United States
Department of
Agriculture

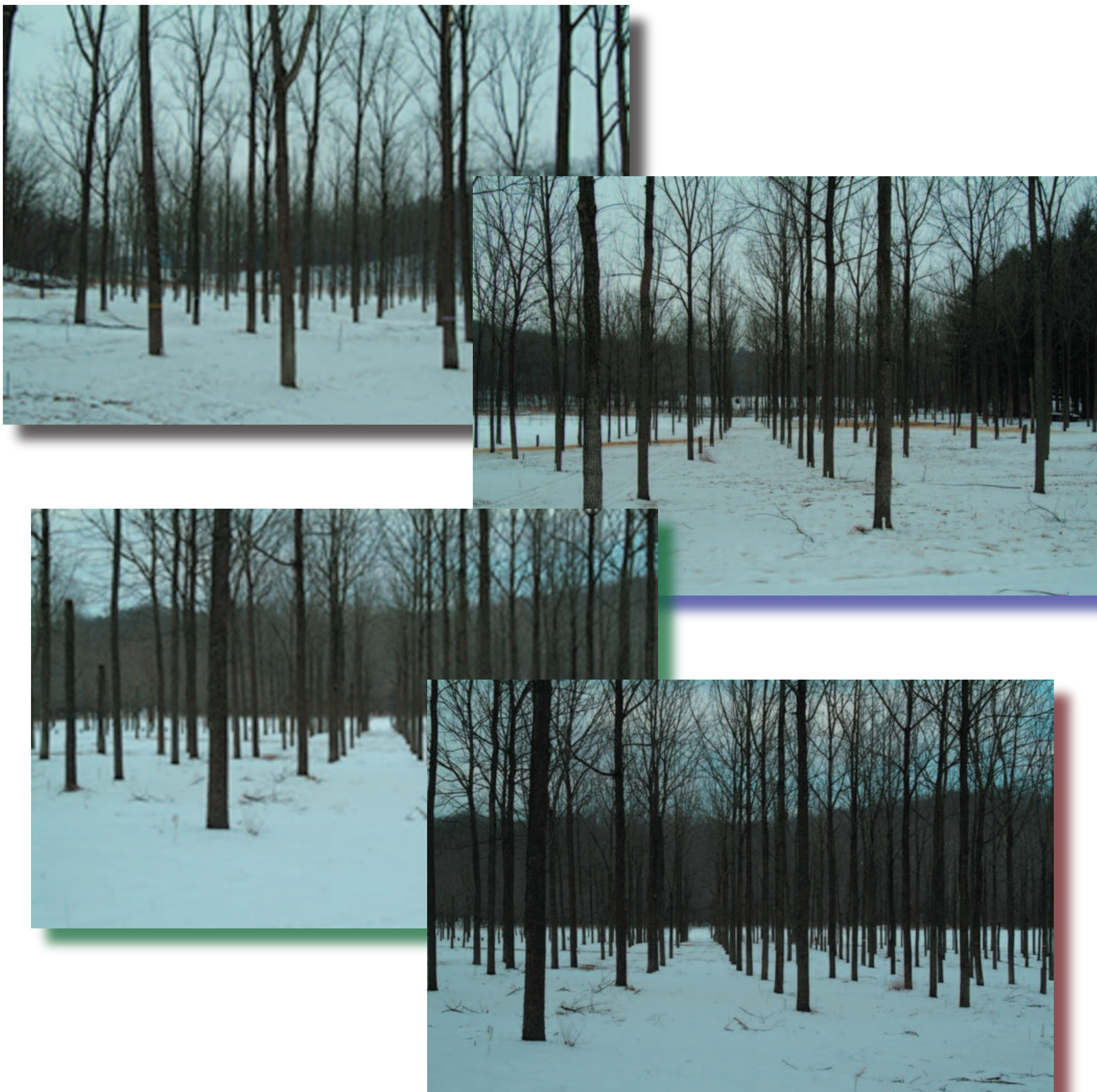
Forest
Service

North Central
Research Station

General Technical
Report NC-243

Black Walnut in a New Century

Proceedings of the 6th Walnut Council Research Symposium



DISCLAIMER

The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture or any product or service.

Papers published in these proceedings were submitted by authors in electronic media. Editing was done to ensure a consistent format. Authors are responsible for content and accuracy of their individual papers.

Published by:
North Central Research Station
Forest Service–U.S. Department of Agriculture
1992 Folwell Avenue
St. Paul, MN 55108
2004

Web site: www.ncrs.fs.fed.us

Cover Photo by: James McKenna

Black Walnut in a New Century

Proceedings of the 6th Walnut Council Research Symposium

Lafayette, Indiana

July 25-28, 2004

Editors:

C.H. Michler

P.M. Pijut

J. W. Van Sambeek

M. V. Coggeshall

J. Seifert

K. Woeste

R. Overton

F. Ponder, Jr.

Contents

Agroforestry and Nut Production

Walnut Pest Management

International Walnut Research

Economics of Walnut Production

Short Note

Poster Session

HISTORY OF BLACK WALNUT GENETICS RESEARCH IN NORTH AMERICA

Erin Victory; Keith Woeste; and Olin E. Rhodes, Jr.[1]

ABSTRACT—Eastern black walnut (*Juglans nigra* L.) is an economically and ecologically important hardwood species that has been used throughout the history of settlement in North America. It was a resource that helped Native Americans in their everyday life, it helped European settlers carve a living out of the wilderness, and it has helped rural farmers and private landowners subsist and invest in the future. Described here is a brief history of black walnut breeding and molecular genetics research. Current genetic research may ultimately lead to the domestication of black walnut, an event that would be a hallmark for forest tree species.

Eastern black walnut (*Juglans nigra* L.) has been a component of North American forests since the Middle to Upper Cretaceous (Elias 1980) and has been used by humanity for centuries. This species has never held a predominant position in temperate forests, but it has been a consistent feature in the Central Hardwood Region. Almost every part of the tree has played some valuable role in helping people subsist, including consumption of the nuts and the use of nut husks for dyes, and use of the wood for fencing, railroad ties, firewood, gunstocks, and fine furniture. Black walnut also is an important tree for forest animals, providing shelter and forage especially for squirrels.

In the 20th century, black walnut researchers began to study the ecology, reproductive biology, and phenotypic variation of this valuable species. Eventually breeding programs were initiated to find black walnuts of sufficient growth rate and architecture to meet the demands of industry. Although much is now known about black walnut biology and natural history, this quest continues today, after over 60 years of effort. The purpose of this is to provide a summary of improvement programs, investigations of vegetative propagation, advances in taxonomic relationships within the Juglandaceae, parentage, gene flow, and studies of genetic diversity made possible by molecular genetics.

NUT CULTIVARS

Landowners have identified and selected walnut trees for propagation since the late 1800s (Zarger 1969). Emphasis on cultivation in the early 20th century was focused on nut tree culture and propagation (MacDaniels 1933, Talbert 1942). Gradually, an awareness grew that quality walnut trees were becoming uncommon due to the clearing of land for agriculture and overharvesting. A tree planting movement began with the goal of restoring the diminishing supply of quality black walnut (Record 1986, U.S. Forest Service 1966). Contests sponsored by the Northern Nut Growers Association and State agencies were conducted with the intent of discovering new cultivars (Crane and Reed 1937).

The Tennessee Valley Authority spent many years, from 1934-1960, identifying trees and cultivars that were well adapted to the valley (Zarger 1969). McKay (1971) published his lifelong breeding program to develop a Persian walnut and eastern black walnut hybrid with superior nut production, with little success. By the end of the 1960s, there were 18 nut cultivars that were widely used for nut production in the Central Hardwood Region (Zarger 1969), but efforts towards an improved timber variety were just beginning.

[1]Graduate Student (EV), Professor (OER), Department of Forestry and Natural Resources, 195 Marstellar St., Purdue University, West Lafayette, IN 47906; and Research Molecular Geneticist (KW), Hardwood Tree Improvement and Regeneration Center, North Central Research Station, United States Department of Agriculture Forest Service, 170 South University St., Purdue University, West Lafayette, IN, 47906. EV is corresponding author: to contact, call (765) 494-3997 or email at erin@fnr.purdue.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

TREE IMPROVEMENT

In the 1960s there was a shift from "mining" walnut trees from natural stands to a conscientious effort towards improving black walnut as a crop. The impetus for this was a perceived decrease in the availability of high quality walnut timber in natural stands, a condition attributed to the long-standing practice of high-grading (Clark 1965, Elias 1980). Reduced supplies of high quality trees increased the price of walnut timber leading growers and industry to recognize the need for high quality, fast-growing trees to meet increasing market demands. The increasing demand for black walnut led the USDA Forest Service to initiate their Black Walnut Genetics Project at Carbondale, IL in 1965 (Funk 1966), a research effort focused on progeny testing, seed sources, and the heritability of apical dominance. The mission was to gather and provide genetic information with which to base a breeding program for superior trees (Caraway 1976).

By the mid-1960s, several provenance studies were already underway, with plans to begin hybridization research (Clark 1965). However, while information gathered at the beginning of the century regarding pollination, vegetative propagation, and seed germination provided a sound foundation for basic research, there were virtually no data on the genetics of the species (Wright 1966). To address questions related to regional adaptability and variability, in the absence of genetic data, seed zones were proposed for the Central States based upon temperature, annual precipitation, occurrence of frost in the spring and fall, the number of frost-free days, latitude, and summer day length (Deneke and others 1981). Seed zones are regions representing a limited range of environmental variance such that the seeds collected anywhere in a zone could be planted in the same zone with some assurance of success.

By the 1970s, black walnut research had been focused on tree improvement for almost a decade, but there remained a lack of high quality product (seeds or seedlings) to meet the demands of a growing industry. In a summarization of what was known about taxonomy, evolution, sexual reproduction, and breeding and improvement programs of black walnut, Funk (1970) stated that black walnut breeding programs should focus on climatic adaptation, vigor and growth rate, form, fecundity, and pest resistance. In describing the importance of the natural phenotypic variation in walnut, both Funk (1973) and Bey (1970, 1973) found that there were more differences among seed sources than within stands or areas. For instance, Bey's research on six year-old provenance studies from six States in the Midwest indicated that the origin of seed collection affected the timing of leaf flush and leaf fall, and tree height, with trees from southern sources flushing earlier, losing their leaves later, and being generally taller and of larger diameter. Bey (1980) again used these provenances to determine that geographic origin was an important component when considering seed sources and recommended choosing trees up to 200 miles south of the planting site as the tallest 20% of the trees in his study were from sources south of the planting site. His results indicated that though sources beyond 200 miles south of the planting site were taller and bigger than trees from northern sources, they also were more prone to injury or death due to frost and cold winters. This finding was again confirmed by Geyer and Rink (1998) when they took diameter, height, and survival measurements on a 17-year-old provenance test in southern Illinois.

An outcome of Bey's (1973) research was his conclusion that future research needed to determine the importance of trade-off's in trait selection, how successful silvicultural manipulations were in addressing problems, and the effectiveness of selecting wild trees based on phenotype.

By 1982, 11 States within the walnut commercial range had established walnut improvement programs, from provenance and progeny testing, to second-generation seed orchards (Rink and Stelzer 1982). For example, nine timber varieties (thought to be superior in growth rate, form, or seed yield [Beineke 1984]) were selected and propagated at Purdue University. Moreover, in the same decade, several regional cooperatives were established: the NC-99 program, and the eight-state Fine Hardwood Tree Improvement Cooperative. NC-99 began in 1960 (then known as NC-51) and focused on determining the range and pattern of genetic diversity, breeding, selection and seed production, breeding zones, and advanced generation breeding for species of economic importance. NC-99 contributed significantly to the knowledge accumulated on breeding black walnut (Stier 1986). The eight-state Fine Hardwood Tree Improvement Cooperative began in 1986 to combine efforts towards genetic improvement of primarily black walnut and northern red oak in the north central region. The optimism that was a hallmark of the early efforts towards black walnut timber breeding was tempered in later years as it became clear that walnut was extremely site sensitive and that significant environment-genotype interactions would confound genetic effects and complicate their interpretation (Geyer and Rink 1998). Today, efforts are still underway to breed a fast-growing, straight-boled, black walnut timber variety. For more detail, see McKenna and Woeste (2004, this volume).

DISEASE MANAGEMENT

Of all the diseases and damaging agents that affect black walnut, only walnut anthracnose, caused by the fungus *Gnomonia lepostyla,* has been the subject of genetic research. Walnut anthracnose can severely damage a nut crop and hinder tree growth. Beginning in the 1970s, Black and Neely published a series of articles on walnut anthracnose and the factors influencing its spread (Neely 1979, Black and Neely 1978a). Resistance to anthracnose was considered an important goal of tree improvement programs, and Black and Neely (1978b) determined that while eastern black walnut was less susceptible than the Hinds (*Juglans hindsii*), hybrids between eastern black walnut and California black walnut varied in their resistance. For example, in a study of the disease susceptibility of walnut hybrids, black walnut crossed with the Hinds was the most susceptible (had the most lesions), while black walnuts crossed with Japanese walnut (*Juglans ailantifolia*) were the most resistant (Black and Neely 1978b). This research suggested that Persian and Japanese walnut were potential sources for resistance genes to anthracnose.

Funk and others (1980) evaluated 62 black walnut families over 4 years for anthracnose incidence by visually estimating incidence of leaflets with one or more anthracnose-caused spots, estimating premature crown defoliation due to anthracnose, and determining the electrical resistance of cambial tissue. They also evaluated individual and family correlations among disease ratings and growth rates. Their results indicated that year to year correlations for anthracnose incidence were stronger for individual trees than family means, and that selecting for faster growth resulted in a 13% decrease in anthracnose incidence, while selecting for low anthracnose incidence resulted in only a 7% growth increase.

TISSUE CULTURE

Vegetative propagation of black walnut became emphasized in the 1980s as a mechanism for circumventing problems associated with conventional propagation (Coggeshall and Beineke 1997). Walnut trees produce fewer seeds per tree than many other species, and once those seeds are harvested, only a portion is viable. Poor seed yield and losses to predation prior to nut collection often result in an undersupply of seeds to sell or plant (Beineke 1982). Vegetative propagation potentially permits the almost limitless multiplication of selected genotypes without additional recourse to seeds. Grafting had long been practiced as a method for propagating black walnut, and a high rate of grafting success provided factors as scion wood storage, rootstock condition, and grafting technique and aftercare were optimized (Beineke 1983). The multiplication of microshoots *in vitro* was pursued as a promising method for producing ramets on their own roots and in association with research into the development of a transformation and regeneration system for black walnut (Van Sambeek and others 1997). Efficient rooting of micropropagated walnuts proved difficult, but by the late 1980's somatic embryogenesis looked promising, and genetic engineering was being considered for black walnut (Rink 1989). Somatic embryogenesis experiments were in progress by 1993 (Van Sambeek and others 1990, Long and others 1995, Neuman and others 1993, Khan 1995). Since then, considerable progress has been made in tissue culture, for more detail, see Michler and Bosela (2004, this volume).

MOLECULAR GENETICS

One of the first molecular-genetic studies of black walnut was the discovery of its chromosome number (n=16, Woodworth 1930). The development of allozyme markers in the 1980s made the study of black walnut population genetics feasible. Kung and Rink (1987) used eight allozyme loci to estimate the outcrossing rate and fixation index for trees sampled within one county in Illinois. The use of molecular tools to answer population level questions and questions related to tree improvement expanded in the 1990's when researchers began to correlate isozyme allele frequencies with traits such as height and diameter (Kung and others 1991, Rink 1997). Additionally, throughout the 1990s allozyme markers were used to examine mating parameters, outcrossing rates, and fixation indices in black walnut (Rink and others 1989, 1994; Zhang 1990; Kung and others 1991; Zuo 1994; Zuo and others 1995; Busov 1996). By the late 1990s questions focused on overall levels of genetic diversity and the partitioning of genetic variation among black walnut populations (Busov 1996, Busov and others 2002).

The 1990s also marked a period in which morphological studies of walnut taxonomy were re-evaluated using molecular techniques. For example, Smith and Doyle (1995) found congruence between taxonomy of the Juglandaceae based on morphological (from literature including Manning's 1978 paper along with subsequent papers) and molecular data (chloroplast restriction site data). Their findings supported Manning's (1978) classifications within the Juglandaceae (based upon inflorescences, flowers, and fruits) with the exception of the subfamilial status of *Platycarya.* Smith and Doyle's findings suggested that either Engelhardieae should be moved to subfamilial status, or that Manning's two subfamilies, Juglandoideae and Platycaryoideae, should be reduced to the tribal level. However, it was clear that Platycarya was grouped with Juglans, Carya, and Pterocarya, and was not a separate subfamily. In 1995, Fjellstrom and Parfitt used species-level phylogenetic trees based on RFLP's (restriction fragment length polymorphisms) to detect clear distinctions between the sections of *Juglans* (e.g., old world walnuts vs. new world walnuts). Fjellstrom and Parfitt (1994b) also used RFLP's to estimate the genetic diversity of 13 *Juglans* species worldwide. Though no linkage maps exist specifically for the eastern black walnut, Fjellstrom and Parfitt (1994a) used the inheritance and linkage of 48 RFLP loci to establish a linkage map for Persian walnut. This linkage map for walnut was expanded by Woeste and others (1996) to total 107 markers in 15 linkage groups.

In the 21st century, Stanford and others (2000) described the phylogeny of *Juglans* using MATK (a chloroplast maturase-encoding gene) and ITS (Internal Transcribed Spacer) sequence data. They found all four sections of the genus branched out as commonly assumed. Rhysocaryon (i.e., old world walnuts vs. new world walnuts such as Persian walnut), to which eastern black walnut belongs, showed eastern and western species distinctions (e.g., *J. nigra* vs. *J. hindsii*), and also a distinction between temperate and tropical species (e.g., *J. nigra* vs. *J. neotropica*). Microsatellite loci were first published for black walnut by Woeste and others (2002); it is clear that microsatellites (SSRs) will replace isozymes as the molecular tool of choice to answer questions pertaining to walnut genetic diversity across regions, studies of parentage, and cultivar identification (Potter and others 2002, Ninot and Aleta 2003, Robichaud and others 2004, this volume).

THE FUTURE?

Domestication, according to Kass (1993), is associated with seven activities: silvicultural manipulation, site enhancement, pest control, natural selection, semi-natural selection, anthropogenic selection, and a correlated response to selection. If Kass is correct, black walnut is well on its way to domestication. The first four activities are undoubtedly common factors to black walnut management, whether on plantations, or in managed woodlots. Semi-natural selection refers to a response by the tree to artificial factors brought about by socio-agricultural circumstances, such as tolerance to grazing animals, local site conditions, and ease of propagation. Human selection, the 6th criterion, involves actively pursuing an ideotype that is a culmination of the desired characteristics in a particular species. In black walnut, this would be traits such as fast growth, straightness, and a high proportion of heartwood in timber varieties, or early nut bearers, consistent yield, and a high proportion of kernel in nut tree varieties. It also includes a component of predictability, which would lead to an increase in crop yield, a characteristic still under development in black walnut. Site conditions and genetic factors are still too unpredictable to strive for, and consistently maintain, a black walnut ideotype. Persian walnut, on the other hand, is much farther along in this process than black walnut and there is probably an ideotype for that species that has more or less been attained.

Kass' last criterion (a correlated response to selection by the organism) also has not been met, as tree breeding and selection programs are too recent. Furthermore, although black walnut responds somewhat to management, it is not dependent upon it for survival. Black walnut has not yet evolved any dependence upon human manipulation to survive (Reid 1997). Persian walnut, though not dependent upon anthropogenic influence for survival, has been cultivated for centuries and the best varieties only grow well in highly modified environments (Ramos 1998). For Weirsum (1997), domestication is realized as increased people-plant interactions that result in morphological and genetic changes in the organism. He makes a distinction between biological and comprehensive definitions for domestication, the former referring to a gradual change in morphological and genetic characters for specific uses and environments, and the latter referring to changes in management and exploitation of the plant that brings about changes in the morphology and genetics of the species.

So far, black walnut has been recognized as a valuable tree species and has been harvested out of natural forests, propagated on farms, in experiments, and in provenance studies and plantations. Its biology, and especially reproductive biology, has been examined, and it has been clonally propagated via tissue culture. The next step in domestication is to genetically modify black walnut (this does not necessarily imply genetic transformation) in some way to make it a better crop species, and more responsive to management activities. This has not yet been accomplished, but it has been on the horizon for some time.

LITERATURE CITED

Beineke, W.F. 1982. New directions in genetic improvement: grafted black walnut plantations. In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; 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. 1983. The genetic improvement of black walnut for timber production. In: Janick, J., ed. Plant breeding reviews. Volume 1. Westport, CT: AVI Publishing Co.: 236-266.

Beineke, W.F. 1984. Characteristics of Purdue University's patented black walnut trees. West Lafayette, IN: Purdue University, Cooperative Extension Service. 4 p. Available online: http://www.ces.purdue.edu/extmedia/FNR/FNR-115.html.

Bey, C.F. 1970. Geographic variation for seed and seedling characters in black walnut. Res. Note NC-101. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 4 p.

Bey, C.F. 1973. Genetic variation and selection. In: Black walnut as a crop: 2nd black walnut symposium; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep. NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 62-65.

Bey, C.F. 1980. Growth gains from moving black walnut provenances northward. Journal of Forestry. 78(10): 640-641, 645.

Black, W.M.; Neely, D. 1978a. Effects of temperature, free moisture, and relative humidity on the occurrence of walnut anthracnose. Phytopathology. 68: 1054-1056.

Black, W.M.; Neely, D. 1978b. Relative resistance of *Juglans* species and hybrids to walnut anthracnose. Plant Disease Reporter. 62(6): 497-499.

Busov, V.B. 1996. Allozyme variation and mating system of black walnut (*Juglans nigra* L) across the central hardwood region. Carbondale, IL: Southern Illinois University. 68 p. M.S. thesis.

Busov, V.B.; Rink, G.; Woeste, K. 2002. Allozyme variation and mating system of black walnut (*Juglans nigra* L.) in the central hardwood region of the United States. Forest Genetics. 9(4): 319-326.

Caraway, C. 1976. A forestry sciences laboratory… and how it grew. St. Paul, MN: U.S. Department of Agriculture, North Central Forest Experiment Station. 77 p.

Clark, F.B. 1965. Forest service research in black walnut. In: Annual report of the Northern Nut Growers Association. 56: 84-87.

Coggeshall, M.V.; Beineke, W.F. 1997. Black walnut vegetative propagation: the challenge continues. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 70-77.

Crane, H.L.; Reed, C.A. 1937. Nut breeding. In: Yearbook of agriculture. Washington, DC: U.S. Department of Agriculture, 75th Congress, 1st Session, House document No. 28. 827-889.

Deneke, F.J.; Funk, D.T.; Bey, C. 1981. Preliminary seed collection zones for black walnut. Gen. Tech. Rep. NA-FB/M-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry. 5 p.

Elias, T.S. 1980. The walnut family. In: Trees of North America, field guide and natural history. New York, NY: Van Nostrand Reinhold Co.: 265-275.

Fjellstrom, R.G.; Parfitt, D.E. 1994a. RFLP inheritance and linkage in walnut. Theoretical and Applied Genetics. 89: 665-670.

Fjellstrom, R.G.; Parfitt, D.E. 1994b. Walnut (*Juglans* spp.) genetic diversity determined by restriction fragment length polymorphisms. Genome. 37: 690-700.

Fjellstrom, R.G.; Parfitt, D.E. 1995. Phylogenetic analysis and evolution of the genus *Juglans* (Juglandaceae) as determined from nuclear genome RFLP's. Plant Systematics and Evolution. 197: 19-32.

Funk, D. 1966. Forest Service begins black walnut breeding research. The Hoosier Kernel. 13(3): 13-14.

Funk, D. 1970. Genetics of black walnut. Res. Pap. WO-10. Washington, DC: U.S. Department of Agriculture, Forest Service. 13 p.

Funk, D. 1973. Genetics and tree improvement. In: Black walnut as a crop: 2nd walnut symposium; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep. NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 59-61.

Funk, D.; Neely, D.; Bey, C.F. 1980. Genetic resistance to anthracnose of black walnut. Silvae Genetica. 30: 115-117.

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(1): 51-58.

Hans, A.S. 1970. Chromosome numbers in the Juglandaceae. Journal of the Arnold Arboretum. 51: 534-539.

Kass, D.C.L. 1993. Tree domestication for agroforestry: present status and future directions. Agroforestry Systems. 23: 195-205.

Khan, S.B. 1995. Micropropagation of adult black walnut (*Juglans nigra* L). Carbondale, IL: Southern Illinois University. 162 p. M.S. thesis.

Kung, F.H.; Rink, G. 1987. Estimating confidence limits for electrophoretic mating system parameters in black walnut. In: Guries, R.P., ed. Proceedings, 6th North Central Tree Improvement Conference: Fargo, ND. 126-131.

Kung, F.H.; Rink, G.; Zhang, G. 1991. Growth and isozyme allele frequency correlations in black walnut. In: Proceedings, 21st Southern Forest Tree Improvement Conference: 258-265.

Long, L.M.; Preece, J.E.; Van Sambeek, J.W. 1995. Adventitious regeneration of *Juglans nigra* L. (eastern black walnut). Plant Cell Reports. 14: 799-803.

MacDaniels, L.H. 1933. Nut-growing in New York state. Bull. 573. Ithaca, NY: Cornell University Agricultural Experiment Station. 24 p.

Manning, W. 1978. The classification within the Juglandaceae. Annals of the Missouri Botanical Garden. 65: 1058-1087.

McKay, J.W. 1971. Walnut hybrids at Beltsville, Maryland. Annual Report of the Northern Nut Growers Association. 62: 34-38.

Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F. Jr., eds. 2004. In: Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Neely, D. 1979. Etiology, epidemiology and control of black walnut anthracnose. In: Walnut insects and diseases. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 58-63.

Neuman, M.C.; Preece, J.E.; Van Sambeek, J.W.; Gaffney, G.R. 1993. Somatic embryogenesis and callus production from cotyledon explants of eastern black walnut. Plant Cell, Tissue and Organ Culture. 32: 9-18.

Ninot, A.; Aleta, N. 2003. Identification and genetic relationship of Persian walnut genotypes using isozyme markers. Journal of the American Pomological Society. 57(3): 106-114.

Potter, D.; Gao, F.Y.; Aiello, G.; Leslie, C.; McGranahan, G. 2002. Intersimple sequence repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. Journal of the American Society for Horticultural Science. 127(1): 75-81.

Ramos, D.E., tech. ed. 1998. Walnut production manual. Publ. 3373. Oakland, CA: University of California, Division of Agriculture and Natural Resources. 317 p.

Record, S.J. 1986. Juglandaceae. In: Timbers of the new world. Salem, NH: AYER Company, Publishers, Inc: 197-201.

Reid, W. 1997. Black walnut. In: Brooks, R.; Olmo, H.P., eds. Register of fruit and nut varieties, 3rd ed. Alexandria, VA: ASHS Press. 744 p.

Rink, G.; Stelzer, H.E. 1982. The status of black walnut tree improvement programs in the north central region. In: Black walnut for the future: 3rd walnut symposium: 1981 August 10-14; West Lafayette, IN. Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 107-112.

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

Rink, G.; Zhang, G.; Jinghua, Z.; Kung, F.H.; Carroll, E.R. 1994. Mating parameters in *Juglans nigra* L. seed orchard similar to natural population estimates. Silvae Genetica. 43(4): 261-263.

Rink, G.; Carroll, E.R.; Kung, F.H. 1989. Estimation of *Juglans nigra* L. mating system parameters. Forest Science. 35(2): 623-627.

Rink, G. 1997. Genetic variation and selection potential for black walnut timber and nut production. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 58-62.

Robichaud, R.; McKenna, J.; Woeste, K. 2004. Finding a walnut in a haystack: How molecular biology can help rediscover lost "elite" black walnuts. In: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Fonder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station: 183.

Smith, J.; Doyle, J. 1995. A cladistic analysis of chloroplast DNA restriction site variation and morphology for the genera of the Juglandaceae. American Journal of Botany. 82(9): 1163-1172.

Stanford, A.M.; Harden, R.; Parks, C. 2000. Phylogeny and biogeography of *Juglans* (Juglandaceae) based on MATK and ITS sequence data. Amerivhcan Journal of Botany. 87(6): 872-882.

Stier, J.C. 1986. An initial assessment of [NC-99] forest tree improvement research in the North Central region. Unpublished final report for Project No. WO-144-83, Cooperative Agreement 58-3159-4-29. 73 p.

Talbert, T.J. 1942. Nut tree culture in Missouri. Bull. 454. Columbia, MO: University of Missouri, College of Agriculture Agricultural Experiment Station. 32 p.

U.S. Forest Service. 1966. Black walnut culture: Proceedings, 1st Walnut symposium; 1966 August 2-3; Carbondale, IL. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 94 p.

Van Sambeek, J.W.; Preece, J.E.; Lindsay, T.L.; Gaffney, G.R. 1990. In vitro studies on black walnut embryo dormancy. Annual Report of the Northern Nut Growers Association. 80: 55-59.

Van Sambeek, J.W.; Lambus, L.J.; Khan, S.B.; Preece, J.E. 1997. In vitro establishment of tissues from adult black walnut. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 78-92.

Wiersum, K.F. 1997. From natural forest to tree crops, co-domestication of forests and tree species, an overview. Netherlands Journal of Agricultural Science. 45: 425-438.

Woeste, K.; McKenna, J.; 2004. Walnut genetic improvement at the start of a new century. In: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.; et al., eds. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28, Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 9-12.

Woeste, K.; Burns, R.; Rhodes, O.E., Jr.; Michler, C. 2002. Thirty polymorphic nuclear microsatellite loci from black walnut. The Journal of Heredity. 93(1): 58-60.

Woeste, K.; McGranahan, G.H.; Bernatzky, R. 1996. Randomly amplified polymorphic DNA loci from a walnut backcross [(*Juglans hindsii* x *J. regia*) x *J. regia*]. Journal of the American Society of Horticultural Science. 121(3): 358-361.

Woodworth, R.H. 1930. Meiosis of microsporogenesis in the Juglandaceae. American Journal of Botany. 17: 863-869.

Wright, J.W. 1966. Breeding better timber varieties. In: Black walnut culture: 1st walnut symposium; 1966 August; Carbondale, IL. St. Paul, MN: U.S. Department of Agriculture, Forest Service. North Central Forest Experiment Station: 53-57.

Zarger, T.G. 1969. Black walnuts as nut trees. In: Jaynes, R.A. ed. Handbook of North American nut trees. Knoxville, TN: Northern Nut Growers Association: 203-211.

Zhang, G. 1990. Estimating black walnut (*Juglans nigra* L.) mating system parameters. Carbondale, IL: Southern Illinois University. 145 p. M.S. thesis.

Zuo, J. 1994. Estimating black walnut (*Juglans nigra* L) outcrossing rates and fixation indices. Carbondale, IL: Southern Illinois University. 90 p. M.S. thesis.

Zuo, J.; Kung, F.H.; Rink, G. 1995. Sampling variation in black walnut (*Juglans nigra* L) outcrossing rates and fixation. In: Weir, R.J.; Hatcher, A., eds. Proceedings, 23rd Southern forest tree improvement conference; 1995 June 19-22; Asheville, NC. Publ. 45. Southern Forest Tree Improvement Committee. 23: 63-69.

Walnut Genetic Improvement at the Start of a New Century

Keith E. Woeste and James R. McKenna[1]

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 1950s and 1960s, 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 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

[1]Research Molecular Geneticist (KEW) and Biologist (JRM), Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, 195 Marsteller St., Purdue University, West Lafayette, IN, 47907. KEW is corresponding author: to contact, call (765) 496-6808 or email kwoeste@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

(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: $P = G + E + (GxE)$; 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 affect 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% 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 10 (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 and others 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 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 in small full-sibling populations (Namkoong 1979). 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% 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% 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% 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 and often grew significantly faster than trees from local seed sources, supporting the 200 mile guideline.

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 20th century (Wright 1966). Most first-generation (F_1) 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_1 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). 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_1 hybrid is a cross between NCB × 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. O lder plantings are more reliable sources of data for selec ting 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

The authors thank Mark Coggeshall and Ron Overton for their critical and helpful reviews of the manuscript.

LITERATURE CITED

Beineke, W.F. 1982. New directions in genetic improvement: grafted black walnut plantations. In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; 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, 15th 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.

Clausen, K.E. 1983. Performance of black walnut provenances after 15 years in 7 Midwestern plantations. Proceedings, 3rd North central forest tree improvement conference; 1983 August 17-19; Wooster, OH. Madison, WI: Deptartment of Forestry, University of Wisconsin: 24-33.

Coggeshall, M.V.; Pennington, S.G. 1982. Indiana Division of Forestry black walnut tree improvement program In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; 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. Gen. Tech. Rep. NA-FB/M-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry. 5 p.

Funk, D.T. 1966. Seed orchards. In: Black walnut culture: walnut workshop; August 2-3. U.S. Department of Arigriculture, Forest Service, North Central Forest Experiment Station: 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.

Hammitt, 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: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. 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.W., 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. Publ. 1347. Ottawa, Canada: Canadian Forestry Service: 1-27.

Namkoong, G. 1979. Introduction to quantitative genetics in forestry. Tech. Bull. 1588. Washington, DC: U.S. Deparement of Agriculture, Forest Service.

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: 4th black walnut symposium; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council: 140–144.

Rink, G.; 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. Res. Pap. NE-569. Broomall, PA: U.S. Deparment of Agriculture, Forest Service, Northeast Forest Experiment Station.

Wright, J. 1966. Breeding better timber varieties. In: Black walnut culture: 1st walnut symposium; 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. New York, NY: John Wiley and Sons, Inc. 505 p.

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

Planting	Planter[1]	Location	Type[2]	Year	Family/ No. Trees	Comments and Recommendation[3]
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]

(Table 1 continued on next page)

(Table 1. continued)

Planting	Planter[1]	Location	Type[2]	Year	Family/ No. Trees	Comments and Recommendation[3]
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.
Missisinewa 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]
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	Fir 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 maintenenace, IDNF nursery. [A]

(Table 1 continued on next page)

(Table 1.—continued)

Planting	Planter[1]	Location	Type[2]	Year	Family/ No. Trees	Comments and Recommendation[3]
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]
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	Van Sambeek	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]
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	Survivial 25%, poor growth repeated deer browse, poor site, great initial management. [A[

[1]Only one investigator is listed although most plantings involved several investigators and collaborators.
[2]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.
[3][M]= Maintained as valuable, [A]= Abandoned, [MM]= Missing maps.

Cultivar Evaluation and Development for Black Walnut Orchards

William Reid, Mark V. Coggeshall, and Kenneth L. Hunt[1]

ABSTRACT—Black walnut is an underdeveloped orchard crop. Hundreds of cultivars have been named but a commercial orchard industry has not developed. The horticultural characteristics of currently available black walnut cultivars are reviewed. Important cultivar traits include: leaving date, flowering date, growth habit, disease susceptibility, yield, and nut quality. Breeding program priorities for developing horticulturally superior cultivars are presented.

Eastern black walnut (*Juglans nigra* L.) trees produce a uniquely flavored nut that is a favorite in many Midwestern kitchens. Each year, American consumers use 2 million pounds of black walnut kernels in cookies, cakes, and ice cream products (Hammons 1998). The current level of black walnut production is supported by the collection and processing of nuts from wild walnut trees. During the fall, rural residents harvest nuts by hand and deliver the nuts to hulling and buying stations set up across the Midwest. An average of 25 million pounds of hulled seedling walnuts are purchased per year at an average price of 10 cents per pound.

The continued reliance of the black walnut industry on the hand harvest of seedling trees will not allow for the future expansion of this nut crop. The wild crop is inherently variable, with wide swings in both total production and nut quality. This unpredictability of supply undermines the industry's ability to increase utilization. Further, increasing family incomes and a continuing population shift from rural to suburban areas is slowly eroding the workforce that has traditionally harvested seedling walnuts. The future of the black walnut industry lies in the establishment of orchards of high-quality, high-yielding cultivars.

Since 'Thomas' was named as America's first black walnut cultivar in 1881 (Corsa 1896), cultivar development has proceeded haphazardly. Throughout the 20th century, black walnut enthusiasts discovered new cultivars in the wild or planted open pollinated seeds of previously named cultivars just to "see what happens". Even at this slow pace of crop development, over 700 cultivars have been named (Berhow 1962, Reid 1997a) and the percent of edible kernel has improved two-fold (seedling walnuts average 17% kernel while some cultivars consistently produce over 34% kernel). But these improvements in kernel quality have not been enough to stimulate large-scale development of black walnut orchards.

In recent years, we have initiated a program to systematically improve black walnut as a nut producing tree. We have started this process by evaluating the horticultural characteristics of existing cultivars, striving to understand the key components that determine yield and kernel quality. This work has also led us to develop key objectives for a systematic breeding effort to improve black walnut as an orchard crop.

HORTICULTURE CHARACTERISTICS OF BLACK WALNUT CULTIVARS

Many of today's black walnut cultivars have been selected based on a single trait, percent kernel (Reid 1990). This has lead to the propagation of hundreds of low-yielding, disease-susceptible cultivars that are able to produce enough nuts

[1] Associate Professor (WR), Pecan Experiment Field, Kansas State University, Chetopa, KS 67336; Research Associate (MVC), Center for Agroforestry, Univ. of Missouri, 10 Research Center Rd., New Franklin, MO 65274; and Research Associate (KLH), Center for Agroforestry, Univ. of Missouri, 203 ABNR, Columbia MO 65211. WR is the corresponding author (620-597-2972 or wreid@oznet.ksu.edu).

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

to satisfy a hobbyist's craving for easy to crack walnuts. In contrast, designing and implementing a commercial black walnut orchard will require detailed cultivar information, enabling growers to choose productive cultivars adapted to their specific growing conditions. Key cultivar traits include: leafing date, flowering dates, growth habit, disease resistance, precocity, productivity, and shelling quality.

