Update on Sweetbay Magnolia breeding at The Ohio State University

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In 2006, Meghan Blake and I were awarded a research grant by the Magnolia Society International. This paper is a report on our progress in 2006 and our plans for the future. Our initial goal is to select for stress-tolerant individuals with tree-like habit and vigorous growth under nursery conditions. Additional traits of interest are winter leaf retention, relative freedom from pests and abundant flower display.

Magnolia virginiana, also known as sweetbay or swamp magnolia, is a fragrant, long-blooming, versatile landscape plant with an open, spreading habit and dark glossy leaves. Sweetbay magnolia grows 15–30ft (3.9–9.1m) in height and bears lemon-scented flowers from June to September. The species is precocious—using production systems developed at OSU, most individuals flower within two years of germination.

Sweetbay magnolia is easily asexually propagated by tissue culture, rooted cuttings, and by grafting. It is reputed to be self-compatible, offering the potential for development of inbred lines and the possibility of commercial cultivar production via hybrid seed. Few controlled pollination studies have been conducted using *M. virginiana*. The few sweetbay magnolia cultivars that exist have been mass selected; little effort has been made to systematically advance desirable characteristics.

The Sweetbay Magnolia breeding program was begun in the fall of 1993 when open-pollinated seeds were collected from superior phenotypes (good looking trees) on the Columbus campus of The Ohio State University. The mother tree identity was not maintained. Seeds were collected from ten trees that tended to be single stem with an upright growth habit, winter leaf retention and vigorous growth. The ten trees were more than 200 yards (183m) apart. A population of 1000 seedlings was grown, initially in quart pots, then 3-gallon and finally in 10-gallon containers.

The best ten individuals in that population were selected for the same traits as the parents and planted at the Waterman Farm Learning Laboratory in spring of 2007. [Waterman Farm Learning Laboratory is a research, teaching, and extension farm located on the Ohio State Campus in Columbus, OH.] A less than ideal planting site was purposely chosen to study performance in stressful conditions. The site was an

exposed, windy, treeless plot adjacent to a four-lane road. The soil was a disturbed silt loam with grass sod cover. After planting, the sod was killed with herbicides and the site mulched with wood chips. Two of the ten trees planted at Waterman Farm died, leaving eight trees. The trees were watered occasionally after planting until the 2005 growing season, when the controlled crosses were performed. The plants were watered during dry periods to facilitate seed set. The initial trees were not selected for floral characteristics. The first flower display revealed some pleasant surprises, such as the wide flowers petals on tree No. 28 (see Figure 1).

In addition to the eight plants at the Waterman site, two additional phenotypically superior plants and one precocious plant were selected as pollen parents (see Figure 2). One of the phenotypically superior plants is the second largest M. virginiana in the state of Ohio, referred to as the Kinnear Road Tree. Another expressed a rapid growth rate and an upright growth habit reaching eight feet in height by September after being propagated from a seed in February. This tree has proven to be winter hardy by surviving two Central Ohio winters. The last individual selected was single stemmed, 9in (23cm) tall and three months old when it first flowered.

The goals of this project are to develop a population from which to select individuals with potentially valuable horticultural attributes, to further our under-

Figure 1. Large flower typical of *Magnolia virginiana* No. 28 used in controlled pollinations. The flower is almost 6in (18cm) in diameter and looked a little different than the others; the petals are not as strap-like, it has large blooms, and is later flowering.

