursery, Bigfoot , TX: "*Acer skutchii* has a great deal of potential in the tree market for the Central and South Texas. It seems to be very tolerant of alkaline soil around San Antonio and the Hill Country. It has been very tolerant of heat and direct sunlight without any burning of the foliage. The growth rate has been 3-1/2' to 4' per year under irrigation."

Mike Arnold, Research Scientist, Texas A & M University, College Station, Texas: "Nice plant in areas without saline soils or irrigation water. We have killed it repeatedly in multiple locations here due to the salts in the irrigation water. Chlorosis due to alkalinity does not appear to be nearly as serious as the salts. It develops the classic marginal necrosis and then dieback and death. It just is intolerant of the salts." In an earlier correspondence, Mike reported *Acer skutchii* had failed to deal with the high salinity irrigation water in College Station (250 PPM Na and 500 PPM bicarbonates). Whether irrigated by drip, sprinkler or microsprinklers, plants performed poorly. Leaves were cupped and took on a bluish cast, and the trees failed to survive in their container trials.

Steve Lowe, Naturalist, Kendall County Park. Boerne, TX: "*Acer skutchii* is remarkably well adapted for this area. After visiting these trees in their home range, it is easy to see their affinity for limestone. They are truly saxicolous. This would explain their tolerance of our high pH soils, outperforming even our native Bigtooth maples. Skutch maples I've planted in this area have consistently grown 3-4+ feet per year with only moderate irrigation. As with the Sierran oaks, new growth is extra early here, pink/brown and sometimes subject to cold damage. Most here attain peak color end of December (citron to canary yellow to pumpkin). Foliage color and texture (more leathery) seems to change as tree matures."

Jim Robbins, University of Arkansas, Little Rock and Hope, Arkansas: "In 2011, twelve one quart plants were donated by SFA Gardens. One plant died at Little Rock in the first growing season and a second in year two. Average height at Hope and Little Rock is 11.2 ft (3.4 m) tall with a trunk caliper at 6 in (15.2 cm) of 5 in (12.7 cm). The growth habit might be a challenge in nursery production since the tree produces extremely vigorous shoots that require pruning and training. Based on observations at Little Rock (Zone 7), we've observed no dieback during this past harsh winter (9 F) low."

Eric Hammond, Heritage Seedlings, Inc., Salem, Oregon: “We have one tree in the grow-out area. It is the only example I know. And though, I *know* Mark Krautmann is enthusiastic about this species because of its broadly adapted nature, I will not lie about our tree. It is hideous in form, growing off at all trajectories like a North Korean rocket test. Marks in its favor are perfectly clean foliage of great texture, though they are puckered much more than I prefer, and no twig damage of any sort after a freeze. So in short I see a tree of little merit for our climate where we have an overabundance of choice but if it is adapted to the SW market I think it is a plant we can produce successfully.”

Matthew Chappell, University of Georgia Horticulture Department, Athens, GA: “It’s doing well in GA - no fruit yet but I have it in container and in-ground. Last year was an establishment year and this year they are jumping out and growing like crazy. We did have some tip dieback but can’t even tell now (we went to 5.5 F)."

Mike Dirr, Plant Introductions, Inc., Watsonville, Georgia: “I’m not sure I can verify hardiness but no issues at 6 F; early fall freezes, 22 F two nights running November 13 and 14, 2013; and a late spring freeze April 16, 2014, 29.5 F. I have three in the garden and all are vigorous (3 ft/year on average) with pretty reddish tinged new shoots. It’s certainly heat tolerant and a good fall colored selection would be a valuable contribution to the southern palette of landscape trees. Species needs to be domesticated like sugar and red maples.”

Tom Cox, Cox Arboretum, Canton, Georgia*: “A. skutchii* has been a solid performer here in north Georgia. It takes the heat and humidity without showing any stress and easily survived 2 F degrees for several days this past January. Leaf retention in the fall is longer than either *A. saccharum*, *rubrum* or *nigrum* 'Green Column' and it colors up later. I like this as it extends fall color in the arboretum. During the spring and summer, it is thus far unremarkable. Its leaf appears pathogen free and is still clean at time of absicission. The leaf is smaller than anticipated so I’m wondering if my two trees might have some mixed parentage. I would rate its vigor as moderate, although this might change as it forms a deep taproot. This suspicion is based on my experience with *Acer nigrum* which took several years before it took off and is now a fast grower.”

Mark Weathington, JCR Arboretum, Raleigh, NC: “We have had mixed success. We lost our oldest plant this winter but it was not a cold issue. That tree had been declining for years, had some canker issues and just generally never looked very good for as long as I've known it. We received a plant from you in December 2007 that was 15 in (38 cm) tall. It was planted spring 2008 and when last accurately measured in 2012 was 20 ft (6.1 m) tall with a 10 ft (3.0 m) spread. It has put on at least 5 ft (1.5 m) since then. It is growing in a very loose soil that has almost perfect drainage for excess water but still retains moisture well but leaves lots of air space. It seems to perform best for us in this type of situation (what doesn't?) but doesn't fare quite as well in very heavy soils where growth is much slower. We had no winter damage at 7.5 F (-13.6 C) this winter and we have not had issues in other winters with it leafing out early and getting hit by later frosts. So far, we haven't had great fall color on any of our trees."

Results and Discussion: Besides conservation of rare maple genetics, there are horticultural reasons to capitalize on skutch maple traits that might benefit progeny. Certainly, fast growth rate and drought tolerance is in line with the long term trend facing the southern USA, a warmer and perhaps drier climate. Alkalinity tolerance may create opportunities in regions where sugar maples often fail. Certainly, cooperator and our own observations reveals trees exhibiting negative branching habit, poor form, multi-leaders, and a training problem for nurserymen. However, there is variety in form and habit; we have selected a dozen for improved form, new growth color, and height/trunk diameter characteristics. Those will be planted for further evaluation. Fall color selections are years away.

Literature Cited

1. Dirr, M. 2009. Manual of Woody Landscape Plants. Stipes Publishing, Champaign, Ill.: 67.
2. Lara-Gomez, G, Gailing, O, Finkeldey, R. 2005. Genetic variation in isolated Mexican populations of the endemic maple *Acer skutchii* Rehd. Allgemeine Forst Und Jagdzeitung 76 (6). Jun-Jul 2005.p.97-103 J D Sauerlanders Verlag, Frankfurt.
3. Efraim Hernandez X, Howard Crum, Wm. B. Fox and A. J. Sharp. 1951. A Unique Vegetational Area in Tamaulipas. Bulletin of the Torrey Botanical Club Vol. 78, No. 6 (Nov. - Dec., 1951), pp. 458-463.
4. Paul S. Martin and Byron E. Harrell. 1957. The Pleistocene History of Temperate Biotas in Mexico and Eastern United States. Ecology, Vol. 38, No. 3 (Jul., 1957), pp. 468-480.
5. Rehder, A., 1936. A new species of Acer from Guatemala. Jour. Arnold Arbor 17: 350-351.
6. Simpson, B. and B. Hipp. 1993. Maples of the southwest. American Nurseryman 177(5): 26-35.
7. Yalma Vargas-Rodriguez and William Platt. 2012. Remnant sugar maple (Acer saccharum subsp. Skutchii) populations at their range edge: Characteristics, environmental constraints and conservation implications in tropical America. Biological Conservation 150: 111-120.
8. Yalma Luisa Vargas-Rodriguez. 2005. Ecology of Disjunct Cloud Forest Sugar Maple Populations (Acer saccharum ssp. skutchii in North or Central America. PhD dissertation, Louisiana State University and Agricultural and Mechanical College, Department of Biological Sciences, Louisiana State University, Baton Rouge, La. http://etd.lsu.edu/docs/available/etd-07152005-083738/unrestricted/Vargas-Rodriguez_thesis.pdf.

Figure. 1. Skutch maple plot at the Science Research Center, Stephen F. Austin State University, Nacogdoches, Texas, June 25, 2014.

Figure. 2. Skutch maple new growth, 2014

Seedling Variations of *Ilex crenata* Thunb. 'Sky Pencil'

Yujie Yang[1,2], Donglin Zhang[1], Jinying Dong[1], and Zhihui Li[2]

[1]Department of Horticulture, University of Georgia, Athens, GA 30602, USA

[2]College of Forestry, Central South University of Forestry and Technology
Changsha, Hunan 410004, China

yujie@uga.edu

Index Words□breeding, diversity, genetic relationship, holly, open pollination, seedling population

Significance to Industry: Nursery industry demands new and improved ornamental plants for the better marketing and gardeners' interest. Open pollination is one of the best ways to breed and select new cultivar. Variations from a seedling population are expected. However, the seedling population from *Ilex crenata* 'Sky Pencil' did not have any sibling with its mother plant's narrow upright habit. To investigate the variations of this small seedling population, both morphological and molecular ISSR data were collected and analyzed. Seedling variations from an open pollinated mother plant were likely from different pollen donors (fathers). The conclusion should better help our ornamental plant breeders for breeding and selecting new cultivars for our nursery industry from open pollinated populations.

Nature of Work: *Ilex crenata* Thunb. is a member of holly family (Aquifoliaceae) native to eastern China, Japan, Korea, Kuril, Sakhalin, Philippines and the Himalayas. It is widely planted as an ornamental plant in the southeastern US for its dense evergreen foliage (Dirr, 2009). Many cultivars have been released for commercial production. Generally, mutation and cross-hybridization are two major ways to get new cultivars. Usually natural mutation is an accidental event, which could not be controlled. Cross-hybridization becomes the major source to select new cultivars. In order to explore open pollination, we studied seedlings variation of *Ilex crenata* 'Sky pencil', which is popular in the landscape for its strongly fastigiated, thin growth habit and lustrous, dark evergreen leaves (Dirr, 2011).

Inter-simple sequence repeat (ISSR) is a polymerase chain reaction (PCR)-based technique with primers composed of microsatellite sequences. It is often preferred over other molecular makers for generating a high number of polymorphism and does not require prior sequence information (Zietkiewicz et al., 1994). It is thought to be particularly useful for studying closely related individuals. It has been applied as a useful alternative to fingerprinting and genetic analysis in ornamental plants, including genetic diversity (Chezhian et al., 2012; Zhao, 2007), genetical relationships (Hu et al., 2006), origins of the cultivars (Lombrado et al., 2012), cultivar discrimination (Monte-Corvo et al., 2001) and fingerprinting of plants (Liu et al., 2012). In particular, ISSR marker was applied to evaluate the genetic diversity of *Ilex integra* (Leng et al., 2005) and the relationship of *Ilex* plants (Zhou et al., 2009). In this study, ISSR was applied to evaluate genetic diversity of *Ilex crenata* 'Sky Pencil' seedlings.

A total of 37 plants were collected in this study. Among them, 36 seedlings were from open pollination from one *Ilex crenata* 'Sky Pencil' mother tree and cultivated at the Horticulture Farm at the University of Georgia (Athens, GA) by Dr. Michael A. Dirr in 2006.

Total genomic DNA was isolated from young leaves using the DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA) following manufacturer's protocols. DNA concentrations were measured using a Nanodrop Lite Spectrophotometer (Wilmington, DE) and DNA samples were diluted to a concentration of 10 ng/µL with double distilled water (DDW) for downstream applications.

ISSR primers were obtained from the University of British Columbia (UBC 801-900). Out of 100 primers, sixteen ISSR primers were used for screening and exhibiting genetic variation among all accessions. PCR reaction was performed in a total volume of 20 µl in a nexus gradient mastercycler (Eppendorf AG, Hamburg, Germany). The reaction mixture included: 10µl Master Mix (Applied Biosysterms, Foster city, CA), 1.2µl primer (12µM), 1.0µl template DNA (0.5ng/µl), 7.8µl DDW. ISSR-PCR amplification procedure included initial denaturation at 94^0C for 5 min followed by 35 successive cycles of denaturation at 94^0C for 30s, annealing at 54^0C for 45s, extension at 72^0C for 90s. Final extension was performed for 7 min at 72^0C and then held at 4^0C. After PCR operation, amplified products were checked by separating on 1.2% agarose gel electrophoresis (with 0.5mg/L ethidium bromide) for 1h at a constant temperature of 120V with 0.5×TBE buffer solution and 100 base pairs (bp) DNA ladder. Gels were visualized via ultra-violet light, photographed, and documented.

Gel image were analyzed using the DNA marker ladder for fragment length calibration. Only the clear bands were scored and the faint bands were ignored. The same size band for a data set was assumed to represent a single locus. For presence or absence of an ISSR band at a particular locus, the data were recorded as 1 for presence and 0 for absence to build binary matrices. The matrix of '01' was analyzed using PAUP (Swofford, 2002) and POPGENE (Yeh et al., 1999).

