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Practical Strategies of Black Walnut Genetic Improvement – An Update*

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**This article was originally published in 1987 in the Walnut Council Bulletin 14(2):3-5. 2017 updates are in italics and provided by J. W. Van Sambeek, Research Plant Physiologist, Columbia Forestry Sciences Laboratory; Phil O'Connor, Tree Improvement Specialist, Indiana Division of Forestry; and Mark Coggeshall, Director, Hardwood Tree Improvement and Regeneration Center at Purdue University.*

The ultimate goal of any tree improvement program is the large-scale production and distribution of genetically improved seedlings. In black walnut, projections based on earlier research indicate that genetically improved seedlings could provide growth improvement of between 15 to 25 percent by using seed or seedlings of the proper geographic origin (Bey 1980; Clausen 1981; *Deneke et al. 1987)* and additional gains of similar magnitude from progeny testing (Rink 1984). Unfortunately, the large-scale production of such genetically improved seedlings has yet to become a reality. The purpose of this paper is to briefly outline how far we've come in walnut improvement programs *between 1967 and 1987* and how far we still need to go.

Large-scale production of improved walnut seedlings will result when nurseries begin using seed collected from seed orchards, although vegetative propagation can also be used to provide improved seedlings *(Coggeshall and Beineke 1997).* If it is ever refined enough to be consistently successful, in vitro culture or micropropagation has the potential to supplement seed orchard production.

Figure 1. Select trees in natural stands or plantations are used to collect scionwood for establishing clonal seed orchards.

Some progress has been made on the invitro culture or micropropagation of walnut; however, procedures to adventitiously root microshoots from explants taken from mature plus trees continue to be a problem. Techniques were developed to establish explants from adult trees using epicormics sprouts initiated on branch segments (Van Sambeek et al. 1997). Using TDZ, a cytokinin-like plant growth regulator, and IBA, a synthetic auxin, explants can be maintained and will undergo rapid axillary shoot proliferation when using frequent transfers between media alternating between with and without liquid overlays. Advances in molecular genetics have now made it possible to

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genetically transform somatic embryo tissues (Bosela et al. 2004); however, techniques to grow transformed embryos into plantlets remain to be developed. Rooting of microshoots continues to be the main challenge although rooting of softwood cuttings has been reported for both black walnut and butternut (Stevens and Pijut 2015; Pijut 2004).

Seed Orchard Approaches

Seed orchards as defined by Wright (1976) are plantations of trees established to produce seed of proven genetic quality. The key words in this definition are **proven quality** for they imply that the trees represented in an orchard have been tested or that their original parents have been rigorously selected to meet the goals of the improvement program, usually a combination of increased survival, faster growth, and better form. Two basic kinds of seed orchards are being used in walnut improvement programs: clonal and seedling orchards. The types of selection and testing for the two kinds of orchards reflect different selection philosophies. The two programs are also at different stages of development and have somewhat different research needs.