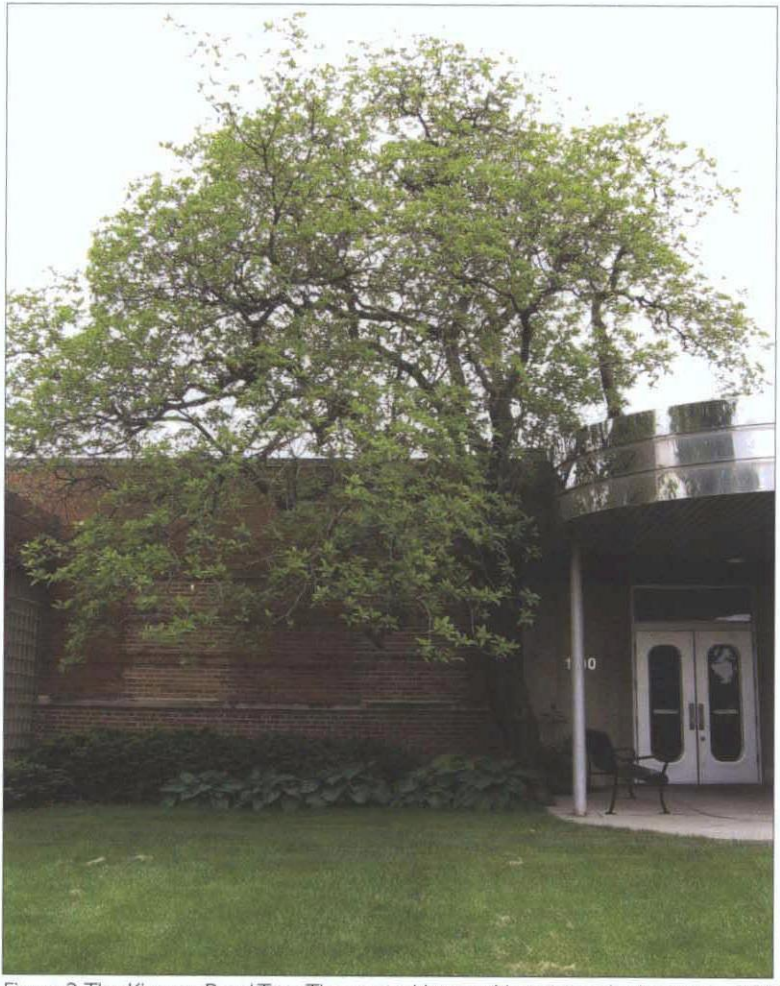

Figure 2. The Kinnear Road Tree. The second largest *M. virginiana* in the state of Ohio.

standing of the inheritance of these horticulturally important traits, and to explore the feasibility of developing homozygous inbred lines for hybrid cultivar development. We purposely did not include any *Magnolia virginiana* cultivars as pollen parents in our crosses. We wanted to make our breeding population genetically different from any commercially available cultivar. Additionally, self pollinations were made to study inbreeding effects on seed germination and growth.

Preliminary pollinations were conducted during spring/summer 2005. Three individuals were identified to be superior in terms of fruit set and number of flowers produced. A flower isolation technique and a pollen storage method were developed and the relative bloom order of the breeding population was documented (Table 1). In the breeding population, there was a two- to three-week difference in spring bloom time among the nine trees.

Table 1. Relative bloom order for the *Magnolia virginiana* breeding population in 2005 and 2006.

Individual	Date of Bloom
36	1st and 2nd week of May
37	
31	
33	2nd and 3rd week of May
35	
34	
29	
Kinnear Road	4th week of May
28	

Between May and August in 2006, Meghan made 462 reciprocal crosses. The spring crosses tended to have better seed set than those made in summer. Of the 462 crosses, 115 resulted in seeds. Fruiting success, defined as one or more one seeds per fruit resulting from a controlled pollination, ranged from 75 to 4.8%, depending on the mother tree (see Table 2). Pollen parent success rate varied from 55 to 0% (see Table 3). The average number of seeds per cross ranged from a high of 33.6 to a low of 1.2 (see Table 2).

Table 2. Reproductive success of 11 *Magnolia virginiana* individuals when used as seed parents.

Seed parent	No. of crosses performed	Percent successful	No. of seeds	Total per cross
28	24	42.1	463	19.3
29	3	14.3	59	19.7
31	28	35.9	523	18.7
33	8	13.8	61	7.6
34	17	56.7	191	1.2
35	5	7.8	168	33.6
36	21	25.9	316	15.0
37	3	4.8	29	9.7
G	1	50.0	8	8.0
K	2	40.0	7	3.5
S	3	75.0	56	18.7

Table 3. Reproductive success of 11 *Magnolia virginiana* individuals when used as pollen parents.

Pollen parent	No. of times used	Percent successful
28	73	43.8
29	20	55.0
31	47	27.7
33	55	21.8
34	5	0.0
35	55	30.9
36	75	6.7
37	64	17.2
G	0	0.0
K	61	23.0
S	7	0.0

The seeds were harvested in September, cleaned, placed in plastic bags with moist peat moss and placed in a 45°F (7.2°C) walk-in cooler to begin a 90-day cold stratification period. In February, the seeds will be germinated and a population grown in containers. Those plants will be lined out for further evaluation. Meghan's crossing structure will allow us to evaluate the genetic worth of our parent trees and to determine the effect of parental genetics on seed germination characteristics and the nature of the inheritance of selected traits in our breeding population.