Results and Discussion: Sixteen inter-simple sequence repeat primers were used to study the genetic variations of *Ilex crenata*. The primer information, the number of bands, number of polymorphic bands and percentage of polymorphism were presented in Table 2. The highest number of fragments (22 fragments/bands) was amplified with the $(AG)_8YC$ primer (UBC835), while the lowest number (11) was amplified with $(GGGTG)_3$ primer (UBC881). The average number of fragments per primer was 15.1. A total of 242 fragments were generated and the fragment size amplified with all 16 primers was similar and ranged from 150-2000 bp. Out of the 242 loci surveyed, 167 were polymorphic (69%). Percentage of polymorphism for primer UBC856 was highest at 85.7% while primer UBC889 the lowest at 47.1%. The results indicated that these 16 ISSR primers used in this study revealed a wide range of genomic DNA diversity in this open pollinated *Ilex* population. Fingerprinting of 24 open pollinated Ilex seedlings DNA band patterns from primer UBC807 were presented in Figure 1.

Binary matrices from the DNA fingerprints produced by 16 ISSR markers used for Ilex were imported into PAUP and POPGENE software for analysis of genetic similarity. Based on all useful ISSR markers, pairwise distances were calculated (data not shown). Pairwise distances ranged from 0.13223 to 0.35124. *Ilex* #8 and *Ilex* #31 showed minimal pairwise distance, while *I. crenata* 'Sky Pencil' mother tree had the highest pairwise distance to *Ilex* #01. The data for observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index for all accessions were analyzed using 16 ISSR primers and their respective values were found as 1.5964, 1.4179, 0.2359 and 0.3444 (Table 3). Compared with that in *Ilex integra* (Leng, 2005), which were 1.421, 1.273, 0.153 and 0.227, Ilex crenata 'Sky Pencil' seedlings had higher variations. It concluded that the genetic diversity of *Ilex crenata* half siblings was much richer than that of *Ilex integra*.

The relatedness between the individuals of *Ilex crenata* seedlings was generated (Fig. 2). Three major groups were observed in the UPGMA tree. *Ilex crenata* 'Sky Pencil' mother tree was clustered as one group. Group 2 had three accessions (*Ilex.* #05, *Ilex.* #15, *Ilex.* #34). The other seedlings were classified into the third group. The mother tree *Ilex crenata* 'Sky Pencil' has a typical character, which is straight narrow habit. Among the seedlings, no one has the same habit as its mother. In Group 2, *Ilex* #05, *Ilex* #15, and *Ilex* #34 were morphological similar. All of them have compact elliptical habit and their leaf margin had serration from the middle of the leaf and entire at the base. The seedlings in Group 2 had uniform leaf size. From both morphological and molecular data, Group 2 and Group 3 might have different pollen contributors. Dr. Dirr listed 61 *Ilex crenata* cultivars (Dirr, 2009). We hypothesed one father was *Ilex crenata* 'Compacta', which had global habit and flat lustrous dark green leaves. The other was *Ilex crenata* 'Beehive', which was a dense, compact mounded form and slightly wider than high with lustrous dark green foliage. It should be not too difficult to speculate that *Ilex crenata* 'Compacta' was the father of Group 2 and *Ilex crenata* 'Beehive' for Group 3. Plants in Group 3 had high divergence. The putative father of Group 3, *Ilex crenata* 'Beehive' originated as a cross of *Ilex crenata* 'Convexa' × *Ilex crenata* 'Stokes' and the leaves were light olive green in Georgia trails. 'Convexa' had round to spreading habit with matte green convex leaves while 'Stokes' rounded habit with glossy leaves. In Group 3, the convex leaves could be inherited from 'Convexa'. Though plants in Group 3 have the same putative father, they still had high divergence in morphology and genetic variation. It indicated that even though they had same gene sources, their genetic contribution from the parent was not half and half. Heritability estimation could be obtained from the partitioning of phenotypic variance into genetic and environmental components or by studying the degree of resemblance between relatives by rather simple measurements made on the population (Falconer et al., 1996). Both morphological and ISSR marker results concluded that the seedling population had high genetic and morphological diversity. Further studies should address the detailed contribution percentage from its father and mother plants. Open pollination has proven to be useful to broaden the genetic base of breeding programs and guarantee that enough variability should be available for cultivar breeding in future generations (Badenes et al., 2011; Kumar et al., 2010). Plant selection should start from F_1 generation and molecular markers are valuable for assessing genetic diversity and relationship in plant breeding.

Literature Cited:

Badenes, M. and D.H. Byrne. 2011. Fruit breeding. Springer, New York.

Chezhian, P., R. Yasodha, and M.G. Dasgupta. 2012. Molecular discrimination of eucalypt clones using the ISSR marker system, Journal of Sustainable Forestry, 31:537-548.

Dirr, M.A. 2009. Manual of woody landscape plants (6th ed). Stipes Pub. Co., Champaign, Illinois.

Dirr, M.A. 2011. Dirr's encyclopedia of trees and shrubs. Timber Press, Portland and London.

Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Longmans Green, Harlow, Essex, UK.

Hu, D.Y., Z.S. Zhang, D.L. Zhang, Q.X. Zhang, and J.H. Li. 2006. Ornamental peach and its genetic relationships revealed by inter-simple sequence repeat (ISSR) fingerprints. Acta Horticulturae 713:113-120.

Kumar, S., R.K. Volz, P.A. Alspach, and V.G.M. Bus. 2010. Development of a recurrent apple breeding programme in New Zealand: a synthesis of results, and a pro- posed revised breeding strategy. Euphytica 173:207-222.

Leng, Xin, Zhongsheng Wang, Shuqing An, Jue Feng, Yuhong Liu, and Guoming Wang. 2005. ISSR analysis of genetic diversity of *Ilex integra*, an insular endemic plant. Biodiversity Science 13(6):546-554.

Liu, B.Y., H. Cheng, Y.Y. Li, L.Y. Wang, W. He, and P.S. Wang. 2012. Fingerprinting for discriminating tea germplasm using inter-simple sequence repeat (ISSR) markers. Pak. J. Bot. 44(4):1247-1260.

Lombrado, G., R. Schicchi, P. Marino, and F. Palla. 2012. Genetic analysis of *Citrus aurantium* L. (Rutaceae) cultivars by ISSR molecular markers. Plant Biosystems 146(Supplement):19–26.

Monte-Corvo, Luisa, Luis Goulão, and Cristina Oliveira. 2001. ISSR analysis of cultivars of pear and suitability of molecular markers for clone discrimination. J. Amer. Soc. Hort. Sci. 126(5):517–522.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321-3323.

Swofford, D.L. 2002. PAUP, phylogenetic analysis using parsimony. Version 4.0b10. Sinauer, Sunderland, Mass.

Yeh, C.F., R.C. Yang, T. Boyle. 1999. POPGENE 32, population genetics software. Version 1.31.

Zhao, K.G., M.Q. Zhou, L.Q. Chen, D.L. Zhang, and G.W. Robert. 2007. Genetic diversity and discrimination of *Chimonanthus praecox* (L.) link germplasm using ISSR and RAPD markers. HortScience 42(5):1144-1148.

Zhou, Xijun, Dongmei Zhang, Yulan Luo, Jinle Su, and Jianxiang Li. 2009. Inter simple sequence repeats ISSR marker analysis of *Ilex* plants species and its application. Journal of Henan Agricultural University 43(2):196-200.

Zietkiewicz, E., A. Rafalski, and D. Labuda. 1994. Genome fingerprint by sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.

Table 1: Morphological data of 36 *Ilex crenata* seedlings and *Ilex crenata* 'SkyPencil'.

No.	Node per 10 cm[z]	Height (cm)	Width (cm)	H/W ratio	Branch angle	Foliage color[y]	Leaf length (mm)	Leaf width (mm)	Compactness rank[x]
0	16	304.8	49.53	6.15	0	D	23	10	5
1	21	152.4	129.54	1.18	50	L	19	9	3
2	21.3	190.5	152.4	1.25	40	D	21.7	8	3
3	21	208.28	121.92	1.71	35	D	17.2	8.2	3
4	18	167.64	154.94	1.08	35	D	20.6	7.8	2
5	18	187.96	109.22	1.33	25	D	17.3	8	4
6	22.3	152.4	142.24	1.18	30	D	15.7	7.7	3
7	18.7	170.18	139.7	1.35	40	D	18	8.3	4
8	22	152.4	160.02	0.95	45	D	15	6.8	3
9	15.3	170.18	93.98	1.81	10	D	22	10.8	5
10	17.3	167.64	147.32	1.14	50	D	16.7	7.8	3
11	15.3	139.7	142.24	0.98	35	L	19.3	8.8	3
12	19	177.8	147.32	1.21	25	D	20.7	8.5	4
13	19.3	205.74	142.24	1.45	25	D	17	7.7	4
14	19.7	203.2	218.44	0.93	50	L	16.7	7.7	1
15	15	195.58	144.78	1.35	25	D	18.7	8.7	4
16	17	218.44	203.2	1.08	30	D	19.7	8.2	3
17	18	246.38	149.86	1.64	25	D	20.3	8.5	3
18	16	195.58	170.18	1.15	45	D	20.3	8	2
19	19	223.52	226.06	0.99	45	D	21	8.7	2
20	21	213.36	160.02	1.33	40	D	19	7.2	3
21	21	185.42	160.02	1.16	50	L	18.3	7.8	3
22	18.3	167.64	152.4	1.10	45	L	19.3	7.5	3
23	13.7	205.74	203.2	1.01	35	D	15.2	7.2	2
24	16	218.44	167.64	1.30	25	D	21.7	9.2	3
25	15	205.74	203.2	1.01	30	D	16.3	7.3	3
26	19.7	228.6	241.3	0.95	60	D	15.3	8	2
27	12.7	185.42	114.3	1.62	20	D	19.3	7.7	5
28	21	170.18	142.24	1.20	55	D	15.3	7	3
29	16.7	152.4	165.1	0.92	65	D	18	8.2	3
30	19	147.32	233.68	0.63	70	L	13	7.3	2
31	26	160.02	190.5	0.84	60	L	17	7.8	1
32	14.7	182.88	167.64	1.09	50	D	18	7.5	3
33	15.7	177.8	142.24	1.25	35	D	22.3	7.7	3
34	14.7	147.32	104.14	1.41	30	L	21	9	4
35	18	147.32	111.76	1.32	25	L	17.7	7.8	4
36	23.7	175.26	142.24	1.23	25	L	16.7	8	5

[z]Node per 10cm: number of leaves on a 10 cm branch.
[y]Foliage color: D for dark green or L for light green.
[x]Compactness rate: 1-5, loose-compact.

Table 2: ISSR results generated by 16 primers in 36 *Ilex crenata* seedlings and *Ilex crenata* 'Sky Pencil'.

Primer code	Sequence (5'-3')[z]	No. of markers amplified	No. of polymorphic markers	Amplicon range (bp)	Percent polymorphism
UBC807	$(AG)_8T$	15	11	240-980	73.3%
UBC 809	$(AG)_8G$	12	8	260-950	66.7%
UBC 810	$(GA)_8T$	18	12	200-1000	66.7%
UBC 811	$(GA)_8C$	15	9	200-1200	60.0%
UBC 814	$(CT)_8A$	12	9	300-1500	75.0%
UBC 825	$(AC)_8T$	14	8	280-1500	57.1%
UBC 827	$(AC)_8G$	13	11	320-2000	84.6%
UBC 834	$(AG)_8YT$	15	9	280-1500	60.0%
UBC 835	$(AG)_8YC$	22	17	150-1200	77.3%
UBC 836	$(AG)_8YA$	13	9	350-1300	69.2%
UBC 840	$(GA)_8YT$	18	12	150-1300	66.7%
UBC 856	$(AC)_8YA$	14	12	350-1800	85.7%
UBC 881	$(GGGTG)_3$	11	7	400-1500	63.6%
UBC 889	$DBD(AC)_7$	17	8	260-1400	47.1%
UBC 890	$VHV(GT)_7$	15	11	280-1100	73.3%
UBC891	$HVH\ (TG)_7$	18	14	220-1000	77.8%
Total		242	167	150-2000	69.0%

[z] Y = (C , T), B = (C , G, T), D = (A , G, T), H = (A , C , T), V = (A , C , G).

Table 3: Genetic variations using ISSR markers in the *Ilex* seedling population.

