Plant Breeding & Evaluation

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'Summer Cascade' River Birch

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Index Words: *Betula nigra* L., New Introduction, Nursery Crop

Nature of Work: River birch (*Betula nigra* L.) are important landscape plants for much of the United States and are valued for their desirable ornamental characteristics (4), heat tolerance (2), tolerance to poor drainage (1), and pest resistance (3). Identification and evaluation of new forms of river birch can expand the utility of this tree for diverse landscape applications.

Results and Discussion: 'Summer Cascade' river birch (PPAF) is a new, weeping form of river birch that was discovered by John and Daniel Allen at Shiloh Nursery in Harmony, NC. The tree is unique in its pendulous habit, with graceful arching branches, that creates an ideal specimen plant or focal point in the landscape (Figure 1). Detailed characteristics of the tree include:

Habit. If untrained, the plant forms a mounded shrub or small tree with successive layers of arching branches. Alternatively, the plant can be staked/trained with a central trunk and pendulous branches. Flexible new growth also makes the tree ideal for use in topiary.

Growth Rate. Growth is fast. A 6 to 8 foot tall branched tree can be produced in one growing season from a rooted cutting.

Size. The original plant, grown free-form, is 6 feet tall and 10 feet wide after 10 years. When staked or trained, it can be grown as a tree to an undetermined height, but could potentially reach 60 feet as is typical for this species.

Adaptability. Typical of river birch, 'Summer Cascade' is extremely adaptable and tolerates a wide range of growing conditions including high temperatures and wet soils.

Pest resistance. As with other river birch, 'Summer Cascade' is expected to have excellent resistance to Bronze Birch Borer and good resistance to Birch Leaf Miner.

Propagation. 'Summer Cascade' is easily propagated by softwood stem cuttings. Terminal and sub-terminal cuttings, from firm wood, can be rooted in high percentages (> 95%) when treated with 3,000 to 5,000 ppm indole butyric acid in 50% isopropyl alcohol.

Licensing. 'Summer Cascade' River Birch PPAF is being released as a joint introduction by Shiloh Nursery, North Carolina State University, and North Carolina Foundation Seed. Funding to assist in developing and releasing this plant was provided by the North Carolina Specialty Crops Program. Parties interested in propagating this tree can contact Thomas Ranney at the following address: Tom Ranney, Professor of Horticultural Science, Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, Fletcher, North Carolina, 28732. Phone: 828-684-3562. email: tom_ranney@ncsu.edu.

Significance to Industry: River birch is an important native species that is used extensively in landscapes across the country. 'Summer Cascade' is a unique, new weeping form of river birch that has potential for use as a specimen tree, focal point, group plantings, or creative design elements including use in topiary. The adaptability and pest resistence of this species further makes this an excellent candidate as a nursery crop and landscape plant.

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3. Santamour, F.S. Jr. 1999. Progress in the development of borer-resistant white-barked birches. J. Arbor. 25:151-162.

4. Weaver, R.E. Jr. 1978. The ornamental birches. Arnoldia 38(4):117-131.Figure 1. 'Summer Cascade' river birch.

Figure 1. ‘Summer Cascade’ river birch.

Callus Production in Anther Cultures of Flowering Dogwood (*Cornus florida*) and Southern Magnolia (*Magnolia grandiflora*)

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Index words: Anther Culture, Callus, *Cornus florida*, Picloram, *Magnolia grandiflora*

Nature of Work: In a preliminary attempt to develop dihaploids for the improvement of flowering dogwood (*Cornus florida*) and southern magnolia (*Magnolia grandifolia* L.), anther cultures were initiated using popular cultivars of these species. Southern magnolias and flowering dogwoods are major landscape trees produced in southern US nurseries. In recent years, both southern nurseries and consumers have experienced a rising trend in tree loss in flowering dogwood due mainly to heat and damaging diseases caused by several fungal species (*Microsphaera pulchra*, *Elsinoe corni*, *Discula destructiva*).

Magnolia grandiflora is a hexaploid. High ploidy is believed to buffer the contribution of diploid species in hybrids to an extent where desirable traits of the diploid species are not visibly expressed. Due to the lack of requisite genetic variation in existing selections, we have set our breeding objectives to encompass mutation breeding, somaclonal variation and anther culture for producton of dihaploids, in an effort to advance knowledge and contribute to the genetic improvement of these two species.

Magnolia grandiflora 'Little Gem' flower buds were collected from field-grown trees in south Mississippi, while dogwood flower buds were collected from 'Junior Miss', 'Cherokee Brave', and 'Cherokee Princess' in south Tennessee. The unopened flower buds were disinfested in 20% Clorox containing a drop of dishwashing soap for 20 min followed by three rinses in sterile distilled water inside a laminar airflow cabinet. Anthers were cultured on B5 (Gamborg et al., 1968) basal salt medium with vitamins supplemented with 7% sucrose and picloram (0, 0.5, 1, 3, 5, 10, 15, 20 mg/L) for *M*. *grandifolia* or 4% sucrose, 0.5g/L casein hydrolysate and a factorial combination of picloram (0.1, 0.3, 1.0 mg/L) and TDZ (0.1, 0.3, 1.0 mg/L) for *C*. *florida*. The pH of both media was adjusted to 5.7 prior to autoclaving at 121°C and 1.1kgcm^{-2} for 15 min. Cultures were incubated at 25-28°C in the dark in completely randomized designs with 7 replications per treatment. After 12 weeks, induced

callus masses were aseptically weighed. Callus fresh weight and frequency data were analyzed statistically.

Results and Discussion: Callus initiation was observed at the cut ends of the filaments and self-ruptured portions of the anther walls of *C*. *florida*, three weeks after culture. As the calli multiplied, three types could be distinguished: 1) yellowish brown friable callus; 2) light yellow friable callus which appeared to emerge from the yellowish brown type; and, 3) compact light green callus. There was a significant difference (p=0.05) between the three cultivars in callus production (Table 1). 'Junior Miss' was the most responsive while 'Cherokee Princess' was least responsive in both the frequency (%) and quantity of callus produced.

Table 1. Callus production in anther cultures of three flowering dogwood cultivars

Cultivar	Callus frequency (%)	Callus fresh weight (g)
Junior Miss	69.35 a*	3.93 a
Cherokee Brave	74.59 a	1.87 b
Cherokee Princess	39.48 b	0.73 c

* Means in the same column with different letters are significantly different at p=0.05

For *M*. *grandifolia*, callus initiation occurred mainly at the attachment ends of the anthers. When two thirds of this portion were cut off, callus initiation still occurred at the cut ends suggesting that wounding, created by detaching the anthers from the mother tissue, facilitated the initiation of callus production. The frequency (62-80%; Fig. 1A) and the quantity (Fig. 1B) of calli produced (Fig. 1B) were significantly influenced by picloram at 10, 15 and 20 mg/L and 15 and 20 mg/L respectively.

Significance to Industry: Plants regenerated via callus from cultured anthers may possess different chromosome numbers that can be exploited for breeding. The haploids can be doubled using colchicine, and incorporated into a breeding program to expedite the development of dogwood and southern magnolia genotypes with greater disease resistance, enhanced flower color, number of blooms or tolerance to abiotic stresses.

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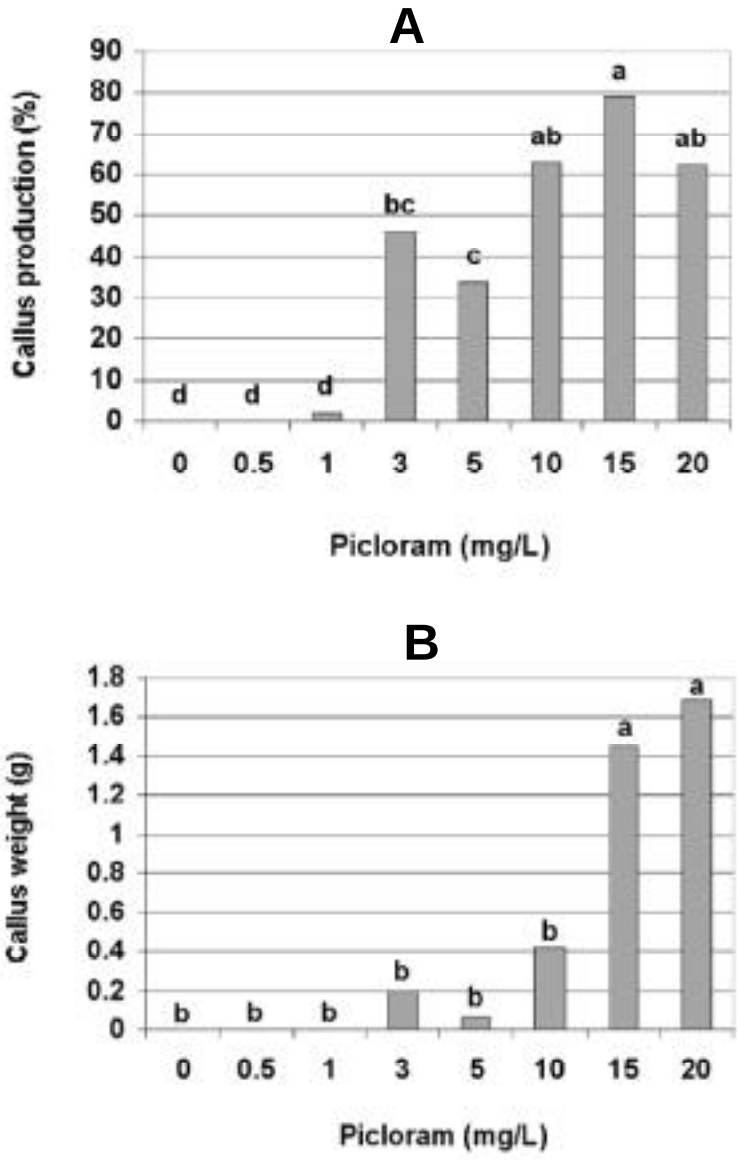

Fig. 1. Effect of picloram on the frequency of callus production (A) and quantity of callus produced (B) in anther cultures of *Magnolia grandiflora*

Buddleja Breeding at the University of Arkansas

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Index Words: Butterfly Bush, *Buddleja davidii*, *Buddleja indica,* Hybridization, Invasiveness

Nature of Work: The *Buddleja* breeding program at the University of Arkansas began in 1999 and has three objectives. The first is to develop sterile *Buddleja* plants through interspecific hybridization. The second is to develop *Buddleja* hybrids with attractive gray pubescent foliage, similar to *B*. 'Lochinch', but with additional flower colors. The third objective is to develop novel *Buddleja* hybrids using species heretofore not commonly used.