Leafing and Flowering Dates

Black walnut trees are among the latest leafing trees in eastern deciduous forests. This late-leafing characteristic ensures emerging leaves, along with developing flowering structures, avoid late spring frosts. Temperatures below -2° C will kill emerging green tissues, destroy floral initials, and eliminate the potential for nut production. Among the cultivars in our collections, 'Davidson' is the first cultivar to break bud. In contrast, 'Thomas/Myers' initiated new growth more than three weeks after 'Davidson' (Table 1). We have also noted significant tree-to-tree variation in bud break date within each cultivar. Although we cannot document the cause of this effect at this time, we have initiated trials to determine rootstock effects on tree phenology and nut productivity.

As expected, late-leafing cultivars flowered later in the season regardless of dichogamy. The variation in flowering date was similar to the variation measured for bud break. (Table 1). The vast majority of cultivars in our collections exhibited the protogynous flowering habit (Table 1). These observations suggest that novel strategies may be needed to ensure pollination in black walnut orchards. In commercial pecan or Persian walnut orchards, adequate pollen is ensured throughout the flowering season by simply planting a protandrous cultivar and a protogynous cultivar in the same block (Grauke 1997, Hendricks and others 1998). In black walnut, providing pollen throughout the pollination season is made more difficult because of a critical lack of protandrous black walnut cultivars. To solve this problem, early-leafing protogynous cultivars could be used to pollinate late-leafing protogynous cultivars. However, at least one early-leafing protandrous cultivar must be included in the orchard to provide pollen for early-leafing protogynous clones.

Growth Habit

Several black walnut cultivars exhibit a branching habit that can be most accurately described as spur-type growth. Previous work has described this growth pattern as lateral bearing (Reid 1997);

Table 1.—Leafing date, flowering type and fruit ripening season for black walnut growing at New Franklin, MO, 2001-2003.

Cultivar	Leafing Date[1]	Flowering Type[2]	Ripening Season[3]
Beck	17.6 ± 2.1	II	mid
Christianson	21.5 ± 4.0	II	mid
Clermont	19.0 ± 5.2	II	mid
Cranz	19.3 ± 4.0	II	late
Daniel	14.6 ± 6.5	II	early
Davidson	0	II	early
Dot	22.5 ± 3.5	II	mid
Drake	17.0 ± 4.4	II	mid
Elmer Myers	18.0 ± 5.4	II	late
Emma K	5.3 ± 2.5	II	mid
Football	11.0 ± 2.1	I	early
Grundy	10.7 ± 2.3	II	late
Hay	22.0 ± 5.3	II	late
Knuvean	8.3 ± 1.2	I	early
Krouse	5.7 ± 0.6	II	late
Kwik Krop	18.3 ± 1.5	II	mid
McGinnis	5.0 ± 1.7	I	mid
Mintle	7.0 ± 1.0	II	mid
Rowher	17.3 ± 2.3	II	mid
Rupert	8.3 ± 1.5	II	late
Sarcoxie	17.0 ± 1.7	II	mid
Sauber	14.7 ± 6.0	II	early
Schessler	2.0 ± 1.1	II	early
Scrimger	16.7 ± 4.0	II	late
Sparks 127	17.0 ± 2.9	II	early
Sparks 129	17.7 ± 3.8	II	late
Sparks 147	22.7 ± 6.4	II	mid
Sparrow	16.8 ± 3.4	II	early
Stambaugh	19.7 ± 3.8	II	mid
Surprise	13.0 ± 5.1	II	late
Ten Eyck	20.3 ± 4.5	II	late
Thatcher	18.3 ± 5.8	II	late
Thomas	21.0 ± 5.8	II	late
Thomas/ Myers	22.7 ± 6.4	II	early
Victoria	15.7 ± 3.8	I	late

[1] *Mean Days after Davidson ± std. dev. (Davidson average leaf burst = Apr. 12)*
[2] *I = protandrous, II = protogynous*
[3] *Ripening dates*
Early = Sept. 1-14
Mid = Sept 15-28
Late = Sept. 29 - Oct 12

however, the growth and bearing habit of spur-type black walnuts is not analogous to the lateral bearing habit found in Persian walnut (*Juglans regia* L.). Lateral bearing in *J. regia* occurs when lateral shoots arise from the current season's new growth and terminate in a pistillate flower cluster (Hendricks and others 1998). This type of bearing habit has not been observed in black walnut. However, several black walnut cultivars develop short, compact branches (compressed internode lengths) along primary branches in a growing habit comparable to spur-type apples. Black walnut spurs are multi-year-old shoots that grow to a length of 8 to 12 inches. These spurs dramatically increase the number of potential fruiting sites on the tree and can spread fruiting throughout the canopy. Spur-type black walnut cultivars are listed in Table 2.

Anthracnose Susceptibility

Anthracnose is a major limiting factor to annual nut production in black walnut (Reid 1995). Although anthracnose can infect stems and fruit, leaf infections prove most damaging to tree performance. The anthracnose fungus (*Gnomonia leptostyla* [Fr.] Ces. & De Not.) can infect leaves shortly after full leaf expansion and completely defoliating the tree by mid-August (Neely 1979). Early defoliation decreases nut quality (Reid 1986) and enhances the trees natural tendency towards alternate bearing (Worley 1979).

To evaluate black walnut cultivars for anthracnose susceptibility, we rate the defoliation of fruiting shoot leaves in mid-August (expressed as a percentage of leaflets lost). By evaluating fruiting shoots only, we ensure that our assessments are made on leaves from all the same age cohort (Reid 1995). We have not found cultivars with complete resistance to this disease, but have noted wide variation in susceptibility. Among the most susceptible clones in our trials are such popular cultivars as 'Football' and 'Surprise'. In contrast, 'Sparrow' and 'Thomas' demonstrated excellent leaf retention. Initial evaluations of anthracnose susceptibility for black walnut cultivars can be found in Table 3.

Table 2.—Black Walnut cultivars exhibiting the spur-type growth habit. Data taken from Reid (1997a, 1997b).

Cultivar Exhibiting Spur-type Growth	
Beck	Kwik Krop
Cranz	McGinnis
Davidson	Rupert
Emma K	Sparks 127
Football	Sparks 147
HPC-120	STW-13
HPC-148	Surprise

Yield and Alternate Bearing

The lack of emphasis in the selection process on nut yield, especially yield of edible kernel/acre, is the primary impediment to the commercialization of black walnut. Potential black walnut growers must be confident that income from nut sales will return a fair profit for investments made in land, labor, trees, supplies, and equipment.

Our initial investigation of black walnut cultivar performance was initiated in 1987 when 21 cultivars were propagated on black walnut rootstocks in a replicated study. Trees were fertilized with nitrogen (100 lbs. N/acre/year) and anthracnose was controlled with fungicides. Nut production began 4 years after propagation but the first significant crop wasn't recorded until 1997. Yield data for these cultivars for the five year period, 1997-2001, is given in Table 4. In this young orchard, Rupert had the greatest in-shell yield but Drake produced the most kernels (Table 5). Emma K and Football exhibited perfect alternate

Table 3.—Anthracnose ratings for black walnut cultivars growing at New Franklin, MO during 2003.

Cultivar	Anthracnose Defoliation Potential[1]	Cultivar	Anthracnose Defoliation Potential[1]
Bowser	higth	Patterson	med
Crosby	low	Rupert	low
Christianson	med	Sauber	med
Clermont	med	Schessler	med
Cochrane	high	Scrimger	low
Davidson	med	Sparks 127	high
Elmer Myers	high	Sparks 147	med
Emma K	med	Sparrow	low
Football	high	Stambaugh	med
Hare	med	Surprise	high
Jackson	high	Thomas	low
Kwik Krop	med	Thomas/Myers	med
Mintle	high	Victoria	med

[1] *High > 30% leaflets defoliated by mid-August*
Med = 16-30% leaflets defoliated by mid-August
Low< 16% leaflets defoliated by mid-August

bearing, producing nuts one year followed by zero production the next year. In contrast, low yielding Cranz produced a small crop of nuts each year, while Rupert demonstrated both high yield and low alternate bearing tendency.

Nut production during this time period was comparatively low even for the highest yielding clones. The black walnut trees in this study were established at a spacing of 30 ft. x 30 ft. or 48 trees/acre. Production of 20 lbs. (a high averages yield in our trial) of hulled and dried nuts per tree would result in a yield of 960 lbs/acre. In contrast, young Persian walnut orchards (years 10-15) yield over 4000 lbs/acre of hulled and dried nuts (Hendricks 1999).

Fruit and Nut Characteristics

Each fall, black walnut enthusiasts collect nut samples of each cultivar in their planting in order to enter nut judging contests held in most Midwestern states. Nuts are weighed, cracked, and then kernels weighed to determine average nut weight and percent kernel. These basic nut quality parameters have been determined for hundreds of black walnut clones and provide much of our current knowledge base for black walnut cultivar performance. The results of 43 years of nut sample evaluation in Kansas are presented in Table 6.

Since black walnut is not marketed to the consumer as an in-shell product, large nut size has not been an important cultivar selection criterion. Ease of shelling has been and will continue to be one of the most important nut quality traits measured. Important nut characters that influence ease of shelling include shell thickness, inner wall partition thickness, and openness of kernel cavity. These characteristics are easily observed by cutting open the nut in cross section with a band saw. We are currently working on methods to quantify these nut traits.

Missing from nut evaluations are critical determinations of husk characteristics. In black

Table 4.—Yield, average yield and alternate bearing index for black walnut cultivars growing at Chetopa, KS.

	Yield (lbs./tree)						
Cultivar	1997	1998	1999	2000	2001	5 Year Average	Alternate Bearing Index[1]
Bowser	1.8	6.1	5.1	1.1	10.6	4.9	0.52
Cranz	10.8	5.5	7.2	7.1	6.8	7.5	0.12
Davidson	23.0	0.0	17.0	27.3	28.9	19.2	0.57
Drake	23.0	6.0	27.8	5.9	36.1	19.8	0.65
Dubois 8415	3.1	0.7	3.8	0.0	3.3	2.2	0.83
Emma K	2.5	0.0	8.1	0.0	13.8	4.9	1.00
Farrington	4.0	2.4	9.6	1.5	11.1	5.7	0.59
Football	24.0	0.0	22.0	0.0	13.0	11.8	1.00
Kwik Krop	2.4	0.3	6.9	0.0	3.4	2.6	0.92
McGinnis	6.0	0.8	12.1	0.0	28.3	9.4	0.91
Mintle	18.9	0.0	6.7	1.5	16.3	8.7	0.87
Rupert	30.9	11.8	19.2	23.8	27.4	22.6	0.22
Scrimger	2.2	2.0	8.7	2.5	15.6	6.2	0.49
Sparks 127	10.0	0.0	4.2	0.9	5.4	4.1	0.84
Sparks 147	2.7	3.4	6.9	0.6	2.4	3.2	0.47
Sparks 177	21.6	7.8	9.8	15.8	1.4	11.3	0.41
Sparrow	15.2	4.2	24.4	7.6	28.2	15.9	0.59
Stabler	4.1	7.6	20.5	0.0	12.4	8.9	0.69
Surprise	7.5	16.1	12.6	4.3	6.2	9.3	0.29
Vander Sloot	12.7	8.6	20.1	1.8	14.5	11.5	0.55
Victoria	22.5	2.3	10.0	0.3	5.4	8.1	0.82

[1] *Alternate bearing index = 1/(n-1) Σ |(*y_n*-*y_{n-1}*)/(*y_n*+*y_{n-1}*)|, where y= yield and n=year.*

Table 5.—Average yield (1997-2001), percent kernel, and kernel yield for black walnut cultivars growing at Chetopa, KS.

Cultivar	Yield (lbs./tree)	% Kernel	Kernel (lbs./tree)
Bowser	4.9	34.03	1.67
Cranz	7.5	32.87	2.47
Davidson	19.2	27.45	5.27
Drake	19.8	30.97	6.13
Dubois 8415	2.2	29.10	0.64
Emma K	4.9	34.28	1.68
Farrington	5.7	32.13	1.83
Football	11.8	32.62	3.85
Kwik Krop	2.6	31.03	0.81
McGinnis	9.4	32.24	3.03
Mintlev	8.7	29.86	2.60
Rupert	22.6	25.55	5.77
Scrimger	6.2	30.94	1.92
Sparks 127	4.1	32.58	1.34
Sparks 147	3.2	38.04	1.22
Sparks 177	11.3	33.46	3.78
Sparrow	15.9	31.51	5.01
Stabler	8.9	30.32	2.70
Surprise	9.3	32.63	3.03
Vander Sloot	11.5	25.70	2.96
Victoria	8.1	24.93	2.02

walnut, kernel color is strongly affected by the length of time the husk remains on the nut following fruit ripening (Chase 1941). As the husk begins to soften (break down), black-staining alkaloids are released from the husk and soak through the shell to darken the nut meat and change the flavor of the nut. This results in the 'strong' flavor of which most consumers of black walnut are familiar. Removing the husk and washing the nut as soon as the fruit becomes ripe results in walnut kernels that are lighter in color and milder flavored. Uniformity of fruit ripening has a major impact on kernel quality. Since most walnuts are harvested following a single tree shaking, trees with long ripening periods will appear to produce kernels that vary widely in color. This stems from the fact that, at the time of harvest, nuts are collected at varying stages of husk ripening and hence varying stages of kernel staining.

Black walnut husks are indehiscent and must be removed mechanically following harvest. However, it has been noted that some cultivars are easier to hull than others. We have observed two types of walnuts: cultivars that could be termed 'cling-stone', where the husk is tightly held by the shell and cultivars that are 'free-stone', where the husk is loosely held or separated from the nut shell at

Table 6.—Average nut weight and percent kernel of black walnut cultivar samples entered in the Kansas Growers Association Annual Nut Show from 1959-2001.

Cultivar	Nut Weight (g) Mean	Nut Weight (g) Std. Deviation	% Kernel Mean	% Kernel Std. Deviation
Beck	15.87	0.23	27.97	1.51
Bowser	15.51	1.42	32.27	3.93
Clermont	16.40	5.70	30.27	3.32
Cochrane	14.05	0.42	32.47	1.94
Cranz	14.18	1.51	30.46	3.62
Davidson	22.31	1.90	26.55	1.57
Drake	19.31	1.82	30.09	2.44
Emma K	16.32	2.57	34.57	1.91
Eureka	15.86	1.96	25.94	0.05
Farrington	19.03	5.93	26.78	5.20
Football	20.29	2.66	29.96	2.30
Hare	21.10	2.31	27.21	1.66
Jackson	14.29	1.85	35.43	1.25
Kwik Krop	17.10	2.89	30.55	2.50
McGinnis	16.66	0.12	31.19	2.90
Mintle	16.06	1.61	30.99	2.30
Myers	14.17	2.53	25.90	8.03
Ogden	19.71	5.93	21.24	5.34
Ohio	16.52	2.21	27.19	4.04
Peanut	19.53	0.32	26.08	5.20
Perry	19.55	1.82	27.41	1.20
Rowher	22.38	3.13	24.30	1.07
Rupert	18.29	1.54	25.12	0.91
Sauber	15.11	2.27	32.03	2.57
Scrimger	19.11	2.56	29.44	2.22
Sol	19.34	2.37	24.13	1.46
Sparks 127	15.00	2.91	32.64	2.99
Sparks 147	16.69	1.51	36.45	4.23
Sparks 177	20.90	2.15	33.07	1.60
Sparrow	18.59	2.25	29.92	2.07
Stabler	16.97	2.91	25.40	4.94
Stangle	18.98	3.30	27.07	3.34
Stark	18.82	3.11	26.56	3.89
Surprise	20.28	2.27	33.20	2.15
Thomas	21.79	4.88	24.39	4.54
Tom Boy	21.66	1.02	27.38	2.15
Vander Sloot	24.35	2.53	27.54	1.77
Victoria	17.73	3.22	24.37	3.60

ripening. We are in the process of collecting data on these important husk characteristics.

SETTING PRIORITIES FOR A BLACK WALNUT BREEDING PROGRAM

Black walnut remains an emerging crop. Will black walnut cultivars be found with the yield potential to cover the high costs of harvest, hulling, washing, and drying nuts? As we work with this crop, we may discover new cultural techniques that promote yield, but a systematic genetic improvement program holds the greatest potential for developing cultivars able to sustain a commercial walnut orchard industry.

Many important genetic traits can be found in today's black walnut cultivars. By producing nuts that average 38% kernel, 'Sparks 147' demonstrates that black walnut can be pushed towards a truly thin shelled nut. Many cultivars produce walnuts on spurs increasing the potential nut bearing surface of the tree. Still other cultivars seem less susceptible to anthracnose. The genetic variation necessary for an effective breeding program is available, but what are our genetic goals?

Goals for Improving Yield

Almost every walnut enthusiast has seen a walnut tree so heavily laden with fruit that the limbs are breaking. Crack open some nuts from one of these over-producing trees and you will find poorly filled kernels. In addition, the tree will not bear another nut crop for at least two years. Do these common observations indicate that black walnut trees are already producing near their genetic limit? We think not.

Black walnut trees expend a huge amount for biological energy to produce both husk and shell. The husk is a waste product that stains many things it touches black. While the shell has some commercial value (Hammons 1998), its value has a minimal impact on the economics of a walnut orchard. In breeding walnuts for nut production, reducing the tree's investment in husk and shell should allow more energy to be directed towards kernel production. The advantage of this strategy can be easily seen by comparing the husk and shell of a modern Persian walnut cultivar to that of a black walnut cultivar, then comparing the yields of these two species (see above). The hybridization of *J. regia* with *J. nigra* has been recorded with few positive results (McKay 1971). Although additional hybridization work may be warranted, we feel that we can meet our objectives of minimal husk and thin shells within *J. nigra*.

Preliminary work with different seed sources for rootstock trees seems to indicate that rootstock may have a dramatic influence on scion performance. We have already observed a rootstock effect on leafing date (above). We have yet to document the impact rootstock has on yield but trials are in place. We are also interested in the potential of interspecific hybrids for rootstocks in black walnut orchards.

Selecting trees that develop fruiting spurs at a young age will enhance precocity, increasing yield during the establishment phase of a walnut orchard. But maintaining the fruitfulness of spurs on mature trees may be the key to maximizing yield. Optimum light penetration into the tree canopy is critical for the stimulation of flowering on spurs (Van Sambeek 1998). By selecting trees exhibiting open tree architecture, we can ensure nut production throughout the canopy.

We have already discussed the critical lack of protandrous black walnut cultivars. Is a lack of early shedding pollen in the orchard contributing to low yields? For some early leafing cultivars, lack of pollen may be a problem; however, we have not documented this or other potential pollination problems in black walnut. In any case, the development of protandrous cultivars should be important to the commercial success of this crop.

Goals for Improving Disease Resistance

As mentioned earlier, anthracnose is a major limiting factor to fruit production in black walnut. Although immunity to this disease has not been identified, we will strive to incorporate some level of natural defense against this disease. Anthracnose can be controlled with well-timed fungicide applications; however, the propagation of extremely susceptible clones will only result in increasing production costs (more fungicide applications) for nut producers. All seedlings in the breeding program will be screened for anthracnose susceptibility. Seedlings demonstrating severe susceptibility to the disease will be culled.

Goals for Improving Nut and Kernel Quality

'Thomas', our oldest black walnut cultivar, averages 24% kernel. Today, we hardly consider planting a new walnut cultivar unless it produces at least 30% edible kernel. Several cultivars regularly produce nuts containing over 35% nut meat. The genetic improvement of percent kernel in black walnut is the one significant advancement made thus far in the evolution of this tree as an orchard crop. But additional improvements can be made. Our thinnest shelled walnuts are only one or two generation removed from wild ancestors. With controlled crosses and additional selection pressure we will strive to develop walnuts with over 40% kernel.

Kernel quality is heavily influenced by husk characteristics. Uniformity in fruit ripening is critical for harvesting uniformly light colored kernels. Ease of hull removal enhances a producer's ability to remove all remnants of kernel-staining hull from the nut. Yet to be discovered are the negative husk characteristics that promote dark veins in the kernel integument. As we continue to work with the collection of cultivars we currently have available, recording husk parameters will provide us with the valuable information we will need to incorporate husk traits into the breeding program.

LITERATURE CITED

Berhow, S. 1962. Black walnut varieties—a reference list. Northern Nut Growers Association Annual Report. 53: 63-69.

Chase, S.B. 1941. Effect of handling methods on the color and flavor of black walnut kernels. Northern Nut Growers Association Annual Report. 32: 34-37.

Corsa, W.P. 1896. Nut culture in the United States. Special report to the Secretary of Agriculture. U.S. Deptartment of Agriculture, Division of Pomology. Washington, DC: Government Printing Office: 144 p.

Grauke, L.J. 1997. Pecan pollination. In: McEachern, G.R.; Stein, L.A., eds. Texas pecan handbook. TAEX Hort. Handbook. College Station, TX: Texas A&M University: 105(3): 7-13.

Hammons, B.K. 1998. Status report on the Eastern black walnut nut industry, nut markets, by-products, and future challenges. In: Jones, J.E.; Mueller, R.; Van Sambeek, J.W., eds. Nut production handbook for Eastern black walnut. Republic, MO: Southwest Missouri Resource Conservation & Development, Inc.: 25-28.

Hendricks, L.C.; Coats, W.W.; Elkins, R.B.; McGranahan, G.H.; Phillips, H.A.; Ramos, D.E.; Reil, W.O.; Syder, R.G. 1998. Selection of varieties. In: Ramos, D.E., ed. Walnut production manual. University of California, Division of Agriculture and Natural Resources: 3373: 84-89.

Hendricks, L.C. 1999. Walnut variety test planting in Merced County. In: Walnut research reports 1999. Sacramento, CA: Walnut Marketing Board: 107-109.

McKay, J.W. 1971. Walnut hybrids at Beltsville, Maryland. Northern Nut Growers Association Annual Report. 62: 34-38.

Neely, D. 1979. Etiology, epidemiology, and control of black walnut anthracnose. In: Kessler, K.J.; Weber, B.C., eds. Walnut insects and diseases. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 58-62.

Reid, W. 1986. Reasons for poor kernel filling. Northern Nut Growers Association Annual Report. 77: 37-38.

Reid, W. 1990. Eastern black walnut: potential for commercial nut producing cultivars. In: Janick, J.; Simon, J., eds. Advances in new crops. Portland, OR: Timber Press: 327-331.

Reid, W. 1995. Understanding black walnut anthracnose. The Nut Kernel. 47(3): 13-14.

Reid, W. 1997a. Black walnuts. In: Brooks, R.; Olmo, H.P., eds. Registry of new fruit and nut varieties. 3rd ed. Alexandria, VA: ASHS Press: 156-160.

Reid, W. 1997b. Black walnut. In: Okie, W.R., ed. Register of new fruit and nut varieties. Brooks and Olmo List 38. HortScience. 30: 785-805.

Reid, W. 1997. Evaluation and management of black walnut for nut production. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 211-216.

Van Sambeek, J.W. 1998. Carbohydrate assimilation, translocation, and utilization. In: Jones, J.E.; Mueller, R.; Van Sambeek, J.W., eds. Nut production handbook for eastern black walnut. Republic, MO: SW Missouri RC&D: 73-78. <www.ncrs.fs.fed.us/epubs/walnut/>

Worley, R.E. 1979. Pecan yield, quality, nutlet set, and spring growth as a response of time of fall defoliation. Journal of the American Society for Horticultural Science. 104: 192-194.

Persian Walnut Breeding in California

Charles A. Leslie and Gale H. McGranahan[1]

ABSTRACT—For over 50 years the University of California Davis Walnut Breeding Program has worked to address the needs of California walnut growers by identifying genetic approaches to problems and developing improved cultivars. The breeding program is a cooperative endeavor that draws on the efforts and resources of university researchers and facilities, USDA germplasm programs, the statewide Cooperative Extension program, grower, and nursery funding sources, and the extensive participation of individual growers, processors, and nurserymen in all aspects of the program.

CALIFORNIA WALNUT INDUSTRY

Production

Persian walnut production in the United States is concentrated almost entirely in the State of California. California growers account for 99% of all domestic Persian walnut production (Walnut Marketing Board 1997). There are now approximately 200,000 bearing acres of Persian walnuts in California, an increase during the last 15 years of about 20%. In that same time period production per bearing acre has increased about 20% to a current statewide average of 1.5 tons per acre and total production of Persian walnuts has increased about 30% to nearly 300,000 in-shell tons annually (California Agricultural Statistics Service 2003).

About 60% of this production is consumed domestically but exports are an important component of marketing the crop and maintaining price to the grower (Siebert 1998). Concerted marketing by grower organizations and processors, in some cases with the assistance of government programs supporting development of export markets (Siebert 1998), have helped increase the proportion of the crop exported from only 25% 20 years ago to about 40% in the last 4 years.

Growers

California walnut growers farm an average of 50 acres of walnuts but farm size varies widely (Hasey 1994). Large growers farming 100 or more acres account for 60% of the acreage but constitute only 12% of the growers. Nearly 60% of growers farm fewer than 20 acres while producing only 10% of the crop. Production over the last several years has averaged 1.5 tons/acre. Prices can vary widely from year to year but recently the crop has returned about $1,000/ton to growers (California Agricultural Statistics Service 2003).

About half of the California walnut crop is produced by growers who belong to Diamond Walnut, the largest growers' cooperative (Beede and Hasey 1998). Diamond has field representatives statewide who work with the member growers, a central shelling and processing facility, and its own product development and marketing programs. Approximately 70 independent shellers and handlers, varying widely in size and services, market the other half of the production. Economic conditions are contributing to increasing numbers of vertically integrated grower-handlers who market their own production.

The ability of cooperative members and independent growers to collaborate on issues of mutual interest such as product standards,

[1]Staff Research Associate (CAL) and Pomologist (GHM), Pomology Department, University of California Davis, One Shields Ave., Davis, CA 95616 CAL is corresponding author: to contact call (530) 752-5604 or e-mail at <caleslie@ucdavis.edu>.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

development of export markets, and investment in research has contributed greatly to the success of the industry. These issues are coordinated through two grower organizations, the California Walnut Commission, responsible for export development (Siebert 1998), and the Walnut Marketing Board.

The Walnut Marketing Board oversees implementation of the Walnut Marketing Order established in 1949 under the 1937 Federal Agricultural Marketing Act (Hasey 1994). The marketing order was established and approved by growers in response to low prices and absence of enforceable quality standards. By law, the board's activities are conducted under USDA oversight but board members are walnut growers and handlers elected by the growers. The board oversees quality and grade standards, monitors collection of industry production statistics, and regulates commodity volume. In addition to these functions, the order established a mechanism for funding marketing and production research. Growers assess themselves at the handler level on merchantable crop tonnage and the board allocates a portion of these funds annually to fund horticultural and post-harvest research (Beede and Hasey 1998).

Nurseries

Commercial walnut nurseries are another key element of the industry. Nurseries vary widely in size and scope of activities from large multi-crop operations with international sales to small family walnut operations and growers who produce seedlings only for their own use. There are no State or other public nurseries.

Persian walnuts are a clonally propagated crop in which production is almost entirely from scion varieties grafted to seedling rootstocks. Rootstocks in California are either seedlings of California black walnut (*Juglans hindsii*) or hybrids derived from black walnuts pollinated with Persian pollen (*J. hindsii* x *J. regia*). These hybrids, known as Paradox, have superior vigor but are more susceptible to crown gall disease (Catlin 1998).

Nurseries collect seed from trees known to produce Paradox seed, establish seed orchards for this purpose, or purchase seed from suppliers. Seedlings are either sold ungrafted after one year for growers to graft on their own or they are grafted or budded in the nursery to the desired scion variety and sold as 2-year-old grafted trees. Recent advances in rooting methods have contributed to very limited production of Persian cultivars on their own roots and clonal rootstock.

Nurseries are regulated by the California Department of Agriculture (CDFA). The CDFA sets standards for nursery stock, oversees production and distribution of certified stocks, and addresses some nursery interests in research. The nurseries, through their organization, the California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (IAB), assess themselves a fee based on plant materials sold and dedicate a portion of these funds to research.

WALNUT RESEARCH FUNDING

Research and technological development is an important component in maintaining a growing, profitable, and competitive walnut industry. Funding for walnut research is derived from a variety of sources.

The State of California, through the University of California, particularly the UC Davis Pomology department, has long contributed support through its funding of faculty, research staff, extension specialists, and county-based farm advisors, infrastructure including research farm operations, greenhouses and laboratories, and funding for equipment.

The USDA-ARS has contributed funding for research positions, some as adjunct faculty, research programs, and particularly funding through the National Clonal Germplasm Repository for an extensive walnut germplasm collection. Some research is also funded through State and university competitive grants intended to support biotechnology development and foster technology transfer to industry.

As State and university funding for agricultural research has come under pressure in recent years, direct grower funding through the Walnut Marketing Board has become increasingly important. Through the Board the growers provide substantial annual funding of research proposals and have endowed a permanent staff position for the Walnut Breeding Program. Nurseries, through the IAB, fund research that addresses their concerns including clean stock, trueness-to-type, and propagation. Contributions from individuals in the industry have also been important and have included funding for a graduate student assistantship specifically for walnut research.

RESEARCH-INDUSTRY INTERACTION

Industry input and participation has been an essential element of walnut research success. The annual Walnut Research Conference has become a key component in coordinating walnut research efforts and industry-researcher interactions in California. This conference originated as an annual training meeting for the UC Cooperative Extension Service county farm advisors. Farm advisors, roughly the equivalent of county agents in other States, are responsible for working directly with growers to solve problems and to disseminate research information from university, USDA, and other sources. They are also expected to conduct some applied research of their own. This meeting was originally an opportunity for university researchers at all levels and perspectives to internally share information, update skills, and address new problems and directions. This meeting also acquired the function of reviewing and recommending proposals to be submitted to the Walnut Marketing Board for funding.

As grower funds became an increasingly important component of research financing, members of the Walnut Marketing Board Research Committee were invited to see the presentation of proposals first hand. Initially they were invited as observers but over the years their role has expanded to full participants, speakers, and members of panel discussions. As proposals began to involve coordinated funding with the nursery industry as well, nursery board members were also invited. USDA-ARS researchers working on walnuts also attend.

The USDA Juglans Crop Advisory Committee, a group with diverse expertise in all species of walnuts and which advises the USDA National Clonal Germplasm Repository on germplasm collection and maintenance issues, generally holds its annual meeting in conjunction with the Walnut Research Conference and committee members with interests in black walnut and butternut have also participated in the conference in recent years.

The result is a 3-day conference with a wide array of participants assembled to review current research results, discuss industry problems, and debate directions for new research. The combination of formal research presentations, structured discussions, and a series of social events supported by industry funds, help cement relationships and define directions for the coming year's work.

Research discussed at this conference covers a broad array of topics. Last year, for example, reports, proposals, and panel discussions included codling moth control methods, husk fly, bacterial blight, crown gall control, integrated pest management research, nematodes, blackline virus, tree training, mechanical hedging, light management, water relations and irrigation methods, replacements for post-harvest fumigants, rootstock selection, propagation methods, tissue culture, genetic engineering, field performance of self-rooted plants, DNA finger-printing, and development of new varieties.

This cooperation and interaction occurs not just at the annual research conference but also throughout the research process. Close industry and researcher cooperation is essential, not just in setting direction, but in implementation and transfer to the growers. Information discussed at the conference is disseminated statewide to growers through a series of annual county grower meetings organized by the farm advisors. In addition, new information is summarized in extension publications and every several years Cooperative Extension organizes a weeklong Walnut Short Course for interested growers. This course is taught by faculty, farm advisors, and innovative growers, includes lectures and field tours, and provides participants with an updated version of the Walnut Production Manual (Ramos 1998), the University of California textbook on Persian walnuts.

This entire process ensures a clear understanding of industry needs in research, a comfort level with progress in the work being funded, and a rapid transfer of results back to the growers. An examination of how the walnut breeding program functions illustrates the importance of this interaction and steps where it occurs.

WALNUT BREEDING PROGRAM

History

The Persian Walnut Breeding Program began at UC Davis in 1948. In the following 30 years under the direction of Gene Serr and Harold Forde crosses incorporated t he lateral bearing trait responsible for precocious high yield into 15 released cultivars (Serr and Forde 1968; McGranahan and others 1990, 1992; Tulecke and McGranahan 1994). Several of these are leading varieties today. 'Chandler', notable for its' light kernel color, high yield of halves, and relatively low input requirements is the leading cultivar in the State.

Objectives

The breeding program has continued since 1982 under the direction of Gale McGranahan. The current breeding program seeks to address a variety of industry concerns by developing both improved scion varieties and clonally propagated rootstocks.

Growers and handlers are interested in obtaining new scion varieties with early harvest dates and reduced need for chemical inputs. Over 60% of current production is from late harvesting varieties (California Agricultural Statistics Service 2003). The most popular variety, 'Chandler', is relatively late harvesting, constitutes 27% of current bearing acres, and is increasing as a proportion of total plantings. Hartley, another relatively late variety, comprises an additional 25% of current acreage. Early varieties (Payne, Ashley, and Serr) represent only 20% of bearing acres and have declined in popularity due to insect and blight susceptibility or, in the case of 'Serr', poor yield. Concentration of crop production at the end of the season results in poor utilization of harvesting equipment and processing capacity. Late harvest also results in increased risk of rain during the harvest operations and a tight time-line for drying and shipping product to Europe before the holiday season.

Development of scion varieties resistant to cherry leafroll virus, the causative agent of blackline disease is of particular concern for growers in the cooler areas of the State where the disease is most prevalent.

Rootstock qualities of greatest interest include vigor, tolerance for blackline virus, and resistance to crown gall, nematodes, and Phytophthora root and crown rots. Developing commercially viable rootstock propagation methods that would allow nurseries to replace the industry's current dependence on unimproved seedlings with clonally propagated improved rootstock is a high priority.

Germplasm

Collection

A breeding program depends in part on a diverse collection of germplasm as a source of raw material from which traits of interest can be identified. Persian walnuts are native to the mountains of Central Asia (Leslie and McGranahan 1998) so considerable effort has been directed towards collecting material from that area. Funding and participation in this work has included a century long plant introduction endeavor by USDA plant collectors, and more recent trips by USDA and university researchers. Collecting has been funded in part by California growers, USAID exchanges, and USDA-ARS Germplasm exploration funds. Material has also become available for use through international research contacts, private breeders, hobbyists, customs confiscations, and observant growers in the State who have noticed useful seedling trees.

Maintenance

Both the University of California and the USDA National Clonal Germplasm Repository maintain walnut germplasm collections. The intent of the USDA collections is to include as broad a diversity of all walnut species as possible and it is maintained for public distribution of material. It will not accept proprietary material and is managed primarily for wood and nut distribution to researchers worldwide. The UC Davis collection includes a representation of California commercial varieties, advanced selections, and some proprietary material and is focused primarily on material of interest for breeding purposes. It is managed for a variety of activities including crossing, breeding evaluations, and graftwood distribution of advanced selections. While there is some overlap of material, duplication is generally avoided and the two collections are used cooperatively.

Evaluation

Germplasm in these collections has to be evaluated and characterized to determine its useful attributes. Some of this work is done specifically for the breeding program and is funded through grower support of the program. Part of this work, particularly evaluation of the broader USDA collection has been funded through USDA-ARS Germplasm evaluation funds. These data are collected and made publicly available on-line through the USDA GRIN database.

Crossing Methods

The UC breeding program has used two distinct procedures for crossing parent material. In the first method, wind-blown pollen is excluded from female flowers of interest by covering them with tightly secured bags with small plastic windows. Pollen is collected from the other parent of interest and stored until use. When bagged female flowers open and are receptive, pollen is applied through the bags with a hypodermic needle. Bags are later removed and nuts marked for collection in the fall. The male parent is known with this method, but the costs are high and seedling production is low.

The second method is to locate geographically isolated young trees to avoid wind-blown pollen. This requires the cooperation of a grower with a recently planted orchard. Any male flowers on these trees are removed by hand before opening to prevent selfing. Once the female flowers begin to open, pollen of the desired male parent or parents is applied by airbrush several times during the bloom period. At harvest the cooperating grower either donates or is compensated for the nuts. This method produces many more seed at lower cost but with low certainty of the male parent. Male parents of selections can be determined later by DNA analysis.

Seedling Evaluation

Seed collected from these crosses is then stratified and grown to produce the next generation of seedlings. These are screened as they mature for traits of interest (Forde and McGranahan 1996). Commercial walnut nurseries have generously donated growing ground, time, resources, and expertise to assist this aspect of the program.

After 1 year in the nursery, trees are dug and replanted on wider spacing for evaluation. At this stage, trees are grown on their own roots, not grafted to rootstock. Most commonly these trees are planted on UC Pomology department ground and farmed by department staff supported by university and grower funding. In some cases growers have assisted the program by donating orchard space for this purpose and have farmed these trees during the evaluation process. This has been done by planting between rows in an existing widely spaced orchard, or more effectively, by interplanting in available open space in a newly established orchard and then removing the breeding program trees as evaluations are completed and the grower's orchard matures to fills the canopy.

As seedling trees mature they are evaluated in the field for traits of interest including leafing, flowering, and harvest dates, yield, and growth habit. When the trees are grown in university orchards they are left unsprayed so that variation in resistance to insects and disease can be observed. When grown within commercial orchards this is not normally possible. Nut samples are hand collected from each tree at maturity. Samples are dried, cracked by hand, and evaluated for percent kernel, kernel quality, kernel weight, shell characteristics, and yield of halves. Data is entered into a database and summarized for multiple years. In addition, samples of promising individuals are sent to the Diamond Cooperative and other interested handlers for their independent evaluation.

Collected data is presented to farm advisors, growers, and nurserymen in several ways. The first is at the annual Walnut Research Conference as part of the Walnut Improvement Program's annual report. Data on selections is presented orally to attendees and published in the annual proceedings of the conference.