	Na[z]	Ne[y]	H[x]	I[w]
Mean	1.5964	1.4179	0.2359	0.3444
St. Dev	0.4915	0.3984	0.2123	0.3021

[z]Na = Observed number of alleles
[y]Ne = Effective number of alleles
[x]H = Nei's (Nei, 1973) gene diversity
[w]I = Shannon's Information index

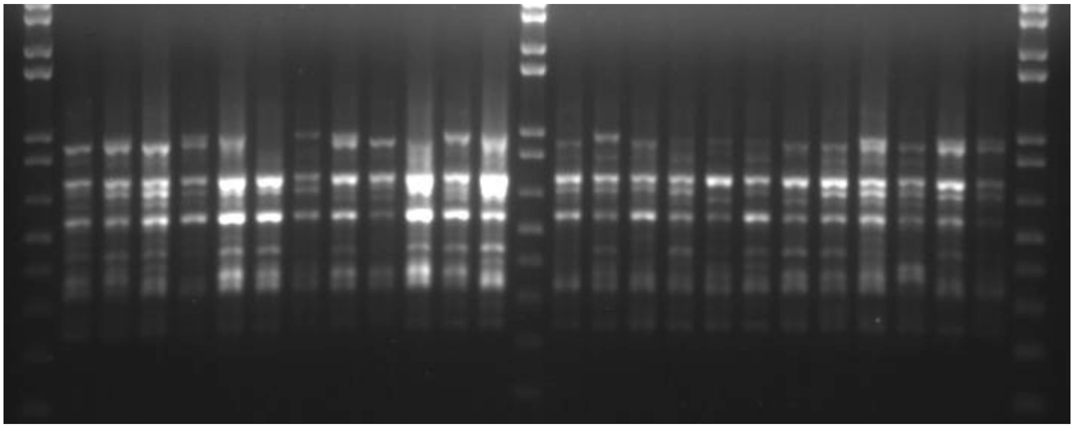

Figure 1: ISSR PCR band pattern of 24 half siblings from Primer UBC807.

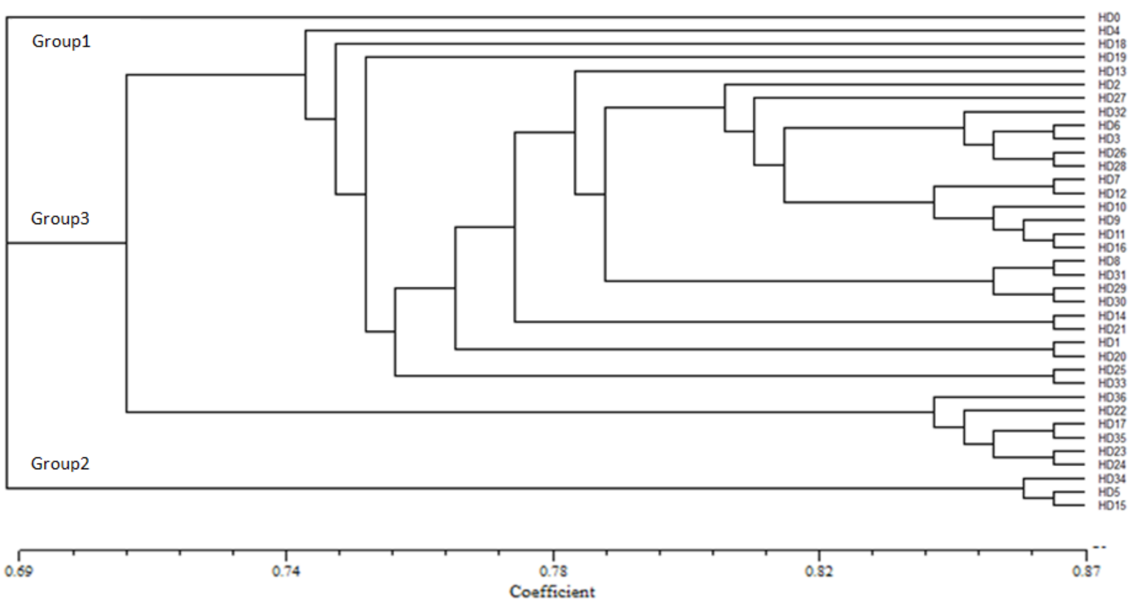

Figure 2: UPGMA dendrogram of 36 *Ilex crenata* seedlings and *Ilex crenata* 'Sky Pencil' (HD0) based on ISSR markers.

Evaluation of Evergreen *Quercus* at the JC Raulston Arboretum

Mark Weathington

JC Raulston Arboretum, NC State University
Department of Horticultural Science
Campus Box 7522, Raleigh, NC 27695

mark_weathington@ncsu.edu

Index words: *Quercus*, oak, landscape trials, species trials, broadleaf evergreen

Significance to the Industry: New plants help fuel the growth of the green industry but nursery professionals must balance new plants with the public's often slow acceptance of the unknown. Entirely new genera often need a longer learning curve before the public accepts them. The public accepts familiar genera, such as *Quercus*, more readily even when the species are novel to them.

Evergreen *Quercus* offer the nursery industry a group of plants that fit many needs of the public. They offer year-round interest, typically form medium sized trees suitable for suburban landscapes although some are shrubby, are generally easy to grow, and are from a familiar genera. Due to their limited hardiness, they will be relegated to warmer southeastern gardens, mostly zone 7b and warmer. Several species are currently available in small numbers from specialty nurseries and provide opportunities to acquire plants for use as propagation stock.

Nature of Work: The JC Raulston Arboretum (JCRA) evaluates a wide diversity of woody plants for suitability to the central piedmont region of North Carolina and the broader southeastern United States. *Quercus* have been an important component of the collections of the JCRA since its inception in 1976. The first plant installed by J. C. Raulston when establishing the Arboretum was *Quercus robur* f. *fastigiata* (Lam.) Schwarz, a farewell gift from his colleagues at Texas A&M University. In recent years, evergreen and semi-evergreen species have gradually been accumulated through wild collections and from cultivated material to add to the existing specimens that pre-dated the Arboretum. Many of these *Quercus* are poorly understood and rarely grown in western gardens but may be suitable for wider use throughout the southern United States.

The genus *Quercus* is comprised of 400−600 species (1, 2, 3, 4) in the Fagaceae with new species and naturally occurring hybrids being described with some regularity. The genus ranges across North America south through Central America to Columbia, Europe, north Africa, and Asia where it crosses into the southern hemisphere (2, 4, 5). The center of diversity is in southern Mexico where the genus is undergoing active speciation (6). Where species overlap throughout their native ranges or in cultivation there can be considerable hybridization. These factors make the taxonomy of the oaks difficult at best.

Most oaks form medium to large trees but can also be shrubby or colony forming rhizomatous sub-shrubs. North American species are often separated into red oak and white oak groups. Red oaks have bristle tips on leaves and seed (acorns) take two years to mature. White oaks lack bristle tips and acorns mature in one year. While this grouping works well with many of the temperate North American oaks, it does not adequately cover the diversity in the genus especially the evergreen Asian species and the subtropical and tropical species. Using the taxonomy of Grimshaw (3), *Quercus* is broken into two subgenera. The strictly Asian evergreen subgenus *Cyclobalanopsis* which is often placed at the genera level by Asian taxonomists (7,8) distinguished by acorn cupule scales fused into concentric rings and the widespread subgenus *Quercus* whose cupule scales are free and spirally arranged. Subgenus *Quercus* is further divided into four sections by Grimshaw (Table 1).

Some classifications admit more or fewer sections in subgenus *Quercus* or more subgenera (9,10). Menitsky (11) breaks subgenus *Cyclobalanopsis* further into eight sections. Recent research indicates the genus *Quercus* is separated into two clades each comprised of three groups (12).

Results and Discussion : Nineteen taxa from the *Cyclobalanopsis* subgenus and two of the sections of the *Quercus* subgenus are currently growing in the JCRA collection and are presented below with brief information on the habit, propagation, and hardiness as presently known. While many of these species have proven to be hardy, some over many decades, others have not been evaluated long enough to be certain of their cold tolerance. Many of the borderline species will show increased hardiness with age so protection when young is advised. The seed (acorns) of oaks are recalcitrant and must be sown fresh. Propagation of oaks by means other than seed is difficult at best for most species although Asian oaks in the *Cyclobalanopsis* subgenus show varying degrees of suitability for cutting production. Most other evergreen oaks root with difficulty or not at all. Published information for these and other evergreen *Quercus* in general can be found in the following resources (2,3,4,6,7,8,9,11,13,14,15,16,17).

Subgenus *Cyclobalanopsis*

Quercus acuta Thunb. syn. *Cyclobalanopsis acuta* (Japanese evergreen oak) (2,9,11,13,14,15,23) – An evergreen tree to 12 m (40 ft) from Japan, South Korea, and Taiwan. Leaves emerge with a brownish pubescence before becoming glossy, dark green and leathery, to 20 cm (8 in) long and about ⅓ to ½ as wide with no lobes or teeth, an undulate margin, and an acuminate tip. *Q. acuta* was introduced to the West by Charles Maries in 1878 (2). It is one of the hardiest evergreen oaks from Japan making a handsome tree with smooth gray bark. It has been much confused in the nursery industry with *Q. glauca* and *Q. myrsinifolia*. Our plant was received from the US National Arboretum in 1994 and is now 9 m (30 ft) tall. It is generally listed as a zone 8 plant (13) but with its distribution, hardiness is likely dependent on provenance. Our plant has proven to be reliable in zone 7b at least. Reports indicate that summer moisture is necessary for adequate growth (14), typically not a problem in the southeastern U.S. Propagation by sowing fresh seed is easy, softwood cuttings, just past the tenderwood stage can be rooted using 1000ppm KIBA (pers. observation) and reports indicate dormant cuttings under mist with bottom heat and medium levels of hormone root in as little as 3−4 weeks (14).

Quercus glauca Thunb. syn. *Cyclobalanopsis glauca* (Japanese blue oak) (2,7,8,9,11,13,14,15,23) – Along with *Q. myrsinifolia*, with which it is often confused in the trade, Japanese blue oak is the most common evergreen Asian oak found in western gardens. It has a relatively wide distribution across east Asia from Japan and Taiwan across south China to the Himalaya below 1525 m (5000 ft). In the garden, it forms an upright pyramidal to conical crown growing to 9 m (30 ft) at maturity although trees can ultimately grow to twice that height. The foliage of *Q. glauca* grows to 15 cm (6 in) long at most and is elliptical to obovate with an acuminate tip and coarse serrations or teeth along the distal ½−⅔ of the leaf. New foliage emerges with a light silky pubescence and waxy coat for a bluish to purplish character which becomes medium green to blue-green as the season progresses. With the typical summer moisture and heat of the Southeast, *Q. glauca* performs very well. It is reliably hardy to zone 8 and there are many plants growing happily in zone 7 gardens. Grow in full sun to part shade. The JCRA plant predates the Arboretum so its source is unknown. In 1985 the record low for Raleigh, NC, -23 C (-9 F) killed the established tree to the ground. It subsequently re-sprouted and is growing now as a multi-stem tree over 12 m (40 ft) tall. In drier locations and windy spots the leaves can yellow somewhat (14), a sign that supplemental water is needed. Propagation is by seed, very softwood cuttings, or dormant hardwood cuttings with bottom heat, mist, and medium to high hormone.

Quercus longinux Hayata. syn. *Cyclobalanopsis longinux* (Taiwan oak) (3,7,8,11) – This endemic Taiwanese tree is sometimes listed as a subspecies of the similar *Q. glauca* (11) but is generally recognized to be distinct. In its native habitat in broadleaved evergreen forests of Taiwan below about 2500 m (8200 ft), it can grow to 15 m (50 ft) often with the smooth gray barked trunk rising branchless to ⅔ of the total height (pers. observation). In the landscape expect a 10 m (33 ft) upright oval to round-headed tree. Foliage is similar to Japanese blue oak, growing to 13 cm (5 in). The JCRA plant was received from the Taiwan Forestry Research Institute index seminum in 2001 and is now over 2.4 m (8 ft) tall growing in sun and rich soil. Our plant has not fruited yet to verify its identity, but the elliptic to lanceolate deep green leaves appear to be correct. The 2014 winter temperatures, -13 C (7.5 F) caused significant tip dieback so this plant will likely be relegated to zone 8 gardens. Fresh seed germinates readily. We have not attempted cutting propagation to date, but based on its close affinity to the *Q. myrsinifolia*/*glauca* group, it may root with bottom heat, mist, and medium to high hormone levels.

Quercus morii Hayata syn. *Cyclobalanopsis morii* (Formosan evergreen oak) (3,7,8,11) – This rarely encountered Taiwanese endemic reportedly grows to 30 m (98 ft) in its habitat (3) often forming pure stands (7) at relatively high elevations, 2400 m (7800 ft). Shiny lanceolate to oblong leaves can grow to about 10 cm (4 in) long and 5 cm (2 in) wide but are often half that width. The distal end of the leaf is serrate and the margins often revolute. New growth often has a bronzy cast. Few plants are found in western landscapes and most of those are quite young. The JCRA plant was received from the

Taiwan Forestry Research Institute in 2011 but has not been grown long enough to evaluate. Plants from the highest elevation should prove hardy to zone 8 at least.