One way to obtain sterile *Buddleja* hybrids is to manipulate the chromosome number. Crosses between a tetraploid and a diploid plant can result in triploid offspring. Plants with an odd chromosome number are oftentimes sterile. For example, *B. davidii* is a tetraploid (2n=76). If crossed to the diploid *B*. *crispa* (2n=38), the F_1 progeny might be triploids (2n=57) and possibly sterile.

Buddleja 'Lochinch' is a commonly-grown, gray-foliaged butterfly bush cultivar that is thought to be the result of a cross between *B. fallowiana* and *B. davidii* (1). The particular selection has light blue flowers over a long period of time in the summer. *Buddleja fallowiana* is characterized by an attractive white pubescence covering the stems and leaves. In this respect, it is similar to *B*. *nivea*. *Buddleja nivea* is less cold hardy than *B*. *fallowiana*, but the pubescence on this species is even more noticeable than it is on *B*. *fallowiana*.

One of the species not commonly used in previous *Buddleja* hybridization is *B*. *indica*, or parlor oak. In the past, this species has been cultivated as a houseplant and has some use for bonsai. Native to Madagascar and the Mascarene Islands, the species is the only African species known to be a tetraploid (2n=76) (2, 3). The most outstanding characteristic of *B*. *indica* is the foliage; it is dark green in color, glaucescent and oak-shaped. The flowers on most cultivated clones of parlor oak are insignificant, a few-flowered cyme borne in the axils of the leaves. Flowers are greenish-yellow and flowering begins in early summer and continues into fall. Unlike most cultivated *Buddleja*, the fruit of *B*. *indica* is a yellow-white, fleshy berry. Having a fleshy berry as a fruit, rather

than a dry capsule, is a characteristic shared by the related species *B. madagascarensis*. Both species are in the section *Nicodemia* of the genus *Buddleja* (2).

Results and Discussion: We are evaluating the F_1 progeny of several *Buddleja* crosses for sterility. These crosses use either the diploid *B*. *alternifolia* or *B*. *crispa* as one parent and tetraploid *B*. *davidii* as the other parent. F_1 seedling populations have been planted for evaluation, both for ornamental characteristics and for the ability to set seed. Reduced seed capsule formation has also been observed in *Buddleja* hybrids with *B*. x *weyeriana* 'Moonlight' as a parent. This was despite the fact that the F_1 seedlings were evaluated within a seedling block that contained many other fertile *B*. *davidii* hybrids.

To enhance the foliage characteristic of *B*. *davidii* selections, we are evaluating a series of *Buddleja* hybrids that are crosses between *B*. *davidii* and either *B*. *fallowiana* or *B*. *nivea*. The F_1 hybrids between *B*. *davidii* 'White Bouquet' and *B*. *fallowiana* have attractive pubescence and long, relatively stiff panicles of blue or white flowers. They were winter hardy in Zone 6b during the winter of 2001-2002. The F_1 hybrids between *B*. *davidii* 'White Bouquet' and *B*. *nivea* were also winter hardy during 2001-2002. Unlike the *B*. *fallowiana* hybrids, the inflorescence on the *B*. *nivea* hybrid is lax and spreading across the top of the plant.

In the fall of 2000, *B*. *davidii* 'White Bouquet' and *B*. *indica* were crossed. Seed was collected from capsules on *B*. *davidii* 'White Bouquet' and sown in February 2001. F_1 hybrids began to flower in September of 2001. Morphological measurements (Table 1) and randomly amplified polymorphic DNA (RAPD) markers were used to confirm the hybrid origin of the progeny. Selected F_1 hybrids were backcrossed to both parents in 2001 and seed collected from these crosses in early 2002. These plants are now being evaluated in the field.

Leaves of the F_1 progeny are opposite, simple, ovate to lanceolate, dentate to sinuate with 6-8 pairs of obtuse teeth. The color of the leaves is dark, almost blue-green. Depending on the plant, pubescence on the leaf can be either tan or white. The growth habit of the plant also varies. Under cultivation the *B*. *indica* clone used in the hybrid has a spreading growth habit. Mature plants are often broader than high and this is especially apparent on plants grown in full sun. Several progeny (for example, 01-20-501) exhibit this characteristic. Others are upright in appearance (for example, 01-20-510) and resemble *B*. *davidii* 'White Bouquet' in that respect.

Significance to Industry: Development of sterile *Buddleja* hybrids will address the problem of invasiveness found with some *B*. *davidii* cultivars. Using unusual species in hybridization will introduce other ornamental characteristics (foliage shape and flower color) not currently seen in commonly cultivated butterfly bush cultivars.

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Table 1. Morphological measurements for the F_1 progeny of the cross *Buddleja davidii* 'White Bouquet' x *B. indica.* All measurements are given in centimeters and are the average of ten individuals. The second leaf below the inflorescence was measured to determine leaf width and height.

	Number of flowers per inflorescence	Flower size	Leaf length	Leaf width	Inflorescence length
B. indica	*4*	*.35*	*4.2*	*3.1*	*13.7*
B. davidii 'White Bouquet'	304	.51	7.8	3.2	- - -
F_1 hybrids:					
01-20-498	41	.46	5.3	2.3	6.4
01-20-499	59	.49	4.5	2.4	5.0
01-20-500	- - -	- - -	- - -	- - -	- - -
01-20-501	35	.50	6.3	2.7	5.3
01-20-502	37	.43	6.6	- - -	6.6
01-20-503	39	.36	6.3	4.6	6.8
01-20-504	47	.38	6.9	3.3	5.3
01-20-505	61	.57	6.7	3.3	8.2
01-20-506	62	.53	5.2	4.0	6.5
01-20-507	- - -	- - -	- - -	- - -	- - -
01-20-508	68	.51	4.5	2.1	7.4
01-20-509	66	.55	6.1	2.1	8.5
01-20-510	41	.52	6.3	2.8	7.8
01-20-511	31	.39	4.6	2.2	5.7
01-20-512	- - -	- - -	- - -	- - -	- - -
01-20-513	27	.40	5.5	- - -	5.5
01-20-514	21	.38	5.4	1.7	6.1
01-20-515	- - -	.28	3.5	1.7	5.5

Rhaphiolepis Evaluations in South Georgia

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Index Words: Defoliation, Disease, Entomosporium, Growth, Indian Hawthorn, *Rhaphiolepis*

Nature of Work: A study was initiated in March, 1996 to evaluate the ornamental characteristics (growth, flowering, etc...) and disease susceptibility of eleven Indian hawthorn selections to Entomosporium leaf spot. The cultivars 'Elizabeth'; a popular selection from Mississippi, and 'Pink Pearl' were added in the spring of 1997. Many new selections of Indian hawthorn have become available since Will Corley published his evaluation work with the genus in the mid 80's. Disease pressure is also greater in the Coastal Plain compared to the Piedmont where Corley's evaluations were conducted.

Plants were established at the Coastal Plain Station (USDA 8a) in Tifton on a Tifton loamy sand. Four single plant replicates were placed on 7.5' centers within the rows and 12.5' between the rows. The plants were fertilized in April of 1996-2001 using IMC 16-4-8 + minors at the rate of 50 lb. N/A. Plants have been watered as needed using drip irrigation (Bowsmith 1.0 gal/hr emitters). Weeds have been controlled by directed sprays of RoundUp herbicide as needed and by preemergent applications of Gallery and Factor in the spring and Gallery and Surflan in the fall. Disease and defoliation were evaluated in May and June of 1997, 1998, and 2001. Enviromental conditions were not suitable for the development of Entomosporium leaf spot in 1999 and 2000. Disease and defoliation were visually evaluated on a scale of 1 to 5 where 1 = no disease, 2 = 1% to 25% of the leaves diseased or defoliated, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = >76%. For simplification, disease and defoliation ratings have been listed as (E) excellent, (G) good, (F) fair, and (U) unacceptable. Disease and defoliation ratings less than or equal to 2 were considered excellent, between 2 and 3 were considered good, 3 to 3.9 was fair, and 4 or greater was unacceptable. A summary of rankings and growth data is shown (Table 1).

Results and Discussion: The selections Minor, Georgia Petite, Olivia, and Georgia Charm have demonstrated excellent to good resistance to leaf spotting and defoliation. Results for these four selections were similar for all years. The selection Pink Pearl shows moderate disease and defoliation. Disease development is unacceptable while leaf drop is fair for Clara, the most commonly used Indian hawthorn in south Georgia

landscapes. Snow White has fair disease resistance but drops fewer leaves than Clara. The selection Eleanor Taber is the best pink- flowered selection for resistance to disease and defoliation. The selections Ballerina, Bay Breeze, Cameo, Elizabeth, and Kathy are considered unacceptable due to extensive disease development and defoliation and should not be recommended for production or landscape use.

Growth data provided in Table 1 should be useful for making landscape selections. Of the plants receiving excellent ratings, Georgia Petite and Olivia are the smallest while Georgia Charm is spreading and Minor is the tallest of the group. The selection Pink Pearl, which has double-pink flowers and large leaves, may be useful as a larger shrub. Snow White grows similar to Clara and may be a better landscape plant with regards to disease and defoliation.

Significance to Industry: The selections Georgia Charm, Georgia Petite, Minor (also known as Gulf Green), and Olivia are superior selections for resistance to Entomosporium leaf spot. Hopefully the selection Georgia Petite will be available to the landscape trade in the near future. The only selection on which I have seen fireblight in south Georgia is Olivia, with damage being minor (personal observation). Cercospera leaf blight occurs in the fall and winter on the selection Minor, but the defoliation caused by this disease has been acceptable. Growers and landscapers wishing to implement IPM programs now have several disease resistant selections to choose from. Trials are being continued with new germplasm in an attempt to find new forms and a dwarf, leaf spot resistant pink selection.

