

## WALNUT GENETIC IMPROVEMENT AT THE START OF A NEW CENTURY

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**ABSTRACT.** —Since the early 1960s, researchers in programs to improve black walnut for timber have struggled to meet some basic breeding objectives. Promising avenues of research had been identified by the early 1950s, and conventional methods to breed forest trees were widely adopted as suitable for walnut. Progress has been slow however, due to practical problems related to field plot establishment and maintenance. Many plantings are of no scientific value because of poor site selection, insufficient experimental blocking, and a lack of long-term care. From good test plantings, we have learned that regional adaptation exists for walnut, and that traits such as growth rate and timber form are genetic and may be improved through selection and breeding. The pace of walnut improvement in the next forty years should be much greater because of lessons learned from the past and because of the availability of new molecular tools to modify genetic backgrounds and track genetic gains. Scientists at the Hardwood Tree Improvement and Regeneration Center are seeking to overcome practical problems in tree improvement research so that we can produce high quality research plots that will provide improved walnut for midwestern hardwood forests throughout the next century.

Walnut improvement has been an important area of forest genetics research in the Central Hardwoods region for over 40 years. However, many of the central issues in walnut breeding identified by researchers in the 1950s remain unresolved. This paper will review the areas of research suggested by scientists working at the start of hardwood improvement in the 1950's and 1960's, and review important issues related to future prospects for improving walnut. Our goal is to evaluate which approaches have yielded valuable data, to learn from past mistakes, to identify future genetic improvement goals, and successful methods to achieve these goals.

Black walnut has a natural tendency to grow crooked and forked, without strong apical dominance, and with a great deal of variability in form and growth rate. To have high timber value, a walnut must be straight and have a bole free of branches. Thus, trees with straight boles and sparse lateral branches that naturally prune themselves; relatively fast growth, strong apical dominance, high percent heartwood, heartwood with good color, and little or no pin knots are the goals of black walnut improvement.

Naturally, walnut grows in rich moist sites with deep, well-drained soils. Foresters have long recognized that walnut is site-sensitive. Thus, wider site adaptability and regional adaptation are additional goals for walnut timber improvement. If the objective of walnut improvement is to produce trees that can be grown anywhere within its range under any level of management, then fast growth and other important traits will be sacrificed for broad adaptability. Few private landowners want these type of trees and it is not possible to breed a tree that will grow well without management (Zobel 1984). It might be theoretically possible to “select specific families

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for specific sites” (Rink and Clausen 1989), but such an approach is impractical without more information about site variation in the Central Hardwoods region.

### **Old Lessons – New Prospects**

#### Plantation Management

Many of the past walnut genetic improvement trials failed to accomplish their goals because of poor site selection and management of field plots (Table 1). Regardless of seed source, walnut can only be grown adequately on a good walnut site and tree improvement is not capable of altering the basic biology of a species. Many genetic trials have yielded little data because they were planted on sites that should not have been used. Furthermore, good site preparation, control of annual weeds, and removal of vines and perennial competition are indispensable if a study is to succeed. It is critical to have clearly drawn maps that are updated (track the loss of trees) with many backup copies. Navigating around many old research plots is difficult because of uncertainty about where rows or blocks begin or end. Under such circumstances, a permanent stake is needed to mark corners of the plot. The use of a species other than walnut to mark border rows and separate blocks is a practice that can keep plots clearly marked for the long-term. Today, GPS technology gives us the opportunity to locate plantings and soon, because of developing technology, every tree can be mapped within a planting. Decisions about which tree tag to use are a compromise among the needs for permanence, low cost, and ease of use.

#### Study Size and Experimental Design

Since mature walnut trees are large, walnut genetic studies have to be large (comprised of many trees) to be certain that differences among families can be statistically determined. In natural stands, walnut does not grow in large, uninterrupted stands, as do many conifers. Good walnut sites are often irregularly shaped, long and narrow or even discontinuous. These features complicate walnut research in particular because such sites are not easily blocked and they may be difficult to manage as experimental plots. Experimental blocking needed to account for the large variability common in good walnut sites is critical, but was rarely undertaken. Experimental methods such as single-tree plots and discontinuous plots can help to remove environmental effects from experimental analysis.

