

# Hardwood Genetics and Tree Improvement – A Midwest USA Perspective

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## Introduction

Fine hardwood trees in the Central Hardwoods region of the United States are an important resource for the furniture, cabinetry, flooring, modular home, and paneling manufacturing industries. Consumers find wood from these trees to be very desirable because of quality factors such as grain, strength and color. To enhance wood production, tree improvement programs can address quantity and quality issues through alterations in genetic traits for growth and vigor, straightness, defects such as pin knots and irregular grain, amount of heartwood and in some cases, wood color.

Tree improvement in fine hardwoods through improved seed production is lagging well behind programs that improve pulp and paper species such as poplar, loblolly pine and Douglas fir. In addition, the majority of hardwood seedlings that are produced by public nurseries are unimproved. Seed is purchased from vendors and collectors and only rarely separated by source. Seed is normally harvested where ease of harvest is the most important factor, thus yard, park, and fencerow trees are often used. Improvement of fine hardwoods has also lagged due to the lack of capital within the industry to fund research and development on the resource. Limited funds tend to be directed towards research in product manufacturing. In the absence of traditional funding sources for tree improvement similar to those that fund conifer programs for pulp production, HTIRC (Hardwood Tree Improvement and Regeneration Center) relies on industry associations, federal agencies, universities and private endowments to generate annual funding for its program.

The HTIRC program intends to address improvement of genetic traits in improved planting stock of black walnut (*Juglans nigra*), black cherry (*Prunus serotina*), butternut (*Juglans cinerea*), and northern red oak (*Quercus rubra*), through (1) tree breeding and genetic modification, (2) developing propagation and seed production systems, (3) improving nursery production methods, (4) developing standards for improvement of seedling quality and (5) developing guides for management of the genetics in small, fragmented stands.

## Early Selection of Improved Black Walnut Genotypes

Several geneticists have researched black walnut improvement in the USA for over forty years. In this time there have been a large number of families identified as phenotypically superior for growth and form, but there has been minimal advanced generation selection. The walnut breeding program at HTIRC is actively evaluating about 350 phenotypically superior black walnuts. A little more than half of these trees were selected from wild populations, the rest were identified in even-age plantations or progeny tests. From this germ plasm, about 40 parents will be progeny tested yearly, including a core set of about 15 elite clones. Some of the progeny tests will be converted to seed orchards as they mature. The best 15 clones will be intermated using controlled crosses, with the goal of identifying superior trees for clonal plantations. How and when the progeny of these trees will be selected is the subject of the remainder of this section.

Studies have suggested that selection for vigor (height and/or diameter growth) could take place as early as age four to six (Rink, 1984b) or at any time after age eight (Rink and Kung, 1995). To provide an empirical validation of the value of early selection in black walnut, we performed a post-hoc analysis of a provenance/progeny test. In 1981, about 1,300 bareroot, 1–0 seedlings derived from open-pollinations of 80 phenotypically superior black walnuts growing in 12 states and Canada were planted at the Southeast Purdue Agricultural Center (SEPAC) in Jennings County, Indiana, USA. We performed a *post-hoc* analysis of the data from these trees to determine the age at which selection for diameter growth would be most effective. We evaluated the effect of setting selection intensity at ten and 20 percent. In each year for which there was data, the selected families were identified and their family mean in the year 2000 (20 years from seed, 19 years after planting, and the last year for which there was data) was compared to the mean of the entire population in 2000. The relative efficiency of early selection as compared to selection of the top ten percent of the families at 20 years of age was calculated as standard deviations of improvement per year.