Quercus myrsinifolia Blume syn, *Cyclobalanopsis myrsinifolia* (Chinese live oak/bamboo oak) (2,7,8,9,11,13,14,15,23) – This Asian evergreen has proven to be one of the best for western gardens, performing well in both droughty West Coast landscapes and wetter southeastern gardens. It forms a medium tree to 10.5 m (35 ft) in the garden although trees can be twice as tall in the wild. It typically grows with a somewhat narrow crown eventually broadening to a rounded or gumdrop-shaped head. The foliage is about 12.5 cm (5 in) long, relatively narrow with a long drip tip and serrations along its edges. Perhaps its most striking attribute is the coloration of new growth which ranges from burgundy to near black before turning deep green with a bluish tinge. Mature trees have been known to survive temperatures well below -18 C (0 F) although its wide native range indicates hardiness may be affected by provenance. Hardy forms should be planted together to produce a good seed source for production. Acorns are produced on very young trees, often after as little as five seasons (pers. observation). Japanese nurserymen have selected several clones which are grafted on seedlings including the splashed variegation of ‘Shima Fu’, cream margined ‘Fun Fun’, and an unnamed clone with a pure white flush of new growth (pers. observation). Trees planted in full sun will develop a very uniform habit and make excellent street trees. Plants will tolerate relatively heavy shade but growth is slower and quite open. Two plants at the JCRA have performed well for many years, one for over 25 years.

Quercus salicina Blume syn. *Cyclobalanopsis salicina* (willow-leaf oak) (3,7,11) – Another evergreen in the Asian Cyclobalanopsis group growing to 18 m (60 ft) in the wild, perhaps ⅔ that height in cultivation. It is found in Japan, North and South Korea. It bears lanceolate foliage to 9 cm (3.5 in) long which is sharply serrate along the distal half. It is reportedly hardy only to zone 8, but germplasm from North Korea likely will prove much hardier. Plants have been growing in the Piedmont of North Carolina for at least a decade (pers. observation) and a specimen has grown well in an exposed location for 5 seasons at the JCRA. Propagation should be similar to other oaks in subgenus *Cyclobalanopsis*.

Subgenus *Quercus*
Section *Lobatae*

Quercus canbyi Trel. (Canby oak) (3,15) – Canby oak makes a 10 m (33 ft) tree, perhaps taller in cultivation, from Nuevo Léon, Mexico (3). This species can be evergreen but is often semi-evergreen or tardily deciduous. It was named in 1924 for Charles Marriott Canby (15) a Delaware businessman and avid botanist. It grows vigorously and easily in cultivation in both dry and moist summer situations but needs a relatively well-drained soil. Foliage is narrow and up to 9 cm (3.5 in) long and about ¼ as wide with a dark glossy green surface and 9−15 lobes with bristle tips, sometimes losing all but the terminal bristle with maturity. It has proven to be hardy at least to zone 6b. The JCRA plant was received in 2007 from Stephen F. Austin Mast Arboretum and is now 4.5 m (15 ft). It retains its leaves through most of the winter in Raleigh, generally going mostly deciduous by mid-February. This species is becoming more widely planted in Texas over the last decade. Propagation is by seed.

Quercus crassifolia Bonpl. (leather-leaf Mexican oak) (2,9) – A tall evergreen species from Mexico and Guatemala growing to 15 m (50 ft) tall although it is also described as a 30 m (100 ft) deciduous species (2, 9). It bears very stiff, leathery, deep blue-green foliage to 16 cm (6 in) , ovate to obovate with widely spaced, bristle-tipped teeth. The adaxial surface of the foliage is rugose while the abaxial is covered in a wooly cream to tan indumentum. New growth emerges brilliant red which is quite showy against the dark green of the previous season's growth. Plants grow stiffly upright with somewhat sparse branching. The JCRA plant has been exceptional with no winter damage after at least 16 years, growing to 10.5 m (35 ft) in that time. While many of the Mexican oaks require a loose, well-drained soil, our plant has thrived in a heavy soil with considerable neglect in full sun. This is certainly a plant which deserves wider consideration as a large tree, perhaps even suitable for urban conditions, we have been unable to root it and it has refused to develop any acorns to date.

Quercus inopina Ashe (Florida oak) (19) – This evergreen from sandy ridges in central Florida forms a mounding shrub that can grow to 4.5 m (15 ft) tall (19). The foliage is typically ovate but can range from elliptic to nearly round or spatulate and grow to 9 cm (3.5 in) long often with several bristle tips. The margins are convex giving even a well-hydrated plant a somewhat parched appearance. The adaxial surface is olive green with distinct yellowish veins while the underside is somewhat tawny tomentose. Florida oak needs well-drained soil, a sunny location, and is drought tolerant in the landscape. The JCRA plants from Woodlanders Nursery, South Carolina and Xenoflora LLC, Florida have only been in the ground for two seasons and have consequently not been fully evaluated but the species is likely of interest only to collectors or for naturalistic settings. Despite the young age of the JCRA plants, both survived -13.5 C (7.5 F) in the garden. Propagation is by seed.

Quercus sartorii Liebm. (Sartor's oak) (3)– A Mexican oak which forms a sizeable tree to 20 m (65 ft) with lanceolate 13 cm (5 in) leaves with entire margins on mature foliage but widely spaced, shallow serrations on juvenile foliage. The glossy green leaves emerge covered in a sparse yellow pubescence which is quickly lost. The JCRA tree from Yucca Do Nursery, Texas has only been in the ground for two seasons but shows promise with no damage in a very exposed situation. Propagation is by seed.

Section *Quercus*

Quercus durata Jeps. (leather oak) (16,17) – This is a low shrub to about 1.5 m (5 ft) native to the West Coast from Monterey to Napa, California (16). Small leaves are oval and convex with wavy margins and sharp equal teeth. It requires full sun and excellent drainage. We received our plant from ForestFarm Nursery, Oregon, in 2007. It was growing relatively well in very well-drained soil but did not survive -13.5 C (7.5 F) during the winter of 2014. Propagation is by seed.

Quercus germana Schltdl. & Cham. (Mexican royal oak) (3) – This eastern Mexican tree makes a semi-deciduous plant to 10.5 m (30 ft) with a pyramidal habit when young, becoming rounded with age in cultivation. Foliage emerges pink to bright red in spring and subsequent flushes during the summer, maturing to deep glossy green, 20 cm (8 in) by 5 cm (2.5 in). With summer heat, it appears to be hardy to zone 7 but in cool summer locations the wood does not harden off causing significant dieback (3). Mexican royal oak is threatened due to habitat loss because of deforestation for coffee plantations (3). At the JCRA, our plants have remained evergreen down to close to -15 C (5 F). One tree procured prior to 1995 is now 7.6 m (25 ft) and another received from Stephen F. Austin Mast Arboretum in 2007 is now 3.6 m (12 ft). This oak has been among the finest of the Mexican oaks at the JCRA and is available in limited landscape sizes and quantities in Texas (D. Creech, pers. comm.). It deserves more attention as a potential landscape specimen for the south. Propagation is by seed.

Quercus ilex L. (holm oak) (2,9,10,14,15) – This native Mediterranean species is widely grown in Europe and Great Britain where it forms large trees to 21 m (70 ft) or larger if grown in a favorable location (2). It develops a bushy crown over time with branches becoming pendulous with age. Small oval to elliptic leaves to 7 cm (2.75 in) are sparsely toothed on immature plants often becoming entire with age. New growth is covered with a fine whitish pubescence which is retained on the abaxial surface often turning gray or tan. Although one of the most prevalent of evergreen oaks in western landscapes, it is rarely seen in the southern U.S. It is well adapted to droughty areas and could prove to be a valuable street tree. It should be perfectly hardy to at least zone 7 (14). It has made a handsome if somewhat shrubby tree at the JCRA but has not shown the growth rate seen in other areas only putting on about 4.5 m (15 ft) of growth in 25 years. The slow growth could be attributable to the heavy clay soil it is situated in since Bean (2) notes it prefers “rather light soil” and a sunny location. Propagation is by seed. There are quite a few clones (18) which have been grafted on seedling stock or rooted.

Quercus marlipoensis Hu & W. C. Cheng (Yunnan maple) (8,11,20) – This exceptionally rare evergreen maple is from low elevations in a restricted locality in south China near the Yunnan–Guangxi provincial and Vietnam border (20). In the wild, it can grow quite tall to 18 m (60 ft) (11). It bears long elliptic to obovate leaves to 22 cm (8.5 in) which are rusty stellate tomentose especially when young and along the midribs of the leathery mid-green leaves. Stout young branches are covered in rusty pubescence with the entire appearance of young plants bringing to mind *Eriobotrya japonica*. The JCRA received this rarity from Dr. Jenny Xiang of North Carolina State University in 2006 and planted it out in 2009. It suffered some winter dieback over the next three years and never increased much in size. The -13 C (7.5 F) temperatures in 2014 killed the plant outright. The mean temperature of the coldest month in its native range is 9.9 C (50 F) (20) so lack of hardiness is no surprise and *Q. marlipoensis* is likely suitable only for sub-tropical landscapes. Propagation is by seed.

Quercus phillyreoides A. Gray (ubame oak) (2,4,7,8,9,11,14,15,23) – This tree is often called the Japanese equivalent of our native *Q. virginiana* but it ranges well beyond Japan into Korea and across much of south and south-central China. It is indeed closely related to our native live oak and grows as an upright tree often branching near ground level unless trained to a single trunk. Reports indicate it may grow to 15 m (50 ft) in the wild but 10 m (35 ft) is more realistic as a landscape tree. Foliage is 7.5 cm (3 in), yellowish olive green, oval, often with fine serrations which may be bristle-like on juvenile growth and leaf tips. It has proved to be quite hardy, to at least zone 7. One plant at the JCRA is reported to be over 50 years old although records pre-dating the Arboretum are sadly lacking. This tree reportedly suffered little or no damage during the devastating freezes of the mid-1980s. The multi-stemmed nature of this plant and its reported 4 m (14 ft) height in the late 1980s indicate it may have been killed back to the ground. In wet seasons plants growing in shade may develop some foliar fungal problems but these have not appeared to affect the health of the trees. Propagate by seed or dormant cuttings with bottom heat and high hormone levels. The JCRA introduced a very upright, nearly fastigiate form as 'Emerald Sentinel' which is reportedly hardy to near zone 6 and is perhaps somewhat easier to root than the species. Several variegated specimens can be found in Japanese specialty nurseries (pers. observation).

Quercus polymorpha Schltdl. & Cham. (Monterrey white oak) (3,15) – This lovely evergreen to semi-evergreen tree ranges from Texas south through Mexico to Guatemala, growing to 20 m (65 ft) in cloud forests. In typical southern gardens expect a mature height closer to 12 m (40 ft) with pyramidal growth when young becoming ovate with age. The long, up to near 15 cm (6 in), leaves are quite variable ranging from elliptic to ovate, typically entire or with some coarse serrations along the margins of the distal half. New foliage emerges shrimp pink to rich red covered in gold hairs before becoming glabrous dark green to deep blue-green. The tree has become quite popular in Texas as a landscape tree (D. Creech, pers. comm.) and deserves much wider consideration across more of the South. Best in full sun, it is hardy to zone 7 but expect it to be fully deciduous in cold winters. Propagate by seed. This is a plant that could possibly benefit from selection of forms with superior new growth coloration.

Quercus pungens Liebm. (sandpaper oak, pungent oak) (9,21,22) – This southwestern species ranges from Arizona, New Mexico, Texas, and into Mexico growing in dry, well-drained soils. It is typically a multi-stemmed evergreen to semi-evergreen shrub to 2 m (6.5 ft) but can grow as a tree to 8 m (26 ft) tall (22). Coarsely toothed lanceolate foliage to 9 cm (3.5 in) is semi-evergreen in Raleigh, North Carolina. where a plant from Woodlanders Nursery grows as a 3 m (10 ft) tall and wide shrub. It likely requires excellent drainage and full sun. In the JCRA's Xeric Garden it suffered no ill effects from -13.5 C (7.5 F). In the wild, it grows on shallow soils and may be a candidate for green roofs in the South.

Quercus rugosa Née syn. *Q. reticulata* (net-leaf oak) (3,12,14,15) – This oak has perhaps the widest distribution of any Mexican oak. It has a quite variable habit, growing as a tree to at least 12 m (40 ft) or as a shrub. The name is notorious for being attached to many of the unidentifiable oaks found in Mexico. A pair of sibling seedlings from a Yucca Do Nursery collection have grown to about 7 m (23 ft) tall with multiple stems at the JCRA. The foliage can grow to 23 cm (9 in) (14) but the JCRA plants rarely grow larger than 10 cm (4 in) long and half or more as wide. The obovate corrugated foliage emerges with a creamy pubescence, the adaxial surface quickly becoming glabrous dark green. The abaxial surface remains covered in a creamy pubescence. The leaf margins are sharply toothed, occasionally forming a pair of lobes, and often curled down in a presumed drought adaptation. It is quite hardy especially where it receives adequate heat to harden the wood, perhaps to zone 6 especially in areas with dry autumns. In the moist Southeast, it may be slightly less hardy but forms collected at high elevations should certainly be hardy to at least zone 7. The JCRA plants have not suffered winter damage but do become semi-deciduous in cold winters. Propagate by seed.