Table 1. Summary of disease and defoliation ratings for 13 selections of Indian hawthorn for the years 1997, 1998, and 2001. Rankings are (E) Excellent, (G) Good, (F) fair, and (U) unacceptable. Growth data during the five year period is also provided.

Selection	Disease	Defoliation	# of years	Height and Width (ft.)
Ballerina	U	U	5	2.6 x 5.7
Bay Breeze	U	U	5	1.8 x 5.0
Cameo	U	U	5	2.3 x 4.5
Clara	U	F	5	2.5 x 5.6
Kathy	U	U	4	3.4 x 7.0
Elizabeth	U	U	4	2.7 x 5.5
Snow White	F	G	5	2.4 x 5.6
Eleanor Taber	U-F	F	5	2.3 x 5.1
Pink Pearl	F-G	F-G	4	4.5 x 6.4
Georgia Charm	G	E	5	3.8 x 8.0
Olivia	E	E	5	2.7 x 6.3
Minor	E	E	5	5.8 x 7.5
Georgia Petite	E	E	5	3.1 x 5.7

Yellow Flowering Magnolias for Florida and the Gulf Coast

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Index words: Magnolia, Flowering Tree, New Crops

Nature of Work: Magnolias are prized worldwide for their spring flowers and have become some of the most widely planted flowering trees. Star magnolia (*Magnolia stellata*) and saucer magnolia *(M. (soulangiana*) are two of the best known deciduous flowering magnolias, with star magnolia valued for its cold hardiness and saucer magnolia planted for its flowering display. However, many new hybrids have larger flowers, later blooming (to make frost damage less likely), and a wide range of flower colors, plant sizes and habits. Particularly noteworthy are the yellow flowering hybrids.

Results and Discussion: The hottest trend in magnolias today is yellow flowering magnolias. Breeders are using the North American native Cucumbertree *(Magnolia acuminata)* as a source of yellow flower color, cold hardiness, and soil adaptability. The smaller stature of *Magnolia acuminata* var. *subcordata* (sometimes called *M. cordata*) has been especially valued in breeding programs. Hybridization with yulan magnolia *(Magnolia denudata)*, lily-flowered magnolia *(M. liliiflora)* and other species combines characters of yellow flower color and flower precociousness. Resulting hybrids range in size from small trees to large trees up to 80 feet tall. Some hybrids are reputed to be cold hardy into USDA Hardiness Zone 3 but most can be grown in USDA Hardiness Zones 5 to 8 and possibly 9.

Results of these initial breeding efforts from the 1950's, 1960's and 1970's include such cultivars as 'Elizabeth' and 'Yellow Bird' (hybridized by Eva Marie Sperber), and 'Butterflies' (from Philip Savage). The more numerous "next generation" of yellow flowering hybrids, now becoming available, often has a broader genetic base. Currently, the Magnolia Society website (www.magnoliasociety.org) and various authors list about 35 cultivars claiming yellow or yellowish flowers, while some growers estimate registrations of new cultivars in the next few years will bring total numbers over 60 (1; 2; 5; 7; P. McCracken, personal communication). Unfortunately, few are widely available due to their novelty and difficulty in propagation. Even fewer have been evaluated for yellow flower color and landscape performance.

Twenty yellow flowering cultivars are planted at the University of Florida's North Florida Research and Education Center in Quincy, Florida (USDA Cold Hardiness Zone 8b; AHS Heat Zone 9) about 20 miles west of Tallahassee, 10 miles south of the Florida-Georgia state border, and 45 miles north of the Gulf of Mexico. They include 'Elizabeth' from Eva Marie Sperber, 'Brenda' from Mike Stansberry, and 'Ivory Chalice', 'Golden Sun' and 'Legend' from the late David Leach. Several cultivars from breeder Philip Savage are planted: 'Gold Star', 'Maxine Merrill', 'Yellow Lantern', and 'Butterflies'. Also planted are a large number of cultivars from the late August Kehr of North Carolina: 'Gold Crown', 'Gold Cup', 'Golden Endeavor', 'Hot Flash', 'Solar Flair', 'Stellar Acclaim', 'Sunburst', 'Sundance', 'Sun Ray', 'Sun Spire' and 'Tranquility'. The purpose of the planting is to screen cultivars for good yellow flower color in USDA Hardiness Zone 8b, with the ultimate goal of formally evaluating promising cultivars in a broader, regional evaluation project.

One of the greatest challenges with yellow flowering magnolias is the tendency of the yellow flower color to vary from year to year or not develop fully in certain climates and regions (e.g., the Coastal Plain of the Southeast U.S.). There is evidence with saucer magnolia that warm winter temperatures improve flower color while reducing flower size (3). Others believe bright sunlight and acidic soils reduce yellow flower color (6). In north Florida, 'Butterflies', 'Golden Sun' and 'Maxine Merrill' have consistently produced flowers with good yellow flower color, but it should be noted that most of August Kehr's hybrids have not been established long enough for full evaluation.

Another challenge of yellow flowering magnolias is propagation with the primary methods being budding, grafting and tissue culture. *Magnolia acuminata* is difficult to root from cuttings, and many of its yellow-flowering progeny also carry this trait. Parentage of many new cultivars includes *M. liliiflora*, *M. (soulangiana* and other species that root more easily. These newer cultivars hold some promise of easier propagation by cuttings. I have tried rooting a small number of cultivars using a 3:1 ratio of K-IBA to K-NAA in rates of 5,000:1,500 ppm, 10,000:3,000 ppm, and 15,000:4,500 ppm as a 5 second quick dip (4). Terminal, 4- to 6-inch cuttings were stuck in 2.25-inch rose pots (Lerio SR-225 plastic cups, Nursery Supplies Inc., Chambersburg, PA) filled with a media of milled sphagnum peat and horticultural perlite (1:1, v/v) and placed under heavy mist (15 seconds every 8 minutes) in an unshaded fiberglass greenhouse providing about 20% light exclusion.

The small sizes of stock plants limited the number and quality of cuttings. However, rooting percentages in 2001 ranged from 50 to 100% for 'Hot Flash', 'Ivory Chalice', 'Tranquility' and 'Sun Ray'. Rooting of other

cultivars was less than 40% ('Maxine Merrill', 'Yellow Lantern', 'Solar Flair', 'Golden Sun', and 'Golden Endeavor'). There was no apparent pattern in rooting responses to rates of rooting compounds. In my limited experience, semi-hardwood cuttings (taken in late April, 2001, in north Florida) rooted better than softwood cuttings, and 16 weeks or more were needed before cuttings were rooted well enough to be potted. Future work will further explore propagation of yellow flowering magnolias by cuttings.

Significance to Industry: Cultivars of yellow flowering magnolias should be more widely planted to evaluate and determine the best cultivars for each region. Demand for these superior flowering magnolias will increase as they become more widely known and planted. The promising future for these magnolias will require growers to have better information about propagation, production and shipping. Landscapers and consumers will need to learn about cultivars best suited for their region and cultivars' flowering characteristics and ultimate size and shape.

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Use of F-AFLP for the Determination of Genetic Relationships in *Cornus florida* Twin-seeds

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Index words: F-AFLP, *Cornus florida*, Twin-seeded Drupes, Flowering Dogwood

Nature of Work: Twin seedlings arise from polyembryonic seeds. Polyembryonic seeds can be haploid-haploid, diploid-diploid, or haploid-diploid twins. In flowering dogwoods, twin-plants do not occur at relatively high rates. The fruit of *C. florida* like those of other *Cornus* species can contain a 2-seeded drupe. This study was carried out to compare the genetic relationship of twin-seedlings from *C. florida* drupes using fluorescent Amplified Fragment Length Polymorphism (F-AFLP) banding patterns. The genetic relationship and origin of twins is discussed according to similarities of polymorphisms of amplified DNA bands. Possible use of F-AFLP techniques in genetic studies of twin-seeds is also discussed.

Ripe fruits of *C. florida* 'Cherokee Princess' were collected in a nursery near Nashville, TN. Fifty fruits were used in this experiment. Fruit pulp was removed and drupes were surface sterilized in alcohol followed by flaming. Drupes were dissected and examined for the presence of twin seeds. Those that contained two fully developed seeds were placed on MS solid basal medium and cultured in a growth chamber at 25C. After four weeks of incubation, two sets of twins that germinated and grew into plantlets were selected for analyses. Shoots were dissected and cultured on MS solid medium supplemented with 0.2mg/l thidiazuron and 0.05mg/l indole acetic acid. Cotyledons were used for genomic DNA isolation. DNA isolation, F-AFLP analysis and data collection was following established procedures (3). Eight MseI (M1=CAA, M2=CTT, M3=CAC, M4=CTG, M5=CAG, M6=CTC, M7=CAT, M8=CTA) and EcoRI (E1= CAA, E2=AAG, M3=ACA, E4=ACC, E5=ACG, E6=SCT, E7=AGC, E8=AGG) selection primers with sixty-four primer combinations were used. EcoRI primers were 3'-end labeled with IRD-800 (LI-COR). F-AFLP composite gels were generated using gene image IR Database Manager 3.56 software (Scanalytics Inc., Fairfax, VA).

Results and Discussion: Only 14% of dissected fruits contained drupes with fully developed twin-seeds, 6% had one fully developed seed and one shrunken one, and 80% contained a single seed. When twin-

seeds were placed on the MS basal medium, both seeds began to germinate after three days at 25C and both seedlings were normal in appearance. F-AFLP analysis of the genomic DNA of twins revealed twenty-nine pairs of primer combinations that amplified clear bands for all pairs. Most bands ranged between 100-270 bp. Each twin member shared the same bands. However, bands that differentiated different pairs were identified with primer combinations m1e4 (ca. 110bp), m1e7 (210bp), m3e1 (135-200bp), m4e5 (200-250bp). Bands shared by one, by both, and those different between twins were identified in primer combinations m1e5 (130-140bp), m8e4 (150bp), m8e7 (135bp, 170bp), m3e8 (250bp), m4e7 (130-150bp), m5e4 (110bp, 140bp, 150bp, 180, 200bp), m5e6 (110bp, 150bp, 260bp), m5e7 (104-200bp, 7 bands), m6e4 (260bp, 440bp), m6e8 (115bp) 120bp, 350bp), m7e4 (250bp), m7e8 (160bp). Bands specific to an individual seedling were identified in primer combinations m2e8 (Seed No. 2-2, band 120bp), m1e2 (seed 2-1, band 204bp), m8e5 (seed No 2-1, band 170bp), m8e7 (seed No.3-1, band 148bp), m3e4 (seed No, 3-1, band 160bp), m3e8 (seed No2-1, 120bp, seed No. 3-1, band 110-115 bp). With primer combinations m2e4, m2e5, m3e7, m3e6, m8e6, most AFLP banding patterns were similar for all members of both sets of twins but some bands were not present in one of the four seeds analyzed.

