

Recent Hybridizations with *Magnolia acuminata* at the National Arboretum

by FRANK S. SANTAMOUR, Jr.*

Magnolia acuminata (L.) is the hardiest of all native-American magnolias, with a northern range extending to the shores of the Great Lakes in western New York and into southern Ontario (Little, 1971). McDaniel (1973b) has reproduced Little's range map and has discussed many of the desirable attributes of this species. Aside from hardiness potential, the most important genetic qualities of *M. acuminata* are its pronounced tree habit and the yellow pigmentation of the flower petals.

In 1803, *M. cordata* Michx. was described as a separate species with yellow flowers and a somewhat shrubby habit. This taxon was relegated to varietal status as *M. acuminata* var. *cordata* (Michx.) Sarg. in 1886. However, Dandy (1964) considered the valid name for this taxon to be *M. acuminata* var. *subcordata* (Spach) Dandy. Hardin (1954) presented a thorough comparison of the varieties and forms of *M. acuminata*. Still, the plant that has been sold in the trade for many years as *M. cordata* appears to be an *M. acuminata* clone with a shrubby or small-tree habit. Newer cultivars of *M. acuminata* with yellow flowers and diverse growth habits have been registered and should, in time, eliminate the spurious association of "yellow flowers" with the epithet "cordata."

Self-incompatibility and unfruitfulness seem to be characteristic of *M. acuminata* (McDaniel, 1963, 1967,) although deliberate self-pollination may result in increased fruit production over natural pollination (Santamour, 1967; McDaniel, 1974b). The first controlled pollination involving *M. acuminata* that was supported by valid publication was made by Oliver M. Freeman in 1943, when he made reciprocal crosses between *M. acuminata* and *M. cordata* at the National Arboretum (Freeman, 1951). In the light of present evidence, this cross should be considered intraspecific or inter-varietal, rather than interspecific.

An interspecific cross between *M. acuminata* (typical variety) and *M. liliflora* Desr. 'Nigra' was apparently made at the Brooklyn Botanic Garden about 1940. The hybrid combination was given the hybrid-specific epithet *M. X brooklynensis* Kalmbacher (Kalmbacher, 1972, 1973). A seedling of this cross was patented in 1968 (Plant Patent 2820), registered with the American Magnolia Society as 'Evamaria' in 1970 (Anon., 1970), and fully described in 1972 and 1973 (Kalmbacher, 1972, 1973). Nowhere does this published work refer to the date that the cross was made, although the "Magnolia Checklist" (Fogg and McDaniel, 1975) contains the entry, under 'Evamaria', "First

* Research Geneticist, U.S. National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Washington D.C. 20002.

flowered in 1959 when 19 years old." McDaniel (1973a) has repeated this interspecific cross, and a selection was registered as 'Woodsmen' (McDaniel, 1974c). McDaniel did not include the year the cross was made in his published accounts.

Another apparently true interspecific hybrid combination exists also without valid published documentation. McDaniel (1974a) stated "The Brooklyn Botanic Garden has another very hardy one (No. 391) from *acuminata* X *denudata* cross".

Thus, until the time that we began to give serious consideration to interspecific hybridization with *M. acuminata*, little information had been published on the potential crossability of this species. Particularly distressing was the lack of critical data on the relative success of the crosses that had been accomplished.

Our goal in this research was the development of hardy, tree-type cultivars that had a peak blooming period somewhere between early precocious-flowering species and *M. acuminata*, and that expressed the yellow petal color either alone or in combination with other petal pigments. The male parents were chosen with this goal in mind, and therefore our attempted crosses did not cover a wide genetic range. The controlled pollinations discussed below include only those made in 1974 and 1975.

Materials and Methods

A few trial pollinations in 1973, using excess pollen of *M. sprengeri* Pampan. 'Diva' indicated that at least one tree of *M. acuminata* was capable of maturing fruit to interspecific crossing. This tree was isolated by more than 100 yards from the nearest magnolia of any species in an abandoned nursery area of the Arboretum. The tree had been grown from seed collected in Lexington, Kentucky, in 1940 and accessioned as seedlot PI 142313 at the U.S. Plant Introduction Station at Glenn Dale, Maryland. Judging from the description of the plant from which the seed was collected, it seems likely that the female parent was var. *subcordata*. At 35 years from seed, our tree was 42 feet in height, 18.5 inches DBH, and of good crown form, with a spread of 28 feet. Its flowers could be considered to show more yellow pigment than the average *M. acuminata*. This tree used almost exclusively as the female parent in crosses attempted in 1974 and 1975. In addition, a young *M. acuminata* with yellow flowers was used to a limited extent in 1975.