Walnut genetic tests were typically planted using a randomized complete block design with four to six blocks, and four seedlings per family planted together in each block. If all trees survived and began as uniform seedlings, this meant that 16 to 24 trees defined each family. This design was practical and sufficient to detect large genetic differences in provenance tests (Clausen, 1983), but it was not appropriate for discriminating small differences among families growing on variable sites.

Hardwoods are most valuable to landowners when they are mature, thus most study sites cannot be re-used. As a consequence, it is difficult to know the extent to which results at one site will be applicable to other similar sites. Beineke (1989) found that on good walnut sites, families from one location also did well in other locations, suggesting that genotype by environment interactions should not be debilitating to a breeding program. This conclusion needs further testing.

Minckler (1953) suggested that because long-term genetic research plots are subject to failure, many small studies should be established to minimize the risk. However Rink (1989) suggested that most studies were too small to provide statistically significant results. Increasing the number of study sites rapidly increases management and labor costs. Large studies are more efficient to manage when programs have small budgets and few staff. A survey of the results of previous walnut plantings (Table 1) suggests that small studies are prone to abandonment,

perhaps because it is easy to find a higher priority than the management of a small stand that will ultimately only produce a small amount of information. Zobel (1984) guessed that as many as half of all genetic studies are abandoned. Test sites that are targeted to committed landowners is the best way to avoid such problems. By including private landowners and forest industries as active participants and cooperators in hardwood research, we are hopeful that new walnut genetic plantings will have better long term care.

## **Selection and Evaluation of Elite Walnut**

### Selection Theory

Selection theory and practice are well developed for many plant breeding systems, but not yet for black walnut. The fundamental equation that underlies genetic selection and evaluation of plants and animals states that:  $P = G + E + (G \times E)$ ; where (P) is the phenotype or the outward appearance of the plant; (G) is the genotype, the underlying genes; (E) represents the environmental factors that effect how a plant looks; and (GxE) represents the interaction of genetic and environmental factors that affect the appearance of a plant. Phenotypic mass selection (picking the best trees based on a comparison with their neighbors) and progeny tests have been the most common tools for the evaluation and selection of superior walnut trees. The efficacy of mass selection when trees are growing in native conditions depends on the traits, species, care taken by the evaluators (Morgenstern 1975), and good fortune. Only by rigorous study of open-pollinated progeny, controlled-pollinated progeny, and replicated clonal trials, can we determine if a given tree really is genetically superior.

### Open-pollinated Progeny Tests

Walnut progeny tests are typically based on seed produced from open-pollination of plus trees. Rink (1989) suggested that a 25% improvement could be obtained by selecting the best trees in the best 50% of the families in a progeny test, but this suggestion is based on a small amount of data and is not likely to be the outcome in practice. Beineke (1989) published more modest results. Our preliminary evaluation of the elite selections at Purdue University indicated that 72 of the 427 trees selected from the wild and past progeny tests are above average and genetically superior. If these values hold true, then phenotypic mass selection was a reliable selection method for walnut 17 percent of the time. In other words, only 17 out of 100 selected walnut trees from natural stands are actually genetically superior.

For the most part, we do not know what test environments are best for which traits, and at what age should selection take place. There has been no empirical test in walnut of gain from early selection for high-heritability traits such as diameter growth (Beineke 1989). Several researchers have suggested that selection among progeny can begin as early as age five (Rink 1984; Coggeshall and Pennington 1982; Beineke 1989). In practice, both Coggeshall and Beineke preferred to wait until at least age ten (personal communication). The best examinations of selection age indicate that early selection (before age 10) is not likely to be effective despite the hopeful enthusiasm of some authors (Wright 1966). It is probably best to select at age 15 or longer (Rink and Clausen 1989, Bresnan and others 1992). Traits, aside from DBH and height, that should be evaluated and the best methods to determine how they are best quantified are not known. Traits such as improved percent heartwood, self-pruning, wound healing, apical dominance and straightness make walnut more valuable, but many of these traits are hard to quantify, and traits that are difficult to measure accurately are difficult or costly to improve (Zobel 1984). Black walnut genomics and genetic modification technologies should improve our accuracy for incorporating genes for quantitative traits into improved families although this research will still be costly. Rink (1989) found that heartwood color is primarily under environmental control and as such not subject to genetic improvement. As past grafted trees reach sufficient size, evaluation of clonal trees from different sites will provide a direct test of the genetics of heartwood color.