In *Arabidopsis,* Shevchenko et al (2) found that twins often arise from faulty cell divisions in zygotes or from splitting embryos. Guo and Mu (1) discovered that some seeds in rice had two embryos. One embryo was formed through normal sexual hybridization and the other from nucellar cells. In our results, most AFLP bands were shared with each twin. However, some bands were different between twins although they were shared with seedlings from different sets of twins. These results suggest that twin-seeds of *C. florida* may not be formed from embryo splitting.

Significance to the Industry: F-AFLP analysis is an ideal technique to investigate the origin of twins and of the genetic loci that can be used to differentiate them. All twin seedlings studied in this research were propagated in tissue culture. Once these plantlets reach suitable sizes, they will be planted out for field evaluation. If differing phenotypic characteristics develop as plants mature, responsible genes could easily be trace back to their genetic background.

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Acknowledgement: This study was partially funded by a USDA Evans-Allen grant.

Composite Gel Profile of F-AFLP Analysis of *Cornus florida* Twin-seeds

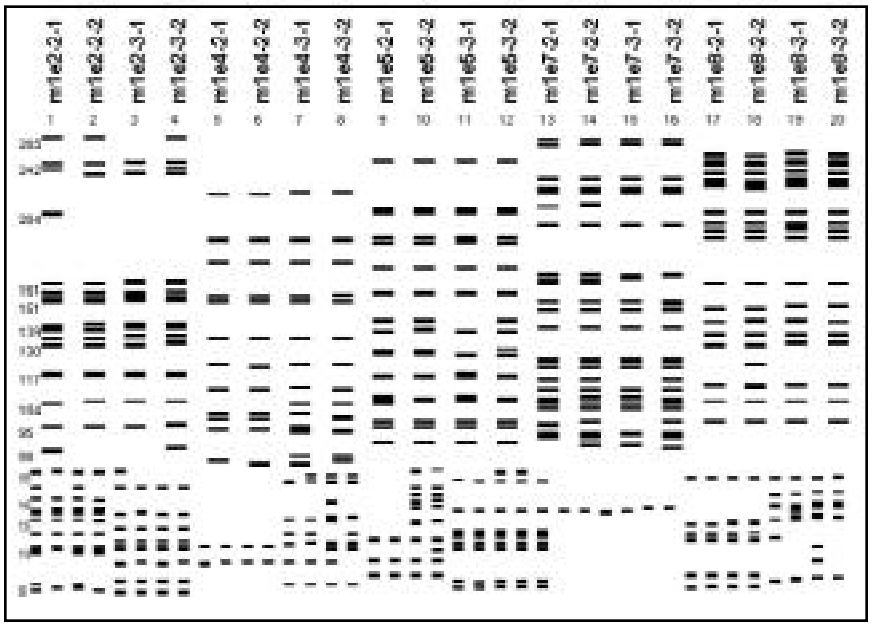
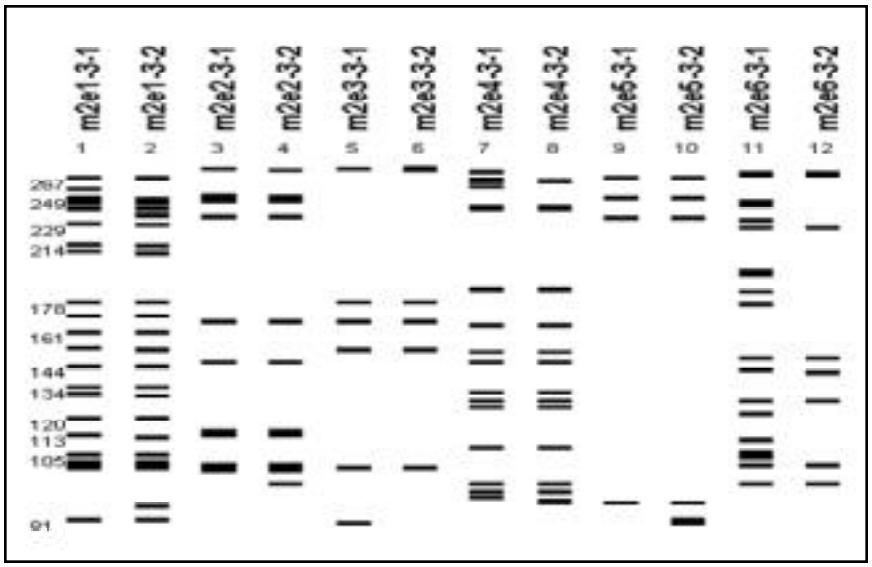


Fig.1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2
3
4
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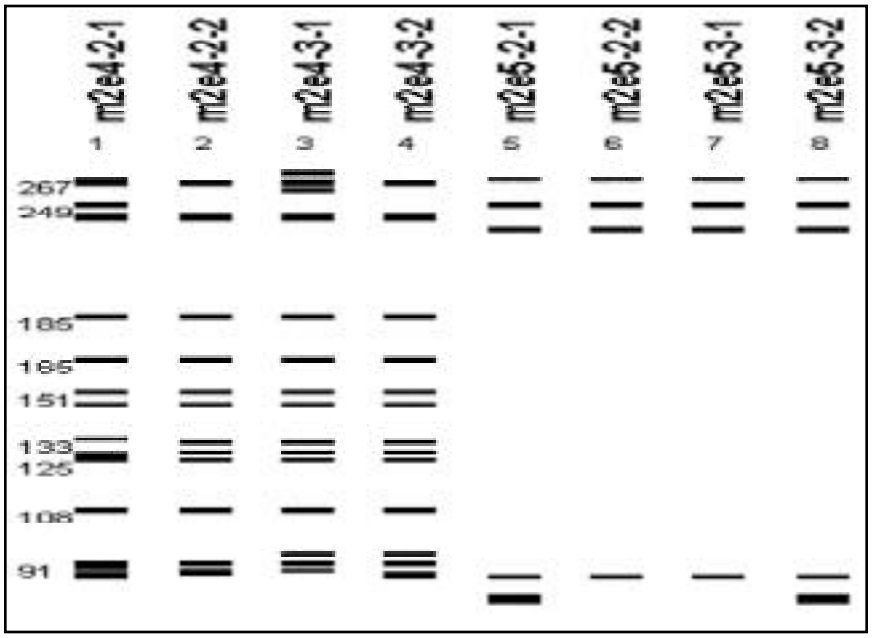
Fig.2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m2e5-3-2
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125
108
91

Fig.3

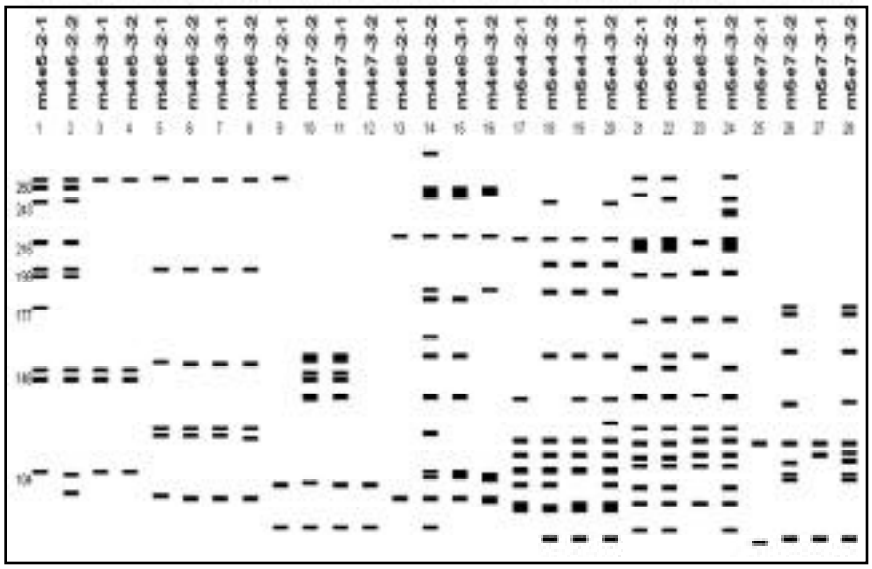
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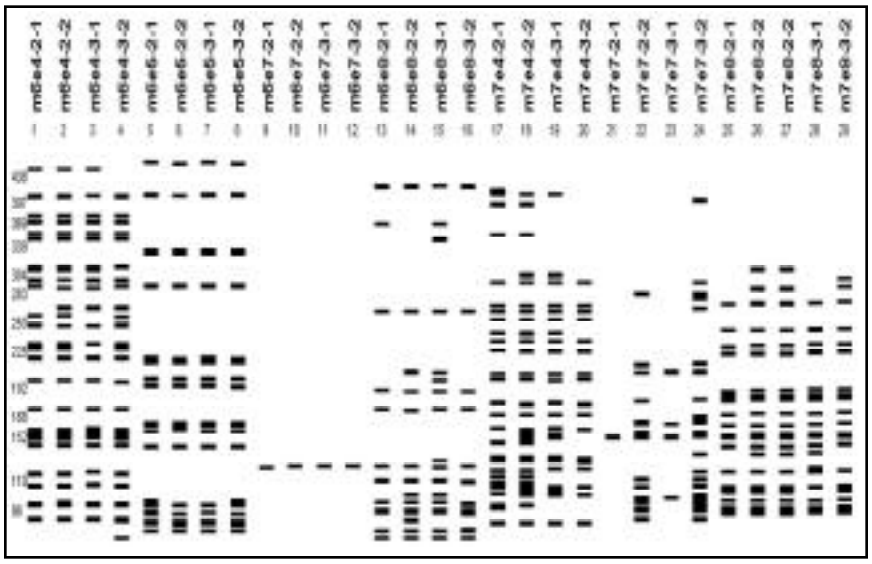
Fig.4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m7e8-2-2
m7e8-3-1
m7e8-3-2

Fig.5

Effect of Cold Treatments on cDNA Differential Display in Japanese Spurge

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Index Words: Japanese Spurge, *Pachysandra terminalis*, Cold Treatment, cDNA Differential Display

Nature of Work: Low and freezing temperatures cause annual yield losses and crop failures wherever cold sensitive crops are grown. Understanding of antifreeze mechanisms in plant species that tolerate sporadic freezing temperatures is needed for the development of strategies that would minimize losses due to low temperature fluctuations. In this study we evaluated the effects of low temperatures on leaf tissue of Japanese spurge (*Pachysandra terminalis)*. Japanese spurge is a cold tolerant perennial in Tennessee as well as in most of the 48 contiguous states. Differential display of extracts from leaf tissue that are subjected to various cold treatments may reveal genes that confer cold tolerance in *P. terminalis*.

