Missouri Eastern Black Walnut Breeding Program

Kenneth R. Tourjee
School of Natural Resources, University of Missouri
Columbia, Missouri

INTRODUCTION

Eastern black walnut (Juglans nigra L.) is native to Missouri and is valued for both the quality of its timber products and nut production for human consumption. A fledgling nut industry based largely on wild germplasm and a few small-scale growers has become established. Generally, harvested nuts are either marketed directly to the consumer or sold to Hammons Products Company for processing. Further commercial development of the industry will require an increased role for the Missouri nursery industry as a supplier of superior cultivars on grafted rootstock.

Developing new eastern black walnut cultivars is a long-term process and its impact on the black walnut industry may not be felt for 20 years after the initiation of a breeding program. Justification for the existence of a breeding program is based on its role in increasing the efficiency of industry integration through the generation of improved cultivars and information pertinent to germplasm recommendations.

Resources need to be applied to three areas to sustain the growth of eastern black walnut as a viable crop: 1) orchard management practices, 2) product development, marketing, and promotion, and 3) cultivar development and evaluation. Simultaneous advancement, in an interactive manner, on all three fronts is essential if the industry is to grow. Development of alliances among nurseries, growers, processors, government agencies, and the University of Missouri will facilitate the rapid economic expansion of this crop.

Immediate impact can be effected through improved orchard management practices and well developed marketing strategies that include all parties from nurseries to consumers. Establishing a black walnut grower's cooperative to serve as a focal point for the dissemination of extension services and product distribution is highly desirable. The cooperative can foster industry integration by improving communication among the nursery industry, growers, and processors. The benefits of industry integration include the standardization to a set of cultivars resulting in increased efficiency from an economy of scale to each entity, and identification of germplasm deficiencies, making it easier for a breeding program to define relevant traits and target audiences. Hammons Products Company strong position in the processing and marketing of nut meats dictates that it take a leadership role in shaping the integration of this industry.

CULTIVAR DEVELOPMENT

Increased yields of a high quality product result from a combination of improved cultural practices and the development of superior cultivars. Cultivar development is a process of engineering design motivated by a need to address production problems through the creation of better adapted germplasm. Implicit in this process is the importance of a precise definition of the target environment. The environment includes not only climatic and edaphic features, but also the end-product use, size of the industry, degree of capital investment, and intensity of management.

The major issues for defining the target environment of a black walnut breeding program center on grower's objectives. The relative importance of nut and timber production must be clearly established and a strategy for achieving these objectives must be delineated. For example, if growers want both nut and timber production they could either 1) dedicate separate acreage to nut or timber trees, 2) intercrop nut and timber trees in alternate rows, 3) high graft superior nut producing cultivars onto superior timber producing understock, or 4) grow trees that are superior for both timber and nut production. Cultural practices are strategy dependent and are an important component of the environment. Therefore, strategy needs to be specified before selection for improved genotypes can proceed. Also, the timber industry must be consulted about their receptivity to purchasing trees grown under each of the strategies.

There is controversy surrounding the fourth strategy. This controversy concerns the feasibility of selecting a cultivar genetically superior for both nut and timber production. Since nut (reproductive) and timber (vegetative) traits represent competing resource sinks, negative genetic correlations may exist between them. Consequently, it would be difficult to optimize both traits simultaneously. However, it is unlikely that the correlation would be perfect. Also, the nut and timber industries currently use wild unimproved germplasm. It is reasonable to expect that cultivars could be developed that are superior, for both nut and timber production, to what is currently used by either the nut or timber industries. A more pressing concern is whether or not the management practices for nut and timber production are compatible. Once suitable management practices for this strategy are identified an appropriate selection scheme could be devised. The long term outlook, though, is that single purpose trees will be better suited for their market niche than dual purpose trees and the industry needs to consider this eventuality.

Germplasm Evaluation

Three tasks critical to the successful initiation of a black walnut breeding program are: definition and prioritization of relevant traits, establishment of a multi-location cultivar evaluation trial, and initiation of a rootstock evaluation trial. Breeding for improved cultivars and rootstock will draw on data obtained from the evaluation trials to set program goals and establish benchmarks.