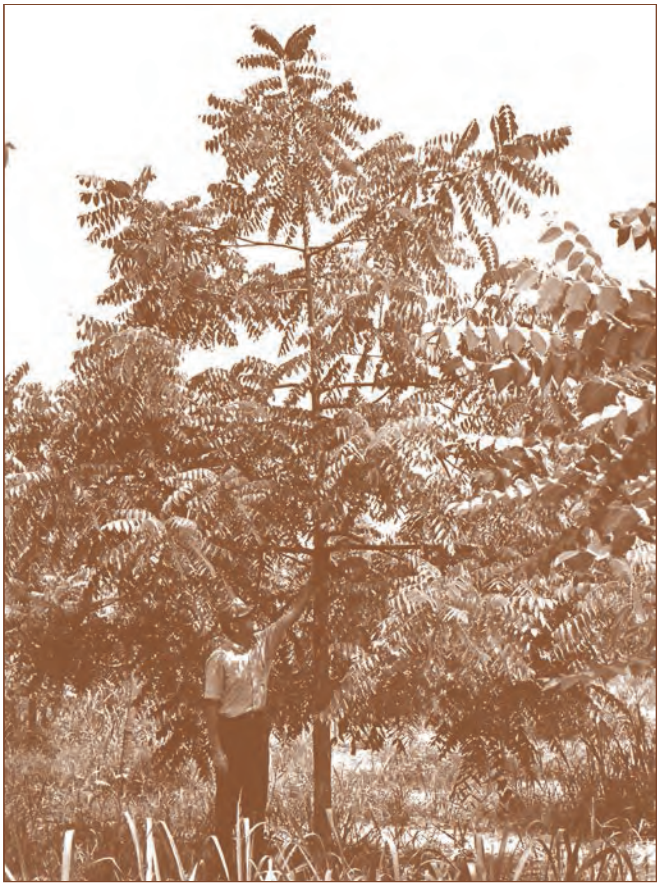

Figure 2. Select trees in progeny tests are used to collect scion wood for second-generation clonal seed orchards.

Clonal Seed Orchards: Clonal orchards are developed by grafting branch wood of selected trees onto unselected rootstock. Fast growing, high-quality individual trees are selected in natural stands (Figure 1) and, more recently, in plantations and are propagated by grafting. The underlying assumptions are that some genetic gain can be made by vegetatively propagating a tree if it is a good phenotype in its native habitat and that the favorable traits of this tree will also be possessed by any seedling offspring. This approach is most commonly taken with tree species growing in even-aged stands. A point system for selecting black walnut in natural stands has been described by Beineke and Lowe (1969). Because black walnut is generally found as an isolated individual tree in stands of uneven-aged and mixed species composition, this approach leaves room for doubt about the effectiveness of selection without further evaluation of the progeny.

In 1984, the North Central Fine Hardwoods Tree Improvement Cooperative with members from eight mid-western states was created. Eventually the cooperators produced more than 3000 walnut grafts from more than 970 plus trees to establish five walnut breeding sublines outplanted in eight state seed orchards. Over the last 10 years on average, 25% of the seed used in the Indiana state tree nursery has come from seed collected out of their genetically improved clonal seed orchard.

Seedling Seed Orchards: The underlying assumption of the seedling seed orchard approach is that it may be more efficient to collect seed from plus trees and planted in a plantation called a progeny test. Because all trees in the progeny test are of the same age and on a similar site, they can be more easily compared and evaluated. At an appropriate age, the progeny test is thinned to remove all but the most desirable trees, thus imposing a regime of rigorous selection on the progeny test for creation of seedling seed orchards.

Progeny tests have shown that seedlings from local sources near the location of future walnut plantings are usually average or above average in survival and growth but are seldom the very best seedlings in new plantings (Overton and Funk 1989). They recommended using seed from good local stands or above average trees in established plantings to

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produce seedlings unless seedlings from nonlocal sources of proven superiority in long-term tests are available. Clausen (1988) recommends using seed that originates within 100 to 200 miles south of the planting site and within 0 to 100 miles if planting sites are near the edges of the natural range for black walnut.

Stimulating Flowering and Fruiting

One major problem of the seedling seed orchard relates to the spacing among trees. Initial spacing is determined by the need to have representatives from a large number of open-pollinated families. An open- pollinated family is defined as wind-pollinated offspring of a single mother tree. Ideally at least 200 families with as many individual trees of each family as possible are used; this need usually dictates relatively close spacing, usually many times more trees per acre than is practical in a seed orchard. Close spacing leads to problems. Unless the progeny test is thinned early, crown size is restricted; and, because the number of flowering and fruiting sites on any tree is directly related to crown size, progeny tests have to be thinned early to allow the crowns room to expand *(Reid et al. 2009)*. Delays in thinning will result in restricted crown growth and delayed fruiting. But thinning too early may result in loss of information and in possibly poor selections due to judgement errors. Theoretically, however, assuming the thinning is done early enough, fruiting will not be delayed. Unfortunately, we lack guidelines for determining when to thin progeny tests to avoid such problems.

In practice, however, none of our progeny test/seedling seed orchards, some as old as 15 years of age, are producing abundant nut crops. Research is needed on stimulating fruiting in seed orchards.