Quercus turbinella Greene (Sonoran scrub oak) (3,17,21) – Growing typically as a shrub but occasionally as a multi-stem tree to 10 m (33 ft), this evergreen desert dweller ranges across much of the Southwest from Colorado to southern California and Mexico. Small leaves to 2.5 cm (1 in) are ovate typically with 3−5 sharp teeth per side but can be entire with maturity. It ranges into high elevations and is reportedly hardy to zone 5 but this is likely dependent on provenance and requiring lean, well-drained soils where late season dry conditions and cold nights ensure it is completely dormant before freezing temperatures. The JCRA plant from Sun Chaser Natives and Specialty Plants, Colorado has survived three years in perfect drainage where the silvery-blue spiny foliage is quite attractive. It is widely considered the hardiest of the American evergreen oaks and is another that may be suitable for green roof culture.

Other evergreen and semi-evergreen oaks growing at the JCRA but too young to evaluate include:

Quercus acutifolia Née
Quercus alnifolia Poech
Quercus castanea Née
Quercus ×dysophylla Benth.
Quercus emoryi Torr.
Quercus gilva Blume
Quercus greggii (A. DC.) Trel. (La Siberia strain)
Quercus guyavifolia H. Lév.
Quercus hypoleucoides A. Camus
Quercus look Kotschy
Quercus rysophylla Weath.
Qurcus sadleriana R. Br. ter.
Quercus sessilifolia Sieb. & Zucc.
Quercus stenophylloides Hayata
Quercus suber L.

Literature Cited

1. Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, June 2008 [and more or less continuously updated since]. 12 July 2014 http://www.mobot.org/MOBOT/research/APWeb/.

2. Bean, W. J. 1976. Trees and Shrubs Hardy in the British Isles. vol 3. 8th ed., 2nd impression. John Murray Ltd., London, UK.
3. Grimshaw, J., Bayton, R., 2009. New Trees: Recent Introductions to Cultivation. Royal Botanic Gardens, Kew. Richmond, UK.
4. Soepadmo E. 1972. Fagaceae. Flora Malenesia. Ser. I. 7. Noordhoff-Kolff. Jakarta, Indonesia.
5. Quercus Portal Website. 12 July 2014. <https://w3.pierroton.inra.fr/QuercusPortal/index.php?p=BIODIVERSITY>.
6. Nixon, K. C., 2006. Global and Neotropical Distribution and Diversity of Oak (genus *Quercus*) and Oak Forests. Ecology and Conservation of Neotropical Montane Oak Forests Ecological Studies. vol 185. Springer. Berlin, Germany.
7. Huang, T. 1996. Flora of Taiwan. 2nd ed. vol 2. Editorial Committee of the Flora of Taiwan. Taipei, Taiwan.
8. Wu, Z. Y., P. H. Raven & D. Y. Hong, eds. 2008. Flora of China. online 12 July 2014 <http://www.efloras.org>.
9. Krussman, G. 1984. Manual of Cultivated Broad-Leaved Trees & Shrubs. Vol. I. Timber Press, Beaverton, OR.
10. Tutin, T. G., et al. 1968. Flora Europaea. University Press, Cambridge, UK.
11. Menitsky, Y. 2005. Oaks of Asia. CRC Press. Boca Raton, FL.
12. Denk, T., G. Grimm. 2010. The Oaks of Western Eurasia: Traditional Classifications and Evidence from Two Nuclear Markers. Taxon. vol. 59:2. Intl. Assoc. for Plant Taxonomy. Vienna, Austria.
13. Dirr, M. 1998. Manual of Woody Landscape Plants. 5th ed. Stipes Publishing LLC., Champaign, IL.
14. Hogan, S. 2008. Trees for All Seasons: Broadleaved Evergreens for Temperate Climates. Timber Press, Inc., Portland, OR.
15. Jacobsen, A. L. 1996. North American Landscape Trees. Ten Speed Press. Berkely, CA.
16. Jepson, W. L. 1963. A Manual of the Flowering Plants of California. Univ. of California Press, Berkely, CA.
17. Pavlik, B., P. Muick, S. Johnson, M. Popper. 1991. Oaks of California. Cachuma Press. Los Olivos, CA.
18. McArdle, A. J., F. Santamour. 1987. Cultivar Checklist of White Oak Species. Journal of Arboriculture, 13:8. Intl. Soc. Of Arboriculture. Champaign, IL.
19. Stein, J. D. Binion, R. Acciavatti. 2003. Field Guide to Native Oak Species of Eastern North America. USDA Forest Service. Morgantown, WV.
20. Fang, J., Z. Wang, Z. Tang eds. Atlas of Woody Plants in China. Higher Education Press. Beijing, China.
21. Correll, D., M. Johnston. 1970. Manual of the Vascular Plants of Texas. Texas Research Foundation. Renner, TX.
22. Elias, T. S. 1980. The Complete Trees of North America: Field Guide and Natural History. Times Mirror Magazines, Inc. New York, NY.
23. Ohwi, J. 1984. Flora of Japan. Smithsonian Institution. Washington, D.C.

Table 1 Subgenus Quercus sections from Grimshaw (3)

Section	EG/Dec.	Identification Characteristics	Nativity
Cerris	EG or Dec.	Leaf margins entire, revolute, or spiny; gray or yellow fascicled hairs on abaxial leaf surface	Asia, Europe, North Africa
Lobatae (red oaks)	EG or Dec.	Leaf margins entire, serrate, or lobed; bristles on leaf tips; seed matures in two seasons (typically)	North America
Protobalanus	EG	Leaf margins entire, dentate, or serrate; glaucous or waxy on abaxial leaf surface	North America
Quercus (white oaks)	EG or Dec.	Leaf margins entire, serrate, or lobed; bristles absent; seed matures in one season (typically)	North America

Figure .1 *Quercus glauca* makes a handsome small to medium tree.

Figure 2. The new growth color can be quite showy on *Q. myrsinifolia*.

Figure 3. *Q. canbyi* foliage.

Figure 4. *Q. crassifolia* showing both last season's growth and bright red new growth.

Figure 5. *Q. durata* foliage.

Figure 6. *Q. ilex* can grow quite large in favorable conditions.

Figure 7. *Q. marlipoensis* has not proven to be hardy.

Figure 8. *Q. phillyreoides* 'Emerald Sentinel' makes a useful screening plant.

Figure 2 *Q. turbinella* has spiny blue-green foliage.

Effect of Oryzalin on Inducing Polyploidy in *Dissotis rotundifolia*

Susan M. Hawkins[1], John M. Ruter[1] and Carol D. Robacker[2]

[1]Department of Horticulture, 1111 Plant Sciences Building, Athens, GA 30602-7273
[2]1109 Experiment Street, Department of Horticulture
Georgia Station, Griffin, GA 30223-1797

darkstar@uga.edu

Index Words: Melastomaceae, Dissotis, oryzalin, polyploidy

Significance to Industry: *Dissotis rotundifolia* (Sm.) Triana is a species in the Melastomaceae with good ornamental potential. Nursery production can be difficult due to the aggressive growth habit of the species. To help reach the goal of obtaining a more compact *D. rotundifolia*, rooted cuttings were treated with oryzalin to induce polyploidy. Flow cytometry was performed to determine the ploidy level of the treated cuttings. One tetraploid was obtained. Growth of the tetraploid is being monitored to determine whether its growth will be more compact than that of diploid plants.

Nature of Work: *D. rotundifolia* is a species in the Melastomaceae. The Melastomaceae family consists of 185 to 190 genera that contain 5,000 species (9). Most Melastomaceae species are tropical or sub-tropical species (8). The genus *Dissotis* is from Africa. *D. rotundifolia* has also naturalized in tropical and subtropical areas such as Puerto Rico and Hawaii (6, 9) .The species is able to live in varying environments, such as waste places and along roadsides, as well as small granite outcroppings in the middle of rain forests (7). *D. rotundifolia* is a diploid (2n = 30) (11).

D. rotundifolia is currently in use as an ornamental. This trailing species flowers abundantly, has attractive dark green foliage, roots easily, and is extremely drought tolerant. It grows so vigorously that it must be pruned back every few weeks. Pruning the plants will cause flowering to cease for several weeks. Vigorous growth and the need for frequent pruning make nursery production of the plant problematic.

Induction of polyploidy will sometimes cause a plant to become smaller and more compact than its diploid form (3). Autotetraploid Brassicas produced 64% less biomass than their diploid counterparts (1). Induced autotetraploids of *Helianthus annuus* L. 'Morden' were reduced in both height and width by one-third compared to the diploid control plants (12).

Induction of polyploidy in ornamental species has been accomplished by application of chemicals such as colchicine, an alkaloid extracted from Colchicum autumnale, or oryzalin (4-(dipropylamino)-3,5-dinitrobenzenesulphonamides). Both substances double chromosomes by inhibiting the formation of mitotic spindles (4). Colchicine is more toxic to humans than oryzalin and must usually be applied at higher rates to induce polyploidy (4). Oryzalin has successfully been used to induce polyploidy in many ornamental species, such as Japanese barberry (*Berberis thunbergii* var. *atropurpurea*), *Hibiscus acetosella* Welw. ex Hiern., and ornamental ginger (*Hedychium muluense* R. M. Smith) (3, 5, 10). The purpose of this study was to induce polyploidy in *D. rotundifolia* through treatment with oryzalin in an attempt to produce a more compact version of the species.

Cuttings of *D. rotundifolia* were taken in December 2013, stuck in Fafard Germination Mix containing processed pine bark, Canadian sphagnum peat, perlite, and vermiculite (Sun Grow Horticulture, Agawam, MA), and placed under intermittent mist for seven weeks in a greenhouse in Athens, GA. All of the cuttings were the same genotype. In February 2014, rooted plants were potted into Fafard 3B consisting of Canadian sphagnum peat, processed pine bark, perlite, and vermiculite in a 38-round flat and placed on a bench in the Riverbend Research greenhouse in Athens, GA . Apical meristems were treated with 50 µM oryzalin solution (supplied as Surflan® A.S.; United Phosphorus, King of Prussia, PA) solidified with agar. The solution was stirred for several minutes and then heated in a microwave oven for 25 s. Before application, the solution was again heated in a microwave oven to melt the agar. Approximately 25 µl of oryzalin or agar solution was applied to the apical meristem and then the meristem was covered with a plastic cap for 24 hours. Following removal of the cap, the meristems were rinsed with tap water. Oryzalin treatments were applied either 1, 2, or 3 days, with each application on consecutive days. A control treatment of one drop of agar only was also used. A total of 64 cuttings were treated, with 16 cuttings per treatment. Treated cuttings were allowed to grow in the greenhouse for three weeks after treatment before being tested for ploidy level.

Flow cytometry was performed to determine the ploidy level of the treated cuttings. Three samples were prepared for each treated cutting. Approximately 1 cm^2 of newly expanded leaf tissue was chopped in a petri dish with 500 µl of nuclei extraction buffer (CyStain PI® Absolute P Nuclei Extraction Buffer; Partec GmbH, Münster, Germany). Tissue was incubated in the solution for 30s. Solution was filtered through Partec CellTrics® disposable filters with a pore size of 30 µm to remove leaf material. Nuclei were stained with a solution of 2.0 ml of CyStain® PI absolute P, 12 µl propidium iodide stock solution, and 6 µl Rnase stock solution. Samples were incubated for at least 60 min on ice. Samples were analyzed with a CyAn™ ADP Analyzer (Beckman Coulter, Brea, CA) to determine mean relative DNA fluorescence. Ploidy was determined by comparing the mean DNA fluorescence of each sample with the 2C peak of a diploid *D. rotundifolia*.

Results and Discussion: No mortality occurred in any of the treated cuttings. One tetraploid (4x) and three mixaploids (2x + 4X) were identified (Figure 1). The tetraploid resulted from treating the cutting with two drops of oryzalin solution. The tetraploid cutting was potted up in a pine- bark based substrate with added nutrients in a 2.8 liter trade container and placed in a greenhouse at the University of Georgia Horticulture Farm, Watkinsville, GA. On 28 March 2014, a flower bud was observed on the tetraploid cutting. The cutting continued to produce flowers after the initial flower bud. Five cuttings of the original tetraploid cutting were taken on 13 May 2014 to produce tetraploid plants. Cuttings were potted into 2.8 liter trade containers in a pine-bark based substrate on 04

June 2014 and placed in the greenhouse at the University of Georgia Horticulture Farm, Watkinsville, GA, for further observation.

Induction of polyploidy did not delay flowering in *D. rotundifolia*. In contrast, flowering in induced polyploids of *Helianthus annuus* 'Morden' was delayed by almost two weeks (12). Induction of polyploidy in *Lilium* L. cultivars by either oryzalin or colchicine caused the polyploid plants to flower from four to ten days later than the diploid plant of the same cultivar (2).