M. acuminata was used only as a female parent in crossing. One of the unique and desirable characteristics of *M. acuminata* (and var. *subcordata*) is the yellow pigmentation in the flower petals. The pigments responsible for the yellow petals are carotenoids. Carotenoid pigments are found only in the plastids, in contrast to the anthocyanins that occur in the cell sap of pink, red, and purple petals. Experience in other plant groups has demonstrated that the inheritance of plastid pigments (chlorophylls, carotenoids) is largely maternal, with the pigment development in hybrids being dependent on the inclusion of pro-plastids in the egg cell. The pollen grain does not normally contain pro-plastids that can be transmitted to the hybrid zygote. Therefore, the chances of obtaining carotenoid petal pigments in the hybrid progenies

were increased immeasurably by using *M. acuminata* as the female parent.

Pollen was collected from various trees by removing the anthers from unopened flowers and allowing them to dehisce while spread on paper on a laboratory bench. The pollen was stored in stoppered glass vials in a desiccator over anhydrous calcium chloride at 2 degrees C. Sepals, petals, and anthers were removed from unopened *M. acuminata* flowers before pollination. Pollen was applied to the stigmas with colored pipe-cleaners. The pipe-cleaners held pollen better than a brush, could be color-coded with the various pollens, and then discarded after use. All pollinations were made during peak flowering periods: April 20-23, 1974, and May 2-4, 1975. We did not bag the flowers immediately after pollination, because past experience indicated that heat build-up within the bags on April days when the air temperature approached 80 degrees F., tended to "cook" the fleshy gynoecium. In contrast, we always bag the pollinated flowers of precocious-flowering magnolias to protect them from freezing and desiccation in our erratic March weather. The complete isolation of the female tree from other magnolias served as a suitable deterrent to fertilization by unwanted pollen.

The lack of bagging protection did not, of course, eliminate the possibility of chance self-pollination. The larger of the two trees used as females normally set fewer than 100 small (mostly 1-to 2-seeded) fruit to natural pollination and could be considered as highly self-compatible. The smaller tree had not matured a single fruit during the previous three years of observations.

Some bagging of maturing fruit was required, however, to protect developing fruit from the depredations of grackles and blackbirds. About May 30 most of the unfertilized fruits had begun to drop from the tree. The fruits that remained were covered with fine nylon mesh bags (made from ladies' hosiery material), which were left over the fruits until maturity.

Mature fruit was harvested in September and allowed to dehisce indoors on a laboratory bench. The seeds were cleaned and immersed in water to distinguish empty seed ("floaters") from good seed ("sinkers"). The good seed was sown immediately in flats of a sphagnum-sand mixture in the greenhouse. Seeds that had not germinated by Christmas were removed from the flats, stratified for 30 days in moist sand in a refrigerator, and re-sown in greenhouse flats. Using this technique, we achieved better than 85 percent germination overall.

Results and Discussion

The results of 188 attempted crosses on the larger *M. acuminata* tree are given in Table 1, along with data on seed germination and progeny hybridity. Aside from the intraspecific "control" cross, no fruit matured to 40 interspecific crosses on the smaller tree, and these data are not included in the table.

The "control" cross, between *M. acuminata* and a highly compatible tree of *M. acuminata* X *cordata* (Freeman origin) parentage, yielded an average of 57 good seed (out of 61) per flower pollinated. If the same degree of cross-compatibility had been expressed in the 184 other crosses, 10,488 potentially

Table 1. Results of Controlled Pollinations on Magnolia acuminata (2n=76) - 1974-1975