Although chemical fertilization has been shown to increase nut production by up to 87 percent (Ponder 1979), this practice is expensive because fertilizer needs to be reapplied annually for maximum effectiveness. *Subsequently, Reid et al. (2009) has recommended annual split applications of 100 pounds of nitrogen per acre on open-grown trees for increasing nut production on improved nut cultivars as late-season fertilization appears to stimulate female flower production.* One of the things we are looking into is the use of nitrogen-fixing cover crops in our plantations to stimulate fruiting and growth (*Van Sambeek 1988*). We are also looking into use of leguminous and actinorhizal shrubs and trees and are finding that proper selection of a woody nurse crop may at least alleviate a part of the fruiting problem (Schlesinger 1984).

Another aspect of the problem is that we may not be thinning our progeny tests early enough. Our thinning recommendations are based on space availability for crown expansion, partly on the assumption of intense competition for light. However, Schlesinger (1986) points out that because horizontal root expansion may be two to three times greater than crown spread, trees may be undergoing intense competition long before crown closure. Furthermore, irregular spacing maybe more of a problem than we had anticipated; within-row crowding has been shown to affect diameter growth and presumably crown expansion. We had assumed that trees could compensate for some crown restrictions by expanding into unrestricted areas.

Although clonally propagated trees generally flower earlier and more abundantly than trees grown from seed (age 3 to 4 vs. age 10 to 15, respectively), clonal orchards also have not been as consistent in seed production as expected. In part, this reflects the fact that our selection criteria are primarily for growth and form characteristics with little, if any, regard for fecundity. Beineke (1984) points out that some clones with outstanding growth and form characteristics are among the poorest nut producers. Inherently faster growth in black walnut may be at the expense of fruitfulness. Although there have been discussions of such a negative correlation (e.g., Van Sambeek and Rink 1981), no concrete data are available for substantiation. An alternative strategy may be to select trees for anthracnose resistance because resistance is correlated with height growth and fecundity. Anthracnose results in premature defoliation, reducing growth and lowering bud set. Anthracnose resistance has been shown to be highly heritable (Funk et al. 1981).

Once our orchards begin flowering, we need to examine the flowering distribution patterns in them. Black walnut has a dichogamous flowering habit, which means that male and female flowers normally mature on differing dates on the same tree. This pattern of flower maturation fosters cross-pollination among different trees and tends to minimize undesirable self-pollination. However, if our orchards consist of a relatively few clones or half- sib families, not all of them may flower synchronously. Under such circumstances, the seed we collect could result from cross-pollination among a small sample of a relatively narrow genetic base, possibly leading to inbreeding or insect and disease problems inherent with monocultures. There are indications that because of our walnut harvesting practices (e.g. high-grading), walnuts remaining in our present forests may already be somewhat inbred (Beineke 1972). Although the distribution of flowering will have to be monitored in both the clonal and seedling seed orchards, this monitoring will be especially critical in clonal orchards because they tend to be comprised of only a very few clones. Monitoring will have to be done for several years because many clones vary in flowering patterns from year to year (Masters 1974).

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Spouse Tours Highlight History and Nature in the Area

Monday, June 12

Spouses will board a bus to visit the small town of Delphi, Indiana. Guides will share adventures and stories of everyday life as it was 150 years ago on the Wabash & Erie Canal. Experience a 35-minute floating trip on The Delphi, a replica 19th century canal boat. After lunch at the canal interpretive center, we will tour the Delphi Opera House, a mid-1800's Italianate building on the National Register of Historic Places.

Tuesday, June 13

The Tuesday tour will explore nearby Battleground, Indiana and Prophetstown State Park, Indiana's newest park. Located at two main rivers, this site was the home of Native Americans and is now a restored prairie. After a bus tour of the prairie, we will move to The Farm at Prophetstown, a living history farm recreating life in the 1920's, including a Sears Roebuck farmhouse from the era.

Please consider signing up for the spouse tours in advance so we can plan transportation and lunch, which are included. Spouse tours are included in full registration or can be reserved by the single day, see registration form.