### Controlled Cross Progeny Tests

Controlled cross pollinations are necessary to efficiently combine traits into a single genotype and to study the genetic regulation of both simple and complex traits. Controlled crosses that produce full-sibling families are expected to be almost twice as efficient as open-pollinated half-sib families for meeting improvement goals (Kung et al 1974). Unfortunately, the biology of walnut makes the use of controlled crosses expensive (Beineke 1989). A problem with using controlled crosses is that the cost of producing a large number of progeny is high, and that if only a relatively small number of progeny are produced; the rate at which genes are fixed is much greater than might be expected from selection alone. Even genes favored by natural selection are at an increased risk of loss (Namkoong 1979) in small full-sibling populations. Open-pollinated half-sib families permit the retention of beneficial genes that have been naturally selected into the breeding population, but at the cost of maintaining large numbers of progeny and large numbers of individuals from each family.

Controlled crossing of elite individual trees is an important method to understand both the specific genetic combining ability of trees, and to develop new superior individuals for seed orchards or clonal propagation. There have been some full sib walnuts produced and planted over the past 40 years, but too few to provide meaningful information. Conventional control-pollination of walnut is labor intensive and on average, results in only 0.5 to 1 nut per bagged shoot. Walnut seed has an average of 50 percent viability, thus many bagged branches and pollinated flowers do not produce seed. Currently, the cost of controlled crosses is so much greater than that of collecting an equal number of open pollinated seed that the value of controlled crosses in walnut improvement is limited to specific applications in research and breeding.

Artificial control-pollination will remain necessary to cross individual trees that do not overlap with time of pollen shed and receptivity of female flowers. DNA markers allow researchers to improve accuracy of selection (marker-assisted selection) and determine propagation mistakes. If the performance of certain full-sib families is significant, then production of select crosses could be accomplished by planting isolated seed orchards with clones that will cross-pollinate naturally.

### Clonal Tests

Clonal trees, whether grafted or own-rooted, can be used to measure and understand local and regional environmental effects. The importance of genotype by environment interactions (GxE) can be determined by placing genetically comparable studies on multiple sites. Unfortunately, grafted trees have been used in only a few black walnut genetic studies. The most important grafted walnut timber breeding collection in the U.S. is at Purdue University. While these plantations were not designed as experimental test plots, they do show that many traits are consistent from grafted tree to grafted tree.

Additional research on vegetative propagation might make rooted clones possible. Own-rooted trees are superior to grafted trees for certain types of genetic tests because the roots and stems of own-rooted trees are genetically identical. An evaluation of the effects of environment on walnut growth will be best answered by long-term trials using clonal (rooted) selections on sufficiently large numbers of sites so that within-region variance can be determined. Own-rooted clones would be particularly valuable as controls in progeny tests for quantifying block and site effects, for quantifying phenotypic plasticity, and for determining the value of various management techniques.

## **The Output of Walnut Improvement**

## Seed Orchards

Black walnut is subject to alternate bearing and low yield (on a pounds of nuts per acre basis), thus seeds are always in short supply. This is especially true of genotypes selected for timber traits rather than nut yield. Seed orchards are a fundamental part of most tree improvement plans (Rink and Stelzer 1981), and they may be the most tangible product of the last 40 years of black walnut improvement. Funk (1966) reviewed some of the practical aspects of seed orchard establishment. Research on black walnut nut production (Jones and others 1998) addressed important issues related to nut orchard management, but research is still needed on treatments for early bearing and seed orchard design. Zobel (1984) suggested that as few as 30 – 40 trees might be sufficient for a production population. Much larger populations (about 300) are needed as genetic reserves. These might be maintained as sublines (see below) and progeny tested over time. Selections from the larger population can be used to supplement genetic deficiencies in the most advanced breeding population or as a long-term buffer against inbreeding.