The breeding program also holds an annual Crackout Meeting in the spring attended by farm advisors, handlers, nurserymen, and growers. Growers, handlers, and nursery attendees generally have an expressed interest in development of new varieties, are interested in assisting with evaluation of material, or are otherwise active in research activities and the marketing board. At this all day meeting the data reports are distributed and kernel samples and intact nuts of the material under evaluation are displayed. Attendees are asked to review the material, examine the samples, and provide written comments. In an ensuing discussion period they provide valuable input on priorities from their varying perspectives, help rank material, and suggest which should continue in the program.

The program also regularly invites interested parties to view selections in the field, either through a formal field day or by scheduling informal visits at their convenience. Progress in the program and information about selections is also presented periodically to a wider range of growers at annual county grower meetings held around the State.

Selection Trials

Once an individual seedling shows promise and is selected for further trials, graftwood is collected from the original seedling and grafted to rootstocks. Nurseries have often provided assistance at this stage by donating rootstock, supplying grafters, and in many cases growing the grafted trees for the program.

Grafted trees of each selection are then planted in test blocks on orchard spacing at diverse locations for further evaluation. Currently these tests blocks are located at the Chico State University Farm in the northern part of the State, on the UC Davis campus in the central region and at the UC Kearney Field Station in the south. These blocks are managed by Cooperative Extension farm advisors and are used to evaluate the performance of selections on rootstocks under a wide range of conditions, obtain a better look at yield, and allow farm advisors and growers to see selections in their local area.

In addition to the university plots, interested growers around the State have volunteered to establish trials ranging in size from several trees to several acres. Farm advisors assist in identifying suitable growers, establishing plots, and observing performance. Graftwood is distributed to these growers under test agreement and they are asked to participate in its evaluation and to attend the crackout meeting. This gives the program valuable input on performance under a variety of conditions and in commercial settings from observers with extensive experience. Growers feel they are assisting the process and get an early look at the material that is most interesting for their situation.

As new selections begin to show promise, commercial nurseries are encouraged to acquire graftwood from the program to test the varieties for themselves and to begin increase-blocks of their own. This ensures nurseries have adequate input into final selection, firsthand knowledge of the material, particularly of its grafting performance, growth habit, and training requirements, and builds an adequate supply of production wood by the time the new variety is released. As with grower trials, nurseries receive wood under test agreement. This allows them to propagate for testing purposes, including grower trials, but trees cannot be produced for sale until they are patented.

Release

Selections that continue to show promise in test blocks and grower trials become candidates for patent and release as new varieties. The patent disclosure process requires an extensive description of the selection, a summary of available data, and identification of attributes distinct from existing varieties.

Once a selection is patented as a new cultivar, nurseries may obtain a commercial license from the University of California that allows sale of trees. A per-tree royalty is assessed at the time of sale from the nursery and returned to the university. After patenting costs are recovered, part of this fee is assigned for overhead and part is returned to the department and breeding program. Patenting provides a return to the inventor and the university but also seeks to protect the growers from unlimited distribution. Patented material is not allowed to be sold or grown outside of California for five years after release. After that period, overseas licensing provides a return to the program that would not otherwise occur.

SUMMARY

In an increasingly globalized marketplace, technological advancement and improved efficiency is essential for survival. The UC Davis Walnut Breeding Program is but one aspect of a long-term, highly cooperative, and broadly based research effort in California, designed to solve problems for the State's walnut growers and to maintain a competitive and profitable industry. The contributions and cooperation of all interested parties has been the cornerstone of success.

LITERATURE CITED

Beede, R.H.; Hasey, J.K. 1998. The History of the walnut in California. In: Ramos, D.E., ed. Walnut production manual. Oakland, CA: Division of Agriculture and Natural Resources, University of California: 8-15.

California Agricultural Statistics Service. 2003. 2003 California walnut objective measurement survey report. 6 p.

Catlin, P.B. 1998. Root physiology and rootstock characteristics. In: Ramos, D.E., ed. Walnut production manual. Oakland, CA: Division of Agriculture and Natural Resources, University of California: 119-126.

Forde, H.I.; McGranahan, G.H. 1996. Walnuts. In: Moore, J., ed. Fruit breeding. New York, NY: John Wiley and Sons: 241-273.

Hasey, J. 1994. Maintaining the competitive edge in California's walnut industry: trends, issues, and challenges. Davis, CA: University of California Agricultural Issues Center. 86 p.

Leslie, C.A.; McGranahan, G.H. 1998. The origin of the walnut. In: Ramos, D.E., ed. Walnut production manual. Oakland, CA: Division of Agriculture and Natural Resources, University of California: 3-7.

McGranahan, G.H.; Forde, H.I.; Snyder, R.G.; Sibbett, G.S.; Reil, W.; Hasey J.; Ramos, D.E. 1992. Tulare Persian Walnut. HortScience. 27(2): 186-187.

McGranahan, G.H.; Ramos, D.E; Forde, H.I.; Snyder, R.G. 1990. Cisco Persian Walnut. HortScience. 25(3): 371-372.

Ramos, D.E., ed. 1998. Walnut production manual. Oakland, CA: Division of Agriculture and Natural Resources, University of California. 320 p.

Serr, E.F.; Forde, H.I. 1968. Ten new walnut varieties released. Califvornia Agriculture. 22: 8-10.

Siebert, J.B. 1998. Marketing California walnuts. In: Ramos, D.E., ed. Walnut production manual. Oakland, CA: Division of Agriculture and Natural Resources, University of California: 23-30.

Tulecke, W.; McGranahan, G.H. 1994. The walnut germplasm collection of the University of California, Davis: A description of the collection and a history of the breeding program of Eugene L. Serr and Harold I. Forde, Genetic Resources Conservation Program, Division of Agriculture and Natural Resources. 39 p.

Walnut Marketing Board. 1997. Walnut Industry Fact Sheet. 22 p.

Effect of Seed Position and Media on Germination of Black Walnut and Northern Red Oak: Implications for Nursery Production and Direct Seeding

Anthony S. Davis, Barrett C. Wilson, and Douglass F. Jacobs[1]

ABSTRACT—Germination of black walnut (*Juglans nigra* L.) and northern red oak (*Quercus rubra* L.) prior to sowing into containers or bareroot nursery beds can help maintain desired crop density and reduce nursery costs. Recommended techniques for germination of black walnut are labor intensive and require that walnuts be completely covered with growing media, periodically removed, and rinsed to identify germination prior to excessive elongation of the radicle. The purpose of this study was to determine if immersing 50% of a walnut into the soil such that the germinating radicle could be readily observed—a successful germination technique for northern red oak acorns—was also viable for germinating walnuts. The effect of media type on germination was also investigated. Percent germination was not influenced by seed position; however, fewer black walnuts germinated in vermiculite than in a soil mix. These findings may benefit research projects, small-scale nursery production, and direct-seeding operations.

INTRODUCTION

Throughout the Central Hardwood Forest Region, production of fine hardwood trees is an important component of the forest industry. In Indiana, landowners typically plant for timber production, creation of wildlife habitats, and to leave a legacy for future generations (Ross-Davis and others *in press*). Northern red oak (*Quercus rubra* L.) and black walnut (*Juglans nigra* L.) have proven to be valuable species for timber production and wildlife utilization, making them among the most abundantly planted species in Indiana (Jacobs and others 2004).

To produce tree seedlings, seeds are collected, stored and stratified (if necessary), and sown into containers, nursery beds, or the field. Standardized nursery practices involve covering the seed with soil immediately after sowing to prevent desiccation (Landis 1990, Auchmoody and others 1994) and, in the case of bareroot stocktypes, to limit seed predation (USDA Forest Service 1948, Auchmoody and others 1994, Kujawski and Davis 2001). Procurement of hardwood tree seed is an expensive undertaking, therefore demanding increased seed viability to ensure uniform crop production and efficient use of nursery growing space. While various tests can be used to ensure that only viable seeds are planted (e.g., float-test and x-ray), germination cannot be guaranteed. For example, viable seed sown under insufficient moisture or temperature conditions may not germinate. Additionally, these tests are expensive and time consuming, limiting their value on an operational scale for many nurseries.

Germinating seed prior to sowing could reduce nursery production costs for bareroot and container grown seedlings and increase the success of direct seeding operations. Bareroot seedling production methods consist of sowing seed in nursery beds immediately after collection or removal from storage. Germination of seed prior to sowing improves the likelihood of maintaining constant density in the nursery bed. In containerized seedling production, the number of containers and the amount of soil media needed are directly dependent upon the number of containers sown. Therefore, an increase in the percentage of cells containing successfully germinated seedlings would reduce production costs. The use of pre-germinated seed would help to ensure that in each container is a viable seed. Direct-seeding operations typically

[1]Graduate Research Assistants (ASD and BCW) and Assistant Professor (DFJ), Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, 195 Marsteller Street, West Lafayette, IN 47907-2033. DFJ is corresponding author: to contact, call (765) 494-3608 or e-mail: djacobs@fnr.purdue.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

broadcast large volumes of seed across the area designated for reforestation (Johnson 1981, 1983). Drawbacks associated with the direct seeding of black walnut include unpredictable germination and seed predation (Van Sambeek 2004). Germination of seed prior to sowing resulted in successful plantation establishment with uniform spacing and survival rates > 90% (Jacobs and Severeid 2004). While pre-germination may not be operationally feasible for many nurseries, it could serve to decrease variation due to germination rate in research projects.

An experiment recently conducted by the Hardwood Tree Improvement and Regeneration Center called for the germination of approximately 10,000 black walnut seeds (Jacobs and others *in press*). To achieve the required number of germinated seeds, walnuts were sown into flats and covered with growing media, following the recommended procedure for *Juglans* spp. of covering nuts to a depth of up to 7.62 cm (USDA Forest Service 1948, Kujawski and Davis 2001, Reid 1998). Seeds were examined regularly for germination, and those that germinated were removed from the flats and stored under moist towels at 2° C until target quantities of germinated walnuts were available. Minimal development of the radicle of germinated seeds was important to maintain uniform seedling development across the project (Fig. 1). Therefore, early detection was vital and extensive time and labor was invested for frequent assessment of seed status.

These intensive requirements raised questions about the feasibility of alternative germination methods. Successful germination of northern red oak results from 50% immersion of acorns into the soil (Fig. 2) (Davis 2003), despite recommendations that seeds be completely covered by soil (USDA Forest Service 1948, Kujawski and Davis 2001). Since moisture conditions can be readily controlled through a combination of timers and mist-irrigation systems, it was hypothesized that germination of black walnuts and northern red oak acorns

Figure 1.—Minimal development of germinated black walnut.

Figure 2.—Northern red oak germinating 50% immersed in soil.

would be similar whether they were placed 50% immersed in the growing media or fully covered by media. Furthermore, given that different media have different moisture holding capacities, a soil media and coarse vermiculite were both selected as variables. Given the ease with which germination could be monitored, successful germination of walnuts and acorns only 50% immersed in the growing media would greatly decrease the traditional labor-intensive process of monitoring germination. The objective of this study was to determine if northern red oak and black walnut seed germination was influenced by seed position or media type.

Materials and Methods

Northern red oak and black walnut were chosen as the trial species based on their importance in the Central Hardwood Forest Region. To reduce genetic variability, all walnuts and acorns used in this study came from a single respective tree. All seeds were collected in autumn 2002. The walnut hulls were removed prior to storage; prior to sowing, acorns were floated for 48 hours with all floating seeds removed.

Seeds were sown April 21, 2003 into individual 75 cm^3 pots (5 cm × 5 cm × 5 cm). Each pot contained either coarse grade vermiculite (Strong-Lite® Sunshine®, Sungro™ Horticulture, Pine Bluff, AR) or Scott's 366-P ScottsCoir™ growing medium (The Scotts Company, Marysville, OH). In their respective growing media, walnuts and acorns were randomly assigned to one of two treatments: (1) 50% immersion in the media, or (2) submersion 1 cm below the surface. To maximize the likelihood of germination, acorns were sown on their side (Trencia 1996) and walnuts were sown with their cleavage line perpendicular to the soil surface (Ciccarese 1995). The pots were then watered to saturation and placed in a mist propagation bench in the Horticulture and Landscape Architecture Plant Growth Facility at Purdue University. Pots were misted every 10 minutes for 10 second durations for 24 hours per day. After 6 weeks, total germination was recorded for each treatment.

The study was established as a completely randomized design (2 × 2 factorial [position × media]). For each species, analysis of variance was used to identify differences in germination percent by treatment. The level of significance was set at $\alpha = 0.10$. SAS® (SAS Institute, Cary, NC) was used for all data analyses.

RESULTS AND DISCUSSION

Neither seed position ($p = 0.9756$) nor media ($p = 0.9759$) had a significant effect on the percent germination of northern red oak acorns (Fig. 3). Position (50% immersed or completely covered by 1 cm of media) did not significantly affect the percent germination of walnuts ($p = 0.7151$). Media had a significant effect ($p = 0.0504$) on the germination percent of walnuts (Fig. 3). It is possible that fewer walnuts germinated in the vermiculite media due to the higher porosity of coarse vermiculite. While coarse vermiculite was used for this study, different grades of vermiculite (i.e., fine or medium textured) may be more suitable to walnut germination given differences in internal porosity (Landis 1990). There was no significant interaction between seed position and media type for northern red oak ($p = 0.9763$) or black walnut ($p = 0.8014$).

Seed position did not influence the germination of black walnut or northern red oak acorns. These findings concur with those reported for Persian walnut (*Juglans regia* L.) (Ciccarese 1995). When the germination of walnuts and acorns prior to transplanting is necessary, this method, compared to covering with soil, will reduce the time needed to identify and collect germinants.

The concept of using pre-germinated seed is not new. DeVelice and Buchanan (1978) recommended investigation into the use of pre-germinated ponderosa pine (*Pinus ponderosa* Laws.) as a means of increasing the likelihood of successful plantation establishment in New Mexico. Germination of seed prior to sowing containerized crops has also proven beneficial in nursery production in South Africa (South and Young 1995). The authors found this method ensures that between 98 and 99% of container cells are filled, and recommend the use of this method in North American nurseries. Adoption of this system in containerized seedling production would likely increase seed use efficiency and crop consistency, decreasing the space needed to produce a crop and costs associated with thinning and transplanting seedlings.

Advantages could also be attained in bareroot seedling production. Since seedling density influences height, root-collar diameter, and dry mass (Tomlinson and others 1996), as well as the number of first-order lateral roots (Schultz and Thompson 1996), using germinated seed to maintain consistent density may assist in producing more uniform seedlings. Black walnut seedlings grown at lower densities were more likely to produce more first-order lateral roots, which increased seedling survival and growth after outplanting (Schultz and Thompson 1996). Therefore, using pre-germinated seed would

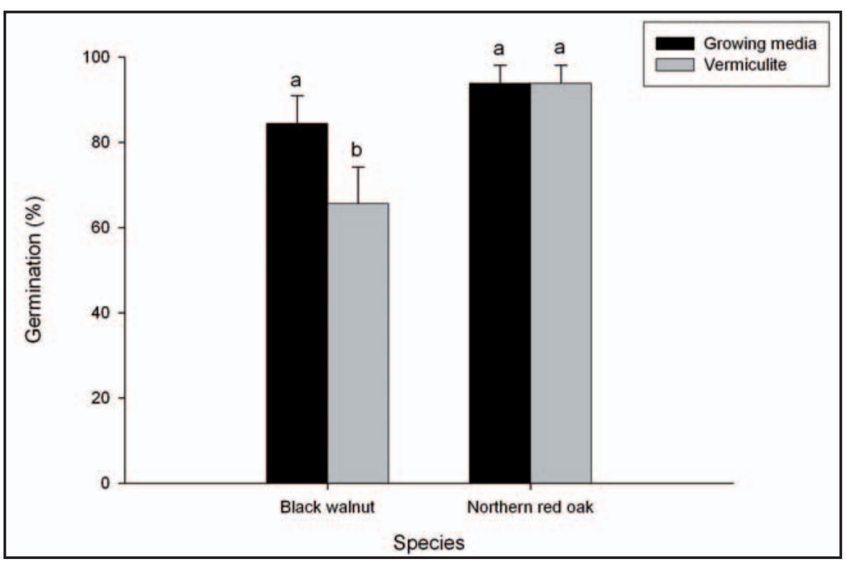


Figure 3.—Percent germination of black walnuts and northern red oak acorns for two media types. Different letters represent differences within species significant at $\alpha = 0.10$.

increase a grower's control over seedling bed density, which could improve outplanting survival and growth.

Successful plantation establishment can be achieved via direct-seeding (Johnson 1981, 1983; Wittwer 1991). Direct-seeded Nuttall oak (*Quercus nuttallii* Palmer) sown at approximately 8,000 acorns per ha yielded 5,657 trees per ha after 10 years (Johnson 1981). Mullins and others (1998) found that there was no significant difference in height or diameter of bareroot, containerized or direct-seeded (not pre-germinated) cherrybark oak (*Quercus falcata* Michx. var. *falcata*) seedlings 5 years after planting.

While attempts to direct-seed black walnut have yielded mixed results (Robison and others 1997), the potential exists to do so successfully (Schavilje 1941, Davidson 1980). Lack of success has been attributed to poor germination and growing conditions in the field (Robison and others 1997). Germination prior to seeding, however, may act to improve the success rate of direct-seeding attempts, given successful plantation establishment with direct-sown pre-germinated seed of northern red oak, black walnut, and American chestnut [*Castanea dentata* (Marsh.) Borkh.] (Jacobs and Severeid 2004). Further research is needed to identify those site conditions and tending requirements that will ensure successful establishment, as well as determining the economic and ecological costs and benefits of this method for reforestation. Additionally, research into herbicides that are suitable for use with direct seeding (Willoughby and others 2003) will lead to improved flexibility in field experimentation.

Direct seeding as a means of plantation establishment could reduce those stresses related to planting. The loss of fine roots of hardwood species during wrenching may retard seedling development. In addition to potential benefits realized in seedling establishment, the cost of transporting seed is lower than that of seedlings (Van Sambeek 2004). Bullard and others (1992) found that on old-field sites in the southern United States the cost of direct seeding oak was approximately 1/3 of those of planting seedlings and that given proper stand management there would be no benefit of planting seedlings over direct seeding.

The potential to use direct seeding of pre-germinated seed as an effective means of enhancing existing plantations should not be disregarded. On non-industrial private forestland plantations in Indiana, survival of hardwood seedlings at age 5 has been estimated at approximately 65% (Jacobs and others 2004). Many landowners establish plantations at a desired density. To maintain the desired stocking with seedlings would require hand planting to fill in where mortality occurred, which can be strenuous and may limit seedling vigor (Jacobs and others 2004, Russell Jr. and others 1998). Germinant sowing to replace dead seedlings may be a viable means of maintaining stocking at a desired level without having to purchase and plant additional seedlings.

CONCLUSION

Pre-germination of acorns and walnuts may be beneficial by increasing uniformity of research projects and small-scale nursery culture and direct-seeding operations. The results of this study emphasize the need to understand the influence of different media types on the production of hardwood seedlings. Identification of media that maximizes the likelihood of black walnut germination will be beneficial to improving walnut production efficiency. Easy detection of walnut and acorn germination can be achieved with sowing seed 50% immersed into soil. This process can save money, time, labor, and materials without losses associated with non-germinated seeds. As consistent seedling density increases the uniformity of a crop and maximizes the use of growing space, crop production efficiency would benefit from this approach. Planting operations that choose to employ the direct-seeding method would also benefit from pre-germinating seed, as reduced sowing density would be needed to achieve the desired spacing. Further trials with a larger sample size and additional treatments may be warranted to affirm our results and identify procedures that further enhance germination success.

ACKNOWLEDGMENTS

We would like to thank Jim McKenna (Hardwood Tree Improvement and Regeneration Center), Rob Eddy (Horticulture and Landscape Architecture Plant Growth Facility at Purdue University), and Patricio Alzugaray for their assistance with seed collection and space management. Marcus Selig, Ron Overton, and Amy Ross-Davis provided assistance with the preparation of this manuscript.

LITERATURE CITED

Auchmoody, L.R.; Smith, H.C.; Walters, R.S. 1994. Planting northern red oak acorns: is size and planting depth important? Res. Pap. NE-693. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 4 p.

Bullard, S.; Hodges, J.D.; Johnson, R.L.; Straka, T.J. 1992. Economics of direct seeding and planting for establishing oak stands on old-fields in the south. Southern Journal of Applied Forestry. 16: 34-40.

Ciccarese, L. 1995. Effects of seed position and planting depth on development of *Juglans regia* nursery seedlings. Monti e Boschi. 46: 56-60.

Davis, A.S. 2003. Performance of northern red oak under adverse conditions. West Lafayette, IN: Purdue University. 111 p. M.S. thesis.

Davidson, W.H. 1980. Direct seeding for forestation. In: Proceedings, Trees for reclamation in the Eastern U.S.; 1980 October 27-29; Lexington, KY. Washington, DC: U.S. Department of Agriculture, Forest Service, Interstate Mining Compact Commission: 93-97.

DeVelice, R.L.; Buchanan, B.A. 1978. Germinant reforestation: a promising new technique. Tree Planters Notes. 29: 3-6.

Jacobs, D.F.; Severeid, L.R. 2004. Relative competitiveness of interplanted American chestnut [*Castanea dentata* (Marsh.) Borkh.]. Forest Ecology and Management. 191: 111-120.

Jacobs, D.F.; Ross-Davis, A.L.; Davis, A.S. 2004. Establishment success of conservation tree plantations in relation to silvicultural practices in Indiana, USA. New Forests. 28: 23-36.

Johnson, R.L. 1981. Oak seeding - it can work. Southern Journal of Applied Forestry. 5: 28-33.

Johnson, R.L. 1983. Nuttall oak direct seedings still successful after 11 years. Res. Note SO-301. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 3 p.

Kujawski, J.; Davis, K.M. 2001. Propagation protocol for production of plug + transplants of *Juglans* nigra plants; Beltsville National Plant Materials Center, Beltsville, MD. In: Native Plant Network. Moscow, ID: University of Idaho, College of Natural Resources, Forest Research Nursery. URL: <http://www.nativeplantnetwork.org> (accessed 16 February 2004).

Landis, T.D. 1990. Volume 2: Containers and growing media: the container tree nursery manual. Agric. Handb. 674. Washington, DC: U.S. Department of Agriculture, Forest Service. 87 p.

Mullins, J.A.; Buckner, E.R.; Hopper, G.; Percell, G.G. 1998. Early growth of sheltered and unsheltered cherrybark oak established by planting 1-0 bareroot and 1-0 containerized seedlings and by direct-seeding. In: Waldrop, T.A., ed. Proceedings, 9th biennial southern silvicultural research conference; 1997 February 25-27; Clemson, SC. Gen. Tech. Rep. SRS-20. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 234-237.

Reid, W. 1998. Propagating eastern black walnut. In: Jones, J.E.; Mueller, R.; Van Sambeek, J.W., eds. Nut production handbook for eastern black walnut. (online). Available: <http://ncrs.fs.fed.us/pubs/misc/walnut>.

Robison, T.L.; Yoder, W.G.; Hoss, G. 1997. Direct seeding and seedling production in nursery beds. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: Proceedings, 5th black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 96-103.

Ross-Davis, A.L.; Broussard, S.R.; Jacobs, D.F.; Davis, A.S. In press. Afforestation behavior of private landowners: an examination of hardwood tree plantings in Indiana. Northern Journal of Applied Forestry.

Russell, D.R., Jr.; Hodges, J.D.; Ezell, A.W. 1998. An evaluation of hardwood reforestation methods on previously farmed lands in central Alabama. In: Waldrop, T.A. ed. Proceedings, 9th biennial southern silvicultural research conference; 1997 February 25-27; Clemson, SC. Gen. Tech. Rep. SRS-20. Asheville, NC: U.S. Department of Agriculture. Forest Service, Southern Research Station: 272-276.

Schavilje, J.P. 1941. Reclaiming Illinois strip mined coal lands with trees. Journal of Forestry. 39: 714-719.

Schultz, R.C.; Thompson, J.R. 1996. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root-graded stock in the central USA. New Forests. 13: 297-310.

South, D.B.; Young, C. 1995. Germinant sowing in South Africa. Tree Planters Notes. 46: 7-10.

Tomlinson, P.T.; Buchschacher, G.L.; Teclaw, R.M. 1996. Sowing methods and mulch affect 1+0 northern red oak seedling quality. New Forests. 13: 191-206.

Trencia, J. 1996. Influence of seed position on first-year survival and growth of directly seeded northern red oak. Tree Planters Notes. 47: 68-75.

USDA Forest Service. 1948. Woody plant seed manual. Washington, DC: U.S. Department of Agriculture, Forest Service. 416 p.

Van Sambeek, J.W. 2004. Direct seeding. Walnut notes. (online). Available: <http://www.ncrs.fs.fed.us/pubs/wn/walnutnotestoc.html>.

Willoughby, I.; Clay, D.; Dixon, F. 2003. The effect of pre-emergent herbicides on germination and early growth of broadleaved species used for direct seeding. Forestry. 76: 83-94.

Wittwer, R.F. 1991. Direct seeding of bottomland oaks in Oklahoma. Southern Journal of Applied Forestry. 15: 17-22.

VEGETATIVE PROPAGATION OF BUTTERNUT (*JUGLANS CINEREA*) FIELD RESULTS

Paula M. Pijut[1]

ABSTRACT—*Juglans cinerea* L. is a hardwood species valued for its wood and edible nuts. Butternut canker disease (*Sirococcus clavigignenti-juglandacearum*) threatens its survival. Vegetative propagation will be required to produce clones of genotypes selected for resistance to butternut canker disease. In 2000, 10 trees were randomly selected from a 6-year-old butternut plantation located in Rosemount, MN. Hardwood stem cuttings were collected in March, April, and May. Softwood cuttings were collected in June and July. Indole-3-butyric acid-potassium salt (K-IBA) at 0, 29, or 62 mM in water and indole-3-butyric acid (IBA) at 0, 34, or 74 mM in 70% ethanol were tested for root induction on cuttings. The basal end of cuttings were dipped in a treatment solution for 10 to 15 s, potted in a peat:perlite mixture and placed in a mist bed for 5 to 8 weeks. Rooted cuttings were gradually hardened off from the mist bed, allowed to initiate new growth, over-wintered in a controlled cold-storage environment, and then outplanted to the field. Rooting was greatest for hardwood cuttings taken in mid-May (branches flushed out), 22% with 62 mM K-IBA and 28% with 74 mM IBA. Softwood cuttings rooted best when taken in June (current season's first flush of new growth or softwood growth 40 cm or greater) and treated with 62 mM K-IBA (77%) or 74 mM IBA (88%). One-hundred and seventy three (173) out of 186 rooted softwood cuttings (93%) survived over-wintering and acclimatization to the field. Average heights and stem diameters were after 1 year (28.6 cm, 12.4 mm) or 2 years (92.8 cm, 18.6 mm). When plants were protected from deer browse, rodent damage, and weed controlled, 91% survived in the field.

INTRODUCTION

Butternut (*Juglans cinerea* L.), also known as white walnut or oilnut, is a relatively slow-growing hardwood species found in bottomlands, moist uplands, and old fields (Leopold and others 1998). Moist, rich loamy soils of hillsides and streambanks are the preferred growing sites, although butternut can grow quite well on dry, rocky soils. *Juglans cinerea* is considered to be one of the most winter hardy (USDA Hardiness Zone range of 3 to 7) of the *Juglans* species (Cathey 2003, Dirr 1998). Butternut is seldom found growing in pure stands, but rather in association with several other tree species such as black cherry, American basswood, white and northern red oak, black walnut, white ash, red and sugar maple, and American elm. It is native to the northeastern United States and adjacent Canada, ranging from New Brunswick to Georgia, and west to Minnesota and Arkansas (Rink 1990). Butternut is valued ecologically and economically for its edible nuts and wood (Ostry and Pijut 2000). The nuts are oily and sweet, and are an important food source for wildlife. Seed production begins on trees around 20 years of age, with good seed crops occurring every 2 to 3 years, based on seedling stands not grafted trees. Because of the limited availability of quality butternut wood, it commands a high market price for many uses including furniture, cabinets, paneling, veneer, and fine woodworking. Its light tan heartwood is very workable with hand and power tools (for specialty carving products) and can be stained to resemble black walnut.

Butternut canker disease, caused by the fungus *Sirococcus clavigignenti-juglandacearum* N.B. Nair, Kostichka and Kuntz (Nair and others 1979) has

[1]Research Plant Physiologist (PMP), North Central Research Station, USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, 195 Marsteller Street, West Lafayette, IN 47907. PMP is corresponding author: to contact, call (765) 496-2162 or e-mail at ppijut@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

caused widespread mortality and is threatening native tree survival (Orchard and others 1982; Tisserat and Kuntz 1984; Cummings 1993; Ostry 1997, 1998; Nair 1999). The fungus, believed to be introduced (Furnier and others 1999), causes perennial cankers on all above ground parts of the tree, even the buttress roots (Sinclair and others 1987). The spores of the fungus are disseminated by rain splash and can travel in aerosols to adjacent trees where infection occurs on young branches in the upper crowns. Several insect species have been found associated with fungus spores on infected trees (Katovich and Ostry 1998). The fungus has also been found on the fruit of butternut and black walnut, causing lesions on the husks of both species (Innes 1998). The cankers cause the wood to turn dark brown to black in an elliptical pattern (Ostry and others 1996) that reduces the quality and marketability of the wood. The girdling effect of multiple coalescing cankers eventually kills the trees.

Butternut is propagated easily from seed (Brinkman 1974), but the canker fungus is also seed-borne (Orchard and others 1981, Orchard 1984). Vegetative propagation will be required to produce clones of genotypes selected for resistance to butternut canker disease. Grafting of butternut to black walnut rootstock (Ostry and Pijut 2000) can be successful, but grafting is a time consuming process with variable success. There are few reports of vegetative propagation of butternut through cuttings (Pijut and Barker 1999, Pijut and Moore 2002). Rooting is reported for other *Juglans* species: *J. hindsii* (Lee and others 1977), *J. microcarpa* (Shreve 1990), *J. nigra* (Farmer 1971, Shreve 1972, Shreve and Miles 1972, Farmer and Hall 1973, Carpenter 1975), *J. regia* (Gautam and Chauhan 1990), *J. sinensis* (Kwon and others 1990), and hybrids (Serr 1964, Reil and others 1998). This paper describes the experimental conditions and results for successful cutting propagation of butternut, and growth and survival data of some of the rooted cuttings after 1- or 2-years in the field.

MATERIALS AND METHODS

Hardwood Cuttings

In 2000, 10 trees were selected from a 6-year-old butternut field plot located in Rosemount, MN. Hardwood cuttings were collected at specific growth stages: dormant (Mar. 29, 2000); budbreak (May 2, 2000); and branches flushed out (May 19, 2000). Six to twelve cuttings (25 cm in length) were taken from each tree at each collection date. Two years of growth were pruned from the remaining branches (after last hardwood collection date) to encourage sprouting. Cuttings were placed in polyethylene bags, held on ice, and transported to the greenhouse, where the cuttings were processed the same day. Stems were recut to between 20 and 23 cm in length. The basal 3 cm of cuttings were treated by dipping for 10 to 15 s in either 0, 29, or 62 mM indole-3-butyric acid-potassium salt (K-IBA) dissolved in deionized water or 0, 34, or 74 mM indole-3-butyric acid (IBA) dissolved in 70% ethanol. Cuttings were inserted vertically to a depth of 5 to 7 cm in Deepots™ (D40) (Stuewe and Sons, Corvallis, OR) containing a moist medium of 1 perlite : 1 peat (v/v). Cuttings in Deepots™ were placed under intermittent mist (15 s every 18 min) on a greenhouse bench with bottom heat maintained at 27° C. Twelve hours of supplementary irradiance (from 0600 to 1800 HR) were provided by high-pressure sodium lamps (60 µmol·m-2·s-1 at bench top), and greenhouse temperature was maintained at 22 $\pm$ 2° C. After 6 to 8 weeks, cuttings were harvested and number of cuttings rooted, number of roots per cutting, and individual root lengths were recorded. Data were analyzed using statistical programs for categorical data (Rugg, unpublished data), log linear modeling (SPSS 1998), and logistic regression (Mehta and Patel 1996). The number of roots per cutting were square root transformed and the data analyzed using linear regression. The data for root lengths were log transformed and the data analyzed using linear regression.

Rooted cuttings were transplanted in Treepots™ (Tall One) (Stuewe and Sons) containing a moist medium of Sunshine SB-40 bark mix (Sun Gro Horticulture, Bellevue, WA), with 14N–14P–14K Nutricote® timed-release fertilizer (Chisso-Asahi Fertilizer Co., Tokyo, Japan) and returned to intermittent mist for 1 week. Rooted cuttings were then acclimatized to greenhouse benches and allowed to initiate new shoot growth. In late October or early November, rooted cuttings were placed in a cooler environment (15° C or less) and lower light to induce dormancy. After a month, containerized cuttings were placed in polyethylene bags and moved to a controlled cold-storage environment (3 to 4° C in darkness) for 4 to 5 months. After overwintering, the pots were returned to the greenhouse (March/April, the following year), and allowed to acclimatize to this environment. Pots were hand-watered as needed until budbreak, after which the pots were placed on an automatic drip irrigation system until they were field-planted in July.

Softwood Cuttings

In 2000, the same 10 trees as used for hardwood cuttings were used for softwood cuttings. Softwood cuttings were collected at specific growth stages: current season's first flush of new growth (June 12, 2000); softwood growth 40 cm or greater (June 23, 2000); shoots beginning to become lignified (July 7, 2000); and shoots starting to set bud (July 19, 2000). Six to twelve cuttings (40 to 45 cm in length) were taken from each tree at each collection date. All but two leaves were removed. Softwood cuttings were handled and processed as described for hardwood cuttings, except no bottom heat was used in the mist bed. After 5 to 6 weeks, cuttings were harvested and rooting data recorded. Data collection and statistical analyses were the same as described for hardwood cuttings. Rooted cuttings were maintained, allowed to initiate new growth, overwintered, and planted in the field as described for hardwood cuttings.

Field Plantings

Rooted cuttings were planted in the field at the Southeast Purdue Agricultural Center (SEPAC) (July 2001; 56 plants) located near North Vernon, IN, and at the Vallonia Nursery (July 2002; 81 plants) located in Vallonia, IN. The butternut plants transported to the Vallonia nursery were kept in the Treepots™ outdoors for one additional year until the site was available for planting in July 2002. Therefore, data on these plants are considered as 1-year-old field results. When feasible rooted cuttings were protected with mesh cages to prevent deer browse and rodent damage, and sprayed with herbicides to control weeds. Percent survival, stem heights (cm), and stem diameter (mm) at ground level (root collar) were recorded in 2003.

RESULTS

Hardwood Cuttings

Rooting success did not vary by growth stage (collection date) ($P > 0.85$). K-IBA or IBA were equally effective in promoting rooting ($P = 0.38$). There was a significant effect of the auxin concentration ($P = 0.007$). The controls [deionized water or 70% ethanol] were not different ($P = 0.11$) from the low concentration [29 mM K-IBA or 34 mM IBA], but did significantly differ ($P = 0.005$) from the high concentration [62 mM K-IBA or 74 mM IBA] (Table 1). The low and high concentrations did not differ ($P = 0.24$) from each other in rooting success, but a logistic regression on these data showed that a linear response to concentration accounts for nearly all of the variability in the data. The greatest rooting success was with 62 mM K-IBA (22.2%) and 74 mM IBA (27.8%) when hardwood cuttings were collected when the branches had flushed out (mid-May). There was no difference in the number of roots regenerated per hardwood cutting ($P > 0.1$). No differences ($P = 0.02$) were observed for root lengths. Six out of six rooted cuttings (100%) survived over-wintering in cold storage and acclimatization to the field.

Table 1.—Effects of time of collection and rooting treatment concentration on rooting percentage, root count, and root len gth of Juglans cinerea hardwood cuttings.[1]

Rooting Treatment (mM)	Date of Collection[2] 2000: 29 March	2 May	19 May
	Rooting (percent)		
0 K-IBA	0 a[3]	0 a	11.1 a
29 K-IBA	9.1 a	6.3 ab	16.7 ab
62 K-IBA	0 a	12.5 b	22.2 b
0 IBA	0 a	0 a	11.1 a
34 IBA	0 a	6.3 ab	11.1 ab
74 IBA	0 a	6.3 b	27.8 b
	Mean number of roots[4]		
0 K-IBA	- -	- -	1.0 a
29 K-IBA	1.0	4.0 a	7.0 a
62 K-IBA	- -	1.5 a	4.8 a
0 IBA	- -	- -	2.0 a
34 IBA	- -	7.0 a	1.0 a
74 IBA	- -	2.0 a	3.1 a
	Mean root length (mm)w		
0 K-IBA	- -	- -	8.0 a
29 K-IBA	22	21.5 a	23.4 a
62 K-IBA	- -	7.3 a	9.4 a
0 IBA	- -	- -	13.7 a
34 IBA	- -	17.9 a	8.0 a
74 IBA	- -	5.0 a	11.2 a

[1]*Average sample size for each collection date and rooting treatment concentration, n =14.*

[2]*Hardwood cutting growth stages: dormant (2000 March 29); bud break (2000 May 2); and branches flushed out (2000 May 19).*

[3]*Mean separation within a column by LSD, P < 0.05. Letters indicate significant differences among means within a column for a given variable. "- -" indicates that no mean was calculated because no observations were available.*

[4]*Means are per rooted cutting.*

Softwood Cuttings

Sample development stages showed no change in rooting success (P > 0.40). Auxin type did not effect rooting success (P = 0.79). However, there was a significant additive effect on rooting success with the auxin concentration of rooting treatment used (P < 0.0001). Using K-IBA (29 or 62 mM) or IBA (34 or 74 mM) improved rooting success over the control, but there was no difference (P = 0.39) between the treatment levels (Table 2). The greatest rooting success of 76.9% (62 mM K-IBA) and 87.5% (74 mM IBA) was achieved when softwood cuttings were taken in June (Fig. 1A). Using K-IBA or IBA produced more roots than controls (P < 0.0001), but the auxin concentrations did not differ in number of roots produced (P = 0.43). There were no differences between the controls (water versus 70% ethanol) in number of roots produced (P = 0.69). In addition, the high concentrations of both K-IBA and IBA produced a similar number of roots (P = 0.11). However, the 34 mM IBA treatment resulted in significantly greater root numbers (P = 0.0001) than in the similar concentration of K-IBA. For K-IBA, the low concentration (29 mM) was not statistically different from either the control (P = 0.14) or the high concentration (P = 0.08). However, the high concentration produced more roots than the control (P = 0.006). Using K-IBA produced a linear dose-response relationship for number of roots produced. Softwood cuttings have a uniformly higher number of roots produced than do hardwood cuttings. Cuttings, from the current season's first flush of new growth (20.8 cm) and shoots beginning to become lignified (24.7 cm) development stages, had shorter roots than the softwood growth 40 cm or greater development stage (34.9 cm). There was no difference in root length from cuttings from the first, third, and fourth sample development stages. Mean root length was similar for all treatments and controls. One hundred and seventy three out of 186 rooted softwood cuttings (93%) survived over-wintering and acclimatization to the field (Fig. 1B).