Since tetraploidy can be induced in *D. rotundifolia* by use of oryzalin, the possibility exists that the growth habit of the plant may be improved if the tetraploid plant proves to be more compact than its diploid counterpart. The five tetraploid plants derived from the original tetraploid cutting are being monitored and compared to the growth habit of the diploid species. Based upon visual observation, we have found no difference in leaf or flower morphology between the tetraploid plants and the diploid *D. rotundifolia*. The study should be repeated with a larger number of cuttings to determine the dose of oryzalin that is optimum for inducing polyploidy and to increase the likelihood of obtaining at least one tetraploid plant that is more compact than its diploid counterpart.

Literature Cited

1. Abel, S. and Becker, H.C., 2007. The effect of autopolyploidy on biomass production in homozygous lines of *Brassica rapa* and *Brassica oleracea*. *Plant Breeding* 126:642-643.
2. Balode, A., 2008. Applying colchicine and oryzalin in *Lilium* L. polyploidisation. *Agronomijas Vestis*:22-28.
3. Contreras, R.N., Ruter, J.M., and Hanna, W.W., 2009. An oryzalin-induced autoallooctoploid of *Hibiscus acetosella* 'Panama Red'. *Journal of the American Society for Horticultural Science* 134:553-559.
4. Harten, A.M.v., 1998. *Mutation breeding : theory and practical applications / A.M. van Harten*. Cambridge, U.K. : New York : Cambridge University Press, 1998.
5. Lehrer, J.M., Brand, M.H., and Lubell, J.D., 2008. Induction of tetraploidy in meristematically active seeds of Japanese barberry (*Berberis thunbergii* var. *atropurpurea*) through exposure to colchicine and oryzalin. *Scientia Horticulturae* 119:67-71.
6. Liogier, A.H. and Martorell, L.F., 1982. *Flora of Puerto Rico and adjacent islands : a systematic synopsis / Henri Alain Liogier, Luis F. Martorell*. Río Piedras, P.R. : Editorial de la Universidad de Puerto Rico, 1982. 1a ed.
7. Porembski, S., Szarzynski, J., Mund, J.P., and Barthlott, W., 1996. Biodiversity and vegetation of small-sized inselbergs in a West African rain forest (Taï, Ivory Coast). *Journal of Biogeography* 23:47-55.
8. Renner, S.S., Clausing, G., and Meyer, K., 2001. Historical Biogeography of Melastomataceae: The Roles of Tertiary Migration and Long-Distance Dispersal. *Am J Bot*:1290.
9. Renner, S.S. and Meyer, K., 2001. Melastomeae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution* 55:1315-1324.

10. Sakhanokho, H.F., Islam-Faridi, N., Kelley, R.Y., and Rajasekaran, K., 2009. Induced Polyploidy in Diploid Ornamental Ginger (*Hedychium muluense* R. M. Smith) Using Colchicine and Oryzalin. *HortScience* 44:1809-1814.
11. Solt, M.L. and Wurdack, J.J., 1980. Chromosome numbers in the Melastomataceae. *Phytologia* 47:199-220.
12. Srivastava, R. and Srivastava, G.K., 2002. Autopolyploids of *Helianthus annuus* L. var. morden. *Cytologia* 67:213-220.

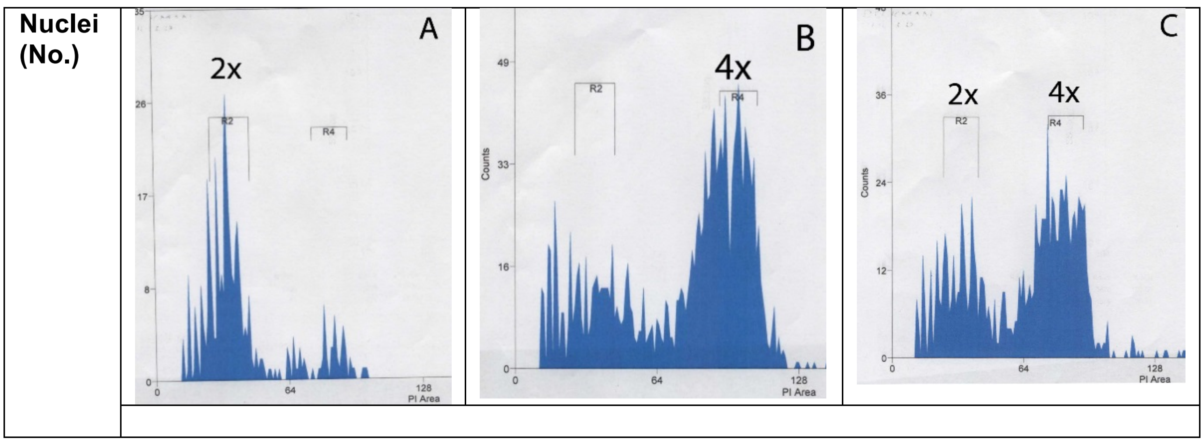

Fig 1. Histograms generated using flow cytometry from leaves of (A) diploid *D. rotundifolia*, (B) induced tetraploid of *D. rotundifolia*, and (C) induced cytochimera of *D. rotundifolia*.

Hybridization Studies in Lilacs (*Syringa* L.)

Jason D. Lattier and Ryan N. Contreras
Department of Horticulture, Oregon State University, Corvallis OR, 97331

Jason.Lattier@oregonstate.edu

Significance to Industry: *Syringa* is a genus in the Oleaceae consisting of 22 to 30 species from two distinct centers of diversity, the highlands of East Asia and the Balkan-Carpathian region of Europe (3). The vast majority of lilacs are native to the Asian center of diversity with only *Syringa vulgaris* and *Syringa josikaea* native to southeastern Europe (2). Hundreds of lilac cultivars have been developed as ornamentals and are ubiquitous in temperate gardens around the world. Historically, the most popular cultivars of lilacs originated from the European species *Syringa vulgaris,* primarily grown for its fleeting spring blooms of purple, pink, blue or white fragrant flowers. The current phylogeny of the genus describes six sections within *Syringa*: *Pubescentes*, *Villosae*, *Ligustrina*, *Ligustrae*, *Pinnatifoliae* and *Syringa* (4).

Each section is separated by significant morphological distinctions. *Pubescentes* is distinct by having pubescent leaves (4), and *Villosae* is distinct by having inflorescences develop from a single terminal bud with lateral buds become vegetative shoots (2). *Ligustrina* is distinct by its privet-like flowers (short, white corolla tubes with long exerted anthers) and its growth habit as a large tree (2). *Pinnatifoliae* is distinct by having pinnately compound leaves (4). *Ligustrae* contains the privets (*Ligustrum spp.*), nested within the lilacs (4). Data on cross compatibility between culitvars, species, and sections within *Syringa* will aid future lilac breeders and lead to new improved cultivars for the nursery industry.

Nature of Work: Intraspecific and interspecific hybridization are proven methods for development of new cultivars in lilac. However, intersectional hybridization has proven more difficult with only several successful hybrids produced from crosses with *S. pinnatifolia* (6). Previous attempts to create intersectional hybrids resulted in highly abortive fruits with no germination of recovered seeds (6). Anatomical studies on *S. villosa*, an Asian lilac with naturally high rates of seed abortion, found that after cross-pollination embryos developed normally through the globular, heart-shaped, torpedo, and cotyledonal stages before embryo and endosperm began to degrade (1). Few embryo rescue studies have been attempted for lilacs. However, Zhou et al. (7) successfully cultured immature embryos on Monnier's Medium (5) supplemented with 1-naphthaleneacetic acid (NAA), 6-benzylaminopurine (BAP), glutamine (Gln), and a high rate of sucrose, indicating that tissue culture may be a platform for rescuing abortive seeds of lilac hybrids.

Though hundreds of improved lilac cultivars have been introduced, fertility and cross compatibility between cultivars, species, and sections have yet to be published in a formal study. The objectives of this study were to 1) investigate cross compatibility of elite cultivars of lilac in intraspecific, interspecific, and intersectional combinations and 2) investigate the potential for intersectional hybridization and embryo rescue of abortive embryos in vitro.

Cross Compatibility Study. During the spring of 2013 and 2014, nearly 20,000 crosses were made between cultivars, species, and sections of lilacs. Parent plants were collected from nurseries, gardens, and arboreta. Representative species and cultivars were obtained for lilac sections *Syringa, Pubescentes*, and *Villosae* focusing on elite cultivars improved for one or more horticulturally important traits. Individual flowers were emasculated prior to anthesis and pollinations were made in a glasshouse with day/night set temperatures of 77/68°F that was kept free of pollinators. Each flower was pollinated two to three times post emasculation. Fruits were counted and collected in the fall and allowed to dry before extracting seed. Seeds were cold stratified for ten weeks at 40°F before being sown in flats filled with Metro-Mix Professional Growing Mix (Sun Gro Horticulture, Agawam, MA) and treated once with Kocide® 2000 (DuPont™, Wilmington, DE) at ½ tbsp/gal. All seedlings were germinated in a glasshouse under conditions described above. In 2013, we counted number of pollinated inflorescences, number of pollinated flowers, number of fruits, number of collected seeds, number of germinated seeds, and number of seedlings. In 2014, we counted number of pollinated inflorescences, number of pollinated flowers pollinated, and number of fruits. Germination percentage and seedlings recovered will be calculated in the fall and winter of 2014.

Intersectional hybridization and embryo rescue. In 2013, intersectional hybrid fruit were collected seven weeks after pollination. Fruits were surface sterilized by rinsing in a 70% ethanol solution for 30 seconds followed by a soak in a 20% (v/v) bleach (6.15% NaOCl) solution with several drops of Tween® 20 (Acros Organics™, Fair Lawn, NJ). Then, fruits were triple-rinsed with filter-sterilized, autoclaved water and seeds were dissected in a sterile hood using a dissecting microscope. Seeds were removed from fruits into sterile petri dishes containing an aqueous solution of L-ascorbic acid at 25 mg/L to reduce oxidation. Dissected seeds were cultured on Monnier's medium according to Zhou et al. (7) and incubated under standard culture conditions.

Results and Discussion: Intraspecific and interspecific crosses in 2013 yielded 1,086 seedlings. The most prolific cross within section *Syringa* was *S. vulgaris* 'Ludwig Spaeth' x *S. vulgaris* 'Angel White', which produced 1.35 seedlings per pollinated flower (Table 1). The most prolific cross within section *Pubescentes* was the interspecific cross *S. meyeri* 'Palabin' x *S. pubescens* 'Penda' Bloomerang® Purple, which produced 1.16 seedlings per pollinated flower. Out of 958 seed collected from cross combinations using *S. pubescens* 'Miss Kim' as the female parent, 160 seed germinated as albino seedlings. No albino seedlings survived, indicating that 'Miss Kim' is not suitable for use as a female parent in the combinations used in our study.

Immature hybrid seed obtained from intersectional crosses in 2013 had low germination percentages in vitro on Monnier's medium (Table 2). The highest number of fruits observed from intersectional crosses occurred with the cross *S. oblata* x *S. pubescens* 'Penda' Bloomerang® Purple yielding 238 fruits. However, fruits from this cross aborted six weeks after pollination and were not collected for tissue culture. Of the 161 seeds cultured in vitro, only three germinated from the cross *S. pubescens* 'Penda' Bloomerang® Purple x *S. vulgaris* 'Ludwig Spaeth' (Table 2). Seedlings failed to grow post germination and tissues subsequently converted to callus. This result may be due to lack of proper transfer media post-germination or lack of proper combination of genotype and embryo rescue medium. Surprisingly, Zhou et al. (7) does not report the genotype used in their protocol. Further research will be necessary to design efficient protocols for embryo rescue of intersectional lilac hybrids.

As more accessions were added to our germplasm in 2013, a wider range of crosses were designed for spring of 2014. Of 9,635 attempted pollinations including 46 cross combinations, 1,111 fruit have been observed from intraspecific, interspecific, and intersectional crosses. The cross combination with the highest fruit set was *S. vulgaris* 'Sensation' x *S. ×hyacinthiflora* 'Old Glory', which has yielded 1.0 fruit per pollinated flower (Table 3). The most promising intersectional cross combination was *S. ×prestoniae* 'Redwine' x *S. pubescens* 'MORjos 060F' Josee™ yielding 174 total fruits (0.3 fruits per pollinated flower, Table 3). This cross between section *Villosae* and section *Pubescentes* represents an intersectional hybridization of less genetic distance, according to Li et al. (4), than intersectional crosses made in 2013. Hybrids between sections may be useful as bridge species for future intersectional crosses between section *Pubescentes* and section *Syringa*. The widest intersectional cross in 2014 to set fruit (between section *Syringa* and section *Pubescentes*) was *S. vulgaris* 'President Grevy' x *S. pubescens* 'Penda' Bloomerang® Purple. Although this cross produced 0.4 fruit per pollinated flower, the fruits are undersized and may lack viable seed (Table 3).