The debate over the use of clonal versus seedling seed orchards divided walnut geneticists for a generation, but in hindsight, clonal orchards seem better when there is no commitment to rogue seedling orchards (Beineke 1989). Beineke (1982) observed gains of 5 to 10 percent for height and diameter using clonal seed orchards containing the best 20 percent of the families. When the seedlings of the entire seed orchard were compared with nursery-run stock the results were much less promising. These results are based on extremely small sample sizes, and Beineke suggested that gains in form were more evident than those for height and diameter. Perhaps the most important reason orchards produce no gain compared to random trees is that the clones in most of the orchards in the Central Hardwoods were not subject to rigorous progeny testing (Beineke 1982; Rink and Stelzer 1981). More recently, a study comparing 'Purdue #1' grafted trees, Tennessee State Nursery improved seedlings, and Missouri state common seedlings showed that after 12 years, improved seedlings were no different in height growth and timber form than the clonal trees (Hammitt, 1996). Both improved sources were significantly better than the common stock for height and form, but they were the same for diameter growth. The HTIRC is planting similar studies to provide data on the gains landowners will receive from planting improved walnut.

Seed orchards at two State-owned nurseries in Indiana produce over 200,000 walnut seeds in a good year; about 25 percent of the total number of walnut seeds needed by the nursery. Seed from the nurseries' seed orchards is mixed with seed purchased from local collectors, expanding the genetic diversity in Indiana hardwood plantations. At present, few private nurseries maintain their own improved seed orchards, but the list of those that do is growing.

It is important for black walnut improvement to improve seed yield from select trees because large progeny tests are necessary to produce the greatest gain. Management techniques that improve the seed yield need additional research. Some of the methods being tested at HTIRC include tree training systems and water and fertility management. Other methods to reduce seedling juvenility, the time period in which a tree cannot flower, will accelerate breeding by permitting more rapid testing of generations. Research on seed handling and storage might provide new possibilities for making seed available to breeders in years when late frosts leave many trees fruitless, and in the years of alternate bearing when seed production is low.

## Seed Zones

Proposed black walnut seed zones (Deneke and others 1981) are based on an educated guess rather than the analysis of experimental data. They seem reasonable based on years of field observations. The existence of locally adapted walnut populations in discrete ecological habitats seems possible, but it has yet to be demonstrated. It also seems likely that for any two seed zones or provenances, the possible variance within the zone could easily exceed the

variance between adjoining zones. Seed zones complicate nursery operations by limiting the bulking of seeds and sometimes by limiting seed availability.

While several studies have investigated stand and provenance level variance (Bey and Williams 1975; Wendel and Dorn 1984; Bresnan and others 1992; Geyer and Rink 1998), the terms do not signify a clear, precise, repeatable type of sample. We were not able to identify any data that indicate exactly – or even approximately -- how many trees from how many locations would adequately characterize the genetic variance of walnut growing in a region. These studies did establish that trees from seed moved a relatively short distance northward often grew significantly faster than trees from local seed sources, supporting the 200 mile guideline (Rink, 1996).

### Sublines and genetic conservation

Sublines can be used for the avoidance of inbreeding depression and the retention of genetic diversity (Namkoong 1979; McKeand and Beineke 1980). Inbreeding depression is a serious long-term concern for certain types of breeding programs. The basic idea behind sublines is that at least two separate breeding populations (or sublines) of trees are maintained, and the best individuals from each subline are included in more advanced breeding populations or seed orchards. Breeders have recourse to the sublines when they need new sources of genetic variability and new genes. Because we do not know which traits will be important in the future, sublines can be used for genetic conservation.