Table 2.—Effects of time of collection and rooting treatment concentration on rooting percentage, root count, and root length of Juglans cinerea softwood cuttings.[1]

Rooting treatment (mM)	Date of Collection[2] 2000			
	12 June	23 June	7 July	19 July
	Rooting (percent)			
0 K-IBA	37.5 a[3]	17.9 a	10.7 a	23.1 a
29 K-IBA	37.5 b	73.1 b	42.9 b	50.0 b
62 K-IBA	44.4 b	76.9 b	42.9 b	57.7 b
0 IBA	37.5 a	25.0 a	10.7 a	19.2 a
34 IBA	37.5 b	67.9 b	39.3 b	50.0 b
74 IBA	87.5 b	75.0 b	46.4 b	46.2 b
	Mean number of roots[4]			
0 K-IBA	3.0 a	3.5 a	1.7 a	3.8 a
29 K-IBA	7.0 b	9.7 b	3.9 b	8.0 b
62 K-IBA	13.8 c	7.3 c	13.3 c	11.2 c
0 IBA	3.0 a	3.1 a	1.7 a	3.0 a
34 IBA	9.7 c	20.6 c	10.7 c	12.2 c
74 IBA	18.7 c	21.0 c	7.4 c	6.0 c
	Mean root length (mm)[w]			
0 K-IBA	29.0 a	42.0 a	35.3 a	24.2 a
29 K-IBA	13.8 a	40.2 a	22.2 a	38.7 a
62 K-IBA	26.5 a	32.5 a	24.2 a	30.9 a
0 IBA	12.7 a	17.5 a	18.0 a	58.7 a
34 IBA	26.2 a	41.0 a	24.1 a	22.1 a
74 IBA	16.7 a	36.1 a	24.5 a	20.9 a

[1]*Average sample size for each collection date and rooting treatment concentration, n = 21.*

[2]*Softwood cutting growth stages: current season's first flush of new growth (2000 June 12); softwood growth 40 cm or greater (2000 June 23); shoots beginning to become lignified (2000 July 7); shoots starting to set bud (2000 July 19).*

[3]*Mean separation within a column by LSD, P < 0.05. Letters indicate significant differences among means within a column for a given variable. "- -" indicates that no mean was calculated because no observations were available.*

[4]*Means are per rooted cutting.*

Field Plantings

In the SEPAC planting, 51 out of 56 plants (91%) survived in the field after 2 years. These plants were protected with mesh cages to prevent deer browse and rodent damage, and routinely sprayed with herbicides to control weeds (Fig. 2). Average stem heights in centimeters (mean number $\pm$ SE) ranged from 66.4 $\pm$ 8.0 to 123.7 $\pm$ 25.6, with a total average of 92.8 $\pm$ 5.9 after 2 years (Table 3). Average stem diameters in millimeters (mean number $\pm$ SE) ranged from 14.1 $\pm$ 0.9 to 21.3 $\pm$ 3.1, with a total average of 18.6 $\pm$ 0.7 after 2 years.

In the Vallonia planting, 54 out of 81 plants (67%) survived in the field after 1 year. These plants were not protected with mesh cages to prevent deer browse. Herbicides were used at this site (prior to planting and once after planting), but several factors (remote site, unavailability of spray equipment, and wet weather) combined to render control of weeds ineffective. Deer browse, drought in the summer, and weed competition in this planting had the greatest overall effect on survival of the butternut plants. Average stem heights in centimeters ranged from 15.7 $\pm$ 6.4 to 46.3 $\pm$ 10.3

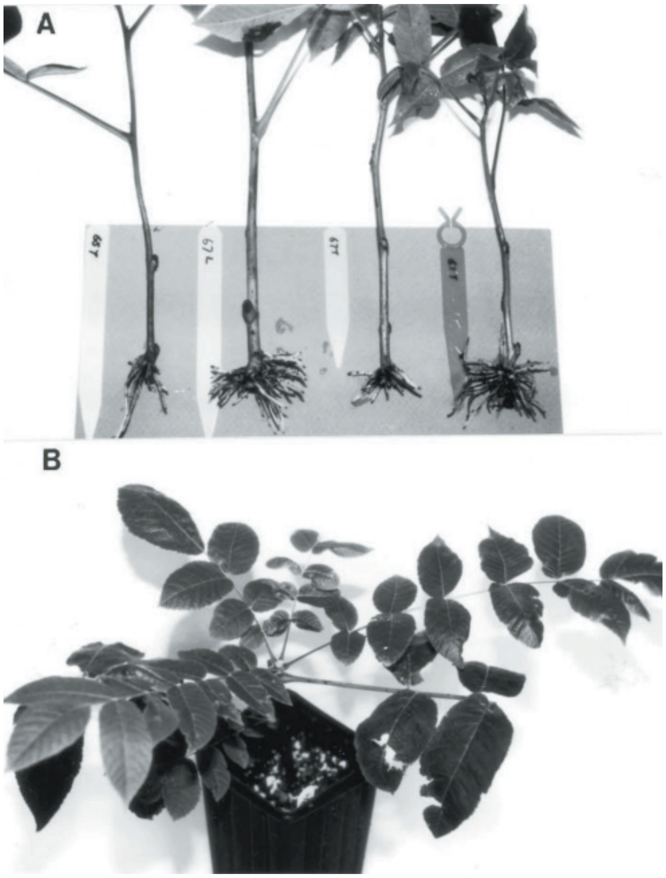


Figure 1.—(A) Rooted softwood cuttings of Juglans cinerea after 4 wk under mist (from left to right: 34, 74 mM IBA, and 29, 62 mM K-IBA). (B) Butternut plant (softwood cutting, June collection, 62 mM K-IBA) ready for transplanting to the field. [Reprinted with permission from HortScience 37(4): 697-700. 2002]

Figure 2.—Butternut plant (rooted cutting) after 2 years in the Southeast Purdue Agricultural Center field. For comparison, the woman in the photo is 160 cm tall.

with a total average of 28.6 ± 1.9 after 1 year (Table 4). Average stem diameters in millimeters ranged from 8.0 ± 1.0 to 14.0 ± 0.8, with a total average of 12.4 ± 0.5 after 1 year.

DISCUSSION

Propagation of 6-year-old *J. cinerea* from hardwood cuttings collected in mid-May (branches flushed out in the spring) was successful (11.1 to 27.8%), although at a low percentage. The greatest rooting success of 22.2% and 27.8% was achieved when hardwood cuttings were treated with 62 mM K-IBA or 74 mM IBA. Rooted hardwood cuttings were successfully over-wintered in cold storage and planted to the field. Propagation of other *Juglans* species by hardwood cuttings has also been investigated. Hardwood cuttings of 'Paradox' walnut (*J. hindsii* x *J. regia*) root with variable success (30 to 80%) after a quick dip in 20 mM to 39 mM IBA or a 24-hr soak in 1mM to 1.5 mM IBA (Serr 1964, Reil and others 1998). Dormant cuttings taken from 4- to 5-year-old hedges of *J. regia* rooted (14.5%) when treated with 74 mM IBA (Gautam and Chauhan 1990). Carpenter (1975) reported 60 to 70% rooting of hardwood cuttings taken from mature black walnut trees (*J. nigra*), when soaked in ethephon for 6 hr, but shoots did not elongate following this treatment and no survival was reported.

Softwood cuttings of butternut rooted better than hardwood cuttings. Softwood cuttings rooted (10.7 to 87.5%) at all collection dates and rooting treatment concentrations tested. Rooting success ranged from 17.9 to 87.5% when June cuttings (current season's first flush of new growth or softwood growth 40 cm or greater) were treated with 0 K-IBA or 74 mM IBA. The greatest rooting success (76.9% and 87.5%) was achieved when softwood cuttings collected in mid- to late-June 2000 were treated with 62 mM K-IBA or 74 mM IBA. Rooted softwood cuttings were successfully over-wintered and transplanted to the field. Propagation of other *Juglans* species by softwood cuttings is also successful. Cuttings taken from adventitious shoots of eastern black walnut, *J. nigra*, (from one to 135-year-old material) rooted 80 to 100% when

Table 3.—Growth parameters of butternut plants (rooted softwood cuttings) after 2 years in the Southeast Purdue Agricultural Center field.

Tree No.	Height (cm)	Stem Diameter (mm)
103	126.0	24.3
103	142.0	28.5
103	158.0	23.8
103	105.0	18.6
103	39.0	11.7
103	93.0	18.3
103	98.0	21.5
Mean number + SE	108.7 + 14.7	21.0 + 2.0
104	51.0	12.4
104	95.0	19.3
104	96.0	20.5
104	89.0	22.1
104	167.0	29.5
104	55.0	14.1
Mean number + SE	92.2 + 17.0	19.7 + 2.5
105	45.0	10.2
105	79.0	16.0
105	70.0	21.2
105	51.0	10.8
105	87.0	14.1
Mean number + SE	66.4 + 8.0	14.5 + 2.0
108	102.0	15.1
108	94.0	15.9
108	31.0	12.1
108	56.0	13.1
Mean number + SE	70.8 + 16.6	14.1 + 0.9
110	47.0	13.0
110	132.0	27.6
110	100.0	16.3
110	153.0	28.6
110	145.0	21.0
Mean number + SE	115.4 + 19.3	21.3 + 3.1
112	80.0	18.9
112	96.0	21.9
112	50.0	14.9
112	90.0	24.9
112	19.0	9.9
Mean number + SE	67.0 + 14.4	18.1 + 2.6
113	69.0	16.2
113	148.0	24.0
113	102.0	20.1
113	32.0	12.5
113	124.0	21.4
113	99.0	14.1
Mean number + SE	95.7 + 16.7	18.1 + 1.8
114	153.0	19.9
114	42.0	11.0
114	148.0	21.4
114	166.0	24.3
114	47.0	23.1
114	186.0	23.9
Mean number + SE	123.7 + 25.6	20.6 + 2.0
115	96.0	17.9
115	70.0	17.3
115	46.0	13.8
115	149.0	26.4
Mean number + SE	90.3 + 22.1	18.9 + 2.7
117	87.0	20.3
117	57.0	14.8
117	72.0	16.3
Mean number + SE	72.0 + 8.7	17.1 + 1.6
TOTAL		
Mean number + SE	92.8 + 5.9	18.6 + 0.7

Table 4.—Growth parameters of butternut plants (rooted softwood and hardwood cuttings) after 1 year in the Vallonia field.

Tree No.	Height (cm)	Stem Diameter (mm)
103	25.0	13.0
103	18.0	16.0
103	34.5	9.0
103	32.0	16.0
Mean number + SE	27.4 + 3.7	13.5 + 1.7
104	27.5	9.0
104	14.0	9.0
104	5.5	6.0
Mean number + SE	15.7 + 6.4	8.0 + 1.0
108	36.0	9.0
108	56.5	13.0
Mean number + SE	46.3 + 10.3	11.0 + 2.0
110	43.0	16.0
110	56.5	19.0
110	43.5	9.0
110 Hardwood	48.5	13.0
110	16.0	13.0
110	26.0	13.0
110	15.0	6.0
110	53.0	16.0
Mean number + SE	37.7 + 5.8	13.1 + 1.5
113	21.0	16.0
113	18.0	16.0
113	4.0	9.0
113	8.0	6.0
113	15.0	13.0
113	11.0	16.0
113	12.0	16.0
113	8.0	6.0
113	15.0	13.0
113	29.0	13.0
113	41.0	16.0
113	42.0	16.0
113	48.0	19.0
113	42.5	16.0
113	38.0	16.0
113	19.0	13.0
113	41.0	13.0
113	42.5	16.0
113	39.0	9.0
113 Hardwood	50.0	16.0
113 Hardwood	39.5	22.0
113	34.5	13.0
113	55.0	13.0
Mean number + SE	29.3 + 3.3	14.0 + 0.8
114	17.0	13.0
114	32.0	9.0
Mean number + SE	24.5 + 7.5	11.0 + 2.0
115 Hardwood	23.0	9.0
115	31.0	3.0
115	28.0	16.0
115	25.0	13.0
115	10.0	13.0
115	23.5	13.0
115	19.0	6.0
115	20.0	6.0
115	26.0	13.0
115	28.0	9.0
115	14.5	9.0
115	22.5	13.0
Mean number + SE	22.5 + 1.7	10.3 + 1.1
TOTAL		
Mean number + SE	28.6 + 1.9	12.4 + 0.5

treated with 25 to 49 mM IBA (Shreve 1972, Shreve and Miles 1972). Rooting (0 to 100%) of juvenile black walnut (2-year-old seedlings) occurs after extensive etiolation and auxin treatments (Farmer 1971, Farmer and Hall 1973). Lee and others (1977) reported that cuttings taken from adventitious shoots of a 5-year-old *J. hindsii* tree had a significantly greater number of roots produced as a result of pretreatment with 2N sulfuric acid prior to a 10 sec dip in 15 mM IBA. Shreve (1990) reported rooting (percentage not stated) *J. microcarpa* (river walnut) softwood cuttings by treatment with 34 mM IBA and without the use of a mist bed.

This is the first report of field survival of *Juglans cinerea* (butternut) plants vegetatively propagated from cuttings of 6-year-old trees.

CONCLUSIONS

Juglans species are, for all practical purposes, recalcitrant to routine, commercial-scale, vegetative propagation. However, successful propagation of *J. cinerea* on a commercial scale can be achieved if the type of cutting (softwood), date of collection (early season), auxin concentration (62 mM K-IBA or 74 mM IBA), and greenhouse parameters (mist bed, supplemental lighting, etc.) are carefully considered. High field survival and good growth parameters can be achieved if plants are protected from deer browse and weeds are controlled.

ACKNOWLEDGMENT

The author gratefully acknowledges Daniel K. Struve and Brian K. Maynard for their constructive reviews and suggestions for improvement of this manuscript; John Seifert, Regional Extension Forester, Purdue University (SEPAC), and Phil O'Connor, Tree Improvement Specialist, Indiana Department of Natural Resources, Vallonia Nursery for outplanting, maintenance, and field data collection of own-rooted butternut plants.

LITERATURE CITED

Brinkman, K.A. 1974. *Juglans* L., Walnut. In: Schopmeyer, C.S. tech. coord. Seeds of woody plants in the United States. Agric. Handb. 450. Washington, DC: U.S. Department of Agriculture, Forest Service: 454-459.

Carpenter, S.B. 1975. Rooting black walnut cuttings with ethephon. Tree Planters' Notes. 26(3): 3, 29.

Cathey, H.M. 2003. The 2003 U.S. National Arboretum Web Version of the U.S. Department of Agriculture Plant Hardiness Zone Map. Misc. Publ. 1475, Issued January 1990. Washington, DC: U.S. Department of Agriculture, Agricultural Research Service, U.S. National Arboretum.

Cummings Carlson, J. 1993. Butternut: Are there any healthy trees left? Woodland Management. Spring: 11–12.

Dirr, M.A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation, and uses. 5th ed. Champaign, IL: Stipes Publishing L.L.C. 1187 p.

Farmer, R.E. 1971. Rooting black walnut cuttings. The Plant Propagator. 17(2): 7-9.

Farmer, R.E.; Hall, G.C. 1973. Rooting black walnut after pretreatment of shoots with indolebutyric acid. The Plant Propagator. 19(2): 13-14.

Furnier, G.R.; Stolz, A.M.; Mustaphi, R.M.; Ostry, M.E. 1999. Genetic evidence that butternut canker was recently introduced into North America. Canadian Journal of Botany. 77(6): 783–785.

Gautam, D.R.; Chauhan, J.S. 1990. A physiological analysis of rooting in cuttings of juvenile walnut (*Juglans regia* L.). Acta Horticulturae. 284: 33-44.

Innes, L. 1998. *Sirococcus clavigignenti-juglandacearum* on butternut and black walnut fruit. In: Laflamme, G.; Berube, J.A.; Hamelin, R.C., eds. Foliage, shoot, and stem diseases of trees; proceedings of the International Union of Forest Research Organizations. Working Party 7.02.02; 1997 May 25-31; Quebec City, Canada: 129-132.

Katovich, S.A.; Ostry, M.E. 1998. Insects associated with butternut and butternut canker in Minnesota and Wisconsin. The Great Lakes Entomologist. 31: 97-108.

Kwon, Y.J.; Youn, Y.; Lee, S.K.; Hyun, Y.I.; Lee, J.J.; Lee, M.H. 1990. In vivo rooting of shoots propagated by bud culture on *Juglans*. Research Report Institute Forest Genetics Korea. 26: 63-68.

Lee, C.I.; Paul, J.L.; Hackett, W.P. 1977. Promotion of rooting in stem cuttings of several ornamental plants by pretreatment with acid or base. HortScience. 12(1): 41-42.

Leopold, D.J.; McComb, W.C.; Muller, R.N. 1998. Trees of the central hardwood forests of North America: an identification and cultivation guide. Portland, OR: Timber Pres, Inc. 469 p.

Mehta, C.; Patel, N. 1996. LogXact for Windows: logistic regression software featuring exact methods. User manual. Cambridge, MA: CYTEL Software Corp.

Nair, V.M.G. 1999. Butternut canker - an internatinal concern. In: Raychaudhuri, S.P.; Maramorosch, K., eds. Biotechnology and plant protection in forestry science. Enfield, NH: Science Publishers, Inc.: 239–252.

Nair, V.M.G.; Kostichka, C.J.; Kuntz, J.E. 1979. *Sirococcus clavigignenti-juglandacearum:* an undescribed species causing canker on butternut. Mycologia. 71: 641–646.

Orchard, L.P. 1984. Butternut canker: host range, disease resistance, seedling-disease reactions, and seed-borne transmission. Madison, WI: University of Wisconsin. 145 p. Ph.D. dissertation.

Orchard, L.P.; Guries, R.P.; Kuntz, J.E. 1981. Butternut canker: screening seedlings for disease resistance. Phytopathology. 71: 247.

Orchard, L.P.; Kuntz, J.E.; Kessler. K.J. 1982. Reaction of *Juglans* species to butternut canker and implications for disease resistance. In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; West Lafayette, IN. Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 27– 31.

Ostry, M.E. 1997. Butternut canker: history, biology, impact and resistance. In: Van Sambeek, J.W. ed. Knowledge for the future of black walnut: 5th black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 192–199.

Ostry, M.E. 1998. Butternut canker in North America 1967–1997. In: Laflamme, G.; Berube, J.A.; Hamelin, R.C., eds. Foliage, shoot, and stem diseases of trees; proceedings of the International Union of Forest Research Organizations. Working Party 7.02.02; 1997 May 25-31; Quebec City, Canada: 121–128.

Ostry, M.E.; Mielke, M.E.; Anderson, R.L. 1996. How to identify butternut canker and mange butternut trees. HT-70. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northern Area State and Private Forestry.

Ostry, M.E.; Pijut. P.M. 2000. Butternut: an underused resource in North America. HortTechnology. 10(2): 302–306.

Pijut, P.M.; Barker, M.J. 1999. Propagation of *Juglans cinerea* L. (Butternut). HortScience. 34(3): 458-459.

Pijut, P.M.; Moore, M.J. 2002. Early season softwood cuttings effective for vegetative propagation of *Juglans cinerea*. HortScience. 37(4): 697-700.

Reil, W.O.; Leslie, C.A.; Forde, H.I.; McKenna, J.R. 1998. Propagation. In: Ramos, D.E., ed. Walnut production manual. Publ. 3373. Oakland, CA: University of California, Division of Agriculture and Natural Resources: 71-83.

Rink, G. 1990. *Juglans cinerea* L., Butternut. In: Burns, R.M.; Honkala, B.H., tech. coords. Silvics of North America. Volume 2, Hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 386–390.

Serr, E.F. 1964. Walnut rootstock. Proceedings of the International Plant Propagator Society. 14: 327-329.

Shreve, L.W. 1972. Propagation of walnut, chestnut, and pecan by rooted cuttings. Proc. 8th Central States Forest Tree Improvement Conference: 20-23.

Shreve, L.W. 1990. Propagating Texas black walnut, *Juglans microcarpa* and Texas pistachio, *Pistachia texana*, from rooted cuttings. Annual Report of the Northern Nut Growers Association. 81: 20-21.

Shreve, L.W.; Miles, N.W. 1972. Propagating black walnut clones from rooted cuttings. The Plant Propagator. 18(3): 4-8.

Sinclair, W.A.; Lyon, H.H.; Johnson, W.T. 1987. Diseases of trees and shrubs. Ithaca, NY: Cornell University Press. 574 p.

SPSS. 1998. SYSTAT 8.0 for Windows: statistics. Chicago, IL: SPSS Inc..

Tisserat, N.; Kuntz, J.E. 1984. Butternut canker: Development on individual trees and increase within a plantation. Plant Disease. 68: 613–616.

GENETIC TRANSFORMATION OF BLACK WALNUT (*JUGLANS NIGRA*)

Michael J. Bosela, Gurpreet S. Smagh, and Charles H. Michler[1]

ABSTRACT—Disarmed *Agrobacterium tumefaciens* strains with binary vectors carrying transgenes for kanamycin resistance (*npt II*) and β-glucuronidase (GUS, *uidA*) were used for the genetic transformation of Eastern black walnut (*Juglans nigra*) somatic embryos. In total, explants from 16 embryo lines, representing 10 genotypes, were used and regeneration was compared both in the absence of kanamycin selection and with different levels of selection stringency (200-250 or 500 mg/L kanamycin). Histochemical GUS expression assays indicated that the rates of T-DNA transfer were high (75-100%), with gene transfer being demonstrated for 14/16 lines. Regeneration was observed for 15% of the infected embryos, compared with frequencies of 50% for the non-infected controls. The regeneration frequencies were nearly two-fold higher when kanamycin was excluded from the culture medium, but most of the secondary embryos appeared non-transgenic. When kanamycin was present, most of the secondary embryos were transgenic, but chimeric, consisting of mixtures of transgenic and non-transgenic cells; and few exhibited *de novo* growth following harvest, even on non-selective medium. Less than 10% of the initial secondary embryos were wholly transgenic, as assessed by GUS assay; however, these embryos appeared essential for the initiation of stable transgenic lines. The establishment of transgenic lines was also facilitated by the use of lower kanamycin concentrations (200-250 mg/L) and by the precocious proliferation of the initial secondary embryos.

INTRODUCTION

In contrast with conventional breeding, which primarily involves gene manipulation at the level of entire chromosomal regions, genetic engineering is more precise. Genetic transformation techniques can be used to alter the expression patterns of individual genes in a predictable manner, with silencing, over-expression, and ectopic expression all being possible. Genes can be transferred across species barriers and genetic transformation protocols can be used for gene discovery.

Genetic transformation is especially attractive in light of the recalcitrance of most trees for conventional breeding. In contrast with agronomic crops, trees are characterized by long sexual generation times, high levels of heterozygosity (Hamrick 2000), and effective progeny screening is hindered by their large size and a frequent lack of juvenile:mature correlations, especially for vegetative characteristics (Ostry and Michler 1993, Tzfira and others 1998). For trees that are routinely grown in plantations, such as black walnut, the introduction genes for herbicide tolerance or pest resistance would be desirable. In addition, over a longer timeframe, strategies for the manipulation of wood quality traits (increased heartwood content, etc.) could be developed.

We are not aware of any previous publications describing the genetic transformation of black walnut; however, the genetic engineering of English walnut (*Juglans regia*), using disarmed strains of *Agrobacterium tumefaciens*, has been widely reported. In fact, *J. regia* was the first plant species (woody or non-woody) to be transformed using somatic embryos (McGranahan and others 1988, Dandekar and others 1989). Although the initial transformation frequencies were low (3%), the authors were able to establish that physical wounding was not necessary for gene transfer and that transformation competence was related to the size of the embryos. The efficacy of the

[1]Assistant Professor (MJB), Indiana University-Purdue University at Fort Wayne, Department of Biology, 2101 E. Coliseum Boulevard, Fort Wayne, IN 46805-1499; M.S. Candidate (GSS) and Director (CHM), Hardwood Tree Improvement and Regeneration Center, USDA Forest Service, North Central Research Station, Purdue University, 195 Marsteller Street, West Lafayette, IN 47907-2033. MJB is the corresponding author: to contact, call (260) 481-6038 or e-mail at boselam@ipfw.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

protocol was improved via the use of selective media (McGranahan and others 1990); however, the initial secondary embryos regenerated were frequently chimeric (i.e., consisting of mixtures of transgenic and non-transgenic cells) or entirely non-transgenic, even when selective media was used (McGranahan and others 1990, Escobar and others 2000). Derivative protocols have been developed in several additional labs (El Euch and others 1998, Tang and others 2000); however, in all cases, the development of efficient selection protocols has proved challenging.

In this research, we used the basic *J. regia* transformation protocol as a model for the development of initial *J. nigra* transformation protocols. However, our research differed from that of our predecessors in that media with multiple kanamycin concentrations were evaluated for selection. In addition, to increase the likelihood of concurrent secondary embryo induction and gene transfer, the embryos used for inoculation were larger (more mature) than those that have typically been used by previous experimenters.

MATERIALS AND METHODS

Plant Material

Somatic embryo cultures of *J. nigra* were induced from the cotyledon explants of immature zygotic embryos during the summer of 1999 (Bosela and Michler 2000). Walnut fruits were collected at 2-3 week intervals from mid-July to the end of August for culture initiation. In total more than 40 embryo lines (differing in genotype or induction media) were obtained, including nearly 20 lines that have been maintained continuously in vitro to date on a 3-5 week transfer cycle. The lines have been cultured primarily on basal (hormone-free) media; however the specific media types (MS or DKW) and sucrose concentrations (20 or 30 g/L) used have varied between the embryo lines.

Somatic Embryo Proliferation

Two mechanisms of proliferation were recognized for the lines, which are termed direct and indirect here, based on the degree of connectivity between the parent embryos and secondary embryos. Direct proliferation is characterized by the production of secondary embryos that are physically attached to the parent embryo (Fig. 1A). Most are produced from the cotyledons and are frequently arranged in rows that may correspond with the position of subtending vascular tissues (Fig. 1B). Cotyledon differentiation is typically observed shortly after embryo emergence (Fig. 1B), and continued proliferation is achieved via the subculture of the initial secondary embryos. However, since proliferation competence and embryo size are positively correlated, one to two culture periods of growth are typically required prior to the production of additional embryo generations.

Indirect proliferation is characterized by the production of secondary embryos that proliferate precociously (i.e., prior to cotyledon differentiation) to form masses of globular embryos, which lack obvious direct connections with the parent embryo (Figs. 1C, 1D). On basal medium, the degree of globular embryo proliferation is typically limited, with most enlarging and differentiating as cotyledonary embryos within 1-2 culture periods of their initiation. However, the continuous proliferation of globular embryos is generally possible upon transfer to MS medium with 2,4-D (0.2 – 2 µM). For the cumulative data set, indirect proliferation was only erratically observed; however, for some embryo lines, greater numbers of embryos were produced via indirect proliferation than direct proliferation. In addition, indirect proliferation appears to be facilitated by the use of MS media, elevated sucrose concentrations (30-50 g/L), and the inclusion of organic nitrogen sources (casein hydroslyate and glutamine).

Agrobacterium Strains

The GV3101/pMP90 strain of *Agrobacterium tumefaciens* (Koncz and Shell 1986) was used in conjunction with two binary vectors; pBI121 (Jefferson and others 1987) and pBISN1 (Narasimhulu and others 1996), depending upon the experiment. pBI121 and pBISN1 are nearly isogenic. Both are derived from pBIN19 (Bevan 1984) and contain a kanamycin resistance gene (*npt II*) on the plasmid backbone. In addition, the T-DNA (transferred DNA) regions are equivalent aside from differences in β-glucuronidase (*uidA*) gene construction (Fig. 2). In pBI121, the *uidA* gene is regulated by the CaMV 35S promoter (Guilley and others 1982), while in pBISN1 it is combined with the “super promoter” of Dr. Stanton Gelvin’s lab (Purdue University, West Lafayette, IN, USA). The super promoter consists of a trimer from the octopine synthase promoter’s upstream activator sequence and a single copy of the mannopine synthase promoter and its upstream activator (Ni and others 1995, Fig. 2). Although both promoters have been generally observed to be “strong” and constitutive in their expression patterns, in tobacco leaves the levels of GUS expression achieved using the super promoter have been shown to be up to 156-fold greater than the equivalent gene

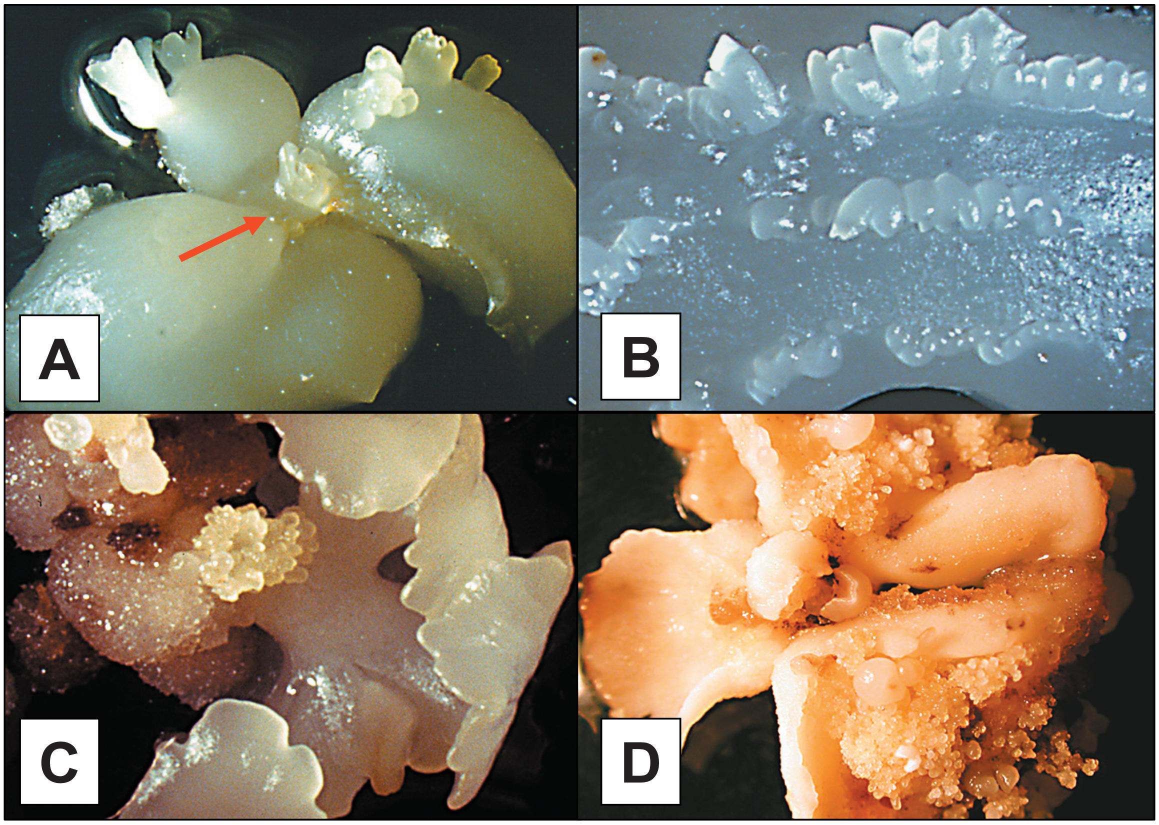


Figure 1.—Embryo proliferation mechanisms. (A) A larger embryo with direct secondary embryos from two of its three cotyledons. The epicotyl of the embryo is indicated with an arrow. (B) A cotyledon with rows of secondary embryos of direct origin. The largest secondary embryos shown are only 0.5-1 mm in size yet most have already differentiated cotyledons. (C) A cotyledonary embryo with a sheath of gelatinous callus and an associated mass of globular to polar embryos from its hypocotyl-root axis. (D) A larger embryo with masses of globular to polar embryos of indirect origin associated with its cotyledons.

expression levels observed for the CaMV 35S promoter (Ni and others 1995). The *uidA* gene of pBISN1 also differs from that of pBI121 in having an intron in its open-reading frame that precludes expression in *Agrobacterium* (Vancanneyt and others 1990, Liu and others 1992).

Transformation

Explants

In total, somatic embryos from the 16 distinct embryo lines, representing 10 distinct genotypes, were used. In general, the inoculated embryos were of direct origin, but embryos of indirect origin were also used. In either case, the embryos were generally isolated at an early cotyledonary stage of development and cultured individually for 3-4 weeks on basal medium prior to inoculation. The infected embryos averaged 7-10 mm in size, but some were significantly larger (up to 20-30 mm in diameter), and were frequently broken either inadvertently during disinfection or intentionally (prior to co-cultivation) to facilitate contact between the explants and antibiotics in the media.

Bacteria Cultures

The *Agrobacterium* cultures were grown in Luria-Bertaini (LB) media [10% (w/v) tryptone, 5% (w/v) yeast extract, 10% (w/v) NaCl, pH 7.0] on an orbital shaker at 28° C at 200 rpm. The initial cultures were prepared in 3 mL volumes and 0.5-1 mL aliquots from these cultures were used to seed larger cultures (25-50 mL) that were used for infection. Gentamycin (25 mg/L) and kanamycin (50 mg/L) were used to select for the disarmed Ti plasmid (pMP90) and binary vectors, pBI121 and pBISN1, respectively. The LB media used for the initial (3 mL) cultures, was also spiked with rifampicin (40 mg/L) to preclude *Escherichia coli* contamination. Once the cultures had grown to an absorbance at 600 nm (A_{600}) of 0.3-0.9, they were pelletted by centrifugation (1000x*g*, 10-15 min) and re-suspended in antibiotic-free media at a density of approximately ≈ 5 x 10^8 cells/mL (Leple and others 1992, Han and others 1996).

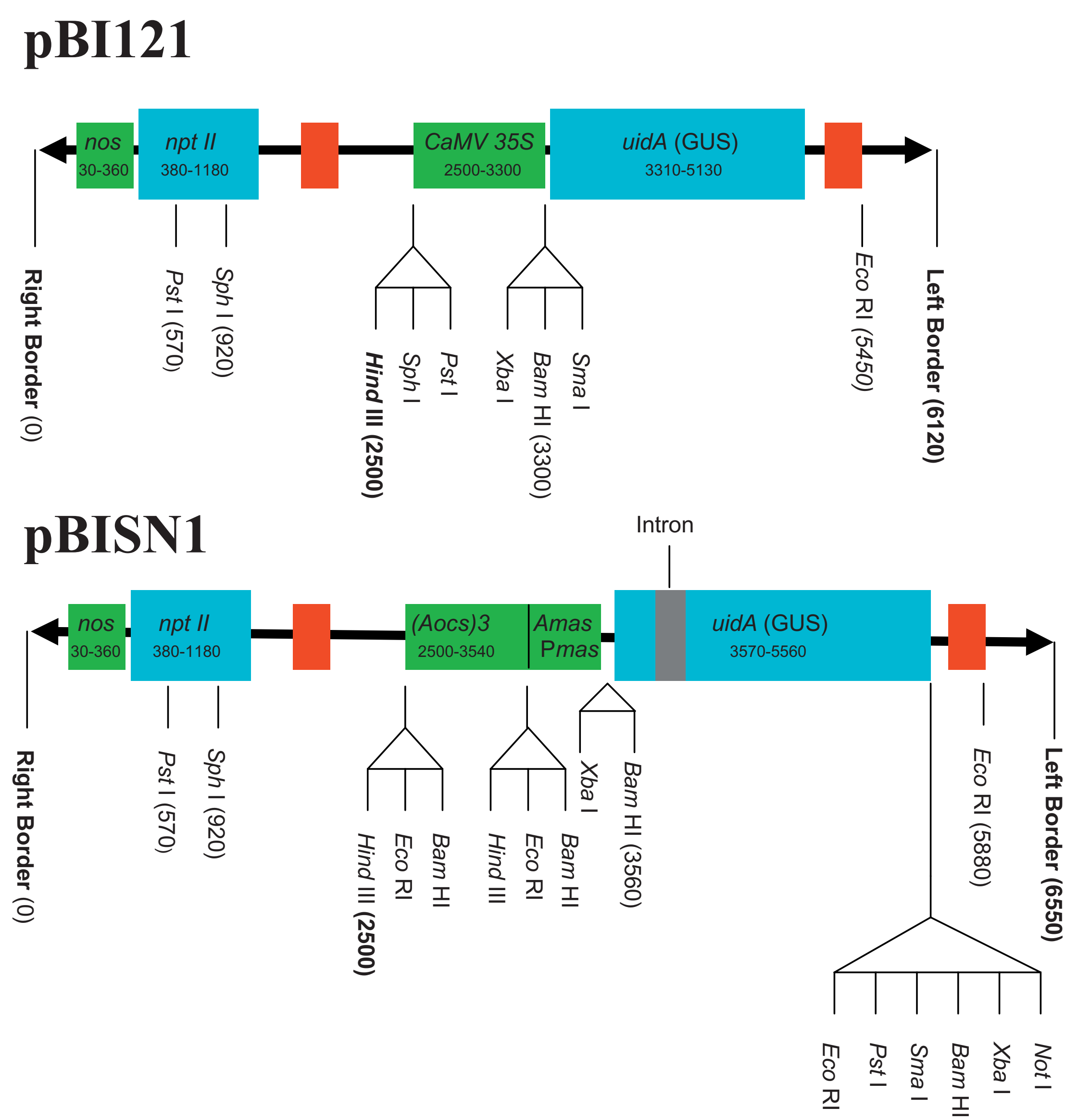


Figure 2.—Physical maps of the pBI121 and pBISN1 T-DNA regions. The T-DNA border sequences are indicated with arrowheads, and colored boxes are used to indicate the positions of promoters, open reading frames, and terminators. Green boxes indicate promoter elements; blue boxes indicate open reading frames; and red boxes indicate transcription terminator sequences. Map positions (± 10 bps) are indicted relative to the position of the right T-DNA border sequences. The cutting sites for several common restriction enzymes are also shown. Note: In pBISN1, the uidA promoter is chimeric, consisting of a trimer upstream activator sequences from the octopine synthase (ocs) promoter and a single copy of the mannopine synthase (mas) promoter and upstream activator (see Materials and Methods).