Trait definition - A breeding program(s) progress is inversely related to the number of independent traits being improved (i.e., for n uncorrelated traits under selection genetic gain will be only $n^{-0.5}$ as great as the gain for a single trait under selection, assuming equivalent genetic parameters). Selection pressure is diluted by the inclusion of each additional trait. Therefore, it is imperative that a breeding program clearly prioritize, and periodically review, those traits being improved. Although the number of traits to which selection pressure is applied must be minimized, many other traits need to be monitored as they also directly impact product quality. Important horticultural traits are: anthracnose resistance, vigor, consistency of nut yield, percent lateral fruitfulness, nut shape, and nut quality (shell thickness, percent crack out and ease of crackability, kernel weight, kernel plumpness, kernel color, flavor and kernel oil content). Timber quality is also a composite of several traits--straightness of bole and specific gravity being the most important traits. Phenological traits are critical to crop productivity. These include leafing date, first pollen shed, last pollen shed, peak female bloom, and harvest date. Ideally, data should be collected on all of these traits for each individual tree in a breeding program. Practically, compromises need to be made because resources are limited.

Multi-location cultivar trial - Baseline data on existing cultivars are needed to provide recommendations to growers and determine limitations of existing germplasm for particular environments. These data should be from a cultivar trial using grafted rootstock of cultivars grown in common orchards and replicated across diverse environments. The primary goal of the variety trial is to determine the extent of genotype stability. Secondarily, the cultivar trial should determine the optimal genotype and cultural practice for the various geographic regions of Missouri. Breeding strategies based upon these data will permit a more efficient targeting of production deficiencies amenable to genetic improvement. Sites chosen for the variety trial should meet the following criteria: they should sample the diversity of environments confronting the Missouri grower, be relevant to the maximum number of growers, and be placed in accessible locations (collection of phenological data is especially sensitive to the last criterion).

The Missouri Department of Conservation (MDC) has divided Missouri into 5 distinct ecological zones related to walnut production. The variety trial should be designed in three sites that captures most of the variability among these five zones. In addition, the trial also should be conducted in an agroforestry environment and compare the effects of cover crops and rootstock selections on cultivar stability.

Rootstock evaluation trial - Converting seedling orchards to plantings of grafted trees transformed the Persian walnut (J. regia) into a major agricultural commodity in California. This transformation occurred about 1915. The resultant uniformity of horticultural traits within orchards increased yields and promoted the development of good horticultural practices that further advanced the industry. Currently, seedling rootstocks represent the state-of-the-art in orchard management practices. However, research is currently underway to develop efficient clonal propagation techniques for rootstocks, which will further optimize orchard productivity.

The Missouri black walnut industry must undergo a similar transformation if it is to reap the benefits of the California model. The availability of proven rootstocks is a prerequisite to the conversion to grafted tree orchards. Although seedling rootstocks are available from nurseries (Starks Bros. promotes open-pollinated seedlings of Kwik Krop), there is very little or no information about performance and sources for superior rootstock. A good rootstock must be hardy and vigorous but not promote vegetative growth over reproductive potential. It must be evaluated across environments so that recommendations may be made with confidence.

The lack of information on black walnut rootstocks dictates that a rootstock evaluation trial be more limited in scope than the cultivar trial. A good strategy for evaluating sources of rootstocks is to examine the general vigor of a broad range of genotype sources at a single site for two years and cull out obviously deficient germplasm. The success of the Paradox rootstock in the California industry suggests that interspecific hybrids (e.g., J. hindsii x J. negri) also should be evaluated.

The next step in a rootstock evaluation trial is to choose sites that sample a variety of environments and evaluate a select group of rootstock sources across these sites. Evaluation at this step should be made by grafting scion from at least two different cultivars to each rootstock source and observing vigor, yield and phenological traits of the grafted trees. Walnuts can be grown on soil types ranging from the extremes of sandy to heavy clay loams and from thin to deep soils. However, it should not be assumed that the same rootstock will be optimal for all soil types. Therefore, site selection should reflect soil diversity. Also, a site that challenges rootstock cold hardiness would provide valuable information for northern Missouri growers. The effect of cover crop on rootstock

performance (e.g., is there variability for production of mycorrhizae among selected rootstock sources? Is such variability reflected in the variability of nut and timber traits?) must be included as a treatment effect at each site .

A complication in evaluating rootstock seedlings is the contribution of the pollen parent to seedling traits. At least some control needs to be placed on the pollen source since distinctly different progeny can result from the same cultivar when it is pollinated by different genotypes. Failure to control pollen source will reduce the stability of rootstock performance and may also confound the evaluation scheme. (The situation is avoided in the cultivar trial because most nut characteristics, excluding the embryo, are determined only by the maternal genotype.) If it is not feasible to do control pollinations or use common seed orchards, then rootstock seed should be collected from a single site for each genotype source.