As fruits continue to develop from 2014 crosses, seed set and germination data from all crosses will be recorded and presented in future publications. Promising intersectional hybridizations that produce abortive fruits or seeds with low germination percentages will be repeated in 2015, and new embryo rescue protocols will be designed using specific cultivars to optimize protocols. Controlled crosses of varying genetic distances (intrasectional vs. intersectional) can be a useful tool for plant breeders in the development of new and improved cultivars that combine traits of distantly related species. Our work provides a foundation to determine the relative ease or difficulty with which a number of cross combinations previously unreported may be made.

Literature Cited

1. Chen, Y., J.-Y. Chen, Y. Liu and S.-W. Zhao. 2012. Anatomical study on seed abortion of *Syringa villosa* under cultivated conditions. Journal of Beijing Forestry University 34(6): 107-114.
2. Kim, K.-J. and R.K. Jansen. 1998. A chloroplast DNA phylogeny of lilacs (*Syringa*, Oleaceae): plastome groups show a strong correlation with crossing groups. American Journal of Botany 85(9): 1338-1351.
3. Kochieva, E.Z., N.N. Ryzhova, O.I. Molkanova, A.M. Kudryavtsev, V.P. Upelniek and I.B. Okuneva. 2004. The Genus *Syringa*: Molecular Markers of Species and Cultivars. Russian Journal of Genetics 40(1): 30-32.

4. Li, J., B. Goldman-Huertas, J. DeYoug and J. Alexander, III. 2012. Phylogenetics and diversification of *Syringa* inferred from nuclear and plastid DNA sequences. Castanea 77(1): 82-88.
5. Monnier, M. 1990. Culture of Zygotic Embryos of Higher Plants. Methods in Molecular Biology 6: 129-139.
6. Pringle, J.S. 1981. A review of attempted and reported interseries and intergeneric hybridization in *Syringa* (Oleaceae). Baileya 20: 49-91.
7. Zhou, L., L.-M. Dai and B.-L. Su. 2003. Embry culture and rapid propagation of *Syringa*. Journal of Forestry Research 14(3): 213-216.

Table 1. Cross compatibility in 2013 lilac hybrids.

Section	Female Parent	Male Parent	Pollinated flowers	Seed	Seedlings per pollinated flower
Syringa	*S. vulgaris* 'Angel White'	*S. vulgaris* 'Ludwig Spaeth'	319	204	0.17
Syringa	*S. vulgaris* 'Ludwig Spaeth'	*S. vulgaris* 'Angel White'	138	222	1.35
Syringa	*S. vulgaris* 'President Grevy'	*S. vulgaris* 'Sensation'	129	16	0.01
Syringa	*S. vulgaris* 'Sensation'	*S. vulgaris* 'President Grevy'	147	1	0.00
Pubescentes	*S. meyeri* 'Palabin'	*S. pubescens* 'MORjos 060F' Josee™	355	40	0.05
Pubescentes	*S. meyeri* 'Palabin'	*S. pubescens* 'Penda' Bloomerang® Purple	239	398	1.16
Pubescentes	*S. meyeri* 'Palabin'	*S. pubescens* 'Bailbelle' Tinkerbelle®	206	155	0.65
Pubescentes	*S. pubescens* 'Miss Kim'	*S. meyeri* 'Palabin'	601	900	0.25
Pubescentes	*S. pubescens* 'Miss Kim'	*S. pubescens* 'MORjos 060F' Josee™	210	0	0.00
Pubescentes	*S. pubescens* 'Miss Kim'	*S. pubescens* 'Penda' Bloomerang® Purple	380	58	0.15
Pubescentes	*S. pubescens* 'MORjos 060F' Josee™	*S. meyeri* 'Palabin'	122	82	0.52
Pubescentes	*S. pubescens* 'MORjos 060F' Josee™	*S. pubescens* 'Penda' Bloomerang® Purple	246	158	0.53
Pubescentes	*S. pubescens* 'MORjos 060F' Josee™	*S. pubescens* 'Bailbelle' Tinkerbelle®	145	42	0.19
Pubescentes	*S. pubescens* 'Bailbelle' Tinkerbelle®	*S. meyeri* 'Palabin'	253	58	0.12

Table 2. Attempted pollinations, recovered seed, and in vitro germination rates from intersectional lilac hybrids in 2013. All seed collected from green capsules and cultured on cultured on Monnier's medium as described by Zhou et al. (7).

Female Parent	Male Parent	Pollinated flowers	Seed	Germinated In vitro
S. vulgaris 'Ludwig Spaeth'	*S. pubescens* 'Penda' Bloomerang® Purple	2206	18	0
S. oblata	*S. pubescens* 'Penda' Bloomerang® Purple	547	0[z]	0
S. meyeri 'Palabin'	*S. oblata*	179	6	0
S. meyeri 'Palabin'	*S. vulgaris* 'Angel White'	91	0	0
S. meyeri 'Palabin'	*S. vulgaris* 'Sensation'	197	39	0
S. pubescens 'Miss Kim'	*S. oblata*	223	0	0
S. pubescens 'Miss Kim'	*S. vulgaris* 'President Grevy'	408	0	0
S. pubescens 'MORjos 060F' Josee™	*S. oblata*	138	77	0
S. pubescens 'Penda' Bloomerang® Purple	*S. vulgaris* 'Ludwig Spaeth'	2098	21	3[y]

[z]Early abortion of 238 fruit occurred 6 weeks post pollination
[y]Radicle, hypocotyl, and cotyledons emerged; seedlings failed to grow post germination and tissues subsequently converted to callus.

Table 3. Fruit set from intraspecific, interspecific, and intersectional lilac hybridizations in 2014.

Female Parent	Male Parent	Pollinated flowers	Fruits	Fruit per pollinated flower
S. vulagris 'Elsdancer' Tiny Dancer™	*S. vulgaris* 'Angel White'	56	0	0.00
S. vulagris 'Elsdancer' Tiny Dancer™	*S. vulgaris* 'Sensation'	36	15	0.42
S. vulgaris 'Monore' Blue Skies®	S. *vulagris* 'Elsdancer' Tiny Dancer™	47	35	0.74
S. vulgaris 'Monore' Blue Skies®	*S. vulgaris* 'President Grevy'	238	82	0.34
S. vulgaris 'Prarie Petite'	*S. ×hyacinthiflora* 'Old Glory'	20	0	0.00
S. vulgaris 'Prarie Petite'	*S. vulgaris* 'Sensation'	74	33	0.45
S. vulgaris 'President Grevy'	*S. ×hyacinthiflora* 'Old Glory'	176	59	0.34
S. vulgaris 'President Grevy'	*S. ×prestoniae* 'Miss Canada'	22	0	0.00
S. vulgaris 'President Grevy'	*S. pubescens* 'Penda' Bloomerang® Purple	68	27	0.40
S. vulgaris 'President Grevy'	S. vulagris 'Elsdancer' Tiny Dancer™	304	3	0.01
S. vulgaris 'President Grevy'	*S. vulgaris* 'Angel White'	182	4	0.02
S. vulgaris 'President Grevy'	*S. vulgaris* 'President Lincoln'	81	0	0.00
S. vulgaris 'President Grevy'	*S. vulgaris* 'Sensation'	111	60	0.54
S. vulgaris 'President Lincoln'	*S. vulgaris* 'Angel White'	135	27	0.20
S. vulgaris 'President Lincoln'	*S. vulgaris* 'President Grevy'	126	0	0.00
S. vulgaris 'Sensation'	*S. ×hyacinthiflora* 'Old Glory'	73	73	1.00
S. vulgaris 'Sensation'	*S. ×prestoniae* 'Miss Canada'	48	5	0.10
S. vulgaris 'Sensation'	S. *vulagris* 'Elsdancer' Tiny Dancer™	95	12	0.13
S. vulgaris 'Sensation'	*S. vulgaris* 'President Grevy'	229	14	0.06
S. ×hyacinthiflora 'Old Glory'	*S. pubescens* 'Penda' Bloomerang® Purple	35	0	0.00
S. ×hyacinthiflora 'Old Glory'	S. *vulagris* 'Elsdancer' Tiny Dancer™	102	20	0.20
S. ×hyacinthiflora 'Old Glory'	*S. vulgaris* 'Angel White'	195	57	0.29
S. ×hyacinthiflora 'Old Glory'	*S. vulgaris* 'Sensation'	82	0	0.00
S. ×prestoniae 'Miss Canada'	*S. pubescens* 'Penda' Bloomerang® Purple	425	80	0.19
S. ×prestoniae 'Miss Canada'	*S. vulgaris* 'Sensation'	142	0	0.00
S. ×prestoniae 'Redwine'	*S. pubescens* 'Penda' Bloomerang® Purple	617	56	0.09
S. ×prestoniae 'Redwine'	*S. pubescens* 'MORjos 060F' Josee™	602	174	0.29
S. sweginzowii	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	237	22	0.09
S. tigerstedii	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	130	12	0.09
S. villosa	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	219	0	0.00
S. wolfii	*S. pubescens* 'Penda' Bloomerang® Purple	176	0	0.00
S. yunnanensis	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	163	0	0.00
S. emodii	*S. pubescens* 'Penda' Bloomerang® Purple	97	0	0.00
S. emodii	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	82	21	0.26
S. josikaea	*S. meyeri* 'Palabin'	58	0	0.00
S. josikaea	*S. pubescens* 'MORjos 060F' Josee™	149	6	0.04
S. josikaea	*S. pubescens* 'Tinkerbelle'	135	10	0.07
S. julianae	*S. pubescens* 'SMSJBP7' Bloomerang® Dark Purple	64	16	0.25
S. meyeri 'Palabin'	*S. pubescens* 'Miss Kim'	522	24	0.05
S. meyeri 'Palabin'	*S. pubescens* 'MORjos 060F' Josee™	351	9	0.03
S. pubescens 'MORjos 060F' Josee™	*S. ×prestoniae* 'Miss Canada'	500	82	0.16

S. pubescens 'MORjos 060F' Josee™	*S. ×prestoniae* 'Redwine'	150	1	0.01
S. pubescens 'MORjos 060F' Josee™	*S. oblata* var. *alba*	329	1	0.00
S. pubescens 'MORjos 060F' Josee™	*S. pubescens* 'Miss Kim'	137	4	0.03
S. pubescens 'Penda' Bloomerang® Purple	*S. ×prestoniae* 'Miss Canada'	482	17	0.04
S. pubescens 'Penda' Bloomerang® Purple	*S. pubescens* 'Miss Kim'	175	2	0.01
S. pubescens 'Penda' Bloomerang® Purple	*S. pubescens* 'MORjos 060F' Josee™	141	28	0.20
S. pubescens 'Penda' Bloomerang® Purple	*S. pubescens* 'Tinkerbelle'	133	0	0.00
S. pubescens 'Tinkerbelle'	*S. oblata*	271	0	0.00
S. pubescens 'Tinkerbelle'	*S. pubescens* 'Miss Kim'	124	0	0.00
S. pubescens 'Tinkerbelle'	*S. pubescens* 'MORjos 060F' Josee™	199	0	0.00
S. pubescens 'Tinkerbelle'	*S. pubescens* 'Penda' Bloomerang® Purple	290	20	0.07

The North American Plant Collections Consortium as a Resource for Breeders and Researchers

Mark Weathington

JC Raulston Arboretum, NC State University
Department of Horticultural Science
Campus Box 7522, Raleigh, NC 27695

mark_weathington@ncsu.edu

Index words: NAPCC, conservation, education, germplasm preservation, collections management

Significance to the Industry: The North American Plant Collections Consortium (NAPCC) is an initiative of the American Public Gardens Association (APGA) in partnership with the United States Department of Agriculture (USDA) tasked with the mission to build a network of public gardens to coordinate a continent-wide approach to plant germplasm preservation and to promote high standards of plant collection management. Participating institutions commit to holding and developing a collection(s) of documented living plants. These collections are used for germplasm preservation, research, and education. Participating institutions commit to allowing access to the germplasm and collections information for external researchers. This continent-wide approach makes efficient use of resources, strengthening collections through combined collaborative activities and has the potential to be a significant asset to ornamental plant breeders and researchers.

Discussion: The American Public Gardens Association (APGA) was founded in1940 as the American Association of Botanical Gardens and Arboreta. APGA is committed to increasing the knowledge of public garden professionals throughout North America via information sharing, professional development, public awareness, and research so these professionals have the tools to effectively serve visitors and members (1). The association is currently comprised of over 500 institutional members and over 5000 individual members. Institutional members include traditional botanical gardens and arboreta as well as zoos, cemeteries, conservatories, historic estates and public parks. APGA has several programs such as the Sentinel Plant Network (SPN), YOUtopia, and the North American Plant Collections Consortium (NAPCC). The SPN contributes to plant conservation by engaging public garden professionals, volunteers, and visitors in the detection and diagnosis of high consequence pests and pathogens (1) through training in monitoring and diagnostics of plant pests, facilitating collaboration between institutions, enhancing participant gardens' outreach, and managing Plant Heroes, a program designed for young learners. YOUtopia is a program designed to help public gardens, gardeners and garden visitors understand the impacts of climate change and provide steps to take at home to participate in solutions.