During December of 2001, sixteen Japanese spurge plants in quart-sized containers were transferred from an unheated greenhouse to a growth chamber set at 24C with a 16hr photoperiod for six weeks. The temperature in the growth chamber was then reduced to 10C for one additional week. The cold treatment study consisted of taking plants from the growth chamber and placing them in incubators set at temperatures ranging from 5C to –10C. The first set of plants was incubated at 5C for 3 days, at 0C for 24 hr, at -5C for 2, 4, or 6hr, and finally at -10C for 30 min or 1 hr. After each cold treatment, young leaves were sampled from each plant and stored at –70 for later analysis. At the end of the experiment, all plants were incubated at 7C for recovery.

For differential display analysis, leaf tissue samples were taken from plants that were incubated at the following temperatures: 5C, 0C (6hr), -5C (2hr), and 24C (control treatment). The total RNA was isolated from all leaf samples using B10101, FastRNAkit-Green (Q-BIO gene, Rutherford, CA). The quality of all isolated RNA samples was evaluated on a formaldehyde agarose gel for freedom of DNA contamination. Following quality evaluation, all samples were used for cDNA synthesis and differential display. The Delta Differential Display PT1173-1 kit (Clontech, Palo Alto, CA) was used for this procedure and 32P-dATP (Perkin Elmer, Albany, MA) for radioactive labeling. After amplification, PCR products

were separated in 5.5% denaturing acrylamide gel and signals were recorded on X-ray film by overnight exposure at –70C. Bands that were present only in specific treatments or showed difference in intensities were marked and considered to contain DNA fragments of putative low temperature responsive genes.

Results and Discussion: After one week at 7C, young leaves treated at 0C for 6hr and —5C for 2 hr recovered and shoots resumed growth. Young leaves that were incubated for 4 and 6 hours at –5C died, but mature leaves did not show any sign of cold damage. After treatment at -10C for 30min, both old and young leaves were killed. No growth was observed on these plants after being incubated at 7C.

The differential display technique can be used to elucidate the expression profiles of known and even novel genes following cellular stimuli (1, 2). In this investigation, cDNA differential displays produced a total of 290 bands. Compared to leaf tissues sampled from plants incubated at 24C, 119 bands showed higher intensity in the 0C treatment and 43 bands from the 5C treatment. Banding profiles at –5C were similar to the 0C treatment. Several bands present after exposure to 24C disappeared after a 5C cold treatment.

Significance to the Industry: Result from this study showed that newly formed leaves of Japanese spurge can survive short period of exposures at -5C and older leaves up to 6 hr at this temperature. Although newly formed leaves and shoots were killed by subzero temperature exposures, these plants resumed growth. This suggests that mature leaves protect auxiliary shoot apexes. However, -10C temperature exposures were lethal to all plants.

Several cDNA fragments that appeared to be related to low temperature fluctuations were observed in this study. Additional research on the identification of these fragments and their characterization may lead us to identify antifreeze genes and assist in the understanding of mechanisms that induce cold tolerance in *P. terminalis*. Methods used in this study could be used to study other plant species.

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Genetic Transformation of Flowering Dogwood Explants with ESF 39A Antimicrobial Peptide Gene

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Index Words: Flowering Dogwood, *Cornus florida*, Antimicrobial Peptide, Genetic Transformation, Callus

Nature of Work: Flowering dogwoods (*Cornus florida*) are important trees to the nursery industry. They are used extensively in residential, recreational, and industrial plantings. In recent years, two fungal diseases (anthracnose and powdery mildew) have decreased their demand (1). Since conventional breeding and selection for resistance is often a lifetime project with trees, introduction of genes for disease resistance through genetic engineering technologies offers hopes for the timely improvement of this plant. Antimicrobial peptide genes can provide a first line of defense against infection by acting as 'natural antibiotics' against fungal and bacterial infections (2). This research project was initiated in order to transform flowering dogwoods with ESF 39A antimicrobial gene to create new cultivars with resistance to powdery mildew and anthracnose.

Ripe fruits were collected from mature trees and surface sterilized by briefly dipping in 90% ethanol followed by flaming. Fruit pulp was removed and seeds were soaked in a 0.2% NaOCl solution for one hour. Seeds were further surface sterilized by a brief dip in 90% ethanol and flamed. Sterilized seeds were cracked open, embryos removed and plated on MS (3) media solidified with 0.3% phytagel (Sigma) for germination and growth.

Explants were prepared by dissecting in-vitro grown plants from germinated seeds. All explants were incubated in a solution containing *Agrobacterium tumefaciens* strain EHA 105 harboring the ESF 39A antimicrobial peptide gene (4). Tissues infected with EHA 105 agrobacterium suspension were used as control. For calli initiation, explants were removed from the bacterial solution and plated onto MS medium supplemented with 50mg/l kanamycin at 25C in the dark for 25 days. Calli that survived three consecutive subcultures on the kanamycin selection medium were considered putatively transformed. These calli were then plated on an embryo initiation medium containing MS basal salt supplemented with zeatin (2mg/l) and IAA (0.05mg/l) and solidified with 0.03% phytogel.

Results and Discussion: Calli were initiated from cotyledon and hypocotyls explants that were infected with *A. tumefaciens* harboring ESF 39A peptide gene. No callus was produced from explants that consisted of leaves or stem. Ten to 15 days old cotyledons produced the most calli. The control explants did not generate callus on the selection media and they died after two subcultures.

Significance to Industry: Transformed poplars expressing the antimicrobial peptide genes have enhanced disease resistance (5,6). In the present study, we have obtained putatively transformed dogwood tissues. Once whole plants are regenerated from transformed calli, they will be evaluated for enhanced disease resistance. Through the use of this technology, we have a good chance to produce dogwoods resistant to most plant diseases

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Effect Of Irrigation Water pH On Gene Expression In Tomato Roots

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Key Words: *Lycopersicon esculentum* 'Micro-Tom', Acid irrigation water, DD-PCR

Nature of Work: Acid soils affect crop yields worldwide (1, 2). Most acid soils are mineral deficient, which reduces crop yields and increases production costs. In acidic soil, minor elements such as Al and Mn often accumulate to toxic levels. Once high levels of Al enter root systems, they inhibit root growth and interfere with the electron transfer system. Additionally, many essential elements become less available when soils become acidic. To identify genes in tomatoes that respond to stress associated with acidic soil conditions, we irrigated tomatoes with water adjusted to pH levels ranging from 4.0 to 10.0. Gene expression related to water pH treatments was identified through the differential display of cDNA from all root systems analyzed. Several acidic up-regulated cDNA fragments were identified.

Tomato (*Lycopersicon esculentum* 'Micro-Tom') seeds were germinated on moist filter paper in petri dishes incubated for 48 hours at 24C and 16 hr photoperiod. Thirty seedlings were transplanted individually in 3.5" square plastic pots containing a synthetic medium consisting of peat and perlite (1:1). Three groups of 10 potted plants were placed in shallow trays (1 tray per group) and irrigated every 2 days with distilled water adjusted to pH of 4.0, 7.0 and 10.0 with HCl or KOH. Plants were maintained in the incubator until fruit maturity (24C, 16 hr photoperiod). Root systems were harvested and washed three times with tap water followed by three rinses in double distilled water. Clean root systems were frozen in liquid N_2 and stored at –70^{o}C until analyzed.

For differential display, RNA was extracted from 250mg of root tissue using B10101, FastRNAkit-Green (Q-BIO gene, Rutherford, CA) for all treatments. Total RNA samples were evaluated for freedom of DNA contaminations using formaldehyde agarose gels and used for cDNA synthesis and differential display. For this procedure, the Delta Differential Display PT1173-1 kit (Clontech, Palo Alto, CA) was used. ^{32}P-dATP (PerkinElmer, Albany, MA) was used for radioactive labeling and products were amplified by PCR and separated in 5.5% denaturing acrylamide gels by electrophoresis. Kodak^{tm} X-ray films were exposed

overnight on each acrylimide gel under refrigeration (–70C). Bands that formed in gels of each sample were marked, cut off and extracted. After elution of the DNA fragments from each gel section, they were re-amplified using the primer used previously and extracted in 1.0% agarose gel using the Wizard PCR Preps DNA Purification Kit (Promega, Madison, WI). DNA fragments were then cloned to pGEM-easy vector (Promega) and sequence analysis was carried out using DYEnamic Direct Cycle Sequencing Kit (Amersham,) and IRDye-800-M13-reverse primer (LI-COR, Lincoln, NE) with a LI-COR DNA analyzer (Model 4200 IR2 Series). Nucleotide sequences obtained were compared with data in the GeneBank database using NCBI Internet service.

Results and Discussion: We have identified cDNA fragments that were present only in root tissues of tomato plants that were irrigated with pH 4.0 water. These fragments were cloned and sequenced. A listing of clones that were obtained from differential display is shown in Table1.

Tomato plants that were irrigated with pH 4.0 water were shorter than those irrigated with less acidic water. Mineral analysis of different tissues showed that tomato leaf and stem tissues from the pH4.0 irrigation treatment contained very high levels of Al. No accumulation of this element was detected in fruits. Manganese accumulations were also higher in these root systems when compared with those irrigated with water adjusted to higher pH levels. The discovery of genes that would allow plants to grow normally in acidic conditions would be beneficial to agriculture worldwide. These genes could be cloned and incorporated into ornamental or crop plants through genetic engineering methodologies.