### Hybrids

Interest in the use of interspecific hybrids (hybrids between two different walnut species) to improve vigor dates back to the early 20<sup>th</sup> century (Wright 1966). Most first-generation (F<sub>1</sub>) interspecific hybrids in the genus *Juglans* show dramatic hybrid vigor, but only small plantings of hybrids have been made because of the difficulty in obtaining F<sub>1</sub> seed (Bey 1969). Hybrids have been most frequently made for nut production (e.g., Persian walnut x butternut) rather than for timber. In the last 20 years in Europe, the black walnut x Persian walnut hybrid has received considerable attention as a potential timber tree ([http://www.walnuttrees.co.uk/timber\\_home.htm](http://www.walnuttrees.co.uk/timber_home.htm)). There are a few examples of other hybrids with potential as a timber crop. *Juglans nigra* x *J. hindsii* (Northern California black walnut or NCB) is known as the "Royal hybrid" and is sold in the Pacific Northwest. Several *J. nigra* x *J. major* (Arizona black walnut) hybrids are growing at the former Tree Improvement Center Arboretum associated with Southern Illinois University. The wood qualities and timber potential of most of these hybrids is not well known yet. HTIRC has identified a few mature hybrids for analysis of their wood quality.

Walnut growers in California purchase 90% of their grafted Persian walnut trees that have a hybrid rootstock called "Paradox." This F<sub>1</sub> hybrid is a cross between NCB x Persian walnut. On very good walnut soils in California, nut yields are similar between NCB and Paradox rootstock. On more marginal sites, Paradox rooted trees out perform NCB. Paradox rootstock is also particularly useful on heavy soils and for fields that are often saturated. In the Midwest, it may be possible to use hybrids to expand the soil types and sites on which black walnut can be grown. Several of the western walnut species may be drought tolerant, and they may be useful as rootstocks for seed orchards.

## **SUMMARY**

The potential for large-scale development of improved walnut seed has never been greater. In the next decade, we will be available to revisit and evaluate many 30 to 40 year old plantings and begin to assess the value and potential of select stock at mid rotation. Older plantings are more reliable sources of data for selecting outstanding parents. We have propagated several new seed orchards at Purdue University and we are planning to expand seed orchards for the

Indiana Department of Natural Resources in the near future. In addition, we can provide material and advice to private companies and individuals that want to establish improved seed orchards. HTIRC orchards will contain better parents than were available in the past, and they will be planted and managed to maximize seed production to provide a steadily increasing quantity of improved walnut seed for landowners through the next century. In addition, molecular genetic tools will be used to access genetic improvement and to speed the rate that genetic improvements are made.

#### **ACKNOWLEDGMENT**

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#### **LITERATURE CITED**

- Beineke, W.F. 1982. New Directions in Genetic Improvement: Grafted Black Walnut Plantations. In: Black walnut for the future: Third Walnut Symposium: Proceedings of the Twelfth Annual Meeting of the Walnut Council; 1981; West Lafayette, IN. Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 64-68.
- Beineke, W.F. 1989. Twenty Years of Black Walnut Genetic Improvement at Purdue University. Northern Journal of Applied Forestry 6: 68-71.
- Beineke, W.; Stelzer, H.E. 1985. Genetic improvement of black walnut: Is it working? Annual Report of the Northern Nut Growers Association 76:26-31.
- Bey, C. 1969. The Forest Service Black Walnut Genetics Project: A Progress Report. In: Schreiner, E.J. ed. Proceedings, Fifteenth Northeastern Forest Tree Improvement Conference; 1967 July 25 - 26; Morgantown, WV. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 31-34.
- Bey, C.F., Williams, R.D. 1975. Black walnut trees of southern origin growing well in Indiana. Indiana Academy of Science Proceedings 84: 122-127.
- Bresnan, D.; Geyer, W.A.; Lynch, K.D.; Rink, G. 1992. Black walnut provenance performance in Kansas. Northern Journal of Applied Forestry 9: 41-43.
- Clark, F.B. 1965. Forest Service Research in Black Walnut. 56<sup>th</sup> Annual Report of the Northern Nut Growers Association. 56: 84-87.
- Clausen, K.E. 1983. Performance of black walnut provenances after 15 years in 7 midwestern plantations. Proceedings, Third North Central Forest Tree Improvement Conference; 1983 August 17-19; Wooster, OH. Dept. Forestry, University of Wisconsin, Madison, WI: 24-33.
- Coggeshall, M.V.; Pennington, S.G. 1982. Indiana Division of Forestry black walnut tree improvement program In: Black walnut for the future: Third Walnut Symposium: Proceedings of the Twelfth Annual Meeting of the Walnut Council; 1981; West Lafayette, IN. Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 61-63.
- Deneke, F.J.; Funk, D.T.; Bey, C. 1981. Preliminary Seed Collection Zones for Black Walnut. USDA Forest Service, NA-FB/M-4. Northeastern Area State and Private Forestry, Broomall, PA. 5 p.