Photo credit, IDNR/Outdoor Indiana Magazine

Prairie plants blaze with color at Prophetstown State Park.

Black Walnut Genetic Improvement

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Early molecular genetic studies using either enzymes or DNA markers concentrated on understanding how much genetic diversity existed within natural stands and seed orchards. The earliest work quantified variation within certain enzymes coded for by a number of alleles (Rink 1997). These studies documented that inbreeding in seed orchards or associated with harvesting should not be a concern (Rink et al. 1994). In the mid-1990's, molecular approaches moved to more direct approaches using markers found on the DNA and subsequently expanded to include black walnut (Woeste et al. 2002; Robichaud et al. 2006). Work by Robichaud et al. (2010) confirmed the early work that inbreeding in black walnut was minimal in harvested stands and thinned plantations.

Future Direction

The most sophisticated improvement programs involve simultaneous progeny testing of half-sib seedlings from selected trees as well as development of clonal orchards in which the selected trees are represented. The progeny testing is critical because it enables us to determine specifically how much genetic gain we are making from our selections *(Rink and Clausen 1989)*. It also lets us evaluate the quality of our selections; during such evaluations some clones not measuring up to standards are removed from the clonal orchard and others are added. Furthermore, the progeny tests become the populations from which we can make second-generation selections. The latter will be used for establishing second-generation orchards for additional growth gains over those achieved with the first-generation orchards (Zobel and Talbert 1984). One of the weaknesses of some black walnut improvement programs is that some clonal selections in them are not adequately progeny tested.

One question that arises with discussions of progeny testing and second-generation selections is at what age is it safe to make selections? Obviously, it would be desirable to be able to make selections as early as possible in the life of a progeny test (Figure 2.) By making the earliest possible selections, we can maximize genetic gain per generation. Such questions are usually answered using statistical correlations of growth measurements at an early age with those at a later age and are called age-age correlations. In black walnut, most age-age correlations calculated thus far have shown that evaluations of height

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made at age 4 hold up well through subsequent measurements, at least through age 10 (Coggeshall and Pennington 1982; McKeand et al. 1979; Rink 1984). Kung (1973) correlated early measurements with stem analysis projections through age 30. This evaluation agrees with the more juvenile correlations. In spite of the high age-age correlations shown thus far, it needs to be shown that these high correlations hold through rotation age. Continued evaluation of progeny tests is, therefore, essential through rotation age, at least in the first generation of progeny tests. *Rink and Kung (1995) subsequently reported age-age correlation coefficients increased from 0.1 at age 1 to 0.8 at age 8 and remained above this level through age 20.*

Most ongoing tree improvement programs for other tree species involve some controlled pollination among selected trees. Such controlled breeding results in trees of known parentage and more precise estimates of statistical genetic parameters. Unfortunately, black walnut has few flowers, especially on younger trees, and poor seed set per flower cluster; one study of controlled pollination resulted in only 24-percent success (Beineke and Masters 1976). Controlled breeding work in this species is too slow and expensive to be practical.

With advances in molecular genetics, it is now possible to fingerprint all trees within an orchard and surrounding areas, collect the seed, and then fingerprint only the winners in a progeny test to determine the paternal parent (Coggeshall 2016). This technique was used to create two mapping populations with more than 135 and 330 full-sib seedlings of the same two parent trees. These mapping populations will be used to define which genetic markers are associated with which measureable traits. In the future we will be able to screen seedlings through marker-assisted selection approaches to identify superior individuals before planting. Material from parents of these mapping populations have also been used to create the first genetic map for black walnut.

I have discussed our current problems in walnut tree improvement, but do not want to leave the impression that no progress was made in genetic improvement of black walnut between 1967 and 1987. Nothing could be further from the truth. For example, the black walnut tree improvement program of the Indiana State Division of Forestry produced its first crop of 25,000-30,000 improved seedlings in 1987. Similarly, Purdue University has patented several clones for commercial distribution of grafted seedlings (Beineke 1984). Genetically improved black walnut seedlings will be readily available in just a few years in several parts of the country. The problems we have today may be more appropriately termed challenges and it is apparent that we are meeting them head on.

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