Significance to the Industry: We have identified cDNA fragments that were induced by the pH 4.0 irrigation treatment. Some fragments shared high homology with other genes reported in tomato and other plants. Some genes identified have not been previously reported in tomato. Further analysis and characterization of these genes may result in the identification of genes that induce tolerance to acidic soil conditions. Such genes have the potential for improving acid tolerance in ornamental plants as well as in food crops or for use in bio-remediation.

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Acknowledgment:
This research was partially funded by a USDA Evans-Allen grant.

Table 1. Identity Of The cDNA Fragments Isolated From Tomato Root Tissues Irrigated With pH 4.0 Water

cDNA fragments	Highest homologous gene	Alignment Scores
TmDDF-1	6692623	58.0
TmDDF-2	14334613	56.0
TmDDF-3	15987770	46.1
TmDDF-4	8547226	100
TmDDF-5	9587171	363
TmDDF-6	12231299	216
TmDDF-7	1785673	79.8
TmDDF-8	20564412	40
TmDDF-9	1785673	80
TmDDF-10	2065530	458
TmDDF-11	2924257	341
TmDDF-12	295354	142

Transformation of Japanese Flowering Cherry with the Antimicrobial Peptide ESF39A Gene

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Index Words: Japanese Flowering Cherry, Genetic Transformation, Antimicrobial Peptide, *Prunus takesimensis*

Nature of work: Japanese flowering cherries (*Prunus* sp.) are of increasing importance in residential, public and industrial plantings. However, they are susceptible to plant pathogens. Bacterial canker limits the nursery production of flowering cherries in southern states. The availability of disease resistant cultivars would substantially increase their production and use.

Transgenic plants that express the ESF 39A antimicrobial peptide gene have enhanced resistance against fungal and bacterial diseases (1, 2). In this study we are working on incorporating this gene into a Japanese flowering cherry explants to produce cultivars with resistance to *Pseudomonas syringae.* pv*. syringae,* the causal agent of bacterial canker.

Fruits of *P. takesimensis* harvested in Washington, DC were surface sterilized and embryos dissected. These embryos were plated onto MS basic solid medium (3) for germination. After seven days in culture in the dark at 25°C, cotyledons were excised and cut longitudinally into three sections and used as explants. These explants were then incubated in a solution containing *Agrobacterium tumefaciens* strain EHA 105 that harbored the ESF 39A antimicrobial peptide gene following an established procedure (4). Infected explants were cultured on a solid callus induction media that contained MS basic salts (5) supplemented with 50mg/l kanamycin and different combinations of cytokinins and auxins. Calli that survived for six weeks on media supplemented with kanamycin were considered putatively transformed. After two subcultures on this selection media, calli were transferred to an embryo induction medium to regenerate new plants.

Results and Discussion: The composition of callus induction media had significant effects on transformation success. When infected cotyledons were cultured on MS medium containing 1 mg/l zeatin and 0.05 mg/

l IAA, no callus was initiated. However, calli were initiated from all explants when cultured on a medium containing 0.6 mg/l zeatin, 0.03 mg/l IAA, 0.05 mg/l 2,4-D, and 0.06 mg/l IBA. If calli were maintained on the same medium for two additional months, they hardened and failed to grow on organo-genesis or embryogenesis media.

When calli induced from infected cotyledons were transferred to the embryo induction medium after two subcultures on callus induction medium (six weeks), they grew into loose and fragile calli after three weeks. After calli were cultured on the same medium for another six weeks, a few somatic embryos began to regenerate.

Significance to Industry: Putative transgenic somatic embryos of Japanese flowering cherry carrying the antimicrobial peptide genes were generated. These embryos are presently being grown in tissue culture. Once these plants have grown into small trees, they will be inoculated with *P. syringae* to determine if they have increased resistance to this pathogen. Since this gene has conferred resistance into other woody plants, there is a good possibility that clones with enhanced resistance to the bacterial canker will be obtained and released to the nursery industry in the near future.

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Acknowlegement: Fruits of Japanese flowering cherry were supplied by Dr.M. Pooler of the USDA National Arboratum. A USDA/CSREES Capacity Building Grant entitled ”Genetic Transformation of Japanese Flowering Cherry” funded this research.

Genetic Diversity Among Red Leaf and Green Leaf *Imperata cylindrica*

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Index Words: PCR, Cogongrass, Diversity, Red Baron, RAPD

Nature of Work: *Imperata cylindrica* (cogongrass) is an invasive exotic species occurring in many areas of the southeastern U.S. The species is reported to be an obligate out-crosser requiring two distinct genotypes for successful seed production (1). The taxonomy of ornamental selections of *Imperata* is not consistent with plants sold in the nursery industry both as the cultivar 'Red Baron' and the variety *rubra*, both with red leaves. Many descriptions of red leaf cogongrass indicate it is unable to produce flowers but the authors have observed flowers on plants growing in Kansas, Florida, and Washington, D.C. Presently it is unknown if ornamental, red leaf selections of cogongrass have the potential to contribute to the sexual reproduction of the invasive species. The objective of the present study was to characterize separate individuals on a genetic level utilizing RAPD/PCR to determine if genetic diversity is present.

Thirteen green leaf selections and sixteen red leaf selections were obtained from sources throughout the U.S. Fully expanded leaves were removed and stored at -80∞C. DNA extraction was performed with Qiagen DNeasy mini plant kits for both the red and green leaf selections. Red leaf selections received an additional ultrafiltration step through the use of Centricon YM-100 membrane filtration tubes. Following DNA extraction, 50.0ml RAPD/PCR reactions were run. The program used a melting temperature of 94˚C for 8 sec., an annealing temperature of 35˚C for 15 sec., and an extension temperature of 72˚C for 30 sec. This cycle was repeated 45 times. The mixture consisted of 0.3µl T*aKaRa Z-Taq*^{tm} polymerase, 4.0ml DNTP's, 5.0ml Z-Taq buffer, 0.5ml primer, 1.0ml DNA, and H_20 to bring the final volume to 50.0ml. This reaction mixture contained 3mM $MgCl_2$. Five individual primers (A02, A07, A09, A19, and B02) were used in the reactions based on a previous report that they produced the most consistent, well-amplified bands for green leaf cogongrass (2). After the amplification reactions were complete, ten ml of 6X loading dye (Promega) was added to each completed 50µl PCR reaction. 20µl samples were then analyzed on 1.5% horizontal agarose gel at 100 V for 4 h. A 1-kb ladder (0.5 mg/well; Promega) was loaded into the first, last, and middle wells of the gel. A 1X Tae buffer (0.5 mg/ml) was used for the electrophoresis and gel buffer. Digital gel images were

captured in TIFF format with a FOTO/Analyst electronic documentation archival system (Fotodyne Inc., Hartland, WI). Image processing was done with the aid of Gel Compar II imaging software (version 2.5; Applied Maths, Kortrijk, Belgium) software on a PC. All of the gel images were processed to separate the individual lanes and then normalized with the 1-kb external standards. All of the lanes were labeled according to sample and primer used and placed into one composite data set. Principal component analysis, Jacquard similarity coefficient, and Jacknife comparisons were performed with Gel Compar II.

Results and Discussion: Extracted DNA produced well-amplified bands for both red and green leaf samples and each of the five primers produced unique banding patterns for each isolate (Figure 1). The cluster analysis indicated four main groups were present in the samples, two green groups and two red groups. This information is represented in the form of a dendogram (Figure 2) and shows the percentage of similarity between samples. Principal component analysis provided a three dimensional representation of the closeness of the relationships between the samples and clearly demonstrated segregation between red and green leaf samples. A majority of the red samples were placed into a single group (Group 1), this was expected since members of this set of samples are thought to be of clonal origin. However three red samples were placed into a group (Group 2) that was distinct from Group 1. Samples within Group 2 exhibited a degree of diversity from plants of Group 1. One selection from Group 2 (Sample 00-045), exhibits a different morphology with a more vigorous growth habit and less red foliage than other red selections. The placement of sample 00-045 within Group 2 suggests this vigorous plant originated from a red leaf selection although at times during the growing season, it also has morphological characteristics commonly associated with green forms. This sample demonstrates that individual red leaf selections of *Imperata* may exhibit morphological characteristics similar to vigorous green leaf forms.

The jackknife comparison accurately predicted that the red leaf selections belonged in Groups 1 or 2 100% of the time while green leaf selections were accurately placed into a green grouping 84.6% of the time (Table 1). The Jackknife comparison placed sample 00-045 in the group with red samples. This indicates that if a sample of red leaf cogongrass with unknown origin were to be analyzed with these methods it could be accurately predicted to belong to the red group.

Significance to Industry: Based on the results of this study, all red leaf *Imperata* were correctly separated from the green leafed selections 100% of the time. This indicates red leaf *Imperata* samples are genetically more similar to each other than to green leaf forms. The genetic

diversity present between the red and green leaf forms indicates a potential for flowering red leaf selections to pollinate or be pollinated by green leaf selections. Additionally, based on the segregation of red leaf samples into more than one distinct group, it is possible that red leaf forms of *Imperata* result from more than one original source. The genetic diversity present between the two groups of red leaf forms indicates the potential for outcrossing of flowering red leaf forms does exist.

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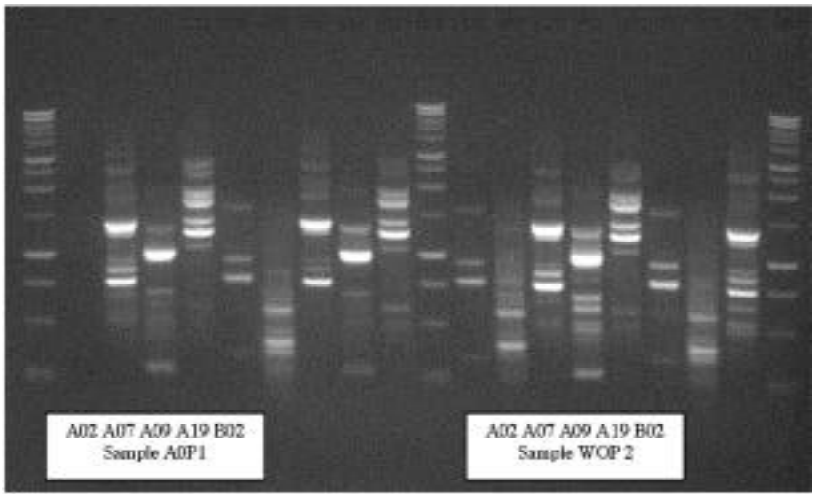

Figure 1. Example of amplified bands for red leaf samples of *Imperata cylindrica* (AOP1 and WOP2) using primers A02, A07, A09, A19 and B02.