Funk, D.T. 1966. Seed orchards. In: Black walnut culture. Walnut Workshop. Aug. 2-3, USDA Forest Service, North Central Forest Experiment Station. pp. 62-65.

Geyer, W.A.; Rink, G. 1998. Interaction effects of seed source, culture, and pest management on growth of *Juglans nigra* in plantations. *Silvae Genetica* 47: 51-58.

Hammit, W.E. 1996. Growth differences among patented walnut grafts and selected seedlings 12 years after establishment. In: Van Sambeek, J.W., ed. Knowledge for the Future of Black Walnut. (Proceedings, 5<sup>th</sup> Black Walnut Symposium). Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 63-68.

Jones, J.E., Mueller, R., Van Sambeek, J.R. (eds). 1998. Nut Production Handbook for Eastern Black Walnut. Southwest Missouri Resources, Conservation & Development (RC&D), Inc. <http://www.ncrs.fs.fed.us/epubs/walnut/>

Kung, F.H.; Bey, C.F.; Larson, J.L. 1974. Nursery Performance of Black Walnut Twins. In: Garrett, P.W. ed. Proceedings, 22nd Northeastern Forest Tree Improvement Conference; 1974 August 7 – 9; Syracuse, NY. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 184–190.

McKeand, E.; Beineke, W.F. 1980. Sublining for Half-Sib Breeding Populations of Forest Trees. *Silvae Genetica* 29:14-17.

Minckler, L.S. 1953. Recent Advances In The Field of Forest Genetics. *Illinois Academy of Science Transactions*. 46: 56–62.

Morgenstern, E.K. 1975. Review of the Principles of Plus-Tree Selection. In: Morgenstern, E.K.; Lolst, A.H.; Teich, A.H.; Yeatman C.W. eds. Plus-Tree Selection: Review and Outlook. Canadian Forestry Service Publication No. 1347. Ottawa, Canada. 1-27.

Namkoong, G. 1979. Introduction to quantitative genetics in forestry. USDA Forest Service Technical Bulletin No. 1588. Washington, D.C.

Rink, G. 1984. Trends in genetic control of juvenile black walnut height growth. *Forest Science* 30:821-827.

Rink, G. 1989. Recent advances in walnut tree improvement. In: Phelps, J.E., ed. The continuing quest for quality: proceedings, 4th Black Walnut Symposium; 1989 July 30 – August 2; Carbondale, IL. Indianapolis, IN: Walnut Council. 140–144.

Rink, G., and Stelzer, H. 1981. The Status of Black Walnut Tree Improvement Programs in the North Central Region. Northern Nut Growers Association Annual Report. 72: 57-60.

Rink, G.; Clausen, K.E. 1989. Site and age effects on genotypic control of juvenile *Juglans nigra* L. tree height. *Silvae Genetica*. 38(1): 17-21.

Wendel, G.W.; Dorn, D.E. 1985. Survival and growth of black walnut families after 7 years in West Virginia. Northeast Forest Experiment Station Research Paper NE-569.

Wright, J. 1966. Breeding Better Timber Varieties. In: Black Walnut Culture; 1966 August; Carbondale, IL. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 5

Zobel, B.; Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley and Sons, Inc. New York. 505 pages.