A two-stage breeding strategy where phenotypic selection is followed by clonal testing or progeny testing is often more efficient than single-stage selection (Danusevicius and Lindgren, 2002). We therefore envision a breeding strategy where selection for diameter growth on family means would be followed by the clonal propagation of families as rooted cuttings or as scions on mature rootstocks. After four years on mature rootstocks the selected families would bear enough flowers to complete one cycle of breeding. Selection before age four would not be advantageous since selected trees need to have enough wood for second-stage propagation. Given the current feasible strategy of early selection, clonal propagation to mature rootstocks, and a delay of four years for production of flowers, the efficiency of early selection as compared to selection at age nineteen was calculated using an age adjustment factor of  $(19 + 4) / [(selection\ age) + 4]$  where the minimum selection age was four years. The age adjustment factor corresponds to the number of breeding cycles possible if selection is in a given year relative to the number of cycles if selection is at age nineteen. The results indicate that selection at or near age eight would have resulted in the greatest gain in diameter per year. We calculated the expected gain from selection of the top 20 percent of the families at age eight as follows: selection differential  $\times$  age adjustment =  $0.65 \times 23/12 = 1.25$ ; gain =  $1.25 h^2\sigma_p$  (the heritability and phenotypic standard deviation of diameter growth in the population) or  $(1.25)(0.35)(3.4\ cm) = 1.5\ cm$ . This is a conservative estimate based on an assumption of heritability for diameter growth in the range of 0.34 to 0.5 (Beineke, 1989; Rink, 1984a). A gain of 1.5 cm is about 0.3 cm more than would be expected from selecting the top ten percent of the families at age nineteen. The advantage of early selection is maintained even under a two-stage breeding strategy where phenotypically selected families are rooted or grafted and evaluated for straightness after 15 years. An important additional benefit of early selection is that families could be selected before stand thinning is necessary, resulting in considerable savings. Early selection permits either savings in land costs or higher selection intensity because young trees need less space.

## **Vegetative and Tissue Culture Propagation**

Clonal reproduction of commercially important hardwood tree species is necessary, in a tree improvement program, in order to provide improved planting stock for use in progeny testing and for production forestry. Vegetative propagation methods (via rooted cuttings) will be required to produce clones of elite genotypes. Many ecologically

and economically important hardwood tree species have a low genetic or physiological capacity for adventitious root formation, and are considered recalcitrant to routine, commercial-scale vegetative propagation. However, successful propagation of difficult-to-root species can be achieved if the type of cutting, date of collection, stock plant or cutting manipulation, rooting treatment, and greenhouse parameters are carefully considered.

Preliminary results with 9-year-old black cherry trees appear promising. We achieved 63 percent rooting when cuttings were collected in mid-June and treated with either K-IBA (12, 29, or 62 mM) or IBA (15, 34, or 74 mM). Fifty-six rooted cuttings are presently being overwintered in a controlled cold-storage environment. After overwintering the rooted cuttings will be returned to the greenhouse, allowed to flush and initiate new growth, and then outplanted to the field.

An example of our improvement effort utilizing tissue culture and genetic modification is with black cherry. In vitro shoot cultures have been established for three genotypes of black cherry from nodal explants obtained from juvenile seedlings growing in the greenhouse. Shoot cultures can be routinely micropropagated for regeneration and rooting studies. Adventitious shoots have been successfully regenerated from in vitro leaf explants and rooting experiments are underway. Once the complete protocol has been established, elite black cherry genotypes will be introduced into in vitro culture. The development of transgenic elite black cherry trees with resistance to pests or engineered for reproductive sterility will potentially have great economic benefits to landowners, lumber mills, and the forest products industry. Genetic gain in black cherry genotypes through this research will complement traditional tree improvement efforts at the HTIRC.

## **Trees grown for wood production**

Once in vitro regeneration systems have been developed, economic traits such as form, wood quality, and stress tolerance can be modified by genetic transformation.

Form. Plant domestication is the conversion of a wild plant, which has become adapted, through the process of natural selection to a plant whose morphology and physiology have been altered by selection to yield useful products when cultivated. Ideally, a fully domesticated tree would be relatively short and have a disproportionately large diameter stem. This could result in less reaction wood, higher harvest indices, improved handling efficiency, and greater unit-area yields. A domesticated tree should also have reduced numbers

and sizes of branches, which should also be favorable for conducting water. Crowns of dwarfed trees will likely be narrower, which, when coupled with reduced sensitivity to competition, will allow a greater number of stems to be planted in a given area. Crown geometry could also be optimized to capture sunlight in tight spacing by altering expression of or introducing genes that affect leaf size and shape, branch allocation, and branch angle (Bradshaw and Strauss 2001).