The NAPCC is the oldest of these APGA programs and has been designated a core program by the APGA board (Casey Sclar pers. comm.). The NAPCC's mission is to coordinate a continent-wide approach to plant germplasm preservation among public gardens and to promote professional standards of plant collections management. Its vision is to be the recognized standard for excellence in plant collections management and demonstrates a garden's enduring commitment to global efforts to save plants. Participant gardens must be members of APGA, have an active collections management program, show a commitment to maintaining the plant group, have the endorsement of a governing body, have a collections policy and a designated curator, and must provide reasonable access to the collection for researchers. Participants in the program commit to holding and developing one or more collections of documented living plants or seed. The collections are to be used for germplasm preservation, education, and research.

The program was begun in the late 1980's modeled on the United Kingdom National Collections Scheme. By 1995 a cooperative agreement with the U.S. Department of Agriculture (USDA) was formalized and in the following year six pilot collections were designated. In 2004, the APGA board recognized the NAPCC as a core program and has re-affirmed this recognition regularly since that time. In 2005 the first multi-site application was piloted recognizing the need for multiple institutions with different growing conditions to adequately hold some of the larger or more widespread genera.

NAPCC collections are predominantly alpha-taxonomic but also include floristic collections (i.e. Mesoamerican cloud forest), thematic (i.e. alpines of Colorado) or historic (i.e. Upjohn herbaceous *Paeonia* cultivars, Dr. Griffith Buck *Rosa* cultivars). Collections cover both wild species and material of horticultural origin. There are currently 70 participating gardens holding 64 single site collections and 4 multi-site collections (*Acer*, *Cycas*, *Magnolia*, and *Quercus*). These collections are being used for *ex situ* conservation, taxonomic research, molecular and genetic analysis, public education, breeding, and conservation of horticultural forms and cultivars.

The NAPCC *Cercis* collection at the JC Raulston Arboretum (JCRA) at NC State University (NCSU) holds 69 accessions representing 56 taxa including 11 species or purported species. This collection is used by former JCRA director and current NCSU researcher Dennis Werner and other nurserymen for breeding work. Dr. Werner's introductions include *Cercis canadensis* 'Pink Pom Poms', 'Merlot', and 'Ruby Falls' among others. The collection has also been used for horticultural selection of improved forms including *C.* 'Big John' selected by Shiloh Nursery, North Carolina from a seedling received from the JCRA and *C. chinensis* 'Kay's Early Hope' from the JCRA collection. Other research work utilizing the JCRA's NAPCC collection include phylogenetic and molecular studies by the California Academy of Sciences and the Hungarian Academy of Sciences, assistance in the development of a U.K. National Collection, and dissemination of propagules to various nurseries for production stock plants and breeding (2).

Other NAPCC collections are being used in a variety of ways. The Dawes Arboretum, Newark, Ohio, holds a NAPCC *Metasequoia glyptostroboides* collection. This collection represents the broadest genetic diversity of the species outside of China and serves as an important *ex situ* conservation collection. Forty-seven wild collected accessions from across *M. glyptostroboides* native range consisting of 320 total trees and eight cultivars are included in this important collection. The collection is currently being used for population genetic analysis and conservation studies. Based on the morphological diversity shown in the collection, there is great potential for horticultural selection and breeding (Fig. 1).

Multi-site collections allow for a wider variety of material to be grown across the country. This allows for more genetic diversity at the species level, more room for larger growing taxa, and different climactic conditions for plants that have different growth requirements. The NAPCC multi-site *Magnolia* collection has 15 sites across North America. This collection represents wild species, hybrids, and cultivars. The combined database now lists 2,793 accessions of 697 different taxa including 145 different species (3) of the approximately 225 species (4). This shared database allows for gap analysis pinpointing taxa that are a priority for collection for conservation and horticultural use. The shared database also allows participant gardens to evaluate the source of wild material and to begin to understand the extent of genetic diversity represented in North America.

The NAPCC program has allowed for a much greater degree of coordination and communication between gardens allowing for a greater degree of germplasm conservation. The high standards of curatorial care required by the program ensures high quality germplasm and associated data for researchers (5). Participant gardens' commitment to allow reasonable access to material for researchers makes these collections valuable resources for phylogenetic study, breeding, taxonomic work, and other basic and applied research.

Current NAPCC particpants (6/2014)

Arboretum at Arizona State University – *Phoenix* (1995) 300 taxa
Arizona-Sonora Desert Museum – *Agave* (2010) 65 taxa
Arnold Arboretum of Harvard University – *Acer* (1996 multisite) 133 taxa; *Carya* (1996) 18 taxa; *Fagus* (1996) 25 taxa; *Stewartia* (2006) 11 taxa; *Syringa* (1996) 201 taxa; *Tsuga* (1996) 59 taxa
Atlanta Botanical Garden – *Acer* (2008 multisite) 82 taxa; *Magnolia* (2011 multisite); *Sarracenia* (2006) 78 taxa
Bartlett Tree Research Laboratories Arboretum – *Magnolia* (2011 multisite); *Quercus* (2010 multisite) 153 taxa
Betty Ford Alpine Gardens – Alpine Plants of Colorado (2010) 87 taxa
Boyce Thompson Arboretum - *Quercus* (2012 multisite) 24 taxa
Cheekwood Botanical Garden – *Cornus* (2012) 61 taxa
Chicago Botanic Garden – *Geranium* (2007) 102 taxa; *Spiraea* (2003) 89 taxa; *Quercus* (2007 multisite) 64 taxa
Cornell Plantations – *Acer* (2001 multisite) 139 taxa; *Quercus* (2007 multisite) 77 taxa
The Dawes Arboretum - *Acer* (2008 multisite) 336 taxa, *Aesculus* (2010) 59 taxa, *Metasequoia* (2009) 9 taxa including 47 DWO accessions, 8 cultivars

Denver Botanic Gardens – Alpines of the World (2012) 223 taxa; *Quercus* (2007 multisite) 61 taxa
Desert Botanical Garden – *Agavaceae* (2010) 346 taxa, *Cactaceae* (2010) 1319 taxa
Donald E Davis Arboretum at Auburn University – *Quercus* (2010 multisite) 34 taxa
Fairchild Tropical Botanic Garden – *Arecaceae* (2006) 625 taxa, 2006 accessions; Cycads (2007) 150 taxa
Fullerton Arboretum, California State University – Citrus (2000) 36 accessions, 20 taxa
Ganna Walska Lotusland – Cycad (2002) 170 taxa including 9 hybrids, 16 undescribed taxa
Green Spring Gardens – *Hamamelis* (2001) 77 taxa, 98 accessions
Henry Foundation for Botanical Research – *Magnolia* (1995) 15 taxa native to US
Highstead Arboretum – *Kalmia* (1995) 82 taxa, 3 spp, 76 cvs
Holden Arboretum – *Quercus* (2007 multisite) 64 taxa
Hoyt Arboretum - *Acer* (2008 multisite) 67 taxa; *Magnolia* (2011 multisite)
Huntington Botanical Garden – *Camellia* (1995) 1,120 taxa
Idaho Botanical Garden – Western *Penstemon* (2012, provisional) 33 taxa
JC Raulston Arboretum – *Cercis* (2008) 40 taxa; *Magnolia* (2011 multisite)
Jenkins Arboretum & Garden – *Kalmia* (2010) 48 taxa, *Rhododendron* (2010) 1,861 taxa
Jensen-Olson Arboretum – *Primula* (2012) 65 taxa
Landis Arboretum – *Quercus* (2002 multisite) 14 taxa
Longwood Gardens – *Nymphaea* (2012) 97 taxa
Matthaei Botanical Gardens & Nichols Arboretum – *Paeonia* (2009)
Mendocino Coast Botanical Gardens – Heath and Heather (2007)
Minnesota Landscape Arboretum – Ornamental Grasses (2012) 184 taxa, *Pinus* (2012) 60 taxa
Missouri Botanical Garden – *Quercus* (2007 multisite) 40 taxa
Montgomery Botanical Center – *Arecaceae* (2007) 358 taxa; *Cycadales* (2007) 222 taxa
Montreal Botanical Garden/Jardin botanique de Montreal – *Rosa* (2008) 1097 taxa
Morris Arboretum of University of Pennsylvania – *Abies* (2001) 35 taxa; *Acer* (2008 multisite) 82 taxa; *Quercus* (2007 multisite) 58 taxa
The Morton Arboretum – *Acer* (2008 multisite) 67 taxa; *Magnolia* (2011 multisite); *Malus* (1995) 185 taxa; *Quercus* (2007 multi-site) 67 taxa; *Tilia* (2014) 45 taxa, *Ulmus* (2001) 78 taxa
Mount Auburn Cemetery - *Quercus* (2007 multisite) 25 taxa
Mt. Cuba Center – *Hexastylis* (1996) 26 taxa; *Trillium* (2001) 71 taxa
Naples Botanical Garden - *Plumeria* (2011) 348 taxa
New England Wild Flower Society's Garden in the Woods – *Trillium* (2013) 28 taxa
New York Botanical Garden - *Acer* (2008 multisite) 114 taxa; *Quercus* (2007 multisite) 46 taxa
Norfolk Botanical Garden – *Camellia* (1997) 525 taxa; *Hydrangaceae* (2001) 190 taxa, *Lagerstroemia* (2013) 75 taxa
North Carolina Arboretum – *Rhododendron* (1995) 15 species, SE US native azaleas

Phipps Conservatory & Botanical Gardens – Cypripedioideae (2014) 352 taxa
Polly Hill Arboretum – *Stewartia* (2005) 19 taxa
Powell Gardens – *Magnolia* (2011 multisite)
Quarryhill Botanical Garden - *Acer* (2008 multisite) 48 taxa; *Magnolia* (2011 multisite, provisional)
Rancho Santa Ana Botanic Garden - *Quercus* (2007 multisite) 27 taxa
Reiman Gardens at Iowa State University – Dr. Griffith Buck Roses (2009) 75 cultivars
Rhododendron Species Botanical Garden – *Rhododendron* subsect. *fortunea* (1995) 24 taxa
Rogerson Clematis Collection (Friends of) – *Clematis* (2009 provisional) 709 taxa
San Diego Botanic Garden - Bamboo (2013) 121 taxa
San Francisco Botanical Garden at Strybing Arboretum – *Magnolia* (2011 multisite); Mesoamerican Cloud Forest (1997) 331 species in 182 genera
Santa Barbara Botanical Garden – *Dudleya* (2002) 52 taxa
Scott Arboretum of Swarthmore College – *Ilex* (1995) 312 taxa; *Magnolia* (2003 multisite); *Quercus* (2007 multisite) 52 taxa
Smithsonian Gardens – Tropical Species Orchids (2014) 2,400 plants
South Carolina Botanical Garden – *Magnolia* (2011 multisite)
Springs Preserve – Cacti & Succulents of the Mojave Desert (2012) 28 taxa
Starhill Forest Arboretum – *Quercus* (2009 multisite) 290 taxa
Taltree Arboretum - *Quercus* (2010 multisite) 112 taxa
Toledo Botanical Garden – *Hosta* (2002) 468 taxa
Tyler Arboretum – *Rhododendron* (2006) 529 taxa
University of British Columbia Botanical Garden - *Acer* (2008 multisite) 116 taxa; *Magnolia* (2011 multisite)
UC Davis Arboretum –*Quercus* (2007 multisite) 92 taxa
University of California Botanical Garden at Berkeley – Cycads (2011) 103 taxa; Ferns (2012) 370 taxa; *Magnolia* (2011 multisite); *Quercus* (2007 multisite) 71 taxa
University of Florida-North Florida Research Center – *Magnolia* (2011 multisite)
University of Washington Botanic Gardens – *Acer* (2008 multisite) 209 taxa; *Ilex* (2002) 47 species; *Magnolia* (2013 multisite), *Quercus* (2007 multisite) 86 taxa
United States National Arboretum – *Buxus* (1995) 137 taxa
VanDusen Botanical Garden – *Magnolia* multisite (2011 provisional)

Literature Cited

1. American Public Garden Association. 14 July 2014. <http://www.publicgardens.org/>.
2. Weathington, Mark. 2009. The NAPCC Cercis Collection at the JC Raulston Arboretum. The Public Garden 24 (1):38-39.
3. Weathington, Mark. 2011. The Magnolia Collection at the JC Raulston Arboretum at NC State University. Magnolia 90:4-7.
4. Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, June 2008 [and more or less continuously updated since]. 12 July 2014 <http://www.mobot.org/MOBOT/research/APWeb/>
5. Taylor, Patricia. 2011. Gardens for the Greater Good. The American Gardener 90 (6) 14-19.

Figure 3. *Metasequoia glyptostroboides* branchlet variation at the Dawes Arboretum.