Table 1.—*Characteristics of 33 typical walnut genetic studies and factors that affected their success.*

Planting	Planter <sup>1</sup>	Location	Type <sup>2</sup>	Year	Families/ No. Trees	Comments and Recommendation <sup>3</sup>
Salamonie Reservoir	Funk	Wabash Co., IN	PP	1963	24/ 840	Study CG-369 (NC-1402), good uniform site, good drainage, no slope, good early management, several companion plantings abandoned very early. [M]
Hoosier NF	Bey	Lawrence Co., IN	IP PR	1967	15/ 360	Fair to good site, flood prone six parents per provenance, pruned for 5 years, fair survival to year 7 (61%), root rot in planting stock, herbicides used for six years, stakes not found in 2003, alder overtopped walnut. [M]
Shidler (Martell)	Beineke	Tippecanoe Co., IN	PT	1968	13/ 170	Good site, no records after age five, growth fair, [A]
C.B. Stems	Beineke	Harrison Co., IN	PT	1969	8/ 80	Measured in 1978, 1982 and 1986. Two of three reps abandoned for poor survival, excellent growth. [MM, A]
Pierson Hollowell Richmond	Beineke	Wayne Co., IN	PT	1969	---	[MM], [A]
Pierson Hollowell	Beineke	Parke Co. IN	PT	1971	9/ 300	Not thinned, vines, slow growth, PVC pipe with rebar at corners, some stakes with tags. [A]
SIPAC Flick	Beineke	Dubois Co., IN	PT	1971	8/ 108	Demonstration plot, excellent site and management. [M]

<sup>1</sup>Only one investigator is listed although most plantings involved several investigators and collaborators.

<sup>2</sup>PP= Provenance/progeny test, IP = Interplanting trial, PR= Provenance study, PT = Progeny test,

CT= Clone test, CS= container study, CC= Controlled crosses, SO= Seed orchards.

<sup>3</sup>[M]= Maintained as valuable, [A]= Abandoned, [MM]= Missing maps.

Table 1.—cont., *Characteristics of 33 typical walnut genetic studies and the factors that affected their success.*

Planting	Planter <sup>1</sup>	Location	Type <sup>2</sup>	Year	Families/ No. Trees	Comments and Recommendation <sup>3</sup>
Hillenbrand Industries	Beineke	Franklin Co., IN	PT	1972	11/ 150	Good site, poor care, hard to find corners and border rows, fair survival. [A]
Jasper Free Tract	Beineke	Orange Co., IN	PT	1972	4/ 158	Good site and growth, good early management, visited 1988 and corners blazed. [A]
Pierson Hollowell	Beineke	Parke Co. IN	CC	1973	16/ 250	Never thinned, could not find corners in 2000. [A]
Pleasant Valley, Sexton Creek	Rink	Alexander Co., IL	PT SO	1973	54/ 2700	FS-NC-1151, CG-425, trees were thinned, disked, vetch seeded in year 11, excellent site, thinned at age 10, three blocks lost to flood. [M]
Martell	Beineke	Tippecanoe Co., IN	PT	1975	12/ 168	Good site, map upside down, good growth, selections made, harvested 2001.
IDNR Harrison-Crawford SF	Beineke	Crawford Co., IN	PT CC	1976	20/ 710	Site also known as “Church Camp”, poor growth, native hardwoods overtopped test, poor management, could not find corners in 2000. [A]
Missisnewa Reservoir	Beineke	Wabash Co., IN	PT SO	1976	11/ 285	Poor site, no maintenance, variable growth, no thinning, grasses suppress growth, IDNR seed source. [M]
IDNR Harrison-Crawford SF	Beineke	Crawford Co., IN	PT CC	1977	36/ --	Site also known as “Cave” Poor site, poor survival and growth. [A]