**Clonability.** Being able to vegetatively propagate forest trees will allow growers to practice clonal forestry, which will permit them to fully capture genetic gains that have been achieved through conventional breeding. The ease with which trees can be vegetatively regenerated shows great diversity within and among species, suggesting that genes and control mechanisms can be isolated that will strongly modify competence for regeneration. One factor that can strongly influence propagation success is the ontogenetic age (maturation state) of the donor plant. The progressive change in a number of traits, including a pronounced decline in rooting ability due to maturation, has been well documented (Greenwood and Hutchison 1993). The existence of differential rooting ability across tree taxa presents an opportunity to conduct comparative studies, contrasting the molecular and developmental events that occur in those that successfully produce adventitious roots with those that do not.

### **Engineering reproductive sterility**

Before genetically engineered trees are deployed commercially, it would be advisable to develop a system to minimize the risk of transgene spread in the environment. One way of accomplishing this objective is to engineer reproductive sterility.

The three most common ways to engineer reproductive sterility involve using: (1) floral tissue-specific promoters to drive the expression of a cytotoxin gene; (2) dominant negative mutations (DNMs), to suppress floral gene function by over-expressing a mutant version of the encoded protein; and (3) double-stranded RNA, a potent inducer of post-transcriptional gene silencing, an approach known as RNA interference (RNAi) (Meilan 2004).

## **Hardwood Seedling Nursery Culture and Plantation Establishment**

For a tree improvement program to be most effective, scientific advances in genetic quality should be complemented by research involving nursery culture and plantation silviculture. Tree improvement serves as an

additional silvicultural tool available to foresters, and its effectiveness is only realized when used in conjunction with other silvicultural practices. In the early stages of a tree improvement program, it is likely that far greater gains in operational plantation productivity will be realized by improving methods to produce high quality nursery stock and nurture seedlings through the plantation establishment phase.

Most hardwood plantations established in the Midwestern USA involve afforestation of private lands, in which seedlings are planted on open fields that were formerly in agricultural production. Quality hardwoods are an important component of these plantations and a recent survey in Indiana identified northern red oak, black walnut and white oak (*Quercus alba* L.) as the three most commonly planted species (Jacobs *et al.* 2004). Although timber production is a motivation for private landowners to establish plantations in Indiana, leaving a legacy for future generations, providing wildlife habitat, and conserving the natural environment rank higher in priority (Ross-Davis *et al.* 2004).

Establishment success of hardwood plantations is highly variable, and plantation failure is not uncommon. A survey of 87 randomly selected operational plantings in Indiana (one to five years of age) found that seedling survival across all sites averaged approximately 65%, with the majority of mortality occurring the first year after planting (Jacobs *et al.*, 2004). After five years, only 49% of surviving seedlings were deemed "free-to-grow". Planting performance is generally associated with the condition of nursery stock, planter experience, control of competing vegetation, and animal browse. These results indicate that substantial improvement in hardwood plantation establishment could likely be made by improving the quality of nursery stock available for afforestation and the silvicultural techniques employed at outplanting.

Seedling quality is a major focus of hardwood seedling nursery production, and may be defined in both morphological and physiological terms. Morphological quality is relatively easy to assess, and hardwood seedlings may be operationally graded for variables such as height, stem diameter, and number of permanent first-order lateral roots (FOLR). Though there is debate as to the relative importance of specific morphological variables for predicting field performance of hardwoods (Dey and Parker 1997, Jacobs and Seifert 2004a), collectively these variables tend to be positively correlated with outplanting performance. Nursery seedling morphology is largely dependent on cultural factors such as bed density,

fertilization, irrigation, and undercutting to manipulate root growth. These practices may be modified to produce a seedling meeting specific morphological standards.