| Male parents | No. flowers pollinated | No. fruit harvested | Total | No. Seed | | Hybrids obtained |
|--|---------------------------|------------------------|-------|-------------|-------------------|---------------------|
| <u>Hexaploids</u> (2n=114) | | | | <u>Full</u> | <u>Germinated</u> | |
| <u>denudata</u> | 12 | 3 | 5 | 5 | 3 | Yes |
| <u>grandiflora</u> | 7 | 0 | 0 | 0 | 0 | ---- |
| <u>sprengeri</u> 'Diva' | 24 | 15 | 31 | 28 | 22 | Yes |
| 'Diva' x <u>denudata</u> | 9 | 2 | 16 | 16 | 16 | Yes |
| ← x <u>veitchii</u> | 2 | 0 | 0 | 0 | 0 | ---- |
| (x <u>veitchii</u>) x <u>denudata</u> | 59 | 9 | 13 | 9 | 9 | Yes |
| <u>Pentaploids</u> (2n=95) | | | | | | |
| <u>liliflora</u> x 'Diva' | 13 | 1 | 1 | 1 | 1 | Yes |
| x <u>soulangiana</u> 'Brozzonii' | 6 | 0 | 0 | 0 | 0 | ---- |
| x <u>soulangiana</u> 'Purpliana' | 13 | 4 | 4 | 4 | 4 | No |
| x <u>soulangiana</u> 'Verbanica' | 22 | 1 | 1 | 1 | 1 | No |
| <u>Tetraploids</u> (2n=76) | | | | | | |
| <u>acuminata</u> x <u>cordata</u> | 4 | 4 | 244 | 228 | 1/ | ---- |
| <u>Diploids</u> (2n=38) | | | | | | |
| x <u>kwensis</u> 'Wada's Memory' | 3 | 2 | 2 | 2 | 1 | Yes |
| x <u>loebneri</u> | 14 | 2 | 4 | 1 | 0 | ---- |

1/ Not all seed sown.

viable seed could have been produced. However, we only obtained a total of 67 good seed or 0.64 percent of the "control" potential. The crosses of *M. acuminata* with *M. sprengeri* 'Diva' and with the hybrid ('Diva' X *denudata* Desr.) were the most successful, with more than one good seed per flower pollinated. Although only one or two different individuals of each species or hybrid were normally used as male parents, a total of nine hybrids of *M. X veitchii* Bean X *denudata* were used in 59 attempted crosses on *M. acuminata*. Even with this wide genetic diversity of male gametes, the yield of good seed was extremely low.

The verification of true hybrid seedlings was based on cytological study of the chromosome number in seedling root tips or the chemical identification of hydrocyanic acid (HCN) in seedling leaves. Hybrids between *M. acuminata* and hexaploid (2n equals 114) males were pentaploids with 2n equals 95 chromosomes. Some seedlings with 'Diva' or ('Diva' X *denudata*) male parents also could be verified as hybrids by the HCN test (Santamour, 1975). The one hybrid seedling of *M. acuminata* X 'Wada's Memory' was a triploid with 2n equals 57 chromosomes.

We anticipated greater difficulty in verifying hybrids derived from crosses with pentaploid male parents. Irregular meiosis in pentaploid *M. X soulangiana* Soul. cultivars can result in pollen grains with variable chromosome numbers (Santamour, 1970) and the hybrid progeny could likewise be cytologically

variable. However, the single seedling of *M. acuminata* X 'Verbanica' had $2n$ equals ca. 76 chromosomes and its leaves were pubescent on the lower surface, as in the "control" progeny. Thus, this plant was judged to be non-hybrid. The one seedling of *M. acuminata* X (*liliflora* X 'Divia') parentage was verified as a hybrid by the HCN test.

Leaf shape and other morphological characteristics were also useful in determining hybridity of the progenies noted above, but cytological and biochemical analyses were the primary criteria.

Unfortunately, we did not make any controlled attempts at self-pollination, although, as noted above, both trees of *M. acuminata* appeared to be highly self-incompatible. We expected to be able to distinguish selfed seedlings by the light-colored pubescence on the undersides of leaves. This pubescence was readily apparent on leaves of the female parent, seedlings of the "control" progeny, and on the vigorous-growing non-hybrid seedling of *M. acuminata* X 'Verbanica'. True hybrids, even with the diploid 'Wada's Memory', verified by cytology and chemistry, did not have pubescent leaves.

We did, however, harvest fruit that had matured after natural (presumably self) pollination. Seedlings derived from such seed grew poorly (six to eight inches in the first year), the atypical leaves were small, mottled, and wrinkled, and there was no pubescence on the leaf underside. Thus, they were far different than the vigorous seedlings of the "control" progeny, which were two to three feet tall after one growing season and had large, pubescent leaves. These spindly, weak seedlings of open-pollinated origin were similar in every respect to all four seedlings of supposed *M. acuminata* X 'Purpliana' parentage, as well as a few seedlings from other putative hybrid seedlots. Chromosome counts on root tips of weak seedlings from open-pollination and from several "hybrid" combinations showed that they were tetraploid with $2n$ equals 76 chromosomes. Thus, hybridity can be ruled out.