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Pierson Hollowell	Beineke	Morgan. Co., IN	PT	1977	18/ 440	Good site, PVC with rebar at corners, map upside down. [M]
Rattlesnake Ferry	Geyer	Jackson Co., IL	IP PP	1977	5/ 1500	Flood prone, fair drainage corrective pruning, good early management, follow- up study published in 2003. [M]
Clark State Forest	Beineke	Clark Co., IN	PT	1978	12/ 534	Fair site, well managed in excellent condition, borders removed, PVC pipe with rebar placed inside corner at row 2 tree 2. [M]
Missisinewa Reservoir	Beineke	Wabash Co., IN	PT SO CC	1978	29/ 802	Off site, growth better than 1976 progeny test, poor management. [CC], [M]
Parsons	Dorn	Tucker Co., WV	PP SO	1978	34/ 1000	Fair site, top dieback, poor growth first 7 years, survival fair to good, no published records since 1985. [A]
Jasper- Pulaski	Beineke	Jasper Co., IN	PT SO	1979	9/ 200	Off site, high site variability 4' x 5' planting too close, no maintenance, IDNR nursery. [A]
Martell	Beineke	Tippecanoe Co., IN	PT	1979	40/ 800	OK site, good map, fair growth, thinned 1987, 1991, 10 selections made. [M]
Vallonia Nursery	Beineke	Jackson Co., IN	PT SO	1979	9/ 200	Managed by IDNR, good site, thinned, good seed source, paired with Jasper- Pulaski test. [M]

<sup>1</sup>Only one investigator is listed although most plantings involved several investigators and collaborators.

<sup>2</sup>PP= Provenance/progeny test, IP = Interplanting trial, PR= Provenance study, PT = Progeny test,

CT= Clone test, CS= container study, CC= Controlled crosses, SO= Seed orchards.

<sup>3</sup>[M]= Maintained as valuable, [A]= Abandoned, [MM]= Missing maps.

Table 1.—cont., *Characteristics of 33 typical walnut genetic studies and the factors that affected their success.*

Planting	Planter <sup>1</sup>	Location	Type <sup>2</sup>	Year	Families/ No. Trees	Comments and Recommendation <sup>3</sup>
IDNR	Beineke	Harrison Co., IN	PT	1980	44/ 876	Off site, poor growth. [A]
Jasper-Pulaski	Beineke	Jasper Co., IN	PT SO	1980	30/ 496	Off site, high site variability, 4' x 5' planting too close, no maintenance, IDNR nursery. [A]
Vallonia Nursery	Beineke	Jackson Co., IN	PT SO	1980	36/ 740	Managed by IDNR, good site, thinned, good seed source, paired with Jasper-Pulaski test. [M]
Big Creek	Rink	Hardin Co., IL	PP	1981	131/ 2500	Flood prone site, good drainage, low survival in some blocks, corners clear in 2003, fair to good growth. 4-tree plots could be discerned, good maps, but access to the site contested. [M]
SEPAC	Seifert	Jennings Co., IN	PP	1981	80/ 900	Companion to Kellogg Forest planting (CG -369) Good site and management. [M]
Merry Lee Nature Center Goshen College	Beineke	Noble Co., IN	PT	1984	15/ 308	High mortality. [MM], [A]
Spurgeon Hollow Lake	Beineke	Jackson Co., IN	PT	1984	58/ 1180	Excellent site and management, thinned twice, frequently visited, collaboration with IDNR. [M]
Clark State Forest	Beineke	Clark Co., IN	PT CS	1988	10/ 546	Site extremely variable, fair growth, collaboration with OSU, primarily potting media study. [M]

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Table 1.—cont., *Characteristics of 33 typical walnut genetic studies and the factors that affected their success.*

Planting	Planter <sup>1</sup>	Location	Type <sup>2</sup>	Year	Families/ No. Trees	Comments and Recommendation <sup>3</sup>
Mount Tabor	Beineke	Monroe Co., IN	PT, IP	1990	53/ 3180	Interplanted pine and European black alder, good site, good growth, corners and row end markers missing. [A]
Wolf Creek	Beineke	Lenawee Co., MI	CT	1993	5/ 800	Survival 25%, poor growth repeated deer browse, poor site great initial management. [A]

<sup>1</sup>Only one investigator is listed although most plantings involved several investigators and collaborators.

<sup>2</sup>PP= Provenance/progeny test, IP = Interplanting trial, PR= Provenance study, PT = Progeny test,

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<sup>3</sup>[M]= Maintained as valuable, [A]= Abandoned, [MM]= Missing maps.