The benefits of using high quality hardwood nursery stock are best realized when integrated with effective silviculture during plantation establishment. Matching species to the appropriate site conditions, consulting with a professional forester, aggressive control of competing vegetation, and minimizing damage from animal browse help to promote vigorous hardwood plantation establishment (Jacobs *et al.* 2004). Hardwood afforestation plantings are susceptible to failures associated with heavy pressures from deer browsing and competing vegetation, and it is critical to promote rapid growth immediately following planting to encourage seedlings to quickly reach free-to-grow status. Competing vegetation in hardwood plantations is most effectively controlled using chemical means, and different herbicides have been tested with a variety of hardwood species to help identify optimal protocols for weed control specific to hardwood plantations (Seifert and Woeste 2002).

Fertilization at the time of planting is another practice that may promote early plantation development. Technical advances in fertilizer technology have resulted in a product more compatible with the nutritional needs of forest trees than traditional agronomic fertilizers (Jacobs *et al.* 2003). In a recent afforestation trial involving three hardwood species, application of 60 g per seedling of a polymer-coated controlled-release fertilizer to the planting hole resulted in significantly greater height (52%) and diameter (37%) growth during the first growing season as compared to non-fertilized seedlings (Jacobs and Seifert 2004b).

## Genetics of Native Stands

In addition to afforestation activities described above that are accomplished through genetically improved plantations, the sustainable management of natural stands is equally important and partly dependent upon knowledge of population genetics and their conservation.

To this end, we are developing molecular genetic markers for black walnut and northern red oak. Microsatellite markers, or short sequence repeats (SSRs), are highly variable and provide sufficient genetic resolution to characterize germplasm, track pollen and seed movement, and evaluate the genetic organization of native and managed populations. We have developed thirty microsatellite markers for black walnut (Woeste *et*

*al.* 2002) and thirty for northern red oak (Aldrich *et al.* 2002a, 2003a). The latter amplify well in several red oak species, and some perform well in chestnut (*Castanea*) and beech (*Fagus*).

Since species are a common unit for management, oaks represent a unique challenge at this level due to unclear species boundaries. As with European white oaks (Muir *et al.* 2000), North American red oak species are thought to hybridize readily (Jensen 1995), and red oak species may have a more recent origin compared to species in the white oak group (Guttman and Weigt 1989). Consequently, it is not always possible to treat their respective gene pools as independent when species co-occur, though genetic differences typically are evident. For example, we examined genetic (SSR) differences between northern red oak (*Q. rubra*), Shumard oak (*Q. shumardii*), and pin oak (*Q. palustris*) in an old-growth stand and found small though significant genetic differentiation, with pin oak the most distinct (Aldrich *et al.* 2003b).

At the regional level, black walnut and northern red oak exhibit high levels of genetic diversity within their populations and relatively low genetic differentiation among populations. This is as expected for long-lived, predominantly outcrossed, woody species (Hamrick and Godt 1989). Currently we are using microsatellite markers to explore the diversity of black walnut in native stands across several states (unpublished data). In northern red oak, a survey of variation in the chloroplast genome (Romero-Severson *et al.* 2002) revealed inter-stand variation across Indiana. Preliminary evidence from a nuclear study using microsatellites (unpublished data) supports this pattern, showing that germplasm collected from sites near one another are more genetically similar in their nuclear genomes compared to those collected far apart. These data may prove useful in directing germplasm sampling for tree improvement and nurseries.

Unfortunately, oaks and other shade tolerant species are experiencing recruitment failure in many forests of the Central Hardwood Region (Abrams 2002). Fire, timber harvest, and grazing have been important factors in the development of these forests, and 20<sup>th</sup> century suppression of these disturbance regimes has altered successional trajectories, favoring shade-tolerant later seral species such as maple and beech. Though the ecological consequences for the less tolerant species like the oaks are well-described, including changes in density and spatial structure (Aldrich *et al.* 2002b), it remains unclear what genetic consequences will hold for the gene pools in the region.