Procedures and observations

Blooming occurred during two periods; in the spring, typically from mid-May to the end of June, and again beginning in the first week of August until mid-September. The second bloom was dependent on the plants producing a second flush of growth. In 2005, the plants flushed and bloomed in relative unison. In 2006, the second flush was non-uniform among and within the trees. In 2005, there were frequent and uniform rains, while in 2006 rainfall was sporadic, which was the likely cause of the differences in flushing and blooming between the years.

The day before pollinations were to be made, the stems immediately below the potential flowers to be pollinated were marked with colored ribbon. This saved time locating flowers on the day of pollination. The flower buds of the flowers to be pollinated were tightly closed with reflexed tepals. Any damaged flowers were left unpollinated due to possibility of pollination by an insect and any stigmas with a hint of color were not pollinated. Anthers were collected from damaged flowers for pollen extraction.

At pollination, petals were removed and discarded (Figure 3) and the anthers collected, placed into coin envelopes, stored in an insulated cooler in a plastic box filled with anhydrous calcium sulfate (Drierite desiccant, W. A. Hammond Drierite

Company Ltd., Xenia, Ohio). Pollen was applied with a #2 natural sable-hair brush with each pollen parent having an individual brush. Between pollinations, the brushes were cleaned in 70% ethanol. After pollination, the stigmas were covered for three days with an isolation "cage" of ankle length panty hose (the type of disposable hose used at shoe stores) and secured at the base of the receptacle with a twist tie (see Figure 3). The flowers were then labeled, including parental information, date of pollen collection, date of pollination and the ribbon color changed to denote a pollinated flower.

After the anthers were severed from the flower, they were laid overnight on filter paper in an open plastic box at room temperature, 75°F (24°C). The filter paper was labeled with the pollen parent, folded and placed in a coin envelope, the envelope placed in a desiccator in a refrigerator at 42°F (5.6°C) until used.

Pollinations performed in early May were not successful (102 pollinations and no seed set). Pollinations made after May 22, 2006 did produce fruit. As a test, at each pollination time, three to five flowers were left unpollinated to develop as a check. No "check" flowers developed fruit until May 22. Beginning May 22, the first insect pollinator, *Diabrotica duodecimpunctata*, the spotted cucumber beetle was seen. It emerges when temperatures reach 65°F (18°C) (Ohioline Extension Factsheet HYG-2139-88). Other possible natural pollinators include the stripped cucumber beetle, bumble bees and honey bees (see Figure 4). During fruit development stink bugs, Family Pentatomidae, were seen feeding on the maturing fruit. These insects were a major cause of poor seed yield in the controlled pollinations. Pollinations made in August were also successful, but were a greater challenge to make. Warmer August temperatures sped flower development, compressing the time individual flowers could be pollinated.

Figure 3. A *Magnolia virginiana* flower after petal removal (left) and the isolation cages (ankle length hose) (right). After removing the petals, the anthers were collected and placed in coin envelopes for later pollen extraction. After anther removal, pollinations were made. Only stigmas that were green-to-white in color were pollinated. The isolation cages remained on the flowers for three days after pollination.

Different stages of fruit maturity can be found on the same plant due to differences in flowering times (see Figure 5). The first fruits were collected on August 3 and continued until September. The seeds were cleaned of their flesh, rinsed in detergent and placed in individual socks like those used as isolation cages. The socks were mixed with moist peat moss and placed in plastic boxes in a 45°F (7°C) walk-in cooler for a 90-day stratification period. Beginning in March, the seeds will be removed, germinated and seedlings produced in a heated greenhouse. In mid-May, the seedlings will be moved outdoors and potted in 3-gallon (11-liter) containers. We hope to have 3- and 4-foot (0.9 and 1m) tall seedlings by October 2007. The seedling population will be transplanted to a field and evaluated over the next three years.

Figure 4. Bees were seen collecting pollen and were potential pollinators of *Magnolia virginiana* flowers.

Figure 5. Ripe *Magnolia virginiana* fruit (far right) ready for collection. Different stages of fruit maturity can be found on the same plant due to differences in flowering times.

All photographs by the authors.