Information obtained from the cultivar and rootstock trials will indicate if there is a significant scion-by-rootstock interaction for black walnut. Whether or not cultivars and rootstock need to be selected in tandem will be based on the magnitude of this interaction.

Selection and Hybridization Program

A breeder must be aware of the biological constraints to developing a successful breeding program. The salient features of eastern black walnut biology are: 1) generation times are long--time to first flower is at least four to six years; 2) it is a heterozygous cross-pollinated species that undergoes a marked reduction of vigor and productivity with inbreeding; 3) flowering is dichogamous and bloom period lasts only a few weeks; 4) flowers are wind-pollinated; 5) pistillate flower abscission (pfa) from an over-abundance of pollen has been observed; 6) seed germination requires 3 to 4 months of cold stratification; and 7) vegetative propagation by cuttings (grafting) is feasible.

The long generation time of eastern black walnut dictates that a selection and hybridization program be initiated concomitant to the cultivar and rootstock evaluation trials. Therefore, the program needs to be flexible enough to accommodate new information generated by these trials without disrupting progress made through selection. A recurrent selection program initiated with a broad base of unrelated superior cultivars, using controlled matings and minimizing inbreeding, has the best chance of long term success. (Such a program is labor intensive and it may be necessary to scale back to a scheme using open-pollination in isolated seed orchards. The drawback to doing so is that progress is reduced and it is more difficult to track inbreeding.) The peculiarities of walnut floral biology reduces the efficiency of controlled crossing. Therefore, it may take several seasons to complete a crossing scheme. Dominant gene action can be captured through vegetative propagation so broad sense heritabilities are appropriate for planning breeding strategies.

Selection - Specification of important traits (see section II.A.1) must be completed before parents can be selected. An attractive breeding approach for perennials with long juvenility periods is to combine independent culling and index selection in a two stage selection procedure. This is accomplished, for example, by first doing a greenhouse seedling screen for anthracnose resistance, culling out susceptible genotypes, followed by a field planting of the resistant types. Field trees are then evaluated for the desired traits (a key assumption is lack of linkage between anthracnose resistance and other traits) and trees ranked based on a phenotypic index of these traits. If necessary, several indices can be constructed to accommodate the requirements of different geographic regions. For example, northern Missouri sites might require a later date of leaf flush (and accompanying flowering) than southern sites. Increasing the selection intensity results in greater

gain per selection cycle but also increases the rate of inbreeding. Consequently, selection intensity needs to be balanced with the program(s) anticipated number of selection cycles.

Mating design - Factorial mating designs (e.g., NCII) serve the dual purpose of combining germplasm in a controlled manner and providing the appropriate family structure for genetic analysis methodology. The main disadvantage of the factorial design is the difficulty in obtaining a balanced set of progeny from each family. Particular sets of crosses may be difficult or impossible to make. If extensive sets of crosses do not produce seed, then entire crossing blocks could be lost to analysis. Large crossing blocks provide more reliable information about each parent but are more difficult to complete. Considering the difficulty in making controlled crosses with black walnut, a 3x3 crossing block size may be optimal. Eight 3x3 crossing blocks, a total of 48 parents, would require three generations to completely introgress the original parents into a common germplasm pool. Progeny from each generation would be evaluated for possible cultivar release.

ANCILLARY RESEARCH

MDC Black Walnut Progeny Testing Program

The MDC has established a wide ranging progeny test covering multiple sites. Data have been collected on several timber quality traits for a varying number of years for this project. Evaluation and analysis of these data could provide valuable information regarding genotype-by-environment interactions of black walnut vigor. Also, some of the MDC selections may be suitable for inclusion in the breeding program.

Other Research

Several lines of research need to be pursued to increase the efficiency of a black walnut breeding program. The following are some examples of research that could directly impact a breeding program:

Anthracnose resistance - Defoliation by anthracnose infection affects nut fill and is implicated in yield reduction of the subsequent years crop. It is the most destructive foliar disease of black walnut in Missouri. Currently, only cultivars resistant to anthracnose infection are suitable for release. The inheritance of anthracnose resistance has been investigated by Dan Neely and others, but individual resistance genes have not been identified yet. Establishing the Mendelian basis of anthracnose resistance can greatly increase the selection efficiency for this trait because it determines the population size of seedlings that needs to be screened. Development of a seedling resistance screening technique suitable for a selection program is another benefit obtained from a genetic study of anthracnose resistance.