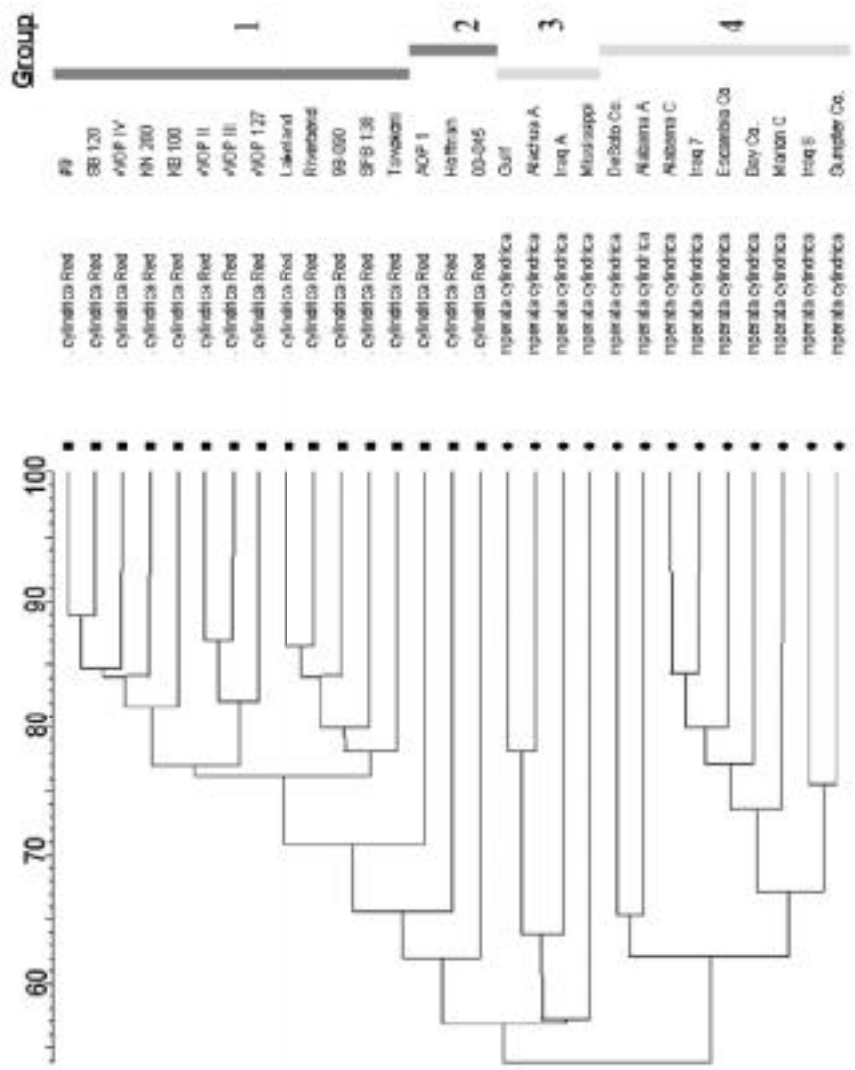

Figure 2. Dendogram of RAPD data for red and green leaf selections of *Imperata cylindrica* segregated into four related groups of samples.

Table 1. Results of Jackknife comparison of red leaf and green leaf samples of *Imperata cylindrica*.

Actual Sample Morphology	Predicted Sample Morphology (%)	
	Green leaf	Red leaf
Green leaf	84.6	15.4
Red leaf	0	100.0

Inheritance of Flower Color and Plant Architecture in Stokes Aster [*Stokesia laevis* (J. Hill) Greene]

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Index Words: Plant Breeding, Hybridization, Cut Flowers, Herbaceous Perennial, Native Plant, Asteraceae

Nature of Work: Stokes aster [*Stokesia laevis* (J.Hill) Greene] is a herbaceous perennial native to the southeastern United States, where it is used primarily as a landscape ornamental or a specialty cut flower. Although classified as hardy in USDA Zones 5-8 (1) this species is reported to thrive in southern Florida (Zones 10B-11) (2). Stokes aster is a versatile plant that grows well in both full sun and part shade in a variety of different soil types and moisture levels. Growth habit is acaulescent, forming a basal rosette of alternate leaves that are elliptic to lanceolate in shape and ranging in size from 4 to 12 inches (10-30 cm) long by 0.3 to 2.0 inches (1-5 cm) wide (4). Flowers of stokes aster are typically found in a range of soft blue to lavender, however cultivars exist with purple, white, pale magenta, or pale yellow flowers. In nearly all cultivars, flowers are borne within, or slightly above, the leaf canopy at 1 to 2 feet (30-60 cm). Thus, most cultivars have a fairly compact growth habit that rarely exceeds 28 inches (70 cm) in height. However, the cultivar 'Omega Skyrocket' has a dramatically different architecture with flowers borne on long, erect scapes that often reach a height greater than 3.3 feet (1m). The "upright" architecture of this cultivar makes it more suitable for use in a larger perennial bed or as a cut flower. Incorporating this growth habit with other flower colors could greatly increase the potential uses of stokes aster in the landscape.

The purpose of this study was to better understand the inheritance of flower color and plant architecture in stokes aster by growing out F1 and F2 families derived from controlled hybridizations. In general, most existing cultivars of stokes aster arose as selections from pre-existing natural populations or from homeowners' gardens. Therefore, no formal documentation exists that describes their individual pedigrees. For this research, controlled pollinations were made by hand in the greenhouse using a camel's-hair artists' brush as previously described (3). All cultivars used were self-incompatible, except for 'Alba' (4). Therefore, crosses were made without emasculation, and all F2 families were created using full-sib (sister-brother) mating. Hybrid seeds were harvested and subsequently germinated in the greenhouse. Seedlings were then transplanted directly to field plots located at the Horticultural Field

Lab in Raleigh, NC or the Sandhills Research Station in Jackson Springs, NC. All progeny were characterized for both flower color and plant architecture in the summers of 2000 and 2001.

In 2000, F1 families derived from separate hybridizations between all combinations of blue, purple, yellow, pale magenta, and white flowered parents were evaluated in order to determine whether one flower color is dominant to the others. Additionally, the F1 family derived from the hybridization of 'Mary Gregory' (normal architecture) x 'Omega Sky-rocket' (upright architecture) was evaluated to determine how plant architecture is inherited. In 2001, F2 families of 'Alba' (white) x 'Blue Danube' (blue), 'Mary Gregory' (yellow) x 'Alba' (white), and 'Mary Gregory' (yellow) x 'Omega Skyrocket' (blue, upright architecture) were evaluated for both architecture and flower color.

Results and Discussion:

Summer 2000-

All F1 progeny derived from different combinations of blue, purple, pale yellow, pale magenta, and white flowered parents were either blue or purple flowered (data not shown). These results indicate that blue or purple flower color is dominant to all other flower colors. The F1 prog-eny derived from the hybridization of 'Mary Gregory' (yellow, normal architecture) x 'Omega Skyrocket' (pale blue, upright architecture) were all very uniform and closely resembled 'Omega Skyrocket' both for flower color and upright architecture (data not shown). These results suggest that the upright architecture of 'Omega Skyrocket' is dominant to the normal wild-type architecture found in the other cultivars.

Summer 2001-

The F2 progeny derived from the hybridization of 'Alba' (white) x 'Blue Danube' (blue) segregated in a 3 (blue) : 1 (white) ratio ($X^2 = 0.2$), suggesting that white flower color is controlled by a single recessive gene (figure 1). The F2 progeny derived from the hybridization of 'Mary Gregory' (yellow) x 'Alba' (white) included 40 blue, 14 yellow, and 7 white flowered plants (figure 2). Although the total population size for this cross is too small to make any significant conclusions about flower color inheritance, it is important to note that the recessive flower colors (yellow and white) did segregate and are, therefore, heritable in this family. The F2 family derived from the hybridization of 'Mary Gregory' (yellow) x 'Omega Skyrocket' (blue, upright architecture) was designed to test both the inheritance of architecture and flower color. Based on our under-standing of flower color inheritance observed in previous families we expected to see both yellow and blue flowered progeny in this family. However, all progeny had blue flowers (figure 3). No yellow flowered plants were recovered. Overall, these results suggest that yellow flower

color is recoverable only in certain genetic combinations, and that more families need to be evaluated before a conclusion can be made about the inheritance of yellow flower color.

Significance to Industry: The information obtained from these families provides a framework for understanding how flower color and plant architecture is inherited in stokes aster. The confirmation that blue or purple flower color and upright architecture are both dominant traits in stokes aster allows breeders to better design future crosses. With this knowledge, breeders now have the ability to select better methods for maximizing expression of recessive flower colors and other recessive traits that are otherwise masked by dominant traits. This approach may provide access to novel flower colors in stokes aster and offer the grower and consumer more choices when selecting a cultivar. Furthermore, by combining new flower colors with the upright architecture of 'Omega Skyrocket', development of upright cultivars that are more appropriate for large perennial beds or cut flowers may be possible.