## Developing Guides for Sustaining Genetic Variation

Forest management can be considered sustainable only if attention is paid to the conservation of the genetic resources of the managed species (Ledig 1992). Such genetic resources are essential since they encapsulate the ability of tree species to adapt to future environmental challenges such as global warming and new insect and disease pests. However, scant attention has been paid to date to conservation of the genetic resources of even the most valuable Central Hardwood species. As a consequence, the gene pools of our most valuable fine hardwoods may be in peril. What was once a vast continuum of forest cover has been reduced to a patchwork of isolated fragments. Furthermore, repeated targeted harvesting of only the very best phenotypes in the remaining fragments, or 'high-grading' (McGuire *et al.* 1999), may have resulted in the loss of some of the most valuable alleles. Moreover, since selective harvest is generally the silvicultural method of choice in the remaining relict woodlots, widespread recruitment failure has been documented for shade intolerant species (Lorimer 1993); our most valuable species – such as black walnut, northern red oak, or black cherry – are shade-intolerant. An additional threat to the genetic resources of our fine hardwoods is a consequence of the extensive establishment of plantations on formerly cleared land. The seedlings that make up these plantations are supplied by a small number of nurseries; in general, these source nurseries pay little attention to the genetic origin of their seedling crops, the bulk of which are derived from locally-collected seed. As a result, seedlings of limited genetic origin are being deployed over vast areas, possibly causing genetic pollution (via pollen flow and seed dispersal) of the remnant native gene pools.

Hence the time is more than ripe for systematic gene conservation efforts to be undertaken for the fine hardwoods of our region. Gene conservation in trees is typically carried out via two complementary approaches, *in situ* and *ex situ* (Yang and Yeh 1992). *In situ* programs attempt to conserve genetic resources in their natural surroundings through management of native stands (Ledig 1988). *Ex situ* programs utilize seed stores, seed orchards, clone banks, and/or progeny or provenance tests (Lipow *et al.* 2002).

Genetic Resource Management Units (GRMUs) have been proposed as an economically realistic means of effectively conserving the genetic resources of valuable tree species *in situ* (Ledig 1988). The concept of GMRUs is to allow some level of timber extraction via partial

logging, provided that this does not interfere with the overarching goal of conserving the local genetic resources. The question then arises as to just how much harvesting should be allowed in GMRUs, or, given that harvest of a certain intensity has taken, what regeneration targets are appropriate to minimize the risk of losing genetic resources? We are developing a computer simulation model to help answer such questions (Glaubitz *et al.* 2004). Our simulation identifies, for a given harvest intensity of a focal tree species, how much regeneration is needed so that there is no more than a ten percent risk of retaining less than ninety percent of the original allelic richness present in the stand. Results to date, based upon a 'virtual' tree population, indicate that such genetic diversity retention targets could be readily achieved via appropriate management (Glaubitz *et al.* 2004). This would be the case even for shade intolerant species – where high harvest intensities would be required to allow regeneration – provided that adequate care is taken to ensure that the regeneration is derived from local germplasm sources. Our simulation was based upon the assumption that the regeneration is produced via pre-harvest random mating among the local adult population. We are currently incorporating the empirical results from our above ongoing studies of population genetic processes in native stands of black walnut and red oaks, in order to make our model more realistic.

For many tree species, *ex situ* gene conservation can be effectively carried out using seed stores (Lipow *et al.* 2002). However, this is not possible for the fine hardwood species of the Midwest, since the seeds of most of these species rapidly lose viability after only one or two years in storage (Schopmeyer 1974). To supplement the genetic resources captured in tree improvement programs, we have proposed the establishment of Gene Conservation Plantations (GCPs). These could be established with the dual purpose of producing veneer or saw-logs while conserving the genetic resources of targeted local regions. The GCPs associated with a particular region (or seed transfer zone) would be established from seed collected from a variety of remnant natural stands within that region. Prior to harvest of GCPs for timber, seed could be collected from the GCPs themselves and then cycled into the establishment of second generation GCPs.

In sum, the genetic research that we are conducting at the HTIRC should facilitate the effective conservation of the genetic resources of fine hardwood species in the Midwest. Perhaps the greatest challenge lies not in developing the scientific knowledge and expertise to carry out efficient *in situ* and *ex situ* conservation programs, but rather in fostering the political will to implement the fruits of our labor in reality.

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