Micro-propagation - Black walnut is considered to be a difficult species to vegetatively propagate by rooting. Developing a convenient and reliable micro-propagation technique for black walnut will greatly enhance our ability to evaluate rootstock and permit the development of clonaly propagated rootstocks. Jerry Van Sambeek is currently developing a micro-propagation technique based on rooting of stem cuttings. Collaboration with him will ensure that the technique is optimized for genotypes important to the breeding program.

Morphology of nut architecture - Ease of crackout is an important trait in nut production, and cultivars vary widely for this trait. Measuring the dimensions of various

morphological characteristics observed in cross- and longitudinal-sections of a sample of nuts from various cultivars may reveal which features are most strongly correlated to crackability. A multivariate statistical analysis (e.g., principal component analysis, canonical correlation analysis, and discriminant analysis) may lead to the development of a selection index for crackability. Additionally, detailing the morphological characteristics of nut architecture may expedite cultivar identification. For example, it may be possible to determine whether or not 'Mintle' and 'Brown Nugget' are different cultivars based on nut morphology.

Genetic variability of wild germplasm - Although the black walnut industry should be encouraged to move from using native seedling selections to elite germplasm, it is still necessary to maintain and catalogue species diversity. This is important because it is sometimes necessary to infuse a breeding population with new germplasm to minimize inbreeding. Isozyme analysis is a useful tool for screening wild germplasm to select the best genotypes for inclusion in a breeding program. The technique has been developed for black walnut by George Rink. Sampling black walnut germplasm throughout its native range and cataloging its isozyme diversity will be a useful addition to the morphological data that has already been collected.

Inter-specific hybrids - Several investigators have initiated studies into the hybridization of the Persian and eastern black walnut (J. regia x J. negri). Potential advantages to this line of research are the development of thin shelled black walnuts and higher yielding black walnut trees. Rootstocks are another potential use for inter-specific hybrids. J. microcarpa is better adapted to high pH and calcareous soils than is J. nigra. A hybrid of these two species, used as a rootstock, may show increased vigor and extend the productive range of J. nigra into thin calcareous soils.

Black walnut juvenility - The long generation times of black walnut are a major obstacle to tree improvement through breeding. Research aimed at developing convenient techniques to reduce the time from seed germination to flowering could pay great dividends to a breeding program. Much research has been done with other crops in this regard, most notably with apple.

Variability of juglone concentration among black walnut genotypes - The phytotoxic nature of juglone (5-hydroxy-1,4-napthoquinone) is well known. However, the magnitude of its role as a phytotoxin in an agroforestry environment has not been demonstrated. Also, the inhibitory effects of juglone on insect populations is not well understood. Investigating the variability of juglone concentration in roots, foliage, and husks among genotypes of black walnut is the first step to understanding its ecological role in the orchard environment. Understanding this role is a critical step in deciding the importance of juglone concentration as a trait to be monitored in a breeding program. An HPLC assay for juglone (and its precursor, hydrojuglone glucoside) in black walnut leaves and husks has been developed by Steven Cline and Dan Neely.

Genetic correlations and heritabilities of black walnut traits - Reliable estimates of genetic parameters require establishing breeding populations of sufficient size and family structure. These estimates are used to predict genetic gain from selection programs, define selection indices, and determine if a program should be infused with new germplasm.

Kernal color measurement - Nut quality and value is directly related to kernal color. Developing an objective, precise, and automated protocol for kernal color measurement based on spectrophotometry will facilitate nut grading by processors. It also could lead to the development of a differential pay scale to growers based on the nut quality of their crop. A breeding program could use this technique to investigate cultivar post-harvest color stability.

Dwarf trees - Size reduction of J. nigra trees can lead to increased yields per hectare and decreased labor costs in close plantings. Smaller trees also may provide advantages in an agroforestry setting by reducing the shading of cover crops. Size reduction can be achieved through the selection of trees of smaller size or through the use of dwarfing rootstocks. J. ailantifolia interstocks have been demonstrated to reduce J. regia scion growth.

Flavor variation among cultivars - Establishing the variability for flavor parameters (e.g., sweetness, astringency, and rancidity) of cultivars is an important first step in the industry's move away from wild germplasm. Depending on the magnitude of the variation, profound changes in product quality may result from this move. It is important for the industry to establish consumer preferences for black walnut flavor as it strives to increase the consumer base for this product.