The major question was whether these weak, atypical seedlings arose as a result of self-fertilization or from some form of apomixis (asexual seed reproduction). Apomixis usually results in seedlings with a marked resemblance to the mother plant. Such a resemblance was not the case, and it must be concluded that self-fertilization in this tree produced progeny that were distinctly different from those arising from cross-fertilization.

There is a warning attached to this finding for magnolia breeders. In the absence of cytological study, it might be concluded that any seedlings that did not resemble the female parent (*M. acuminata*) were definite, although poor-growing, hybrids. It is possible that our particular *M. acuminata* parent was unusual in producing inferior selfed seedlings, but our experience shows that judgements on hybridity, based solely on "difference", may be in error.

Conclusions

The production of superior interspecific hybrids with *M. acuminata* may be difficult and erratic. An obvious first requisite, and this is largely a matter of luck is the discovery and identification of a *M. acuminata* tree that will produce mature fruit and some viable seed to controlled interspecific pollina-

tion. The second requisite is the accurate determination of true hybridity at an early age. Cytological analyses may be the best method for hybrid verification in most crosses, because only *M. liliflora* has the same ($2n$ equals 76) number of chromosomes as *M. acuminata*. Morphological comparisons of putative hybrids with seedlings resulting from controlled intraspecific crosses, controlled selfing, and from non-pollination may be an acceptable alternative. The third requisite is patience, while the trees are growing to a flowering age and size. The fourth requisite, and this applies only to the naming and release of such hybrids, is the maturity of judgement to refrain from propagating and promoting a mediocre plant as a cultivar merely because it is a hybrid.

Literature Cited

- Anonymous 1970. New Cultivar Names. Newsl. Amer. Mag. Soc. 7(1):7.
- Dandy, J.E. 1964. *Magnolia acuminata* var. *subcordata* (Spach) Dandy. Amer. J. Bot. 51: 1056. (footnote in Tucker, Shirley C. 1964. Terminal idioblasts in magnoliaceous leaves. A.J.B. 1051-1062).
- Fogg, John M., Jr. and Joseph C. McDaniel (Eds.) 1975. Check list of the Cultivated Magnolias. Amer. Hort. Soc. 54pp.
- Freeman, Oliver M. 1951. New *Magnolia* hybrids. Nat. Hort. Mag. 20: 132-135.
- Hardin, James W. 1954. An analysis of variation within *Magnolia acuminata* L. Jour. Elisha Mitchell Sci. Soc. 70: 298-312.
- Kalmbacher, George 1972. *Magnolia* X *brooklynensis* 'Evamaria' Newsl. Amer. Mag. Soc. 8(2): 7-8.
- Kalmbacher, George 1973. More on *Magnolia* X *brooklynensis* 'Evamaria' Newsl. Amer. Mag. Soc. 9(1): 12-13.
- Little, Elbert L. Jr. 1971. Atlas of United States Trees. Vol. 1 Conifers and important hardwoods. U.S. Dept. Agr. Misc. Pub. No. 1146.
- McDaniel, Joseph C. 1963. Securing seed production in *Magnolia acuminata* and *M. cordata*. Internat. Pl. Soc. Proc. 13: 120-123. (also reprinted with postscript in Newsl. Amer. Mag. Soc. 2(1): 4-6, 1965).
- McDaniel, Joseph C. 1967. Self-unfruitfulness of some magnolias. Univ. Penna. Morris Arb. Bull. 18(3): 64-69.
- McDaniel, Joseph C. 1973a. Illinois clones of *Magnolia* X *brooklynensis*. Newsl. Amer. Mag. Soc. 9(1): 13-14.
- McDaniel, Joseph C. 1973b. Sharpening our sights on *Magnolia acuminata*. Newsl. Amer. Mag. Soc. 9(2): 9-14.
- McDaniel, Joseph C. 1974a. Get in on the "ground floor" of *Magnolia* hybridizing. Newsl. Amer. Mag. Soc. 10(2): 13-14.
- McDaniel, Joseph C. 1974b. 1974 observations on *Magnolia acuminata*. Newsl. Amer. Mag. Soc. 10(2): 21-22.
- McDaniel, Joseph C. 1974c. *Magnolia* registrations - 1974. Newsl. Amer. Mag. Soc. 10(2):23.
- Santamour, Frank S., Jr. 1967. A plea for quantification of breeding data in *Magnolia* hybridization. Newsl. Amer. Mag. Soc. 4(2): 6-7.