For the 2002 experiments (n = 3), LB medium was used for *Agrobacterium* re-suspension. In contrast, the cultures from the 2003 experiment were resuspended in liquid plant culture media (MS vs DKW). Acetosyringone (20 µM) was added and the cultures were grown for an additional 20-30 min for virulence gene induction.

Infection, Co-cultivation, and Disinfection

For infection, the somatic embryos were divided into sterile 50 mL centrifuge tubes with aliquots (3-5 mL) from the virulence-induced *Agrobacterium* cultures and placed on a rotary shaker at room temperature (23 ± 2° C). After 20-30 min the embryos were removed, blotted dry, and transferred to plates of basal medium with 100 µM acetosyringone in the dark for co-cultivation. The embryos were removed from the co-cultivation plates after an additional 50-65 hr and “washed” five to six times, with the final three washes containing antibiotic (500 mg/L cefotaxime for the 2002 experiments or 500 mg/L timentin for the 2003 experiment) to prevent the growth of residual *Agrobacterium* cells. The explants were washed in sterile 50 mL centrifuge tubes on a rotary shaker with 15-20 min/wash. The medium used for washing (sterile water or liquid plant culture media) varied between experiments.

Regeneration

Following washing, the embryos were blotted dry on sterile paper towels and transferred to plates of basal medium containing the same antibiotics as had been used for washing, (i.e., 500 mg/L cefotaxime or 500 mg/L timentin). For each embryo source, the embryos were divided between selective and non-selective media. In general, only 4-6 embryos were assigned to the non-selective medium, and the rest were assigned to selective medium. For most embryo sources, only a single replicate of each treatment was prepared; however, when enough explants were present, multiple replicates of the kanamycin treatments were prepared (5-10 embryos per replication).

The explants were transferred to fresh media every 3-4 weeks. Embryos assigned to the selective media were maintained for three culture periods, while those assigned to non-selective media (and the controls) were maintained for only two culture periods. Secondary embryos were harvested at the end of the same culture period when they first became apparent and sorted into transformation events based on their spacing (i.e., embryos found within 5 mm of one another were considered to be derived from the same transformation event, while embryos spaced further apart were presumed to represent separate transformation events). Depending on the number of transformation events and embryos per transformation event, the secondary embryos were subcultured (to one or more media types), stained for GUS activity, or both.

The labeling system of McGranahan and others (1988) was used to distinguish between embryo generations (i.e., the inoculated explants were designated as E_0 embryos, the initial secondary embryos were termed E_1 embryos, and the subsequent generations of embryos were assigned progressively higher order subscripts [E_2, E_3, etc.]). Proliferation was generally direct with the embryo generations being separated by 1-2 culture periods of growth. However, when the initial secondary embryos proliferated indirectly (at the globular stage), embryo generations were impossible to distinguish.

Selection

For the 2002 experiments, media with lower (250 mg/L) and higher (500 mg/L) kanamycin concentrations were tested in separate experiments. However, for the 2003 experiment, the effects of differences in kanamycin concentration were evaluated more directly. The basic selective medium contained 500 mg/L kanamycin; however, for lines with sufficient numbers of embryos for the preparation of three more replicates, the embryos were divided between media with 500 mg/L kanamycin (2 replicates) and 200 mg/L kanamycin (single replicates). The 200 and 500 mg/L kanamycin media were also compared using E_1 and E_2 embryos, especially when multiple embryos were available from the same transformation events.

The sensitivity of non-transgenic walnut somatic embryos to kanamycin was evaluated in a series of preliminary, supplemental experiments. Kanamycin was tested at concentrations of 25, 50, 100, 250, 500, and 1000 mg/L, using both small (2-5 mm) and large (10-20 mm) cotyledonary embryos. Embryos from multiple lines were evaluated; however, replicates of the treatments were not prepared on a per embryo line basis. After 4-6 weeks, the embryos were evaluated for proliferation. The health of the embryos was also assessed and the small cotyledondary embryos were evaluated for de novo growth.

GUS (*uidA*) Expression Assays

Somatic embryos were screened for GUS activity by histochemical assay (Jefferson and others 1987). The staining solution contained 1mM X-Gluc, 10 mM EDTA, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 0.1 M sodium phosphate buffer (pH 7.2), 0.1% Triton X-100, and 2% dimethyl sulfoxide (DMSO), as recommended by Stomp (1992). Staining was carried out at 37° C for 16 to 20 h, followed by clearing in 70% ethanol. Samples with blue staining (indigo precipitate) were scored as positive for GUS gene activity.

RESULTS AND DISCUSSION

Initial (E_1) Regeneration

Secondary Embryo Formation

For the 2003 experiment, the regeneration competence of the E_0 embryos was adversely affected by co-cultivation (Table 1), as has been previously observed for *J. regia* somatic embryos (Tang and others 2000). Similar differences in regeneration frequency between the infected and non-infected embryos were not apparent for the 2002 experiment, presumably as a result of the recalcitrance of the embryos for regeneration in general, with secondary embryo formation being observed for only 15% of the control embryos (Table 1), compared with more typical regeneration frequencies of 40-75% as assessed during culture maintenance (data not shown). Several factors, including differences in binary vector type, antibiotic type, and embryo line representation may have contributed to the contrasting regeneration responses. However, the use of different media for *Agrobacterium* resuspension (i.e., LB medium for the 2002 experiments and plant medium for the 2003 experiments) is thought to have played a critical role.

For the infected embryos, the regeneration frequencies were approximately twice as high when non-selective media was used (Table 1), and the differences were statistically significant ($\propto$ = 0.05) for both data sets (2002 and 2003) as evaluated by Chi-Square analysis. The regeneration frequencies were also highly dependent on the concentration of selective agent, with the frequencies of secondary embryogenesis being two- to three-fold greater when lower (200-250 mg/L) rather than higher kanamycin concentrations (500 mg/L) were used (Table 2). The number of transformation events and secondary embryo yields per responsive explant (E_0 embryo) were also much higher for the 200 mg/L kanamycin treatment (Table 2).

Table 1.—Regeneration frequency by experimental treatment and calendar year. For each treatment, the data is combined across embryo lines and experimental replicates. The direct, indirect, and cumulative (total) regeneration frequencies are indicated.

Year[1]	Treatment[2]	N	Regeneration (%) Total	Direct	Indirect
2002	Control (not infected)	20	15	15	0
	No selection	54	14	10	4
	Kanamycin selection	215	8	7	1
2003	Control (not infected)	101	57	54	3
	No selection	85	30	24	6
	Kanamycin selection	346	17	16	1

[1] *Experimental conditions varied between years. For the 2002 experiments, the GV3101/pMP90/pBI121* A. tumefaciens *strain was employed, the bacteria were resuspended in LB medium prior to infection, and cefotaxime was used for Agrobacterium elimination. For the 2003 experiment, a different binary vector (pBISN1) was employed, plant media (MS or DKW) was used for resuspension, and timentin was used for Agrobacterium elmination.*

[2] *The control embryos were subjected to the same infection, co-cultivation, and disinfection regimes as were typical for the experimental treatments; however, "blank"* Agrobacterium *resuspension medium was used for inoculation and antibiotic-free media were used for regeneration.*

Table 2.—Relationship between kanamycin (Kan) concentration and transformation efficacy.

Year[1]	Kan (mg/L)	% Regeneration	Transformation Events Per Responsive Embryo[2]	Secondary Embryo Yield Per Responsive Embryo[3]
2002	250	18	N.D.	N.D.
	500	5	N.D.	N.D.
2003	200	33	2.9	5.7
	500	13	1.4	1.4

[1] *In 2002, the 250 and 500 mg/L kanamycin media were tested in separate experiments, employing different combinations of embryo lines. In 2003, the 200 and 500 mg/L kanamycin media, were tested in a single experiment using explants that were genotypically and physiologically equivalent. Note: The regeneration frequencies presented here are not directly comparable with those shown in Table 1, since the 2003 data presented here is only for the subset of embryo lines that were tested on both types of kanamycin media.*

[2] *The mean number of transformation events per embryo for the subset of E_0 embryos with regeneration (i.e., secondary embryogenesis). Note: The E_1 embryos from the 2002 experiments were not sorted by transformation event.*

[3] *Mean yield of secondary embryos per embryo (combined across transformation events, see above) for the subset of E_0 embryos with regeneration, as calculated for the 2003 experiment.*

GUS Expression Assays

To assess the frequency of T-DNA transfer and integration, several E_0 embryos that had remained sterile and relatively healthy, as assessed by their limited degree of discoloration, were stained for GUS activity. For the 2002 experiments nearly 85% (21/25, data not shown) of the E_0 samples tested positive. Since the uidA gene used for the 2002 experiments did not contain an intron it is impossible to exclude the possibility that the blue staining may have resulted from T-DNA expression by residual, interstitial *Agrobacterium* cells. However, similarly high staining frequencies were obtained for the 2003 experiment (Table 3).

The preferential staining of only the healthiest E_0 embryos may have biased the staining results, especially if the rates of embryo discoloration and T-DNA transfer were inversely related. However, since the E_0 embryos evaluated in this study were stained primarily at the end of the second or third culture periods, their degree of browning (senescence) was frequently quite advanced, and selection of the least discolored embryos was thought necessary to ensure the visualization of cells exhibiting a positive staining response. In fact, it could be argued that T-DNA transfer rates reported are lower than the staining frequencies that would have been obtained if the embryos had been stained by the end of the first culture period. However, perhaps even more telling than the high frequencies of GUS-positive staining observed for the cumulative data set, is the consistency with which positive staining results were observed across embryo lines; i.e., stable *uidA* gene integration was demonstrated for 14 of 16 embryo lines evaluated, representing 9 of 10 genotypes.

For most E_0 embryos the staining observed was primarily distributed as discrete blue spots, the smallest of which were estimated to be only 20-25 µM in size, and thought to represent single transgenic cells (Figs. 3A, 3B). However, for some of the E_0 embryos, a subset of the blue spots were distinctly larger (0.2-0.5 mm in diameter) and were presumed to represent clones of daughter cells from the same transformation event (Fig. 3C). In other cases patches or bands of blue were apparent, especially in the vicinity of regions, such as the root axis or cotyledon bases, that would be expected to show rapid and directional growth during embryo development (Fig. 3D). The density of blue foci (spots and patches) was highly variable between embryos (Fig. 3) and appeared to be related to differences in embryo growth rate (i.e., the number of transformation events detected per embryo was greatest for embryo sources that exhibited the most vigorous growth as non-infected controls [data not shown]).

Table 3.—Cumulative X-Gluc staining data for the 2003 experiment. Data are presented only for the subset of E_1 and E_2 embryos that were cultured on the same media type (selective vs. non-selective) as had been used for the E_0 embryos.

Treatment	Embryo Type[1]	N	GUS Positive N	GUS Positive %	Staining Distribution (%)[2] Small spots	Larger spots	Patches	Entire
Kanamycin Selection	E_0	44	44	100	89	18	25	0
	E_1 – Direct	39	37	87	82	12	3	16
	E_1 – Static	52	34	65	100	3	6	0
	E_1 – Growth	4	3	75	0	0	0	100
	E_2 – Direct	9	9		0	0	0	100
	E_2 – Growth	8	8		0	0	0	100
No Selection	E_0	38	28	74	61	21	29	0
	E_1 – Direct	27	8	26	43	14	0	43
	E_1 – Static	9	3	33	33	0	33	33
	E_1 – Growth	32	0	0	-	-	-	-
	E_2 – Direct	6	0	0	-	-	-	-
	E_2 – Growth	0	-	-	-	-	-	-

[1] The E_1 and E_2 embryos are distinguished based upon whether they were stained directly or after one or more months of individual culture. Those that were subcultured are further distinguished based upon whether they exhibited a positive growth response.

[2] Four basic staining patterns were recognized for the subset of embryos that tested GUS-positive: 1) chimeric staining consisting of smaller blue spots (< 0.5 mm diameter), 2) chimeric staining consisting of larger spots (> 0.5 mm tviameter), 3) chimeric staining consisting of discrete patches or sectors of blue (at least 2-3 mm in size), and 4) continuous blue staining (wholly transgenic embryos). The percentage of embryos that were scored positive for each staining pattern is indicated. Note: Since some of the chimeric embryos scored positive for multiple categories, the percentages frequently add up to more than 100%.

[A] All of the E_2 embryos evaluated were derived from the same two transformation events.

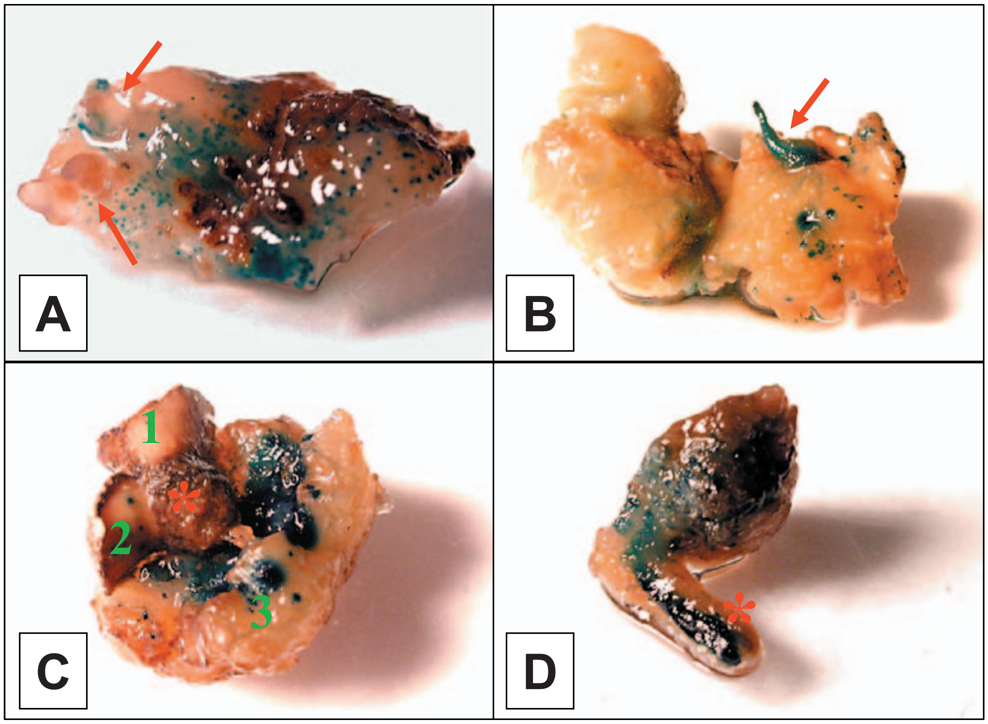


Figure 3.—T-DNA transfer and GUS expression patterns in the infected embryos (E_0 generation). (A) An E_0 explant stained at the end of the first regeneration culture period. Notice the variable size of the blue spots. The smallest spots, which were estimated to be 35-50 micrometers in diameter may have represented single transgenic cells. Two secondary embryos (E_1) are visible on the left side of the explant (arrows). One of the embryos is entirely unstained, while the second (upper) contains a few blue spots and differs little in staining pattern from that of the E_0 embryo. (B) An explant stained at the end of the second culture period. The number of blue spots is more limited here; however, a wholly transgenic E_1 embryo (with a tubular morphology, see arrow) has developed from one of the spots of GUS-positive cells. (C) An E_0 embryo with three distinct cotyledons as viewed from below. Note the larger diameter of the blue spots for the cotyledon, which has grown in size since co-cultivation, compared with the size of the blue spots on the smaller (ungrown) cotyledons (labeled as 1 and 2). (D) An E_0 embryo with spots of GUS positive cells on its cotyledon and a longitudinal band of GUS-positive cells along its hypocotyl-root axis, suggesting that most of the axis elongation has occurred since gene transfer (co-cultivation). Note: Asterisks indicate the root axes of the embryos (when visible).

The frequency of GUS-positive E_1 embryos varied widely between experimental treatments. When non-selective media was used for regeneration, only 16% (11/68) of the E_1 embryos tested positive for GUS expression (Table 3; cumulative data, combined across embryo types). In addition, the degree of E_1 embryo growth and the frequency of staining appeared to be inversely related (i.e., nearly 30% of the embryos that were stained at an early cotyledonary stage, tested positive for GUS activity; however, none of the embryos assayed after one or more culture periods of growth exhibited definite staining (Table 3). When kanamycin was included in the regeneration medium, positive staining results were obtained for nearly all of the E_1 embryos evaluated directly (87%, Table 3), irrespective of the specific kanamycin concentration used (data not shown). The percentage of GUS-positive embryos was lower for the subset of embryos that were stained after one or more months of culture (65-75%, Table 3). However, since most of the embryos had failed to exhibit de novo growth, the lower staining frequencies may have been a consequence of embryo quiescence rather than a lack of gene transfer.

The effects of the presence or absence of selection agent on the frequency of transgenic embryo regeneration has also been evaluated in *J. regia*. For the study in question (McGranahan and others 1990), the EHA105/pBI 121 *Agrobacterium* strain was used, 100 mg/L kanamycin was used for selection, and the E_1 embryos were stained directly. However, only small samples from the E_1 embryos were stained and the differences in staining frequency were even more dramatic than those observed here (i.e., only 0.05% of the embryos regenerated on non-selective media testing positive for GUS activity, compared with GUS-positive frequencies 31% for those regenerated on kanamycin medium).

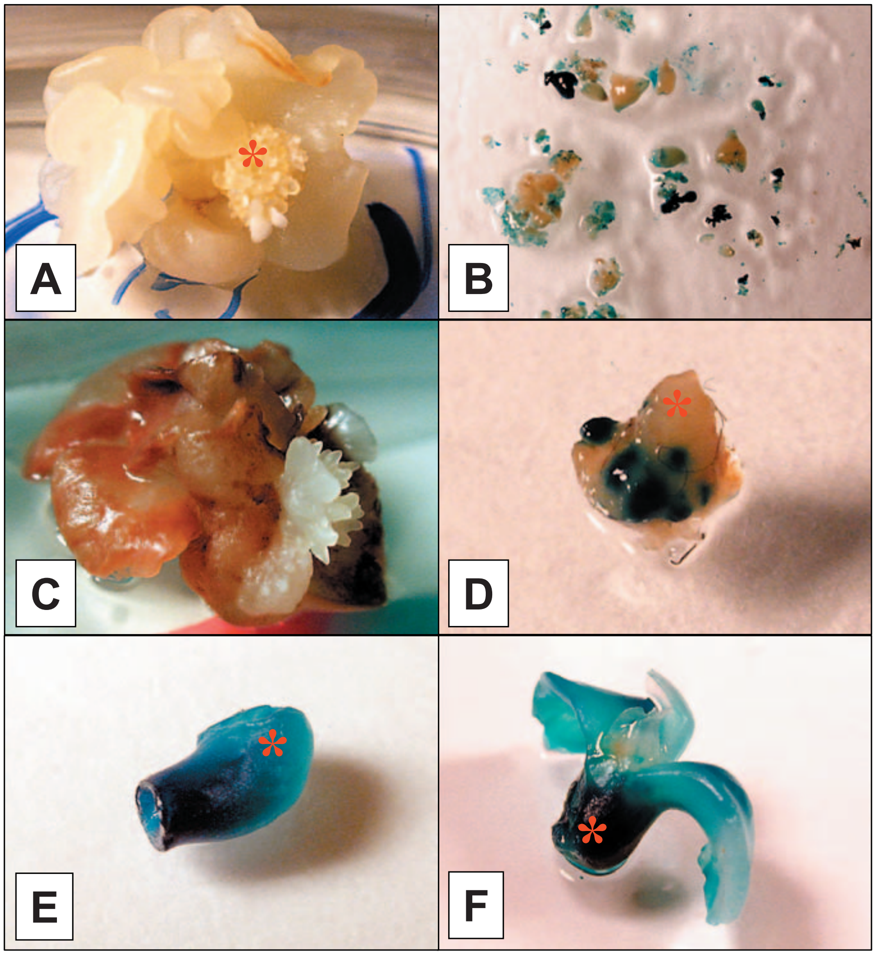


Figure 4.—Secondary embryo regeneration and GUS staining phenotypes. (A) A mass of globular to polar-shaped embryos of indirect origin from its root pole of an E_0 embryo on non-selective medium. (B) The X-Gluc staining patterns that were observed for a similar group of secondary embryos harvested from an explant on selective medium (500 mg/L kamanycin). Most of the embryos appeared either wholly transgenic or wholly non-transgenic. (C) A row of secondary embryos of direct origin from an E_0 embryo cultured on 200 mg/L kanamycin medium. (D) An E_1 embryo of direct origin following X-Gluc staining. Notice the larger spots of blue, some of which appear to be associated with spots of localized tissue growth which may represent early secondary embryos. The asterisk indicates the position of the embryo's root pole (facing upwards). (E) An E_2 embryo that appears wholly transgenic as evidenced by its extensive blue coloration. (F) A larger, well-differentiated cotyledonary embryo from an established transgenic line. Note: Asterisks indicate the root axes of the embryos.

Despite the higher frequencies of transgenic embryo regeneration observed in the current study, most of the E_1 embryos that tested positive for GUS staining were chimeric (Fig. 4D), irrespective of the experimental treatment (Table 3). Although some of the E_1 embryos may have been present prior to co-cultivation, in which case chimeric staining would have been expected, very few of the E_0 embryos bore secondary embryos at the time of inoculation, as assessed by visual inspection (data not shown). For the subset of E_1 embryos regenerated on kanamycin medium, only 6/39 (16%) of the embryos that were directly stained appeared fully transgenic (100% blue). However, three additional transformation events that had produced fully transgenic E_1 embryos, but had been overlooked at the point of data collection, were identified in association with the evaluation of the stained E_0 embryos. The percentages of GUS-positive E_1 embryos that stained wholly blue was greater for the no-selection treatment (33-43%, Table 3); however, since the sample sizes of GUS-positive embryos were so small (n = 3-8), these percentages may not be representative of the population-level means.

For the direct selection treatment, the percentage of wholly transgenic E_1 embryos was related to the mode of origin of the secondary embryos and

Table 4.—Relationships between secondary embryo origin, frequency of transformation events yielding wholly transgenic E_1 embyros[1], and the regeneration timecourse for the 2003 experiment direct selection treatment.

			Regeneration Time Course[4]		
Origin[2]	N[3]	% Wholly GUS Positive	Culture Period[1]	Culture Period[2]	Culture Period[3]
Direct-cotyledon	92	7	47	90	100
Direct-root	5	40	20	80	100
Indirect	4	75	0	25	100

[1] When regeneration was indirect, the initial harvested embryos presumably consisted of mixtures of E_1 embryos and subsequent embryo generations (E_2, E_3, etc). Wholly transgenic E_1 embryos were defined as those staining 100% blue when tested with X-Gluc (see Table 3)

[2] The transformation events were distinguished based on whether the E_1 embryos were of direct or indirect origin, with those of direct origin being further distinguished based on whether the secondary embryos were derived from the cotyledons or root of the parent (E_0) embryo.

[3] The number of transformation events. Each transformation event consisted of one or more E_1 embryos that were located together on the surface of an E_0 embryos (i.e., within 5 mm of one another)

[4] For each class of E_1 embryos, the percentage of the total number of transformation events that were apparent at the end of each regeneration culture period is indicated.

the timing of embryo formation (Table 4). For most of the transformation events evaluated, the initial secondary embryos produced were of direct cotyledonary origin. The rate of secondary embryo formation was rapid, with 90% of the transformation events being apparent by the end of the second culture period; however, fully transgenic E_1 embryos were noted for only 7% of the transformation events. In contrast, for the subset of transformation events that involved the production of secondary embryos of either root or indirect origin, the frequencies of wholly GUS-positive E_1 embryos were much higher (40-75%) and the rate of regeneration was slower (Table 4).

Similar relationships between the frequency of GUS positive E_1 embryos and the rate of embryo regeneration have been observed for *J. regia* somatic embryos, as assessed via the staining of samples from the cotyledons of the E_1 embryos (McGranahan and others 1990). For the study in question only 5% of the embryos harvested within 6 weeks of co-cultivation tested positive for GUS activity, compared with GUS-positive frequencies of 11-40% for the subset of embryos harvested within 7-13 weeks of co-cultivation, and 55-65% for the subset harvested 14-16 weeks after co-cultivation. In addition, staining assays involving the evaluation of later generation embryos derived from the initial E_1 regenerants, indicated most of the embryos that tested positive at the E_1 stage had been wholly transgenic.

The relationships between the rate of embryo regeneration and percentage wholly transgenic secondary embryos observed here and by McGranahan and others (1990) can be interpreted in terms of differences in the degree of embryo development at the time of gene transfer. In particular, secondary embryos transformed at a multicellular stage of development, which would exhibit chimeric staining, would be expected to appear prior to the emergence of embryos transformed at the unicellular stage, which would stain wholly blue. However, the higher frequencies of fully GUS-positive embryos that were observed for the transformation events involving the production of E_1 embryos of root or indirect origin that were observed in this study cannot be fully accounted for by differences in the rate of embryo regeneration. In fact, the frequency of transformation events yielding fully transgenic E_1 embryos was significantly lower for the transformation events involving direct embryo formation from the cotyledons of the infected embryos, even when the data was compared only for the subset of transformation events, where regeneration was not apparent until the end of the third culture period (data not shown). For the transformation events involving indirect embryo proliferation, the regeneration of stable transgenic lines may have been facilitated by the precocious proliferation of chimeric E_1 embryos prior to the onset of a state of embryo quiescence (see below).

E_1 Proliferation and Establishment of Transgenic Lines

For the 2003 experiment, none of the E_1 embryos that were regenerated on kanamycin-free media continued to grow or proliferate following transfer to kanamycin media for either of the concentrations tested. *De novo* growth and proliferation were observed for most of the embryos kept on non-selective medium (Table 5); however, none of the E_2 embryos produced tested positive for GUS activity (Table 3). The number of embryos evaluated was limited (6-35 depending on the parameter); however, similar results were obtained for *J. regia* by Tang and others (2002) (i.e., when non-selective regeneration media was used, the frequency of GUS-positive E_2 embryos ranged from 0-2% depending on the antibiotic used for Agrobacterium elimination). In contrast, when kanamycin (100 mg/L) was used for selection, 14-22% of the embryos tested GUS positive.

The recalcitrance of the E_1 embryos regenerated on kanamycin medium for continued growth and proliferation was unexpected. Irrespective of the type of media used (selective or non-selective), less than 10% of the subcultured E_1 embryos exhibited *de novo* growth (Table 5). *De novo* growth was observed for three of the embryos transferred to non-selective medium (3/34) and two of the three produced additional generations of secondary embryos; however, none of the E_2/E_3 embryos tested positive for GUS activity (0/14) and none were kanamycin tolerant (0/12). In contrast, for five of the seven E_1 embryos that were maintained on selective medium and exhibited *de novo* growth, stable transgenic lines (representing two distinct transformation events) were obtained. Although only two of the five E_1 embryos that yielded transgenic lines were evaluated by X-Gluc assay, both stained fully blue. In contrast, none of the E_1 embryos from the selective media plates that had failed to grow or proliferate following harvest stained 100% blue (Table 3).

For the combined data sets (2002, 2003) three distinct transgenic lines, representing separate embryo genotypes, were obtained. However, all three of the lines were derived from E_0 embryos that had been cultured on regeneration media with 200-250 mg/L kanamcyin, suggesting that some or all of the kanamycin concentrations used in this study (200-500 mg/L) may have been supraoptimal for line establishment. In a

Table 5.—Growth and proliferation responses of E_1 embryos from the 2003 experiment. The data is sorted by experimental treatment and media type, but is combined across embryo lines and kanamycin (Kan) concentrations.

Treatment	Media	N	% Growth[1]	% Prolif[2]	% Producing Stable Lines[3]
No Selection	Kan	15	0	0	0
	No Kan	35	63	55	0
Kanamycin	Kan	114	7	5	5
Selection	No Kan	34	9	6	0

[1] *Percentage with definite cotyledon enlargement (to at least 5 mm in maximal dimension).*

[2] *Percentage producing secondary embryos, typically observed after 2-3 culture periods.*

[3] *Percentage giving rise to stable kanamycin-resistant lines.*

Table 6.—Kanamycin (Kan) sensitivity of non-transgenic walnut somatic embryos. The data presented is pooled from two experiments. For each experiment embryos from 4 to 8 lines were evaluated, with two to four embryos from each line being evaluated per media type. The cumulative data (collected after 5-6 wks of culture and pooled across embryo sources) are presented. Proliferation data are presented only for the subset of embryo lines that had produced secondary embryos on control medium (without kanamycin).

Media	Small Cotyledonary[1]			Large Cotyledonary[2]	
(mg/L Kan)	N[3]	% Grown[4]	% Prolif[5]	N	% Prolif[5]
0	29 (18)	79	75	16	44
25	21 (14)	81	64	16	50
50	28 (19)	75	16	16	31
100	29 (18)	72	0	16	0
250	29 (18)	21	17[A]	24	12[A]
500	29 (18)	3	0	24	0
1000	29 (18)	0	0	24	0

[1] *The embryos were 2-5 mm in total length with cotyledons 1-3 mm long.*

[2] *The embryos were 10-20 mm in dimension with cotyledons 7-15 mm long.*

[3] *The sample size for the % proliferation data is indicated in parentheses.*

[4] *% embryos with cotyledon enlargement as visually assessed.*

[5] *% embryos producing secondary embryos.*

[A] *For two-thirds of the embryos that exhibited proliferation on 250 mg/L kanamycin medium, the secondary embryos produced were of indirect, rather than direct, origin.*

series of preliminary kanamycin dose-response experiments, embryo proliferation was only erratically observed at kanamycin concentrations of 100 mg/L or greater (Table 6), and for several embryo lines, the secondary embryos produced in the presence of kanamycin exhibited retarded cotyledon differentiation and would not have been expected to produce additional generations of embryos of direct origin. Similarly, sub-lethal doses of kanamycin have been observed to antagonize shoot regeneration in several plant species (Yepes and Aldwinkle 1994, Tosca and others 1996, Peros and others 1998, Humara and Ordas 1999, Kapaun and Cheng 1999). In addition, in some studies, kanamycin has been found to interfere with plant regeneration even following NPT II gene incorporation (Mullins and others 1990, Robertson and others 1992, Ellis and others 1993, Aldemita and Hodges 1996, Azhakanandam and others 2000).

For *J. regia*, 100 mg/L kanamycin have routinely been used for the selection of transgenic embryos (McGranahan and others 1990; Dandekar and others 1989, 1998; Escobar and others 2000; Tang and others 2000); however, this medium has been shown to be ineffective for the exclusion of non-transgenic secondary embryos in multiple labs (McGranahan and others 1990, Tang and others 2000). In addition, for certain *J. regia* x *J. nigra* lines the regeneration of non-transformed escapes has been observed even at kanamycin concentrations of up to 500 mg/L. However, in contrast with the results obtained in this study, when media with 100 mg/L kanamycin were used, the continued growth and proliferation of chimeric E_1 regenerants was possible (McGranahan and others 1990, Tang and others 2000).

The delayed kanamycin sensitivity that was exhibited by the chimeric secondary embryos regenerated in this study suggests that the degree to which populations of transgenic black walnut cells are able to protect an embryo from the inhibitory effects of kanamycin exposure may be related to the size of the embryo and/or its level of tissue differentiation. During the initial stages of somatic embryogenesis, the transgenic sectors of the chimeric E_1 regenerants were apparently able to cross-protect adjacent non-transgenic cells. However, with continued (symmetric) embryo growth, the ability of the transgenic cells to provide immunity for the entire E_1 embryo decreased, presumably as a result of the increased spacing between the transgenic and non-transgenic cell groups and/or changes in NPT II diffusion/transport characteristics, to such a degree that the competence of the embryos for continued growth and proliferation was ameliorated even prior to the point of embryo harvest.

CONCLUSIONS AND FUTURE WORK

Despite the high rates of gene transfer and integration that were documented across multiple genotypes, robust protocols for the selection of transgenic *J. nigra* embryos and subsequent establishment of transgenic lines remain to be developed. When kanamycin (200-500 mg/L) was used for selection, nearly all of the initial regenerants were transgenic; however, most were chimeric and appeared incapable of continued growth and line establishment, at least via a direct proliferation pathway. Future investigations in our lab will focus on a more systematic evaluation of the kanamycin sensitivity of black walnut somatic embryos (both in the presence and absence of a heterologous NPT II gene), with the primary objective of identifying kanamycin concentrations that could be used to block non-transgenic regeneration without inhibiting the subsequent growth and proliferation of chimeric secondary embryos, which accounted for the majority of the regenerants in this and other studies. Other research possibilities include an evaluation of the effects of E_0 embryo size on the transformation response. The use of smaller (younger) embryos for infection would presumably decrease the likelihood of secondary embryo formation prior to gene transfer, resulting in reductions in the frequency of chimeric embryo regenerantion. However, for high transformation rates to be achieved, the initial transgenic cells would need to be able to divide relatively freely, so as to produce populations of transgenic cells (i.e., entire blue sectors or patches) from which embryos were subsequently regenerated.

LITERATURE CITED

Aldemita, R.R.; Hodges, T.K. 1996. *Agrobacterium tumefaciens*-mediated transformation of Japonica and Indica rice varieties. Planta. 199: 612-617.

Azhakanandam, K.; McCabe, M.S.; Power, J.B.; Lowe, K.C.; Cocking, E.C.; Davey, M.R. 2000. T-DNA transfer, integration, expression, and inheritance in rice: effects of plant genotype and *Agrobacterium* super-virulence. Journal of Plant Physiology. 157: 429-439.

Bevan, M. 1984. Binary *Agrobacterium* vectors for plant transformation. Nucleic Acids. 12: 8711-8721.

Bosela, M.J.; Michler, C.H. 2000. Comparative studies of somatic embryogenesis in *Quercus rubra* and *Juglans nigra*. *In Vitro* Cellular and

Developmental Biology – Animal. 36: 3, pt. II: 60-A. 2014.

Dandekar, A.M.; McGranahan, G.H.; Leslie, C.A.; Uratsu, S.L. 1989. *Agrobacterium*-mediated transformation of somatic embryos as a method for the production of transgenic plants. Journal of Tissue Culture Methods. 12: 145-150.

Dandekar, A.M.; McGranahan, G.H.; Vail, P.V.; Uratsu, S.L.; Leslie, C.A.; Tebbets, J.S. 1998. High levels of expression of full-length *cryIA*(c) gene from *Bacillus thuringiensis* in transgenic somatic walnut embryos. Plant Science. 131: 181-193.

El Euch, C.; Jay-Allemand, C.; Pastuglia, M.; Doumas, P.; Charpentier, J.P.; Capelli, P.; Jouanin, L. 1998. Expression an antisense chalcone synthase RNA in transgenic hybrid walnut microcuttings: effect on flavanoid content and rooting ability. Plant Molecular Biology. 38: 467-479.

Ellis, D.D.; McCabe, D.E.; McInnis, S.; Ramachandran, R.; Russel, D.R.; Wallace, K.M.; Martinell, B.J.; Roberts, D.R.; Raffa, K.F.; McCown, B.H. 1993. Stable transformation of *Picea glauca* by particle acceleration. Bio/Technology. 111: 84-89.

Escobar, M.A.; Park, J.-I.; Polito, V.S.; Leslie, C.A.; Uratsu, S.L.; McGranahan, G.H.; Dandekar, A.M. 2000. Using GFP as a scorable marker in walnut somatic embryo transformation. Annals of Botany. 85: 831-835.

Guilley, H.; Dudley, R.K.; Jonard, G.; Balazs E.; Richards, K.E. 1982. Transcription of cauliflower mosaic virus DNA: detection of promoter sequences, and characterization of transcripts. Cell. 30: 763-773.

Hamrick J.L.; Nason J.D. 2000. Gene flow in forest trees. In: Young, A.G.; Boshier, D.; Boyle, T.J., eds. Forest conservation genetics: principles and practice. Collingwood, VIC, Australia: CSIRO Publishing: 81-90.

Han, K.H.; Gordon, M.P.; Strauss, S.H. 1996. Cellular and molecular biology of *Agrobacterium*-mediated transformation of plants and its application to genetic transformation of *Populus*. In: Stettler, R.F.; Bradshaw, H.D. Jr.; Heilman, P.E.; Hinckley, T.M., eds. Biology of *Populus* and its implications for management and conservation. Ottawa, Canada: NRC Research Press, National Research Council of Canada. 9: 201-222.