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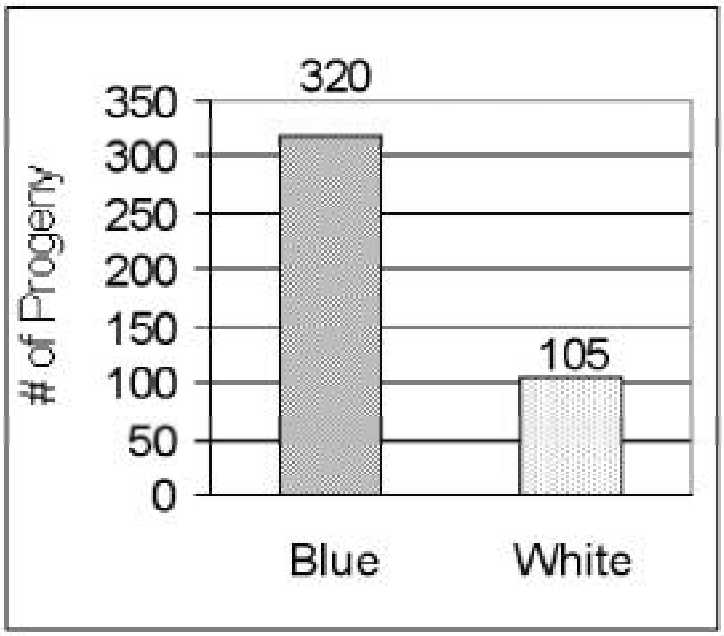

Figure 1 – Segregation for flower color in a F2 family derived from 'Alba' (white) x 'Blue Danube' (blue)

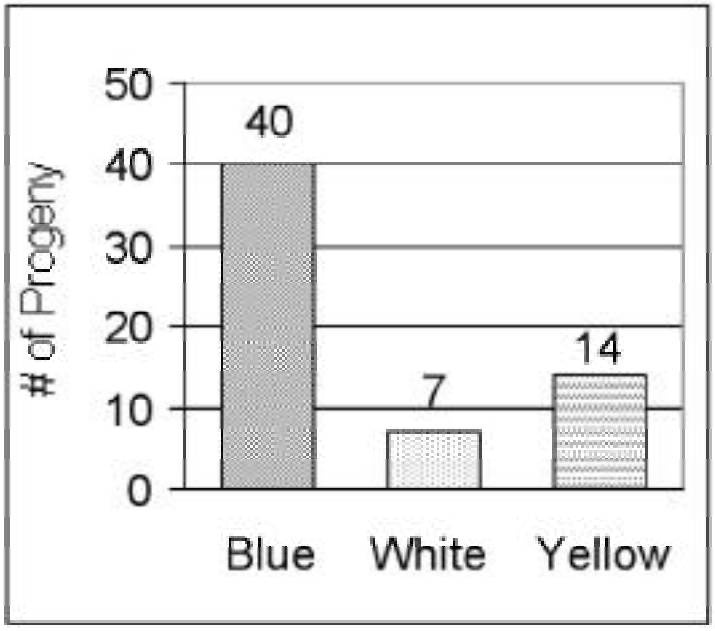

Figure 2 – Segregation for flower color in a F2 family derived from 'Alba' (white) x 'Mary Gregory' (yellow)

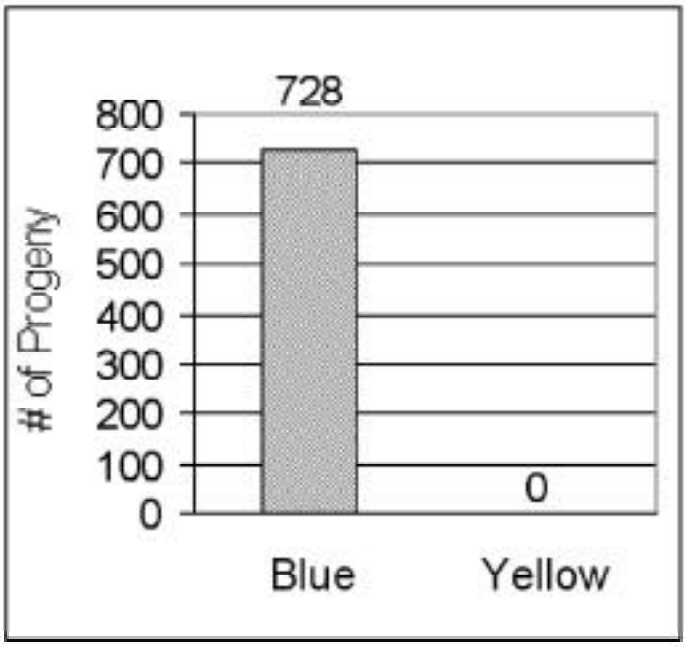

Figure 3 – Segregation for flower color in a F2 family derived from 'Mary Gregory' (yellow) x 'Omega Skyrocket' (blue)

Determining the Mechanism Controlling Yellow Variegated Foliage in *Abelia*

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Index Words: *Abelia chinensis, A. (grandiflora* 'Francis Mason', Foliage Variegation

Nature of Work: *Abelia (grandiflora* (Andr†) Rehd. and its cultivars have been widely used in landscapes for nearly a century because of their profuse pinkish-white flowers, lustrous dark semi- evergreen to evergreen foliage, and tolerance to heat, drought, and pests (Bean, 1970). Among its cultivars are several variegated selections. Variegated plants are often irresistible in the landscape because of the presence of distinct markings of different colors on their foliage and/or flowers. Variegated foliage contributes aesthetic value to the landscape throughout the year as opposed to the brief effectiveness of flowering (Elbert & Elbert, 1987). Nurseries and gardeners have expressed interest in new *Abelia* cultivars with unique foliage colors (Dirr, 1998).

Foliage variegation is caused by a number of different factors, and depending on the controlling mechanism, the trait may or may not be transmissible through seeds. Understanding the mechanism controlling a trait is beneficial if one is attempting to incorporate the characteristic into a new cultivar. A heritable trait governed by a single gene can be readily transferred to seedlings, but if the characteristic is controlled by more than one gene, integration can become increasingly difficult. A complexly controlled characteristic may require larger seedling populations, and consequently more resources to manage the populations and develop the cultivar.

'Francis Mason' originated as a variegated branch sport of *A. (grandiflora* at Mason's Nurseries in New Zealand in the 1950s (Dirr, 1994). The evergreen to semi-evergreen foliage of 'Francis Mason' is yellow to yellow-green with a mottled appearance, and the young shoots are copper colored. *Abelia chinensis* is deciduous with dark green leaves that are pale beneath and less lustrous than *A. (grandiflora*. In the present study, segregation ratios of progeny from reciprocal crosses between *A. chinensis* and *A. (grandiflora* 'Francis Mason' and their

backcross progeny were evaluated to determine if yellow variegated foliage is 1) seed transmitted and 2) controlled by a single gene or more than one gene.

Reciprocal crosses between *A. chinensis* and 'Francis Mason' were performed in June-July 1998 in a greenhouse. The F_1 progeny were backcrossed reciprocally to both *A. chinensis* and 'Francis Mason' in June-July 1999. Seeds were sown on milled sphagnum peat and grown under mist with bottom heat in the greenhouse. The F_1 progeny and backcross progeny were grown in the greenhouse.

The F_1 and backcross progeny were visually evaluated for the presence or absence of variegated foliage. Ratios were calculated for all crosses and the data used to determine the mechanism of inheritance. Hypotheses were evaluated by the chi-square goodness of fit test for observed segregation ratios.

Results and Discussion: No reciprocal backcross differences were found, and data were combined. Progeny of *A. chinensis (* 'Francis Mason' and the reciprocal cross segregated in 1:1 ratios of green to yellow seedlings, indicating that yellow variegation is transmitted through the seed. Segregation of the progeny provides evidence that one parent was heterozygous (Yy) and the other parent was homozygous recessive (yy) for foliage color (Table 1). Because green foliage color is generally considered to be dominant to yellow variegation, our initial hypothesis was that foliage color in *Abelia* is controlled by one gene with *A. chinensis* heterozygous. However, backcrosses between *A. chinensis* and green F_1 plants yielded only green seedlings with the exception of one yellow seedling (BC_1 in Table 1). The data indicated that *A. chinensis* is homozygous recessive (yy) and yellow variegated foliage is dominant to green foliage. Support for the one-gene hypothesis was provided by progeny of green F_1 plants backcrossed to 'Francis Mason' and yellow F_1 plants backcrossed to *A. chinensis* segregating in ratios of 1:1, yellow to green. Variegation governed by a single gene has been reported in *Dieffenbachia* Schott. with variegation dominant to non-variegation (Henny, 1982).

Based on the one-gene hypothesis, yellow F_1 seedlings backcrossed to 'Francis Mason' were expected to segregate in a 3:1 ratio of yellow to green. However, the seedlings (cross BC4, Table 1) segregated in a ratio of 1:1, yellow to green, suggesting that foliage color is controlled by more than one gene. Variegation governed by more than one gene is not unusual (Lawrence, 1974). However, due to low seedling numbers, a model depicting the number of genes and the mode of action governing those genes could not be clearly defined.

Despite the need for additional research to clarify the genetic model, preliminary data from this study has demonstrated that yellow variegated foliage is dominant to green foliage and the trait can be readily transferred to seedlings for use in development of new cultivars.

Significance to the Industry: Widely used in the landscape, *Abelia* is highly important economically to the nursery industry. Although several cultivars are commercially available, they vary little with exception of a few variegated varieties. Interest in the development of new cultivars with unique foliage attributes has been expressed by gardeners and nursery owners. Variegation is controlled by several factors that may or may not be transferrable to seedlings. Preliminary research indicates yellow variegated foliage in *Abelia* is dominant to green foliage and the characteristic can be seed transmitted allowing development of new variegated cultivars.

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Table 1. Segregation data from 1999 for yellow and green foliage among seedlings from crosses involving *A. chinensis, A. (grandiflora* 'Francis Mason' and backcross progeny.

Cross	Code	Total	Yellow	Green	Ratio tested	x^2	*P*
CH[z] (FM[y]		64	34	30	1:1	0.25	0.75-0.50
FM (CH		73	39	34	1:1	0.34	0.75-0.50
CH (Green F_1[xw]	BC1	98	1	97	0:1	0.01	0.95-0.90
CH (Yellow F_1[xv]	BC2	56	26	30	1:1	0.29	0.75-0.50
FM (Green F_1[x]	BC3	46	18	28	1:1	2.18	0.25-0.10
FM (Yellow F_1[x]	BC4	27	12	15	3:1	13.44*	<0.005

[z]CH - *A. chinensis*
[y]FM - *A. (grandiflora* 'Francis Mason'
[x]Includes reciprocal in count data.
[w]Green F_1 - Includes green F_1 progeny from *A. chinensis (* 'Francis Mason' and 'Francis Mason' (*A. chinensis.*
[v]Yellow F_1 - Includes yellow F_1 progeny from *A. chinensis (* 'Francis Mason' and 'Francis Mason' (*A. chinensis.*
*Indicates significance.