Humara, J.M.; Ordas, R.J. 1999. The toxicity of antibiotics and herbicides on in vitro adventitious shoot formation on *Pinus penea* L. cotyledons. *In Vitro* Cellular and Developmental Biology - Plant. 35: 339-343.

Jefferson, R.A.; Kavanagh, T.A.; Bevan, M.W. 1987. GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO Journal. 6: 3901-3907.

Kapaun, J.A.; Cheng, Z.M. 1999. Aminoglycoside antibiotics inhibit shoot regeneration from Siberian elm leaf explants. HortScience. 34: 727-729.

Koncz, C.; Schell, J. 1986. The promoter of the T_L-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. Molecular and General Genetics. 204: 383-396.

Leple, J.C.; Brasiliero, A.C.M.; Michel, M.F.; Delmotte, F.; Jouanin, L. 1992. Transgenic poplars: expression of chimeric genes using four different constructs. Plant Cell Reports. 11: 137-141.

Liu, C.-N.; Li, X.-Q.; Gelvin, S.B. 1992. Multiple copies of virG enhance the transient transformation of celery, carrot, and rice tissues by *Agrobacterium tumefaciens*. Plant Molecular Biology. 20: 1071-1087.

McGranahan, G.H.; Leslie, C.A.; Uratsu, S.L; Martin, L.A.; Dandekar, A.M. 1988. *Agrobacterium*-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. Bio/Technology. 6: 800-804.

McGranahan, G.H.; Leslie, C.A.; Uratsu, S.L.; Dandekar, A.M. 1990. Improved efficiency of the walnut somatic embryo gene transfer system. Plant Cell Reports. 8: 512-516.

Mullins, M.G.; Tang, F.C.A.; Facciotti, D. 1990. *Agrobacterium*-mediated genetic transformation of grapevines: transgenic plants of *Vitis rupestris* schelle and buds of *Vitis vinifera*. Bio/Technology. 8: 1041-1045.

Narasimhulu, S.B.; Deng, X.; Sarria, R.; Gelvin, S.B. 1996. Early transcription of *Agrobacterium* T-DNA genes in tobacco and maize. Plant Cell. 8: 873-886.

Ni, M.; Cui, D.; Einstein, J.; Narasimhulu, S.; Vergara, C.E.; Gelvin, S.B. 1995. Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes. Plant Journal. 7: 661-676.

Ostry, M.E.; Michler, C.H. 1993. Use of biotechnology for tree improvement in Populus model systems. In: Ahuja, M.R., ed. Micropropagation of woody plants. Dordrecht, Netherlands: Kluwer Academic Publishers: 471-483.

Peros, J.P.; Torregrosa, L.; Berge, G. 1998. Variability among *Vitis vinifera* cultivars in micropropagation, organogenesis, and antibiotic sensitivity. Journal of Experimental Botany. 49: 171-179.

Robertson, D.; Weissinger, A.K.; Ackley, A.K.; Glover, S.; Sederoff, R.R. 1992. Genetic transformation of Norway spruce (*Picea abies* L. Karst) using somatic embryo explants by microprojectile bombardment. Plant Molecular Biology. 19: 926-935.

Stomp, A.M. 1992. Histochemical localization of β-glucuronidase. In: Gallagher, S.R., ed. GUS Protocols: using the GUS gene as a reporter of gene expression. New York, NY: Academic Press: 103-114.

Tang, H.; Ren, Z.; Krczal, G. 2000. An evaluation of antibiotics for the elimination of *Agrobacterium tumefaciens* from walnut somatic embryos and regeneration of transgenic plants. Plant Cell Reports. 19: 881-887.

Tosca, A.; Pandolfi, R.; Vasconi, S. 1996. Organogenesis in *Camellia x williamsii*: Cytokinin requirement and susceptibility to antibiotics. Plant Cell Reports. 15: 541-544.

Tzfira, T.; Zuker, A.; Altman, A. 1998. Forestry tree biotechnology: genetic transformation and its application to future forests. Trends in Biotechnology. 16: 439-446.

Vancanneyt, G.; Schmidt, R.; O'Conner-Sanchez, A.; Willmitzer, L.; Rocha-Sosa, M. 1990. Construction of an intron-containing marker gene: Splicing of the intron in transgenic plants and its use in monitoring early events in *Agrobacterium*-mediated plant transformation. Molecular and General Genetics. 220: 245-250.

Yepes; L.M.; Aldwinckle; H.S. 1994. Factors that affect leaf regeneration efficiency in apple, and effect of antibiotics in morphogenesis. Plant Cell Tissue and Organ Culture. 37: 257-269.

Production and Early Field Performance of RPM® Seedlings in Missouri Floodplains

Daniel C. Dey, Wayne Lovelace, John M. Kabrick, and Michael A. Gold[1]

ABSTRACT—A new nursery culture process has been developed to produce large container RPM® seedlings in an effort to improve the success in artificially regenerating hardwoods. Major features of the process include air root pruning of seedlings grown in a well aerated soil medium to encourage a dense, fibrous root system. Production has focused on native bottomland tree, shrub, and herbaceous species. Field plantings of RPM® seedlings have been done in agricultural floodplains throughout Missouri. In one such planting, the survival and growth of pin oak and swamp white oak RPM® and bareroot seedlings are evaluated when seedlings are established with soil mounding and a redtop grass cover crop.

It is challenging to regenerate hardwoods such as oak (*Quercus* sp.), black walnut (*Juglans nigra* L.), and pecan (*Carya illinoinensis* [Wangenh.] K. Koch) on productive floodplains because competing vegetation, flooding, and animal damage retard seedling growth and decrease survival (Stanturf and others 1998). In addition, slow (shoot) growth rates are characteristic of oak, hickory (*Carya* sp.), and pecan reproduction, which further complicates efforts to regenerate these species, and usually necessitates controlling competing vegetation. Early growth of planted bareroot seedlings can be disappointing for black walnut (von Althen and Prince 1986, Van Sambeek and others 1987) and upland oaks (Johnson 1984). However, regeneration success can be improved by planting large, big-rooted seedlings and promoting early hardwood growth through such practices as fertilization, vegetation management, and protecting seedlings from wildlife herbivory.

Johnson and others (2002) stress the importance of planting large oak seedlings with well-developed root systems to promote regeneration success and increase dominance probabilities. Kormanik and others (1995, 1998) and Schultz and Thompson (1997) demonstrated that upland oak (i.e., northern red oak [*Quercus rubra* L.] and white oak [*Q. alba* L.]) and black walnut 1+0 bareroot seedlings are more successful in open agricultural fields and forest clearcuts if they have a threshold number of first-order-lateral roots (FOLR) greater than 1 mm in diameter. Schultz and Thompson (1997) recommended that northern red oak 1+0 bareroot seedlings should have a minimum of five FOLR and black walnut at least seven FOLR.

Nursery managers can increase the number of FOLR on bareroot seedlings by undercutting the taproot during the first or second year, or by transplanting 1+0 seedlings for a second year in the nursery to produce 1+1 transplant seedlings (Johnson 1988). Undercutting hardwood seedlings is now a common practice in the production of 1+0 bareroot seedlings. Pre-plant treatments with auxins have also been used to promote enhanced root regeneration in black walnut (Van Sambeek and others 1982). Air pruning the roots of seedlings grown in open-bottomed containers is another way to promote lateral root growth and a dense fibrous root system. Based on 50 years of research in container production and hardwood regeneration, the Forrest Keeling Nursery in Elsberry, MO has developed a culture system, known as the Root Production Method (RPM®), to produce high quality hardwood seedlings that have large caliper and height, and a substantial fibrous, root system.

For the past 5 to 7 years, private landowners, public land managers and scientists have been planting RPM® seedlings to regenerate primarily agricultural bottomlands. Bottomland oak species such as pin

[1]Research Forester (DCD and JMK), North Central Research Station, USDA Forest Service, 202 ABNR Bldg. Columbia, MO 65211; Vice President – General Manager (WL), Forrest Keeling Nursery, P.O. Box 135, Elsberry, MO 63343; Associate Director (MAG), Center for Agroforestry, University of Missouri, 203 ABNR Bldg. Columbia, MO 65211. DCD is corresponding author: to contact call (573) 875-5341 ext. 225 or e-mail at ddey@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

oak (*Quercus palustris* Muenchh.), swamp white oak (*Q. bicolor* Willd.) and bur oak (*Q. macrocarpa Michx.*) are often planted as RPM® seedlings in Missouri. To a lesser extent, black walnut, pecan, shellbark hickory (*Carya laciniosa* [Michx. f.] Loud.), sycamore (*Platanus occidentalis* L.) and other native bottomland species are planted as RPM® seedlings. Information on the growth and survival of RPM® in floodplain plantings is lacking. In this paper, we summarize the RPM® nursery culture process and present early growth and survival of pin oak and swamp white oak RPM® seedlings in a study of regeneration methods for afforesting agricultural crop fields along the Missouri River.

PRODUCTION OF THE RPM® SEEDLING

The root production method, or RPM® (patent no. pending 60/312593) is a nursery culture process to grow seedlings with dense, fibrous root systems in large (e.g., 11- to 19-liter) containers (Lovelace 1998). Using oak as an example, acorns are collected from trees growing in floodplains within 161 km of the bottomland planting site. Acorns are graded and sized using an aspirator and gravity table. Only the largest and heaviest seeds are used to produce RPM® seedlings. Acorns are placed 4 cm deep in mesh-bottomed trays filled with a composted rice hull, pine bark and sand medium (4:4:2 by volume). The shallow depth of media with 35 to 40% air space promotes air pruning of the tap root near the root collar, production of first-order lateral roots near the soil surface and development of a fibrous root system. Slow release fertilizer, micronutrients and a wetting agent are added to the soil medium and trays are enclosed in plastic to maintain proper humidity during cold stratification at 1° C for the recommended period depending on species. Next, trays of acorns are moved into a heated greenhouse usually in early February to initiate germination.

One to two months after emergence, when seedlings have completed their first shoot flush, they are transplanted into individual plastic, 10 cm deep bottomless band containers and placed on chicken wire and wood-framed benches in the greenhouse to permit continued air root pruning. Seedlings are graded based on height, stem caliper, and root development. Typically only the largest 50% of the seedlings continue through the RPM® process. In early May, seedlings are transplanted into 11 or 19 liter plastic containers and placed outside under mist irrigation for 48 hours to acclimatize them to the outdoor environment. Shallow containers are used to concentrate root growth in the upper 15 to 20 cm soil surface. The larger container is used for seedlings that will be grown a second year in the nursery.

After one to two growing seasons in the nursery, RPM® seedlings develop large root systems, have basal stem diameters exceeding 2.0 cm, and grow to over 1.5 m in height. Dey and others (2003) reported that root dry weight of pin oak and swamp white oak RPM® seedlings averaged 117 and 101 g for 11- and 19-liter container plants, respectively. In contrast, root dry weight of 1+0 bareroot oak seedlings averaged 18 g. Similarly, root volumes (by water displacement) of RPM® oak seedlings were substantially larger than 1+0 bareroot seedlings. Nineteen-liter swamp white oak and pin oak RPM® seedlings averaged 252 and 222 ml root volume, respectively, whereas root volume of swamp white oak and pin oak bareroot seedlings averaged 33 and 26 ml, respectively.

SMOKY WATERS AND PLOWBOY BEND RPM® PLANTING

In the fall of 1999 a study was established to evaluate methods for regenerating pin oak and swamp white oak on former agricultural crop fields in the Missouri River floodplain at Smoky Waters (Sec. 5, T 44 N, R 9 W and Sec. 1, T 44 N, R 10 W; Cole County, MO) and Plowboy Bend (Secs. 24 and 25, T 47 N, R 14 W; Moniteau County, MO) Conservation Areas. The study fields had been in crop production for years before this study.

Soils at the Plowboy Bend site were mapped as Sarpy Fine Sand (mixed, mesic, Typic Udipsamments). These soils are formed in sandy alluvium and consequently are excessively-drained. Soils at the Smoky Waters site were mapped as Haynie Silt Loam (coarse-silty, mixed, superacitve, calcarious, mesic Mollic Udifluvents), Leta Silty Clay (clayey over loamy, smectitic, mesic, Fluvaquentic Hapludolls), and Waldron Silty Clay Loam (fine, smectitic, calcareous, mesic Aeric Fluvaquents). These soils are formed in silty or clayey alluvium and range from somewhat-poorly drained (Leta and Waldron) to moderately well drained (Haynie). The Plowboy Bend site is also protected by a levee and the Smoky Waters is not protected by levees.

Dey and others (2003) provided a detailed explanation of experimental design and study establishment. In general, both 1+0 bareroot and 11- and 19-liter RPM® seedlings were planted to evaluate the effect of seedling size and nursery stock type on the survival and growth of pin oak and swamp white oak seedlings. Seedlings were planted in soil mounds created with a rice plow or in unmounded soil; and with either a cover crop of redtop grass (*Agrostis gigantea* L.) or with natural vegetation that normally colonizes abandoned bottomland crop fields.

Natural Disturbances

In 2 of the 4 four years (2001, 2002) the study site at Smoky Waters was flooded for up to 3 weeks in June. Also, every winter after the first year, cottontail rabbits (*Sylvilagus floridanus* Allen) have girdled and shoot clipped oak seedlings and oak sprouts, which profoundly affected oak survival and growth. The amount and severity of rabbit damage to planted oaks varied greatly between the cover crop treatments (i.e., redtop grass versus natural vegetation fields).

Rabbit Herbivory

In the natural vegetation fields, the composition and structure of winter cover provided by forbs promoted higher rabbit densities (7.4 rabbits per ha) than in the redtop grass fields (2.5 rabbits per ha) (Dugger and others 2003). In the winter, the dead tops of forbs and clumps of Johnsongrass (*Sorghum halepense* [L.] Pers.) remained somewhat erect providing cover that was 1.0 m tall. However, redtop grass matted down to 0.20 m and provided rabbits little hiding cover from predators. Thus, rabbits were able to move freely across the natural vegetation fields causing damage to nearly all of the seedlings each winter. Rabbits clipped the shoots of all bareroot seedlings and severely girdled (more than half of the circumference of the stem) 90% or more of the RPM® seedlings in the natural vegetation fields by the end of the second winter. In comparison, only 8% of the bareroot seedlings and 26% of RPM® seedlings in the redtop grass field at Plowboy Bend Conservation Area had herbivory damage from rabbits. Similarly, but to a lesser extent, 12% of the bareroot seedlings and 23% of the RPM® seedlings in the redtop grass field at Smoky Waters Conservation Area were damage free. Moreover, the severity of damage to RPM® trees in redtop grass fields was less than in the natural vegetation fields. These differences in winter habitat between the cover crop treatments affected rabbit densities and movements, which in turn, contributed to the significant differences in oak seedling survival, growth, and acorn production between the cover crop treatments.

Survival

Survival of oak RPM® seedlings remained high (i.e., > 94%) during the first 3 years (Fig. 1), while survival of bareroot seedlings continued to decline for both swamp white oak and pin oak. After 3 years in the field, survival of swamp white oak bareroot seedlings was significantly higher ($P < 0.01$) than pin oak bareroot seedlings, based on an analysis of variance of treatment effects on survival (Dey and others 2003). Swamp white oak bareroot survival averaged 76%, while survival for pin oak bareroot seedlings was 54%. There was no significant difference ($P = 0.24$) between 11- and 19-liter RPM® seedlings, nor between swamp white oak and pin oak RPM® seedlings ($P = 0.87$). Survival of oak seedlings was not significantly affected by soil mounding or cover crop treatments. However, an assessment of the survival of individual trees that were not damaged by rabbits indicated that redtop grass cover crop did significantly increase survival over that of trees grown with natural vegetation (Fig. 2) based on logistic regression analysis conducted by Dey and others (2003). There were no significant differences in survival among trees on mounded and unmounded soils.

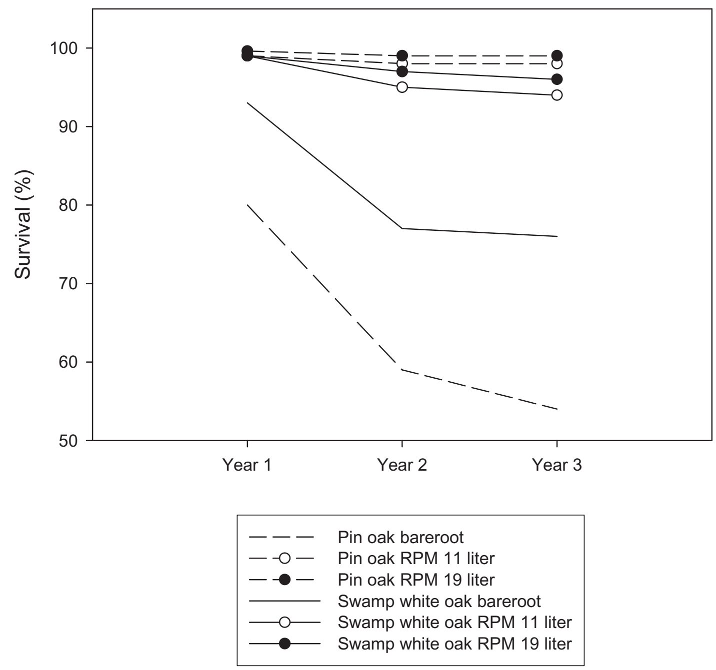


Figure 1.—Annual survival of oak seedlings by species and nursery stock type.

Basal Diameter Growth

Basal diameter increment after 3 years was significantly greater ($P < 0.01$) for RPM® seedlings than bareroot stock, regardless of species (Fig. 3), based on an analysis of variance (Dey and others 2003). The average basal diameter of all RPM® oak seedlings increased 0.8 cm in the first 3 years, whereas bareroot seedlings increased only 0.3 cm. There was no significant difference ($P = 0.34$) in basal diameter increment between the 11- and the

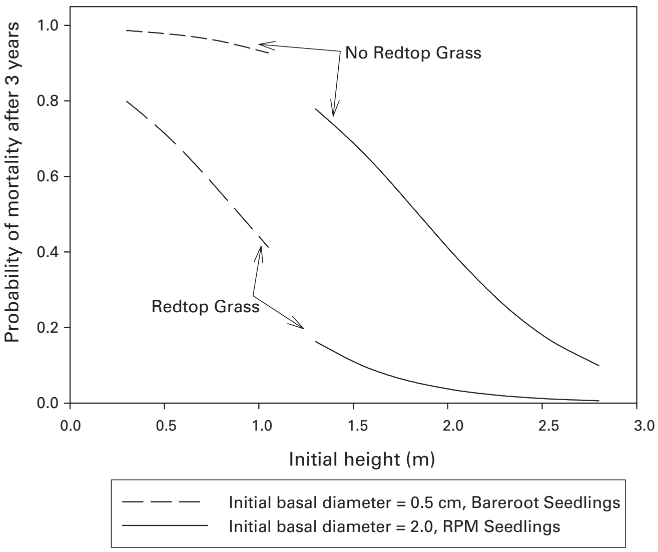


Figure 2.—The probability of mortality after three years in relation to initial seedling height of pin oak seedlings planted with a cover crop of redtop grass or in natural vegetation that develops in abandoned bottomland crop fields at Smoky Waters Conservation Area. In this illustration, mortality is estimated for oak seedlings with initial basal diameter = 0.5 cm (representative of the average pin oak bareroot seedling in our study); and for seedlings with initial basal diameter = 2.0 cm (representative of the average RPM® pin oak in our study).

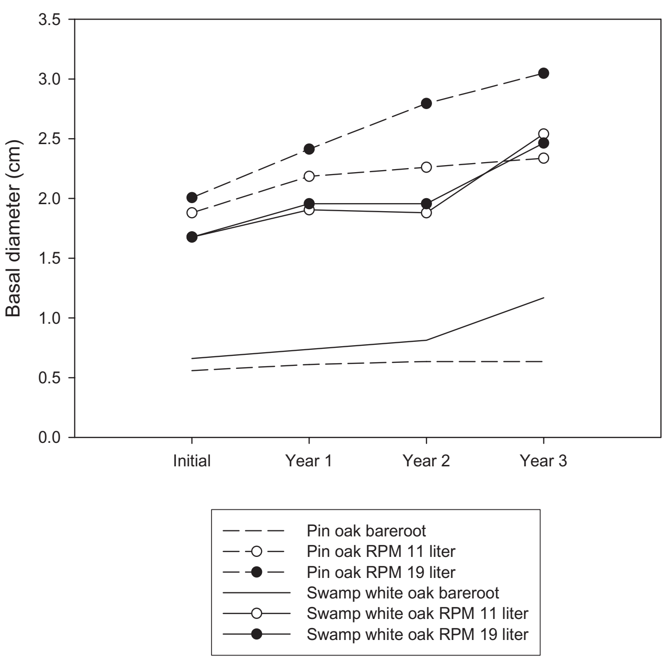


Figure 3.—Average initial and annual basal diameter (measured 2.5 cm above the ground) of oak seedlings by species and nursery stock type.

19-liter RPM® seedlings. The basal diameter of pin oak 19-liter RPM® seedlings increased the most during the first 3 years, averaging 1.0 cm of new growth. Basal diameter increment was least (0.1 cm in 3 years) for pin oak bareroot seedlings. The above analysis includes rabbit damaged and undamaged trees. By removing the rabbit damaged trees, average basal diameter increment was 1.6 cm for RPM® seedlings and 0.2 cm for bareroot trees.

Although soil mounds functioned as anticipated by improving drainage and aeration, diameter growth was not significantly affected by soil mounding. Basal diameter increment of all trees combined was substantially larger in redtop grass fields (1.4 cm) compared to natural vegetation fields (0.2 cm); however, no significant differences can be reported as yet ($P = 0.08$). For undamaged trees, average basal diameter increment was 1.6 cm for RPM® seedlings in redtop grass fields and 0.4 cm in natural vegetation fields, while the basal diameter of bareroot seedlings increased by 0.3 cm in redtop grass but decreased by 0.2 cm in natural vegetation fields.

Height Growth

Average height increment after 3 years was negative for most species and nursery stock types because cottontail rabbits caused extensive damage by girdling the stems of RPM® seedlings, or by clipping the shoots of bareroot seedlings at ground-level, which caused shoot dieback and loss of height (Fig. 4). Three year height increment was significantly less ($P < 0.01$) for RPM® seedlings than bareroot, based on an analysis of variance (Dey and others 2003). For bareroot seedlings that had been shoot-clipped by rabbits, annual sprout growth came close to, or slightly exceeded the initial height, resulting in small negative or positive increments in height. In contrast, net height increment was much lower in RPM® trees because rabbit girdling, which occurred in the lower 0.30 m of the stem, caused shoot dieback to near ground-level, and annual sprout growth was not enough to recover the original height. In addition, trees were often repeatedly damaged by rabbits each winter. Three year height increment averaged -0.50 m for the RPM® seedlings. Despite rabbit browsing, RPM® trees remained taller than bareroot seedlings three years after planting. Undamaged trees in the redtop grass fields had slightly positive average height growth (0.10 m for bareroot and RPM® seedlings), but net growth was negative in natural vegetation fields, averaging -0.53 m for RPM® and -0.13 m for bareroot seedlings.

Height growth of undamaged RPM® seedlings may be low because these trees were planted on a

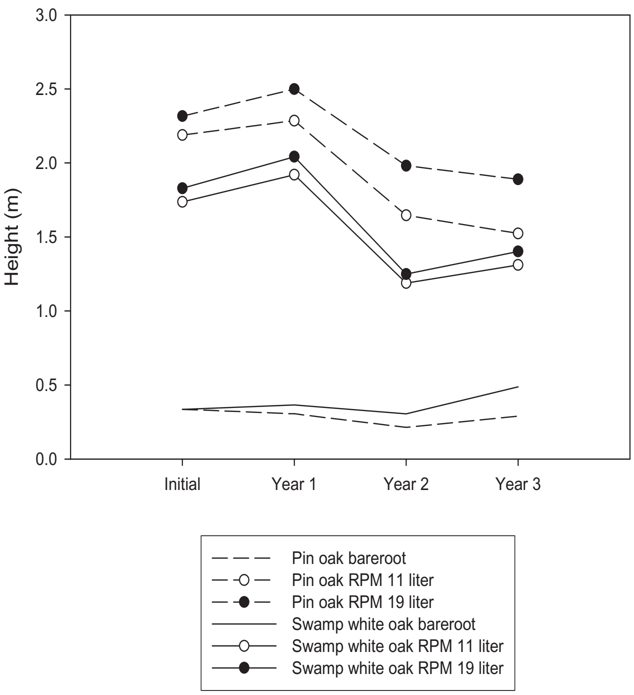


Figure 4.—Average initial and annual height of oak seedlings by species and nursery stock type.

9 x 9 m spacing; and widely spaced, open-grown trees often experience reductions in height growth, especially trees with weak epinastic control such as the oaks (Oliver and Larson 1996). Also, height growth of oak reproduction is slow at first because seedlings characteristically allocate photosynthates to root growth often at the expense of shoot growth (Johnson and others 2002). Height growth of RPM® and bareroot seedlings may also be limited by low levels of foliar nitrogen, which averaged 2.05% at Smoky Waters and 1.71% at Plowboy Bend Conservation Areas (J.W. Van Sambeek and N. Sullivan, Research Physiologist and Research Forester, U.S. Forest Service, North Central Research Station, personal communication).

An analysis of all trees by cover crop treatment showed that 3 year height increment was significantly higher (P = 0.02) for oak seedlings growing in the redtop grass fields than those trees competing with natural vegetation. There may be less light competition in the redtop grass fields during the growing season than in the natural vegetation fields. Redtop grass typically grows to a height of 0.4 to 0.6 m whereas herbaceous ground cover in the natural vegetation fields grew to over 2 m in height, overtopping many of the oak seedlings. Also, rabbit densities were less in the redtop grass fields than in the natural vegetation fields. There was no significant difference in height growth among trees on mounded and unmounded soils.

Acorn Production

Swamp white oak RPM® seedlings that were 18 to 24 months old at time of planting produced acorns in each of the first 4 years following outplanting (Table 1). Acorn production occurred in a small proportion (3.5%) of the 2,522 swamp white oak RPM® seedlings their first year in the field. Most of the production (60%) occurred in oaks from 19-liter containers, but larger 11-liter container RPM® trees also produced acorns. During the first 4 years in the field, average RPM® acorn production increased from 4.3 to 12.5 acorns per tree. Individual trees were able to produce as many as 125 acorns. A single pin oak RPM® seedling produced acorns for the first time in year 4.

The probability of a RPM® swamp white oak seedling producing at least one sound acorn in the first year after planting was significantly (P < 0.001) related to initial basal diameter and height of the seedling, based on logistic regression analysis by Grossman and others (2003). Acorn production was more likely to occur in the first year for large diameter (> 1.3 cm), tall (> 1.5 m) RPM® seedlings. For example, the probability of producing a sound acorn after 1 year is 2% for a 1.5 m RPM® seedling with a basal diameter of 1.8 cm, but increases to 15% for a 2.5 cm basal-diameter tree of similar height. Consistent, early production of acorns was surprising considering that open-grown oaks do not begin producing seed until they are 20 to 30 years old (Burns and Honkala 1990). In contrast, no bareroot oak seedlings have produced acorns after four growing seasons.

Table 1.—Acorn production by swamp white oak RPM® seedlings planted at Plowboy Bend and Smoky Waters Conservation Areas.

Years After Planting	Number of Trees With Acorns	Mean Number of Acorns Per Tree	Standard Deviation	Range
1	86	4.3	4.4	1-21
2	29	5.2	9.2	1-45
3	70	6.3	7.5	1-42
4	151	12.5	17.0	1-125

SUMMARY

Large container RPM® seedlings had significantly greater survival and basal diameter growth than bareroot seedlings after three years. Three-year height increment for RPM® seedlings was negative and significantly less than that of bareroot seedlings largely due to the loss of initial height from rabbit herbivory on oak seedlings. There was no difference in growth or survival to-date between the 11- and 19-liter RPM® trees.

A redtop grass cover crop benefited oak regeneration by controlling competing vegetation and reducing the amount and severity of rabbit damage to oak seedlings. Redtop grass was effective in preventing the development of much of the forb and woody growth that normally forms on abandoned bottomland crop fields, thereby reducing plant competition for light and the quality of winter habitat for rabbits. Oak seedlings growing in redtop grass fields had significantly greater height increment and substantially larger basal diameter growth after 3 years than trees competing with natural vegetation.

After 3 years, and two June floods at Smoky Waters Conservation Area, soil mounding did not improve height and diameter growth, or survival of oak seedlings at either site. Use of soil mounding to improve drainage, reduce flooding effects on trees and improve soil environments for root growth may be worthwhile on soils that are more poorly drained, and clayey than those of our study site but this remains to be tested.

One- to two-year-old swamp white oak RPM® seedlings produced acorns in the first year after planting in Missouri River floodplain crop fields. The number of acorn bearing trees and production per tree has increased during the first four years. Acorn production did drop in year two because rabbit girdling caused shoot dieback in RPM® trees in the natural vegetation field, taking them out of production. But production rose in years 3 and 4 as more trees came into production. Most acorn bearing trees are located in the redtop grass fields. Pin oak RPM® have begun producing acorns in year 4. Larger RPM® seedlings are more likely to produce acorns within the first few years of plantation establishment.

Under controlled conditions in the nursery, the RPM® process produces seedlings with very large root systems that have numerous large diameter lateral roots (or multiple taproots), and a high density of fine roots. These are desirable root characteristics that many have recognized as being essential for successful regeneration of species such as the oaks. Early results from this study indicate that planting RPM® seedlings of large size in a redtop grass ground cover appears to be a successful formula for regenerating oaks and restoring acorn production in agricultural floodplains. It is critical to successful oak regeneration on productive bottomlands that high quality nursery stock be planted in combination with vegetation management practices that control competing vegetation and animal damage. Further research is needed to evaluate the how RPM® seedlings, soil mounding, and cover crops may enhance black walnut establishment in bottomlands.

ACKNOWLEDGMENT

This work was funded, in part, through the University of Missouri Center for Agroforestry under cooperative agreements 58-6227-1-004 with the ARS and C R 826704-01-2 with the US EPA. The results presented are the sole responsibility of the P.I. and/or MU and may not represent the policies or positions of the ARS or EPA.

LITERATURE CITED

Burns, R.M.; Honkala, B.H., eds. 1990. Silvics of North America. Vol. 2, Hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service. 877 p.

Dey, D.C.; Kabrick, J.M.; Gold, M.A. 2003. Tree establishment in floodplain agroforestry practices. In: Proceedings, 8th North American agroforestry conference; 2003 June 23-25; Corvallis, OR (in press).

Dugger, S.; Dey, D.C.; Millspaugh, J.J. 2003. Vegetation cover effects mammal herbivory on planted oaks and success of reforesting Missouri River bottomland fields. In: Proceedings, 12th biennial southern silvicultural conference; 2003 February 25-27; Biloxi, MS. Gen. Tech. Rep. SRS-. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: (in press).

Grossman, B.C.; Gold, M.A.; Dey, D.C. 2003. Restoration of hard mast species for wildlife in Missouri using precocious flowering oak in the Missouri River floodplain, USA. Agroforestry Systems. 59: 3-10.

Johnson, P.S. 1984. Responses of planted northern red oak to three overstory treatments. Canadian Journal Forest Research. 14: 536-542.

Johnson, P.S. 1988. Undercutting alters root morphology and improves field performance of northern red oak. In: Worrall, J.; Loo-Dinkins, J.; Lester, D.P., eds. Proceedings, 10th North American forest biology workshop; Vancouver, BC: University of British Columbia, Department of Forest Science: 316-323.

Johnson, P.S.; Shifley, S.R.; Rogers, R. 2002. The ecology and silviculture of oaks. New York, NY: CABI Publishing. 503 p.

Kormanik, P.P.; Sung, S.S.; Kormanik, T.L.; Zarnock, S.J. 1995. Oak regeneration – why big is better. In: Landis, T.D.; Cregg, B., tech. coords. National proceedings, Forest and Conservation Nursery Assocations. Gen. Tech. Rep. PNW-GTR-365. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: 117-123.

Kormanik, P.P.; Sung, S.S.; Kass, D.J.; Schlarbaum, S. 1998. Effect of seedling size and first-order-lateral roots on early development of northern red oak on mesic sites. In: Waldrop, T.A., ed. Proceedings, 9th biennial southern silvicultural research conference; 1997 February 25-27; Clemson, SC. Gen. Tech. Rep. SRS-20. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 247-252.

Lovelace, W. 1998. The root production method (RPM) system for producing container trees. Combined proceedings of the International Plant Propagators Society. 48: 556-557.

Oliver, C.D.; Larson, B.C. 1996. Forest stand dynamics. New York, NY: John Wiley and Sons, Inc. 520 p.

Schultz, R.C.; Thompson, J.R. 1997. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root-graded stock in the central USA. New Forests. 13: 301-314.

Stanturf, J.A.; Schweitzer, C.J.; Gardiner, E.S. 1998. Afforestation of marginal agricultural land in the Lower Mississippi River Alluvial Valley, U.S.A. Silva Fennica. 32(3): 281-287.

Van Sambeek, J.W.; Rietveld, W.J. 1982. Preplant inolebutyric acid treatment of outplanted black walnut seedlings. In: Proceedings, 7th North American forest biology workshop; 1982 July 26-28; Lexington, KY: University of Kentucky Press: 272-280.

Van Sambeek, J.W.; Williams, R.D.; Hanover, J.W. 1987. Comparison of planting methods for nursery- and container-grown black walnut seedlings. In: Hay, R.L.; Woods, F.W.; DeSelm, H.R., eds. Proceedings, 6th Central Hardwood forest conference; 1987 February 24-26; Knoxville, TN: The University of Tennessee: 69-73.

von Althen, F.W.; Prince, F.A. 1986. Black walnut (*Juglans nigra* L.) establishment, six-year survival and growth of containerized and 1+0 seedlings. Tree Planters' Notes. 37: 11-14.

Facilitating Nutrient Acquisition of Black Walnut and Other Hardwoods at Plantation Establishment

Douglass F. Jacobs and John R. Seifert[1]

ABSTRACT—Bareroot hardwood seedlings typically undergo transplant shock immediately following planting before root systems are established. Fertilization at planting may act to minimize transplant shock by reducing nutrient stresses. However, previous work with fertilization of hardwoods at planting has generally relied on fertilizers with nutrient forms immediately available. These fertilizers have relatively low rates of fertilizer use efficiency, as the vast majority of applied nutrients are not available for plant uptake. Thus, response of hardwood seedlings to fertilization at planting has traditionally been minimal and is rarely recommended operationally. Controlled-release fertilizer (CRF) is a relatively new technology available for reforestation in which nutrients are slowly released over the course of one to two growing seasons. We tested the use of Osmocote® Exact 15N-9P-10K plus minors (16 to 18 month release) polymer-coated CRF applied to the root zone at planting with three hardwood species: black walnut, white ash, and yellow-poplar in southern Indiana. Application of 60 g per seedling accelerated first-year height growth by 52% and diameter growth by 37% compared to controls (averaged over species). These preliminary results indicate that CRF may provide a previously untested means to improve hardwood afforestation planting success.

Bareroot seedlings typically grow slowly during the first 1 or 2 years following planting until root systems can establish and proliferate through the soil to exploit site resources (Rietveld 1989). While water limitations are often noted as a major barrier to transplanting success (Burdett and others 1984), nutrient limitations may also slow initial seedling growth. Fertilization at the time of planting has been recommended for conifer seedlings as a means to minimize transplant shock and facilitate rapid growth above competing vegetation and the level of deer browse (Carlson and Preisig 1981).

Fertilization at planting is rarely recommended (Ponder 1996) and often discouraged (Beineke 1986) for hardwood seedlings, likely due to previous studies reporting neutral or negative effects from the practice. Williams (1974) reported reduced survival and growth of planted black walnut (*Juglans nigra* L.) seedlings that received fertilization. Black walnut fertilized at 1, 2, and 6 years after planting on a good site exhibited no differences in height or diameter at breast height after 12 years (Braun and Byrnes 1982). Previous studies, however, have generally relied upon surface application of traditional agronomic fertilizers (e.g., urea or ammonium nitrate), which release nutrients immediately upon application. These fertilizers typically have relatively low rates of nutrient recovery by crop species, termed fertilizer use efficiency. Additionally, surface application of fertilizer may act to stimulate growth of competing vegetation which is a predominant reason that fertilization at planting has been discouraged (Ponder 1996).

Controlled-release fertilizer (CRF) offers an alternative to standard agronomic fertilizers. With a single application, CRF may provide plants with enhanced nutrition for extended periods, typically ranging from 3 to 18 months. This acts to provide a more consistent and sustained nutrient supplies that may better coincide with the developmental needs of tree species (Donald 1991). The gradual

[1]Assistant Professor of Forest Regeneration (DFJ), Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907-2033; and Extension and Research Forester (JRS), Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, PO Box 216, Butlerville, IN 47223. DFJ is corresponding author: to contact, call (765) 494-3608 or e-mail at djacobs@fnr.purdue.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

release pattern of CRF may also minimize nutrient leaching, reduce plant damage, and improve overall fertilizer use efficiency. Hangs and others (2003) reported that < 1% of nitrogen from Polyon®, a polymer-coated CRF, applied to conifer seedlings at planting was lost to leaching after two growing seasons. CRF may be particularly effective when applied to the seedling root zone, as CRF applications of 5 to 7 cm from roots resulted in substantially greater fertilization use efficiency than surface application (Hangs and others 2003). Many different types of CRF are available, primarily differing in terms of nutrient formulations, estimated product longevities, and mechanisms of nutrient release (Jacobs and others 2003b).

Although use of CRF has traditionally been limited to the horticultural industry, interest in using these fertilizers for tree plantings has increased in the past decade (Haase and Rose 1997). Some impressive results have been reported when incorporating CRF into tree plantings with conifer species (Carlson 1981, Carlson and Preisig 1981, Nursery Technology Cooperative 2001). However, we know of no reports examining the use of CRF in hardwood afforestation plantings in the eastern United States. Thus, the purpose of this trial was to study the response of three hardwood species to CRF applied to the root zone at planting. In this paper, we present preliminary, first-year observations indicating that CRF accelerated initial plantation establishment of these three hardwood species.

MATERIALS AND METHODS

Bareroot (1+0) seedlings of three species [black walnut, white ash (*Fraxinus americana* L.), and yellow-poplar (*Liriodendron tulipifera* L.)] were grown under standard nursery cultural regimes at the Indiana Department of Natural Resources' Vallonia Nursery during 2001. Following lifting, seedlings were cold-stored at 2° C until delivered to the field planting site. The outplanting site for this study was located at the Southeastern Purdue Agricultural Center (SEPAC) in southeastern Indiana (39°01'N, 85°35'W). The site was formerly in agricultural production and was tilled prior to planting.

The CRF examined in this study was Osmocote® Exact Lo-Start 15N-9P-10K plus minors. Nutrients in this CRF are encapsulated within multiple layers of a polymeric resin, which acts to slow the dissolution rate of nutrients into the soil solution. This CRF was designed by the manufacturer to release over 90% of its nutrients within 16 to 18 months following application, assuming a soil temperature of 21° C at position of fertilizer placement. At this longevity rating, we assumed that seedlings would receive enhanced nutrition for two growing seasons since fertilizer release during the winter months should be slowed. To estimate rates of nutrient release, we placed 75 g CRF within PVC rings covered in nylon. These were buried at the approximate depth of fertilizer placement (30 cm) and excavated periodically during the experiment (8 sampling intervals × 5 replications). The excavated fertilizer was dried at 70° C for 48 hr and re-weighed.

Seedlings were planted on either 2002 April 10 (three blocks) or 2002 April 24 (remaining three blocks) using a tractor-hauled coulter with trencher and packing wheels. Fertilizer was applied at six rates (0, 15, 30, 45, 60, and 75 g per seedling) using a modified funnel system installed onto the machine planter (Fig. 1). For ease of application, rates were converted to volume estimates from lab trials prior to planting. For each seedling, the appropriate CRF rate treatment was measured by volume and applied into the planting trench (approximately 30 cm depth) through the funnel system. The 75 g per seedling rate encompassed a length of about 15 cm along the trench. Seedlings were planted directly over fertilizer with roots extending to just above the fertilizer layer. Herbicide applications were made prior to and following planting, and weed competition was minimal throughout the first growing season.

Seedlings were measured for initial height and root collar diameter on 2002 April 29 and 30. In early May 2002, an electric deer fence was installed around the perimeter of the study and maintained for the duration of the experiment. Seedlings were re-measured in November 2002 for height, diameter, and incidence of shoot dieback when terminal bud died with a lateral bud becoming the new leading shoot.

Figure 1.—Modified funnel system on machine planter for fertilizer application.

The study was established as a randomized complete block design with six blocks and factorial treatments (3 species × 6 CRF rates). Environmental conditions differed among blocks but conditions were relatively homogenous within blocks. Within a block, each of the 18 treatments was randomly assigned to a row in which 20 seedlings representing a treatment combination were planted. The sampling unit was each individual seedling and the experimental unit used for data analysis was the mean value of the 20 seedlings within a row for each treatment. Data were analyzed using analysis of variance (ANOVA). When significant ($P \leq 0.05$) effects were detected in the ANOVA, Fisher's Protected Least Significant Difference test was used to identify differences among treatments ($\alpha = 0.05$).

RESULTS

Fertilization (averaged over species) affected both seedling height ($P = 0.0019$) and diameter ($P = 0.0002$) growth during the first growing season. Fertilizer effects on first-year growth did not vary among species, as indicated by the lack of a significant species × fertilizer rate interaction for both height ($P = 0.3358$) and diameter ($P = 0.6188$) growth. Height growth was increased by 52% as compared to the control at the 60 g per seedling fertilizer rate (Fig. 2). The 60 g per seedling rate was also the most effective rate for diameter growth, with a 37% increase as compared to the control (Fig. 3). For both height and diameter growth, mean values increased along the continuum in fertilizer rate from 0 to 60 g, until decreasing at 75 g (Figs. 2 and 3). Fertilization had no significant effect on survival ($P = 0.3594$) with all treatments resulting in ≥ 91% survival or on the incidence of terminal bud dieback ($P = 0.3387$).

When examining species independently of fertilizer treatment (i.e., averaged over fertilizer rates), there were significant differences among species for both first-year seedling height ($P < 0.0001$) and diameter ($P < 0.0001$) growth. For height growth the ranking was white ash > yellow-poplar > black walnut (Fig. 2), while for diameter growth the ranking was yellow-poplar > white ash > black walnut (Fig. 3). There were differences in survival ($P < 0.0001$) among species, with yellow-poplar having significantly lower survival (85%) than either white ash (100%) or black walnut (97%). There were also differences in incidence of terminal bud dieback among species ($P < 0.0001$), with black walnut having a significantly greater percentage of trees with dieback (22%) than either white ash (10%) or yellow-poplar (13%).

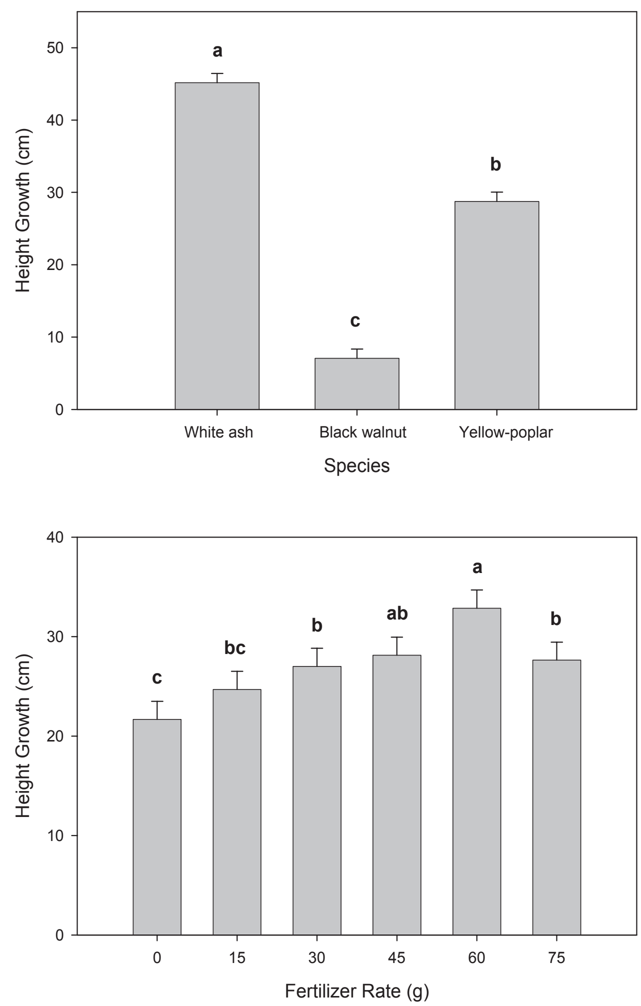


Figure 2.—First-year height growth for species ($P < 0.0001$) and fertilizer rate ($P = 0.0019$). Species × rate was non-significant ($P = 0.3358$). For either species or fertilizer rate treatments, similar letter groupings did not differ significantly at $\alpha = 0.05$.

Analysis of changes in residual fertilizer weight indicated that fertilizer weight decreased to 68% of the original weight by the end of the first growing season. Approximately 33% of fertilizer weight (primarily comprised of residual polymer prill materials) typically remains at the end of the designated release period with polymer-coated CRF (Jacobs and others 2003a). Thus, we expect that additional fertilizer nutrients should still be available for plant uptake during the second growing season.

DISCUSSION

Application of CRF at plantation establishment clearly accelerated first-year seedling growth of

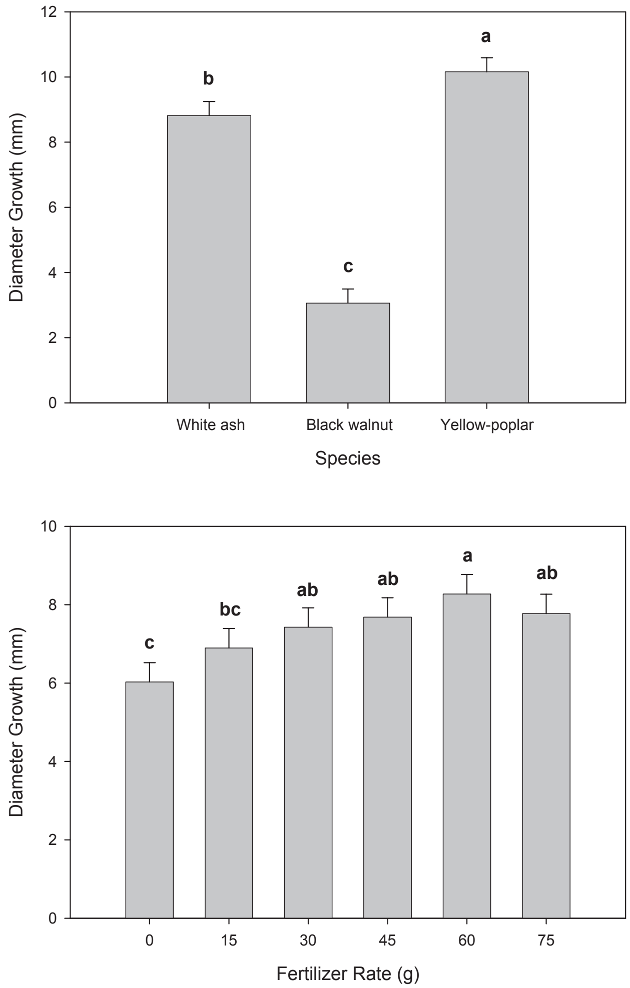


Figure 3.—First-year diameter growth for species ($P < 0.0001$) and fertilizer rate ($P = 0.0002$). Species × rate was non-significant ($P = 0.6188$). For either species or fertilizer rate treatments, similar letter groupings did not differ significantly at $\alpha = 0.05$.

each of these three hardwood species. Regardless of species, fertilization at 60 g per seedling increased height growth by 52% and diameter growth by 37% compared to controls without CRF fertilizer applications (Figs. 2 and 3). Although we know of no published observations examining use of CRF at the time of planting with hardwood species, similar results have been reported for conifer species. After two growing seasons, total height was increased by 42% in Douglas-fir (*Pesudotsuga menzeiseii* (Mirb.) Franco) (Carlson and Preisig 1981) and 27% in western hemlock (*Tsuga heterophylla* Raf. Sarg.) seedlings with application of 21 g of Osmocote CRF at time of planting. Incorporation of Osmocote 18N-5P-12K into the growing media of container seedlings at 30 kg per m^3 resulted in a doubling of stem volume compared to controls after three growing seasons (Nursery Technology Cooperative 2001).

These results suggest that CRF may offer a means to enhance initial plantation growth of hardwood seedlings, which may act to improve the establishment and early productivity of hardwood afforestation plantings. Hardwood plantations have historically been relatively difficult to establish, with a high degree of variability in success. A random survey of 88 recently-established (ages 1 to 5) plantations throughout Indiana indicated that seedling survival averaged approximately 65%, with the majority of mortality occurring the first year following planting (Jacobs and others in press). This survival rate contrasts dramatically with operational conifer tree plantations in the Pacific Northwest and the southeastern United Steas where plantation survival is routinely above 90%.

The majority of hardwood plantation failures in the eastern United States are attributed to vegetative competition and damage from animal browse incurred during the first 1 or 2 years after planting (see citations in Jacobs and others in press). Application of CRF at planting may provide hardwood seedlings with a means to rapidly accelerate over the level of browse and competing vegetation, allowing seedlings to reach a free-to-grow status in a shorter time frame. It should be noted, however, that weed control on this site was excellent and is likely a necessary prerequisite for attaining positive seedling response from any fertilizer application. Although this study was not intended to provide a financial assessment of incorporating CRF technology into forest tree plantation establishment, we estimate the cost of 60 g (the most effective rate per seedling in this study) of this CRF at approximately $0.20 - $0.30 per seedling.

Estimates of fertilizer release in our study indicated that approximately 50% of nutrients had actually released from CRF prills during the first growing season, suggesting that additional nutrients may be available for plant uptake during the second growing season. Additionally, growth of seedlings is largely dependent on the re-translocation of nutrients from stored reserves (Salifu and Timmer 2003). The increased growth of fertilized seedlings compared to seedlings in the control treatment was likely associated with greater nutrient uptake, and implies that enhanced levels of nutrients were also stored by fertilized seedlings which may act to fuel growth during the second season. We expect to conduct long-term monitoring of this plantation to assess whether the growth responses observed during the first year continue to be maintained over time.

ACKNOWLEDGMENTS

Indiana DNR Vallonia Nursery donated seedlings, and O.M. Scotts Co. donated fertilizer for this experiment. The following assisted with field and lab work: Patricio Alzugaray, Anthony Davis, Bill Maschino, Amy Ross-Davis, Barrett Wilson, and Jewell Yeager.

LITERATURE CITED

Beineke, W.F. 1986. Black walnut plantation management. FNR-119. West Lafayette, IN: Purdue University, Cooperative Extension Service. 11 p.

Braun, J.M.; Byrnes, W.R. 1982. Growth of black walnut in a fertilized plantation. In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; West Lafayette, IN. Gen. Tech. Rep. NC-74. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 74-104.

Burdett, A.N.; Herring, L.J.; Thompson, C.F. 1984. Early growth of planted spruce. Canadian Journal of Forest Research. 14: 644-651.

Carlson, W.C. 1981. Effects of controlled-release fertilizers on the shoot and root development of outplanted western hemlock (*Tsuga heterophylla* Raf. Sarg.) seedlings. Canadian Journal of Forest Research. 11: 752-757.

Carlson, W.C.; Preisig, C.L. 1981. Effects of controlled-release fertilizers on the shoot and root development of Douglas-fir seedlings. Canadian Journal of Forest Research. 11: 230-242.

Donald, D.G.M. 1991. Nursery fertilization of conifer planting stock. In: Van Den Driessche, R., ed. Mineral nutrition of conifer seedlings. Boca Raton, FL: CRC Press: 135-167.

Haase, D.L.; Rose, R., eds. 1997. Forest seedling nutrition from the nursery to the field (Symposium proceedings). Corvallis, OR: Oregon State University, Nursery Technology Cooperative. 161 p.

Hangs, R.D.; Knight, J.D.; van Rees, K.C.J. 2003. Nitrogen accumulation by conifer seedlings and competitor species from ^{15}nitrogen-labeled controlled-release fertilizer. Soil Science Society of America Journal. 67: 300-308.

Jacobs, D.F.; Rose, R.; Haase, D.L. 2003a. Development of Douglas-fir seedling root architecture in response to localized nutrient supply. Canadian Journal of Forest Research. 33: 118-125.

Jacobs, D.F.; Rose, R.; Haase, D.L. 2003b. Incorporating controlled-release fertilizer technology into outplantings. In: Riley, L.E.; Dumroese, R.K.; Landis, T.D., eds. National proceedings: Forest and Conservation Nursery Associations-2002. Proceedings RMS-P-28. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 37-42.

Jacobs, D.F.; Ross-Davis, A.; Davis, A.S. 2004. Establishment success of conservation tree plantations in relation to silvicultural practices in Indiana, USA. New Forests. In press.

Nursery Technology Cooperative. 2001. Field performance of Douglas-fir container stock grown with fertilizer-amended media. In: Nursery Technology Cooperative 2000-2001 Annual Report. Corvallis, OR: Oregon State University, Nursery Technology Cooperative: 7-11.

Ponder, F., Jr. 1996. Walnut fertilization and recommendations for wood and nut production. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 128-137.

Rietveld, R. 1989. Transplanting stress in bareroot conifer seedlings: Its development and progression to establishment. Northern Journal of Applied Forestry. 6: 99-107.

Salifu, K.F.; Timmer, V.R. 2003. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15. Soil Science Society of America Journal. 67: 309-317.

Williams, R.D. 1974. Planting methods and treatments for black walnut seedlings. Res. Pap. NC-107. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 5 p.

SOILS AND NUTRITION MANAGEMENT FOR BLACK WALNUT

Felix Ponder, Jr.[1]

ABSTRACT—Although walnut may survive when planted in unsuitable soils, most likely growth will be slow and the trees will be of poor quality. Sufficient time should be spent locating quality sites for this valuable and high-site demanding species. Undoubtedly, only ideal soil conditions will allow for adequate root expansion and for soil levels of nutrients and water to be sufficient for consistent growth and nut production. Nitrogen (N) is often the limiting nutrient to tree growth. Fertilization of young walnuts for growth is usually offset by an increase in vegetation competition from weeds. Benefits from fertilization are more likely before crown closure, after thinning, and for active growing large-diameter trees a few years before harvesting. Greater payoffs from fertilization can likely be expected from nut production, especially for trees of nut varieties with larger and more consistent nut crops.

Black walnut (*Juglans nigra* L.) is perhaps the most valuable hardwood tree species in North America. It is prized for manufacturing high quality furniture and for carvings because of its hard wood, dark luminous color, and ease of processing. Its nutmeat is used for human consumption and is important for wildlife. In addition, the shells are ground for use in many products. Black walnut occurs as a minor component in mixed deciduous forests in eastern United States and in the deciduous forest region of southern Ontario (Burns and Honkala 1990). Quality black walnut logs for veneer and lumber are declining faster than they can be grown as harvesting continues and fewer quality sites are being regenerated to the species. In some plantings, the management objective is production of wood and nuts or just nuts. Many of the acres regenerated to black walnut and other high value species lack sufficient cultural treatments required for sustained good growth and high quality logs. In most cases, except for correcting soil pH, early fertilization is not required or recommended. Several years of weed control is needed. If nut production is the management goal, even on good sites, bearing trees will require fertilization.

The poor performance of black walnut on marginal sites can often be explained by reduced potential for rooting and consequent water stress impacts on photosynthesis and leaf abscission (Pallardy and Parker 1989). Walnut seedlings had poor root growth in soils that had high clay content and poor internal drainage (Dey and others 1987). In this paper I will briefly summarize the available literature on soil and nutrient relations for the species. Readers wanting more specific information on experimental results with fertilization should see Ponder (1997) and Pallardy and Parker (1989).

SOILS ON THE PLANTING SITE

Most of the minerals used by plants are derived from the soil; therefore, because black walnut is a high site-demanding tree, considerable attention should be placed on soil selection for the species, and on management practices that increase the opportunity for its roots to be in a healthy environment. Particular attention should be given to soil drainage (both surface and internal) and soil depth. Walnut grows best on land of good quality. Throughout its range, walnut generally reaches its greatest size and value along streams and on the lower portion of north or east facing slopes (Table 1). It needs a fertile soil. Black walnut needs a soil that provides an adequate supply of moisture throughout the growing season while at the same time provides adequate aeration for roots. Soils with medium to fine textures such as loam, sandy clay loam, silt loam or clay loam with good internal drainage are ideal for the species. The combined

[1]Research Soil Scientist (FPJr), USDA Forest Service, North Central Research Station, 208 Foster Hall, Lincoln University, Jefferson City, MO 65102. To contact, call 573-681-5575 or 573-875-5341, ext. 234 or e-mail at fponder@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Table 1.—Significant soil /site characteristics for black walnut growth.

Soil/site Characteristics	Suitable	Questionable	Unsuitable
Slope exposure	North or east		South or west
Position on slope	Middle, or lower with good drainage		Upper, or lower with poor drainage
Slope	0 to 15 %	15 to 30 %	>30 %
Depth to bedrock, gravel or clay layer	> 3 feet	2 to 3 feet	< 3 feet
Drainage class	Well to moderately well drained	Somewhat poorly drained	Excessively, poorly, or very poorly drained
Duration of flooding	Standing water up to 4 days in early spring		Standing water more than 4 days
Soil texture	Loam, silt loam, silty clay loam, silt, clay loam, sandy loam, sandy clay, fine sandy loam	Silty clay	Clay, sand, loamy sand, loamy fine sand

effect of soil texture and depth determines, to a large degree, available soil moisture, which can become critically low during some parts of the growing season.

The effective soil rooting depth should be 3 to 5 feet or more and not restricted by a sand or gravel layer, compacted till, massive clay layer or bedrock. Soils that are poorly drained, droughty, and sandy should be avoided. Suitable soils may be found on bottomlands that are not prone to excessive flooding (floods lasting more than 3 days) and low uplands including terraces.

With an increase in the development of plantations of grafted, high-producing nut cultivars, comes the increased need for supplemental fertilization. Research is being done to improve the benefits of nutrient fertilization by studying nutrient application rates for mineral and organic fertilizer amendments based on soil test results. Fertilization recommendations for black walnut do exist (Parker and others 1992). Soil fertility levels should be in the range of pH 6.5 to 7.2, 2.0 to 3.5% organic matter, 0.25 to 0.3% nitrogen (N), 60 to 80 lbs/ac phosphorus (P), 225 to 275 lbs/ac potassium (K), 2,000 to 3,000 lbs/ac calcium (Ca), and 250 to 300 lbs/ac magnesium (Mg). However, in most cases, the correlation between soil nutrient supply and tree growth and nut production is poor.

It is recommended that a soil test for nutrient levels be done as part of the site selection process and as part of the maintenance program to maintain growth and nut production. Care must be used in the interpretation of the results and laboratory recommendations because most soil tests have been calibrated to agronomic crop needs rather than for trees or horticultural crops. However, pH is a good indication of lime needs and the availability of some nutrients. For example, in soils with pH of 7.0 or higher, zinc (Zn) is held very tightly by the soil and may be unavailable for plant uptake.

Leaf analysis is a much better indicator of N, P, K and Zn deficiencies than soil test. Leaf nutrient concentrations give a much clearer indication of the tree's actual nutrient status. Black walnut leaf samples should be collected from several trees between the last 2 weeks of June and first 2 weeks of July from current year's growth. Leaves that are dirty or that have been damaged by insects or disease should be discarded. Sampling should be delayed for several days after heavy rains. Leaflets from the mid portion of the leaf omitting the first two to three leaflets at the bottom and at the top of the leaf should be collected with sufficient leaflets (75 to 100) to yield a 5- to 10-gram sample after leaflets are processed (dried and ground).

The nutrient-testing laboratory selected should be suitable for your needs. Most leading state universities that have agriculture programs maintain and operate soil and plant testing laboratories. If the laboratory does not make fertilizer recommendations for black walnut, you may need someone to interpret the results. The Soil and Plant Analysis Laboratory in Madison, WI (University of Wisconsin, Soil and Plant Testing Laboratory, 5711 Mineral Point Road, Madison, WI 53705, phone 606/262-4364) can analyze soil and plant samples and make recommendations for walnut based on the soil's pH, organic matter, and nutrient content (Parker and others 1992). Visit with personnel at your local Natural Resource Conservation office, County Extension office, or District Forester office for help in locating plant tissue testing laboratories and for other helpful suggestions.

Once a leaf analysis program has been established, leaves should be collected from the same trees and branches during the period as in previous years. Although, the grower will decide how often trees will be tested, it is best to test trees annually, especially if trees are being fertilized for nut production. The benefits of fertilization are limited by the tree's ability to convert nutrients into wood and nuts. The grower must remember that factors other than nutrition, such as inadequate moisture during the growing season and other weather related problems as well as inherent soil/site problems could limit good annual growth and nut production.

While the leaf analysis is a representation of the tree's actual nutrient condition, the combination of soil test and leaf analysis gives better information on what may be limiting the tree's productivity. The two tests provide additional information for making management decisions. Table 2 contains leaf nutrient values from walnut trees growing on a good soil for wood and nut production. Also, the change in nutrient levels during the growing season demonstrates the importance of collecting samples the same time as in previous years for comparing nutrient changes.

FERTILIZATION

Nitrogen can be supplied either in an organic form from legumes growing on the site (plantation) or by applying inorganic nitrogenous fertilizers. Legumes can supply in excess of 150 pounds of N per acre in one growing season. Nitrogen fixed by legumes is released to the soil and is free to be taken up by the trees or other vegetation. Because N is released from the legume over the growing season, it may be more beneficial than annual fertilizer applications for tree growth (Ponder 1994). However, an application of commercial N fertilizer can be done without the complications that may be associated with establishing and growing legumes. Diameter growth of 11-year-old walnut trees treated with slow-release fertilizer increased by 33 to 87% the first year and had more diameter growth 5 years later than tree fertilized with ammonium nitrate.

Phosphorus and K can be applied in combination with N or applied individually when inadequate levels are indicated by leaf analysis. Diammonium phosphate contains both N and P in concentrations of 18 and 46%, respectively. Nutrient sources such as triple superphosphate and muriate of potash can be used to supply P and K when applied separate or in combination with each other.

Table 2.—Mean concentrations of N, P, K, Ca, and Mg in leaves from 8-year-old black walnut trees at different times of the growing season for 2 years and suggested sufficiency range.[1]

Date	Nutrient Element				
Sampled	N	P	K	Ca	Mg
			Percent		
First year					
June 11	3.11	0.32	1.07	1.96	0.51
July 6	2.91	0.25	0.97	1.97	0.42
August 11	2.84	0.24	1.02	2.36	0.44
Second year					
June 11	3.52	0.25	0.98	1.28	0.37
July 6	3.02	0.20	0.80	1.61	0.36
August 11	2.73	0.21	0.83	2.15	0.41
Suggested sufficiency range[2]	2.47 – 2.98	0.16 – 0.24	1.32 – 1.47	1.90 – 2.01	0.51 –0.64

[1]*Ponder and others 1979.*
[2]*Mills and Jones 1996, sampled in summer.*

Fertilization of newly established walnut plantings has not proven very beneficial, especially if ideal soils were on the site. In most cases fertilizers depress the growth of seedlings and young trees by stimulating the growth of competing weeds. Controlling competing weed can increase walnut growth. In natural stands, Clark (1967) and Phares (1973) determined that only minor improvements in diameter growth could be obtained by reducing competition (release) and fertilization of walnut trees larger than poles. Ponder (1998) and Ponder and Schlesinger (1986) reported that while there was some benefit from fertilization, release increased diameter growth more. The diameter growth of 18-year-old plantation walnut trees was better when P and K were combined with N than when N was applied alone or P and K together.

Nutrient sufficiency ranges for various nutrients have been developed for black walnut to help determine nutrient needs based on leaf analyses (Mills and Jones 1996). As a guide, nutrients are added as leaf tissue levels approach the lower end of the range or below and nutrients are removed from the fertilizer program as the leaf tissue level of a given nutrient approaches the upper end of the range or higher. The nutrient sufficiency range for N, P, K, Ca, and Mg are presented in Table 2. The sufficiency range for some other nutrients in parts per million are: manganese (Mn), 207 to 274; iron (Fe), 69 to 129; Zn, 33 to 55; boron (B), 66 to 81; copper (Cu), 10 to 12; molybdenum (Mb), 0.10 to 0.30; and sulfur (S), 1500 to 1600. In general, to satisfy N needs for nut production, an application of 90 to 100 lbs of actual N per acre should be applied in March to early April and again in early August depending on leaf analysis. Both P and K should be applied at a rate of 60 to 100 pounds of actual ingredient in the spring. In cases of severe deficiency larger quantities can be applied.

Jones and others (1993) provided evidence that nut production was increased with an application of 6.4 oz of N, P, and K (13-13-13)/cm of tree diameter late in the summer compared with spring a spring application (Table 3). In another study, nut production was significantly increased following annual applications of N, P, and K fertilizers combined (Ponder 1998).

When legumes are used in the management of trees, soil test should be used to indicate the need for P and K rather than leaf analysis. Excessive P and K applied for legume nutrition will not adversely affect trees. By effectively taking care of P and K requirements for legumes, the trees need for these nutrients are also met.

While many black walnut growers are interested in producing wood and nuts, some are mostly interested in producing nuts. Nut producing cultivars are being grafted onto rootstocks of plantation trees that lack many of the nut producing characteristics of the cultivars (Hanson 1999). Several walnut plantations in Missouri are being managed primarily for nut production. Grafted trees of improved varieties are producing good nut crops annually. One reason for these growers success is likely due to the attention they give their trees and not to the soils the trees are planted on which are typically not suited or would not be recommended for growing black walnut for wood production.

Table 3.—Mean 5-year nut production of black walnut trees fertilized at different times of the year[1].

Treatment		
Time	Fertilizer Applied	Nut Production
		#/year/tree
Spring	Yes	127.5
Spring	No	125.7
Late summer	Yes	187.0
Late summer	No	122.1

[1]*Jones and others 1993.*

For example, the Knaust planting, which is approximately one-half acre in size with 35 trees, is being managed for nut production (Table 4). The deep silt loam soil has a fragipan at 18 to 27 inches of the soil surface, which severely limits available water during some parts of the growing season. The grower spreads 21 to 28 pickup loads of cow manure around trees annually in early spring. In mid April, the grower applies 450 pounds of 13-13-13 fertilizer. The orchard floor is covered with "cheat", a grass that dies by late June to form a mulch mat that conserves moisture and stops the invasion of other plants. Although restricted by water pressure and only with a garden hose available for use, some trees are watered. For this site, the difference in nut production in dry years may mean few or no nuts if trees were not watered. Nutritionally, under good management, the soil quality of the orchard exceeds that of a soil on a good site (Table 4). The grower summed up nut production, as "I am kept busy from fall into the winter cracking nuts, which I sell for by mail-order at $8.50 a pound."

The Gardner planting is another profitable nut operation (Table 4). Trees were, also, planted on soils typically not recommended for walnut wood production because the silt loam soil is poorly drained. The trees were later severed and stumps were grafted with several nut-producing cultivars. However, the grower maintains cheat grass for weed suppression and soil fertility, including the addition of minor nutrients, for good nut production (Table 4). The grower said that in some years he has all the nuts he can process without completely harvesting the nut crop.

While these two success stories are practical and not scientifically replicated, they do indicate that taking care of the nutritional needs of black walnut can increase nut production. These examples also suggest that providing good nutrition on marginal soils can make a difference for nut production.

In summary, planting black walnut on a good site is the first step to having a successful plantation. Soils that are 3 to 5 feet deep, well-drained, and have good moisture availability are important site criteria for the species. Sites with good soil moisture are candidates for fertilization. However, some sites with limiting site factors such as less than ideal soil depth, can respond to fertilization. The largest fertilization response will occur where nothing other than nutrient deficiencies limit the site's productivity. Annual testing of soil and leaves will serve as the best guide for determining the frequency of fertilizer application.

Table 4.—Soil chemistry for the upper six inches of soil at Marcules, Knaust, and Gardner black walnut sites[1].

	Depth (in)			Depth (in)			Depth (in)		
Sites	**0-2**	**2-4**	**4-6**	**0-2**	**2-4**	**4-6**	**0-2**	**2-4**	**4-6**
	pH			*EC (mmhos cm^{-1})[2]*			*% Carbon (OM)[3]*		
Marcules	7.2	6.7	6.6	127	23	14	2.1	1.0	0.9
Knaust	7.5	7.5	7.4	259	173	109	5.2	2.6	1.1
Gardner	7.5	7.5	7.4	152	144	97	3.5	3.0	2.0
	P (lbs/A)			*K (lbs/A)*			*NO_3-N (lbs/A)*		
Marcules	195	118	119	648	339	242	12.7	6.3	4.2
Knaust	427	259	114	1339	907	755	25.8	14.7	9.7
Gardner	186	154	148	644	523	426	9.3	8.8	4.7
	Ca (lbs/A)			*Mg (lbs/A)*			*Na (lbs/A)*		
Marcules	3627	2565	2571	271	108	89	17.8	13.8	17.3
Knaust	7691	5105	2949	639	373	220	33.3	23.3	23.6
Gardner	4889	4392	3893	536	432	386	18.3	17.5	16.0
	S (lbs/A)			*Fe (lbs/A)*			*Mn (lbs/A)[4]*		
Marcules	19.5	12.6	10.7	124	129	126	183	165	123
Knaust	67.7	23.7	14.0	99	88	69	163	170	185[5]
Gardner	22.0	20.8	16.3	73	69	59	233	216	198
	Zn (lbs/A)[4]			*Cu (lbs/A)*			*B (lbs/A)*		
Marcules	62.3	46.0	39.7	7.2	6.2	7.2	1.4	0.6	0.5
Knaust	42.3	25.7	13.3	4.8	4.6	5.7	2.7	1.4	0.6
Gardner	93.8	90.5	118	6.4	5.7	5.5	2.0	1.6	1.1

[1]Mean of four random samples. The Marcules plantation is on a bottomland site with deep, nearly level, well drained Huntington silt loam soil. The Knaust plantation is on a bottomland site with deep, moderately well drained silt loam soil with a firm and brittle 15 inch thick fragipan less than 28 inches from the soil's surface. The Gardner plantation is on a bottomland site with deep, somewhat poorly drained silt loam soil.

[2]Electrical conductivity used as an index of the total concentration of dissolved salts.

[3]OM=organic matter, P=phosphorus, K=potassium, NO_3-N=nitrate nitrogen, Ca=calcium, Mg=magnesium, Na=sodium, S=sulfur, Fe=iron, Mn=manganese, Zn=zinc, Cu=copper, B=boron.

[4] High Mn and Zn levels are due to their application to the soil.

[5] Samples are in the correct order.

LITERATURE CITED

Burns, R.M.; Honkala, B.H., tech. coords. 1990. Silvics of North America. Volume 2, Hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service. 877 p.

Clark, F.B. 1967. Pole-size black walnut responds quickly to crown release. Journal of Forestry. 65: 406-408.

Dey, D.; Conway, M.R.; Garrett, H.E.; Hinckley, T.M.; Cox, G.S. 1987. Plant-water relationships and growth of black walnut in a walnut-forage multi-cropping regime. Forest Science. 33: 70-80.

Hanson, B.L. 1999. Commercial black walnut orchard in Iowa (Ben's black walnuts). Annual Report of the Northern Nut Growers Association. 90: 119-126.

Jones, J.E.; Garrett, H.E.; Haines, J.; Lowenstein, E.F. 1993. Genetic selection and fertilization provide increased nut production under walnut-agroforestry management. Annual Report of the Northern Nut Growers Association. 86: 28-34.

Mills, H.A.; Jones, J.B. 1996. Plant analysis handbook II. Athens, GA: MicroMacro Publishing. 442 p.

Pallardy, S.G.; Parker, W.C. 1989. Water and nutrient relations of black walnut. In: Phelps, J.E., ed. The continuing quest for quality: 4th Black walnut symposium; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council Inc.: 46-66.

Parker, D.; Bockheim, J.; Meyer, D. 1992. Soil and site selection. Fact Sheet 68, Walnut Tips. Wisconsin Walnut Council.

Phares, R.E. 1973. Managing immature trees for more high-quality logs and released products. In: Black walnut as a crop: 2nd black walnut symposium; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep. NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 49-54.

Ponder, F., Jr. 1997. Walnut fertilization and recommendations for wood and nut production. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 128-137.

Ponder, F., Jr. 1998. Fertilizer combinations benefit diameter growth of plantation black walnut. Journal of Plant Nutrition. 21: 1329-1337.

Ponder, F., Jr. 1994. Comparison of slow- and quick-release fertilizers on black walnut growth. Annual Report of the Northern Nut Growers Association. 85: 40-43.

Ponder, F., Jr.; Schlesinger, R.C. 1986. Release and fertilization of natural black walnut. Northern Journal of Applied Forestry. 3: 153-155.

Site Relationships and Black Walnut Height Growth in Natural Stands in Eastern Kansas

Wayne A. Geyer and Felix Ponder, Jr.[1]

ABSTRACT—Prediction of forestland productivity is needed for proper species selection in tree planting. By relating site quality to site and soil characteristics, potential productivity can be estimated for non-forested areas. Our study measured the growth potential of black walnut in natural stands in southeastern Kansas. We looked at over 200 stands on unglaciated soils. Numerous environmental factors were evaluated within the following broad categories: site, soil chemical, and soil physical. These environmental factors were related to tree height at the age of 50 years. Simple correlation and multiple regression analyses were run against site index using over 60 variables of soil and topographic characteristics. Depth to a restrictive layer explained 74% of the variation in height.

The natural range of black walnut (*Juglans nigra* L.) extends westward into central Kansas (Williams 1990). Walnut is found as a major component of the prairie-forest fringe environment in eastern Kansas. Although nearly pure stands of black walnut are uncommon, it is found on a wide variety of ecological sites. Most often, black walnut occurs as small patches along the river valleys, side drainages and adjacent slopes, and is often bordered by abandoned cropland and abused pasture-land (Grey and Naughton 1971).

Generally black walnut is found on deep, moist, well-drained areas having good soil structure (Auten 1945, Carmean 1970) and near neutral in reaction (Spurway 1941, Wilde 1934). Soil reaction and nutrient levels were related to walnut growth in 15-year-old walnut plantations in southeastern Iowa (Thompson and McComb 1962). Prediction equations, using important soil and topographic variables, have been generated in an earlier study by Geyer and others (1980) for northeastern Kansas.

Our study attempts to relate tree growth differences as indicated by site index at 50 years to many physical and chemical site factors. Generally, factors relating to moisture availability are of utmost importance to tree growth.

METHODS

Description of the Study Area

The study area is located within the Central Lowlands physiographic province (Keys and others 1995). Elevations range from 800 to 1200 feet. The surface soils are mostly of silt loam to clay loam texture, often with limestone and shale outcroppings on the slopes. Most are of Mollisol origin (soils that developed under prairie vegetation). The climate is typical continental with most of the precipitation in the warm season. The study range covered over 135 miles north and south and 115 miles east to west with moisture patterns of 28 inches annual precipitation in the northeast to 45 inches in the southeast portions of the study area (Fig. 1).

Field Plots

Our investigation evaluated over 210 plots in the unglaciated area of southeastern Kansas on all topographic positions (upland, bottomland, lower slopes, and intermittent streams) and generally well distributed on all four-direction quadrants. About two-thirds of the study plots were given detailed

[1]Professor of Forestry (WAG), Division of Forestry, Throckmorton Hall, Kansas State University, Manhattan KS and Research Soil Scientist (FPJr), North Central Research Station, USDA Forest Service, 208 Foster Hall, Lincoln University, Jefferson City, MO 66506. WAG is the corresponding author: to contact, call 785-532-1409 or e-mail at wgeyer@oznet.ksu.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

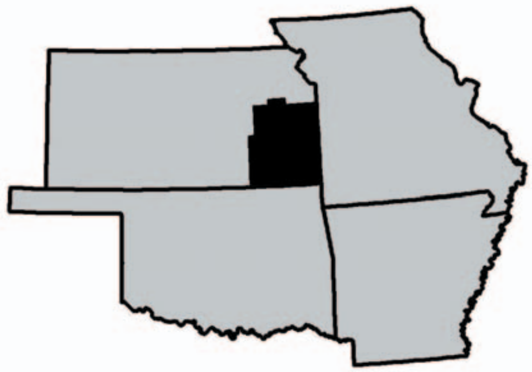


Figure 1.—Study area for location of black walnut field plots.

analysis. The remaining plots were discarded because of discrepancies in tree numbers, tree quality, and missing data. All plots were located in natural hardwood timber stands containing significant amounts of black walnut.

Trees selected for analysis were in dominant and/or codominant crown classes and had no visible signs of stagnation or suppression. We measured two to four walnut trees in each plot. Total height of each tree was measured with a Blume-Leiss altimeter. Tree age was determined from core samples of wood taken at about 4 inches above the ground. Using Kellogg's site index curves for walnut plantations (Kellogg 1939) and plot averages of height and age, we evaluated site index. Site index is the average height of dominant and codominant trees in a stand at age 50 years. Taller trees at a given age indicate a site of higher productivity.

Site Variables

We classified each site by topographic characteristics and each soil profile by soil parent material, either alluvial (bottomland) or residual (upland), and assigned values of +1 or −1, respectively. Landform was classified as bottomland, intermittent stream, lower one-third slope or upland, and assigned values of 1, 2, 3, or 4. Azimuth and slope were determined with a compass pointed down slope through plot center and an altimeter, respectively. Assuming the northeast aspect as the most favorable for tree growth and southwest as the least favorable, we tested two transformations of aspect. First, the cosine (azimuth – 45 deg.) +1 as described by Beers and others (1967) and second, the corrected azimuth from the southwest as described by Munn (1974) along with true azimuth to see if they would correlate with walnut growth. Slope steepness was expressed as slope percentage, less than 10%, less than 5% or equal to or exceeding 10%.

Other variables tested were depth to a restrictive layer (defined as textural B horizon—a massive rock layer, gravel layers of at least 75% rock, a water table, mottling, or 60 inches, whichever occurred at the least depth), and effective depth to a restriction (defined as rock percentage in inches removed from depth to restriction). Also total soil depth, effective soil depth (defined as rock percentage in inches removed from total soil depth), (Steinbrenner 1965) and water holding capacity of total soil depth or water-holding capacity to the restrictive layer. Both are sums of available water-holding capacity calculated by textural horizons.

Soil Analysis

Soil pits were located in between the trees and were dug to a maximum of five feet and the morphological characteristics of each soil profile described. One soil sample from each horizon was analyzed in the laboratory for both chemical and physical characteristics. Soil textures were determined by the Bouyoucos method (1951), except sodium hexametaphosphate was used instead of Calgon as a dispersing agent. Chemical tests were run for both the surface and subsurface horizons. Lime (ECC/ac) pounds needed for crop production, pH, Bray 1 phosphorus, potassium, calcium, magnesium, sodium, and organic matter were done by the Kansas State University Soil Testing Laboratory using standard agricultural testing procedures. The relationship between site index and soil factors was evaluated using simple correlations. Correlation coefficients for the 60 independent variables were calculated with the independent variable, site index, to find the best for generating a multiple regression equation using the stepwise backward elimination procedure (Barr and others 1976).

RESULTS AND DISCUSSION

Analyses of the data showed the largest simple correlation of site index was with soil depth to a restrictive layer (r = +0.85). Other highly correlated factors were rooting depths, expressions of water holding capacity, and soil parent material. Three of the four factors (soil depth, rooting depths, and expressions of water holding capacity) are very closely related. Deep soils can hold more water and have more space for root development. Soil moisture is likely the underlying factor associated with the high correlation of soil depth and site index. Even though black walnut is deep rooted, almost 80% of its root system occupies the upper 24 inches of the soil (Pham and others 1978). Water needs for black walnut are greatest during the growing season at a time when soil water content within the rooting zone decreases due to plant uptake and evapotranspiration. Carpenter and Hanover (1974) reported that for black walnut seedling height growth slows after mid June and ceases by late July, suggesting that seasonal height growth is completed early for the species. These authors also suggested that black walnut is typical of those deciduous species with preformed seasonal shoot growth.

Black walnut is believed to function as drought avoidance species as opposed to drought tolerant species (Lucier and Hinckley 1982). As a drought avoidance species, black walnut is able to maintain plant water content near optimum levels during periods of low soil moisture availability and high atmospheric evaporative demands by initiating stomatal closure restricting photosynthesis and depending on the duration of unfavorable conditions, shedding its leaves. An investigation of black walnut site quality in relation to soil characteristics in northeastern Kansas indicated that site index increased as effective soil depth increased (Geyer and others 1980).

Surprisingly, neither aspect ($r = -0.07$, $p = 0.484$), nor the amount of sand in either the surface or subsurface horizons was correlated with site index (surface $r = 0.059$, $p = 0.563$; subsurface $r = 0.05$, $p = 0.618$) or silt + clay (surface $r = -0.07$, $p = 0.487$, subsurface $r = -0.010$, $p = 0.329$). Neither was organic matter (surface $r = 0.47$, $p = 0.001$, subsurface $r = 0.36$, $p = 0.0004$). Aspect and silt plus clay were important to black walnut site quality in northeastern Kansas (Geyer and others 1980). Soil texture was a major determining factor in the growth of planted walnuts in Kentucky (Kalisz and others 1989). The growth rates of young black walnut plantations were significantly greater on suitable compared to questionable soils. In the Kentucky study, approximately 75% of the soils judged to be questionable for walnut management were limited by the presence of clay subsoil, 10% by shallowness to bedrock, 10% by imperfect drainage, and 5% by the presence of a fragipan.

Soil organic matter had a negative relationship when greater than 4.3%, but thickness of the A horizon had an “r” value of 0.66 and $p = 0.0001$ (Table 1). However, it is important to take soil texture into account in this layer because texture affects soil water storage and availability. Another study (Geyer and others 1980) found that coefficients for soil texture in the A horizon decreased with an increase in clay and increased when the silt content of the clay increased.

Soil nutrient concentrations were not positively correlated with site index for any of the tested nutrients (data not presented). However, optimum walnut growth has been suggested to occur when soil pH values range from 6.0 to 8.0 (Spurway 1941). In the present study, calcium and potassium seem to be the overriding factors. We ran a range of surface pH values and found pH was negatively related to site index when greater than 7.4.

Table 1.—Simple correlations with site index (20 thru 80 years age).

Variable	Mean	Range	Simple Correlation (r)
Depth to restrictive layer (DRL)	31.9″	2.0-60.0	+ 0.85
Estimated soil depth to DRL	31.4	3.0-60.0	+ 0.82
Water holding capacity to DRL	6.2	0.4-14.9	+ 0.82
Soil origin (upland/bottomland)	--	+1, -1	+ 0.67
Landform (bottomland/intermittent/ lower 1/3rd, upland)	--	1,2,3,4	- 0.67
Thickness “A” horizon	20.0	5.3-60.0	+ 0.66
Total soil depth	44.1	6.0-60.0	+ 0.66
Estimated soil depth	41.0	5.3-60.0	+ 0.64
Water holding capacity all horizons	8.1	1.0-14.9	+ 0.63

CONCLUSION

Depth to restrictive layer was a good predictor of height growth for black walnut stands in eastern Kansas. The positive coefficient in the equation for this variable indicates that site index increased as the effective soil depth increased. Many factors used in this study were related to each other and we selected those most apparent and easy to recognize for developing our multiple regression equations. Our best equation, site index = 43.84 + 0.443 depth to restrictive layer + 1.98 soil type, explained 74% of the variation in height. Adding the other soil factors improved predictability by only 5%. Avoiding sites with shallow soils and other properties that reduce water availability and concentrating management on good sites will save resources and increase the growth and value of the trees. Although, data for this study was collected in southeastern Kansas, these results should be useful and applicable for areas in surrounding states.

LITERATURE CITED

Auten, J.T. 1945. Some soil factors associated with site quality for planted black locust and black walnut. Journal of Forestry. 43: 592-598.

Barr, A.J.; Goodnight, J.H.; Sall, J.P.; Helwig, J.T. 1976. A user's guide to SAS. Raleigh, NC: SAS Institute, Inc. 329 p.

Beers, T.W.; Dress, P.E.; Wensel, L.C. 1967. Aspect transformation in site productivity research. Journal of Forestry. 65: 22-24.

Bouyoucos, G.J. 1951. A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy Journal. 43: 432-437.

Carmean, W.H. 1970. Tree height–growth patterns in relation to soil and site. In: Tree. Corvallis, OR: Oregon State University Press: 499-512.

Carpenter, S.B.; Hanover, J.W. 1974. Comparative growth and photosynthesis of black walnut and honey locust seedlings. Forest Science. 20: 317-324.

Geyer, W.A.; Marquard, R.D.; Barber, J.F. 1980. Black walnut site quality in relation to soil and topographic characteristics in northeastern Kansas. Journal of Soil and Water Conservation. 35: 135-137.

Grey, G.W.; Naughton, G. 1971. Ecological observations on the abundance of black walnut in Kansas. Journal of Forestry. 69: 741-743.

Kalisz, P.J.; Stringer, J.W.; Hill, D.B. 1989. Growth of young black walnut plantations in Kentucky. Northern Journal of Applied Forestry. 6: 17-20.

Kellogg, L.F. 1939. Site index curves for plantation black walnut in the Central States Region. Note 35. Columbus, OH: U.S. Department of Agriculture, Forest Service, Central States Forest Experiment Station. 3 p.

Keys, J., Jr.; Carpenter, C.; Hooks, S.; Koenig, F.; McNab, W.H.; Russell, O.; Smith, M.L. 1995. Ecological units of the eastern United States—first approximation (map and booklet of map unit tables). Atlanta, GA: U.S. Department of Agriculture, Forest Service.

Lucier, A.A.; Hinckley, T.M. 1982. Phenology, growth, and water relations of irrigated and non-irrigated black walnut. Forest Ecology and Management. 4: 127-142.

Munn, L.C. 1974. Predicting height growth of yellow-poplar from soils and topography on a Muskinggum-Berks-Neotoma soils complex in southeastern Ohio. Columbus, OH: Ohio State University. 61 p. M.S. thesis.

Pham, C.H.; Yen, C.P.; Cox, G.S.; Garrett, H.E. 1977. Slope position, soil water storage capacity, and black walnut root development. In: Balmer, W.E., ed. Soil moisture-site productivity symposium; Myrtle Beach, SC. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station: 326-335.

Spurway, G.W. 1941. Soil reaction (pH) preferences for plants. Bull. 306. East Lansing, MI: Michigan Agricultural Experiment Station.

Steinbrenner, E.C. 1965. The influence of individual soil and physiographic factors on the site index of Douglas-fir in western Washington. In: Youngberg, C.T., ed. Forest soils relationships in North America. Corvallis, OR: Oregon State University Press: 261-270.

Thompson, G.W.; McComb, A.L. 1962. Growth of plantation black walnut in relation to pH and certain chemical factors of the soil. Forest Science. 8: 322-333.

Wilde, S.A. 1934. Soil relation to forestry, and its determination by simple tests. Journal of Forestry. 32: 415.

Williams, R.D. 1990. *Juglans nigra* L. black walnut. In: Burns, R.M.; Honkala, B.H., tech. coords. Silvics of North America: 2. Hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 391-399.

Managing Black Walnut in Natural Stands: The Human Dimension

H.E. "Hank" Stelzer[1]

ABSTRACT— In managing black walnut, or any forest tree species, the human dimension is often overlooked. As a result, both the number of landowners managing their land and the number of forested acres under management has not significantly increased over the past 30 years. Elements of the human landscape are explored and a roadmap for engaging landowners is proposed.

When asked to write on the subject of managing black walnut (*Juglans nigra* L.) in natural stands, my first response was, "Why?" What had changed since the last overview was presented at the Fifth Black Walnut Symposium (Slusher 1997)? Other than a few new herbicides to control competing vegetation, the answer was "Not much". During my search for new information, I realized just how much information was available to the landowner, especially with respect to managing hardwood stands. Much of this information is contained within the published proceedings from past Central Hardwood Forest Conferences. All papers from the first twelve conferences are now available on-line through the table of contents viewed at http://www.ncrs.fs.fed.us/pubs/viewpub.asp?key=1065 (Fralish 2002).

But, how extensively has this wealth of knowledge been applied? Forest statistics yield a sobering answer. Within the naturally occurring range of black walnut non-industrial private forest (NIPF) landowners own 86% of the timberland area with black walnut present; 13.2 million acres (Schmidt and Kingsley 1997). But, only an estimated 5% of NIPF landowners have a written management plan (Reed 1998). While many forestland owners indicate that they have a plan, it often is not documented in a way that would allow its easy sharing with others. What is most distressing is that the number of managed acres has not changed in the past 30 years!

Why hasn't the acreage of managed forests increased? Among the most striking feature of NIPF land is its concentration in small tract sizes. More than 90% of these parcels are less than 100 acres and of those more than 70% less than 10 acres (Reed 1998). Current information additionally suggests that the number and proportion of NIPF landowners who own small parcels will only increase in the coming years.

While some appreciation for the economic value of fine hardwoods in natural stands has been passed from one generation to the next, it is painfully obvious that there is a growing lack of appreciation for this economic value among the new landed gentry. One just has to look around and observe the indiscriminant clearing of small-diameter black walnut and other fine hardwood species as drainages and fencerows are "cleaned out" or in other cases, no management activity occurs as the land sits in a descendant's trust.

THE HUMAN LANDSCAPE

So, today's challenge is actually the same challenge from days past; but, with a twist. The time-honored challenge is how to interest private landowners with walnut in their woodlots to manage this resource. The twist is that the target audience is growing larger each day as land ownership continues to fragment, but the number of professional foresters to generate interest in forest management has remained relatively flat over the past 20 years.

To gain the most effect from limited resources invested in NIPF assistance, identification of submarkets for focus may help accomplish more

[1]Extension Forester (HES), Department of Forestry, University of Missouri, 203 ABNR, Columbia, MO 65211. HES is corresponding author; to contact, call (573) 882-4444 or e-mail at stelzerh@missouri.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

immediate results. Black walnut growing in natural stands, for example, is an appropriate model submarket. Stands that are nearing harvest age could focus landowner attention on the economic value of fine hardwoods for lumber and veneer markets. Immature stands could provide opportunity for showcasing even larger economic return through simple management activities such as thinning and pruning.

By determining who owns or manages land that could contain natural stands of black walnut, foresters could concentrate their outreach efforts on selected audiences. Three distinct groups come to mind: long-time woodland owners, new woodland owners and trust officers. Long-time landowners are older, have more practical experience and rely upon personal relationships in learning new skills. They usually reside on property that has been in the family for generations. Of these three groups, the long-time landowner stands the best chance of recognizing the value of timber, since in all likelihood they have sold timber from their land at some point in time. These individuals are also more likely to know, or at least recognize, named and branded organizations like the Walnut Council or the American Tree Farm System.

New landowners tend to be younger, better educated, and use technology to access and manage information. They also tend to be absentee landowners. Trust officers comprise a new segment of forest “ownership”. While they do not own the land, they serve the interests of their clients. With the need to serve many clients simultaneously, these individuals tend to have many of same attributes of new landowners. Individuals belonging to both of these groups have a much more casual relationship with trees and as such do not fully appreciate the many benefits of a managed forest, let alone see the forest’s economic potential. Mention of the name “Walnut Council” would in all likelihood generate a blank stare.

Even within these narrow subgroups, one size does not fit all when it comes to learning. While there are many learning styles, behavioral scientists have divided these styles into two types: analytical and rational. Individuals possessing an analytical learning style have long attention spans and can resist distractions. They tend to be highly reflective and can concentrate on the activity at hand. They prefer complexity. These individuals also prefer a more formal learning environment and see the instructor as an information giver. They tend to view the learning experience as a nonsocial event.

On the other hand, individuals having a rational learning style have relatively short attention spans and are easily distracted. Simple tasks and concepts appeal to them. They value learning centered in the self that has personal relevance. People in this group prefer the informal learning setting and see the instructor first as a person.

Up to now, the focus has been on target audiences. What about instructors and their message? While long a foundation of forestry outreach and education programs, foresters need to shift away from one-dimensional prescriptions of basically timber management information and advice. Due in part to the huge and increasing diversity of owners, broader management approaches are needed. Natural resource disciplines can work together. Wildlife biologists and foresters can deliver an integrated, value-added package to landowners so that they see multiple benefits, both in the short-term (improved wildlife habitat) and long-term (higher quality timber). These benefits in turn make their land more valuable in real dollars.

A ROADMAP FOR ENGAGING LANDOWNERS

What do submarkets, learning styles and multi-dimensional delivery programs have to do with managing black walnut in natural stands? Plenty! Foresters and Walnut Council members alike must use their limited resources to speak to the right audiences. If they do not, then as older landowners die and land fragmentation increases, the number of acres of natural black walnut and other fine hardwood stands under management will not increase, but decrease.

How can the number of natural black walnut stands that are actively managed be increased? There are undoubtedly many ways to reach this goal. From my perspective I offer the following four recommendations to motivate the collective “us” to work smarter AND harder. The old cliché, “work smarter, not harder” is a misnomer—it has allowed our society to take the easy way out. We will have to do both if we want to be successful.

First, identify landowners who own natural hardwood stands that might include black walnut. Geographical information systems are becoming more widely used by state and county governments for tracking a wide array of information, including property and timber sale transactions. In many cases, this information can be gathered free of charge; in other cases, data analysts provide this information for a nominal fee. Outreach activities can target these landowners with the primary goal of raising their level of awareness as to the total value of the natural resources on their property. The aforementioned trust officers must not be forgotten. Efforts need to be made to network with property appraisers and financial institutions and identify those officers who are responsible for managing these land trust accounts.

Second, refine both low- and high-tech information vehicles and make sure they are accessible and relevant to individuals managing hardwood stands. Newsletters need to deliver information in a timely manner that is practical and easily digested so landowners can apply this new knowledge to their land. An emphasis on access to information will offer opportunities to reach a greater number of motivated owners in contrast to the more typical delivery of structured programs. This will be especially helpful for those new, younger landowners and trust officers.

Web sites need to be easily navigable with sophisticated graphics kept to a minimum in order for rapid downloading of information from the site. An excellent example of an easy-to-navigate Web site is Walnut Notes at http://www.ncrs.fs.fed.us/pubs/viewpub.asp?key=103. A visitor can quickly download a series of short PDF files on a wide variety of black walnut management topics. However, if net surfers have to wait for dazzling images to appear on their screens or the information they seek is contained in exceedingly large document files, chances are very good that they will not re-visit the site. Case in point, the last proceedings from the 1996 Walnut Symposium is available on-line at http://www.ncrs.fs.fed.us/pubs/viewpub.asp?key=255. If one wants information on, for example, top-working black walnut then they would have to download the entire proceedings—all 59.3 MB. This might not appear to be a big issue to residents of metropolitan areas with high-speed internet access. But, to individuals living in rural areas who are constrained by 56K modems, downloading web pages with large embedded files is a big and very real frustration.

Providing technical assistance to NIPF owners has clear results—more active forest management. However, important questions remain about the origin, quality and cost of such assistance. With the number of landowners increasing and the number of resource professionals holding constant at best, the days of multiple one-on-one on-site consultations are clearly numbered.

The third recommendation is shift the paradigm where both landowners and foresters see the role of the local forester less as a "county agent" and more of a "coach". Among the most innovative technical assistance tools being extensively tried these days is coached planning. This outreach effort redefines technical assistance to utilize professionals as advisers of work that is actually done by the landowners themselves. However, to implement this team approach, each state will have to come to its own set of terms about the appropriate roles and mix of services by the private consulting sector and public service foresters, as well as the prospective role played by NIPF owners themselves.

The role of NIPF owners as teachers is increasingly recognized. A growing number of successful volunteer programs, such as Master Gardener, have only begun to tap the potential of using private citizens to advance information in their own groups of influence, as well as teach professionals about emerging interests or challenges. Walnut Council members are instrumental in this type of outreach effort. The learning and knowledge based that is accumulated and shared among landowners empowers them to become even more active in the management of their land. It also gives them the confidence to teach other landowners.

The fourth, and final, recommendation is think outside the box when "recruiting" either clients or fellow landowners. Most natural resource professionals have been trained to approach "the big picture" from their own, sometimes rather myopic, perspective. Sure, most professionals endured some cross-training in their collegiate years and they might even interact with others outside their discipline. But, for the most part they still think the best way to approach the problem is through the "enlightenment" that comes through their discipline.

My epiphany came when a consulting forester and avid walnut grower was asked to speak about managing timber for wildlife at a local Quality Deer Management meeting in central Missouri. Over 200 people from the local area came out to learn how they could harvest bigger deer. Wildlife management was the hook, the motivator to get them managing their woods. At the meeting there were large land plat maps posted along one wall. One by one, landowners approached the maps, took the highlighter and colored in their property indicating their willingness to participate in the program. This simple exercise accomplished three things. First, it gave each landowner a view of the bigger picture that managing his or her land would have some impact on neighboring property. Second, it gave them a sense of ownership as they took pen in hand and colored in their parcels. Third, it put subtle pressure on those landowners who were not participating to manage their woods.

The Walnut Council was formed in 1970 by a small group of landowners, foresters, forest scientists and wood processors to promote the proper management and utilization of black walnut. Over the years the Council has grown into an international organization that reaches private landowners through newsletters, and chapter, state and national meetings. In that time, professional foresters and forest scientists delivered information in a timely manner as it became available from Federal and State agencies, and universities. Yet, after 33 years there are still less than 1,000 members; of which even fewer are landowners if you subtract professional members.

So, the background information and strategy presented here is extremely relevant if we are serious about increasing both the number of individuals actively managing their woodlands and in turn the number of acres under good forest management. Of all the dimensions of cultivating black walnut that have been researched and published over the years, it is time we turn our attention to a key piece of the puzzle often overlooked: the human dimension.

LITERATURE CITED

Fralish, J.S., ed., comp. 2002. The Central Hardwood Forest conferences 1976-1999: Title, subject, author, and species indexes for the proceedings. Gen. Tech. Rep. NC-225. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 227 p.

Reed, A.S. 1998. Targeting extension programs to nonindustrial private woodland owners. In: Johnson, J.E., ed. Proceedings, Extension forestry: bridging the gap between research and application. Blacksburg, VA: Virginia Polytechnic Institute and State University, College of Forestry and Wildlife Resources: 213-220.

Schmidt, T.L.; Kingsley, N.P. 1997. Status of black walnut in the United States. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 9-22.

Slusher, J.P. 1997. Principles of managing for black walnut in mixed hardwood stands. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 148-154.

GROUND COVER MANAGEMENT IN WALNUT AND OTHER HARDWOOD PLANTINGS

J.W. Van Sambeek and H.E. Garrett[1]

ABSTRACT—Ground cover management in walnut plantings and established stands can include (1) manipulating the resident vegetation, (2) mechanical control, (3) chemical control, (4) mulching, (5) planting cover crops, or (6) interplanting woody nurse crops. Data from over 110 reports were used to compile a database that compared growth of black walnut and other hardwoods under different ground cover treatments as either a percentage of tree growth in the absence of ground cover vegetation or with little or no management of the resident vegetation. Overall, ground cover treatments with the best tree growth were application of organic mulches and annual cultivation. Ground covers associated with the slowest tree growth were grass sods and unmanaged or mowed resident vegetation. Black walnut tended to be slightly more sensitive to ground cover management practices than other hardwoods. The choice of a vegetation management system is largely controlled by management objectives, site characteristics, equipment costs, and, most importantly, labor availability.

Vegetation management, especially weed control, is second in importance only to site selection in establishing plantations of black walnut (*Juglans nigra* L.) as no silvicultural practice can rescue a black walnut plantation on an unsuitable site (Burde 1988, Burke and Pennington 1989). Unlike many other hardwoods, walnut in managed plantings will not dominate a site sufficiently to exclude a ground layer of semi-shade tolerant vegetation. Because they have thin, open crowns, walnut trees absorb less than 60% of the incoming solar radiation in fully stocked stands (Smith 1942). In addition, walnut is one of the last tree species to leaf-out in the spring and one of the first to defoliate in the fall. This makes it possible to grow a wide variety of shade-tolerant forbs and grasses within plantings and agroforestry practices that use walnut. The questions addressed in this paper are (1) does the type of ground cover management affect the growth of hardwood saplings and pole-sized trees and (2) is the response of black walnut to ground cover management similar to that for most other hardwoods? If the latter is true, we can use the research information from other hardwoods to recommend alternative management scenarios for walnut without actually testing them.

The wide variation in growth rates both within and among hardwood species such as that reported by Hansen and McComb (1955) for Iowa makes it difficult to use measurements such as annual height, diameter, or volume growth to directly compare tree response to different ground cover treatments. One alternative is to calculate the growth under different ground cover treatments as a percentage of the growth to a treatment commonly used in most ground cover studies. A quick survey of the literature suggests there are two commonly used treatments that have this potential: unmanaged plots and vegetation-free plots. For example, Schlesinger and Van Sambeek (1986) reported that walnut saplings in a tall fescue sod grew 0.56 inches in DBH over a 5-year period while walnut saplings in cultivated plots grew 1.85 inches over the same period. In this case, walnut diameter growth in tall fescue was only 30% of that in the vegetation-free control. As another example, Van Sambeek and others (1986) reported that walnut saplings planted with hairy vetch on a bottomland site averaged 4.7 feet in height after 3 years compared to only 3.7 feet for walnut saplings in unmanaged plots growing with a normal succession of weeds. Similarly, height of walnut with hairy

[1]Research Plant Physiologist (JWVS), USDA Forest Service, North Central Research Station, 202 Natural Resource Bldg.-UMC, Columbia, MO 65211-7260 and Director (HEG), Center for Agroforestry, University of Missouri, 103 Natural Resource Bldg.-UMC, Columbia, MO 65211-7270. JWVS is corresponding author: to contact, call (573) 875-5341 ext 233 or e-mail at jvansambeek@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

vetch on an upland site averaged 6.4 feet compared to that of 5.3 feet in the unmanaged control. In this case, height growth of walnut planted with hairy vetch was 127% and 121% of that in the unmanaged control for the upland and bottomland plantings, respectively.

For this paper, we examined the methods and results from over 110 reports (52% of which included walnut) resulting in over 2,500 comparisons that examined effects of different ground cover treatments on growth of hardwood saplings and pole-sized trees. To create the ground cover management database, we compiled the following information into a spreadsheet: literature source, planting location by state, the year experiment was initiated, tree species, growing space and percent of growing space in experimental treatment, initial and final tree ages, control treatment (resident vegetation or vegetation free), experimental treatment(s), initial and final measurements (height, diameter, volume, fruit yield, or foliage nitrogen) for both control and treatment trees, and whether or not the authors reported statistical differences ($P < 0.05\%$). (The spreadsheet titled "BW_GCM_Database_Dec2003.xls" is available electronically from the FS-NC-4154-02-03 study files of the Ecology and Management of Central Hardwoods Project of the U.S.Department of Agriculture, Forest Service, North Central Research Station.)

Criteria for being included in the database were that (1) one or more hardwoods or broad-leaved tree species were included in the study, (2) at least one treatment was left unmanaged or one or more treatments were maintained relatively free of competing vegetation, and (3) reported measurements spanned two or more years to minimize responses to transplant shock or atypical climatic conditions.

We calculated tree growth response to each ground cover treatment as a percentage of either the unmanaged or vegetation-free control for each hardwood species included in each paper. We assume the vegetation-free control is an estimate of maximum tree growth in the planting and is the average of one or more treatments with mulches, mechanical and/or chemical weed control. Preliminary analysis of the resultant percentages showed responses were normally distributed with only a few outliers. Outliers primarily occurred in studies where trees in the experimental treatment showed excellent growth with little or no growth in an unmanaged control (resident vegetation). For this paper, treatment responses as a percent of the control exceeding two standard deviations of the group mean were treated as missing values. Because of unequal variances and degrees of freedom, adjusted variances and tabulated t-values (t') were calculated according to Steel and Torrie (1960) to determine appropriate degrees of freedom and probability of statistical differences.

Ground cover management treatments can be divided into six broad categories that include (1) manipulation of the resident vegetation (mowing, nitrogen fertilization, irrigation, or applying selective herbicides), (2) bare ground maintained by mechanical methods (scalping, disking, or rototilling), (3) bare ground established using various chemicals, (4) mulching (organic or inorganic), (5) cover crops (legumes or grass sods), and (6) woody nurse crops. Average percentage + standard deviation (the range within which two thirds of all results are expected to occur) were computed for each category for walnut and for all other hardwoods. We used analyses of variance to test for differences among the categories and for differences between black walnut and other hardwoods within the six broad categories and several subcategories (SAS Institute, Cary, NC). The following sections report the average response of black walnut and of other hardwoods to each ground cover approach. The advantages and disadvantages of using each approach in hardwood plantings are also discussed in the following sections.

RESIDENT VEGETATION

Resident vegetation is the forbs and grasses that naturally emerge following site preparation or disturbance and are left to grow with minimal management (Ingels and others 1998). On previously cropped lands or following intensive site preparation, resident vegetation will consist of a succession of plant species dominated first by annual forbs and grasses, giving way to, presumably, more competitive perennial forbs, and then eventually perennial grasses. To minimize competition in new hardwood plantings, perennial vegetation should be minimized. The best time to do this is during site preparation before the trees are planted. During site preparation, landowners can choose from a wider range of post-emergent herbicides and tillage equipment that will be easier to implement and be more effective than after trees are planted.

There are disadvantages to using resident vegetation as a ground cover in hardwood plantings. When hardwood plantings are established using resident vegetation as living mulch (without spot or strip weed control), seedling and sapling growth is typically as little as 60% of the growth found in plantings maintained free of vegetation through cultivation or use of herbicides (Fig. 1). Growth response of walnut to resident vegetation is

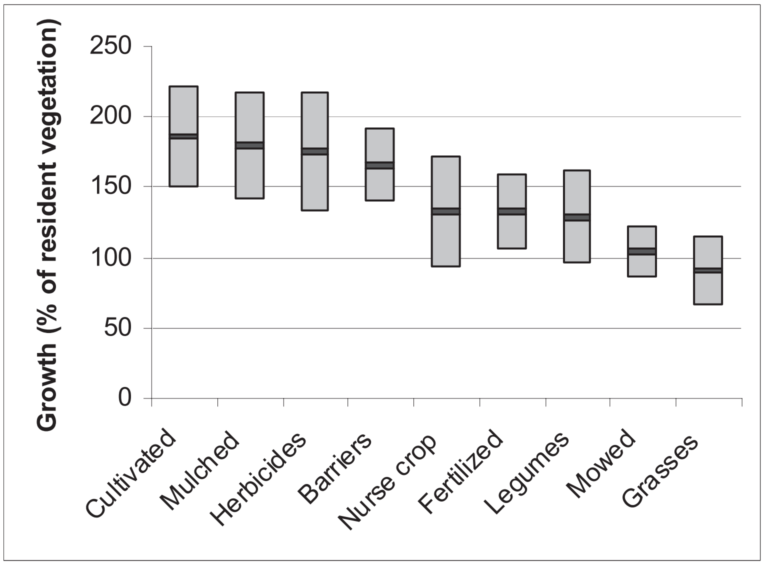


Figure 1.—Average hardwood growth (dark band) for nine ground cover management approaches as a percentage of growth for black walnut and other hardwoods in unmanaged controls or resident vegetation. Approximately two-thirds of all reported values occur within one standard deviation as shown within the shaded area.

statistically similar to that of most hardwoods (56 and 63% of vegetation-free control, respectively; $t' = -1.22$, 37 *df*, $p < 0.30^{ns}$). With resident vegetation there is little control on what the succession of species will be or whether it will include noxious weeds. In addition to competing for light, nutrients, and water, several weeds including tall fescue (*Festuca arundinacea* Schreb.), quackgrass (*Agropyron repens* (L.) Beauv.), yellow nutsedge (*Cyperus esculentus*), and goldenrod (*Solidago* spp.) have been shown to secrete toxins into the soil which stress and slow the growth of trees (Larson and Schwarz 1980; Rink and Van Sambeek 1985, 1987; Rice 2001).

There are some advantages to growing unmanaged ground covers in hardwood plantings. It is the least expensive and least labor intensive method for establishing a ground cover. During the summer, resident vegetation provides shade that can reduce soil temperature maximums and daily fluctuations. A vegetative cover also slows surface run-off and results in greater infiltration of water.

On excellent walnut sites where vegetation is controlled, we should expect annual height growth around 2.5 feet per year for which there would be an expected diameter growth of 0.5 inches per year. If this rate of diameter growth is sustained throughout the rotation, we should expect a rotation length of 45 years to produce an 8-foot veneer log with a 20-inch top diameter or 55 years for a similar length veneer log with 20-inch core of heartwood. In contrast, walnut trees competing with resident vegetation would have rotation lengths of 75 and 85 years or more, respectively. Because of poor site selection, growth rates for walnut plantings are typically 0.2 to 0.3 inches DBH per year which effectively doubles rotation lengths. Although the ground cover approach using resident vegetation involves the least cash outlays, it is unlikely that the high initial costs to establish the planting can profitably be carried over rotation lengths of 75 or more years.

MANIPULATION OF RESIDENT VEGETATION

Presumably, reduced growth of walnut in resident vegetation compared to vegetation-free plantings is due to competition for soil nutrients, especially nitrogen, and competition for limited available soil moisture during the summer. A number of approaches can be used to reduce competition when using resident vegetation as a cover crop. The most common approaches to manipulating the resident vegetation include mowing to reduce transpiring biomass, irrigation to increase available soil moisture, fertilization to increase available nitrogen, and grass-selective herbicides to eliminate grass competition.

Mowing

Mowing of the resident vegetation does not significantly improve tree growth over that of trees growing in unmanaged resident vegetation (Fig. 1). Walnut growth in frequently mowed plantings averages 16% less (84% of the unmanaged control) than that of walnut with unmanaged resident vegetation. In contrast, growth of other hardwoods in mowed plantings is increased by 10% (110% of unmanaged control) over that of hardwoods in resident vegetation. Tree responses to mowing are statistically different between walnut and other hardwoods (t' = -3.60, 20 *df*, $p < 0.01$**). Similar reductions in growth are also found for studies with vegetation-free controls where walnut growth is 43% of the controls compared to 61% for other hardwoods (t' = -2.58, 23 *df*, $p < 0.02$*). Rice (2001) indicated the practice of mowing shifts the competitive advantage away from mostly forbs in which growing points are sheared off with each mowing to grasses which have their growing points near the soil line. Davies (1985) and Ponder (1991) also suggest that reduced tree growth is a consequence of shifting competition to grasses and the renewal of vegetative growth following mowing.

For the small growth gains achieved with other hardwoods, mowing is an expensive, labor intensive management practice that must be completed 2 to 5 times a year for 3 to 5 years before hardwood saplings will dominate the site. In addition to little improvement in tree growth, mowing leads to "mower blight" or the inadvertent damaging of tree stems when accidentally hit by tractor or mowing equipment. Also, repeated mowing can lead to compaction of upper soil layers where walnut feeder roots are located. Mowing, however, has the advantages of preventing noxious weeds from going to seed, exposing rabbits and mice to predators, maintaining a ground cover to protect the soil from wind and water erosion, aesthetically creating a more pleasing appearance to the planting, and facilitates the harvesting of nuts. To reduce risks of wildfires and to slow plant succession from forbs to grasses, we recommend mowing only in the fall after a hard killing frost.

Irrigation

Too few reports were found to indicate if irrigation could be used to increase available soil moisture sufficiently to increase tree growth. Dey and others (1987) and Van Sambeek and McBride (1991) found little or no increased growth of walnut in response to irrigation on their sites with grass ground covers. It is possible that the resident vegetation and grass sods may benefit more from irrigation than will the trees.

Fertilization

On most sites, especially old field sites, available soil nitrogen tends to be the nutrient limiting hardwood growth. The published literature shows a slight increase in tree growth with the addition of nitrogen fertilizers (Fig. 1). Walnut may not compete as successfully with ground covers for added nitrogen as other hardwoods because growth increases for walnut average 24% compared to 52% for other hardwoods (124 and 152% of unmanaged control, respectively; t' = -2.37, 27 *df*, $p < 0.05$ *). Similar patterns exist when nitrogen fertilizers are applied to plantings where ground covers have been eliminated. In this case, growth increases for walnut averaged 14% compared to 31% for other hardwoods (114% and 131% of vegetation-free control, respectively; t' = -2.68, 41 *df*, $p < 0.02$*). Ponder (1997) previously did an in depth review on walnut fertilization and reported responses to added nitrogen can be quite variable and concluded that it is not uncommon to see little or no response to fertilization, especially on good walnut sites.

Selective Herbicides

Grasses tend to be more competitive than broad-leaved legumes or other forbs (Fig. 1); thus, selective removal of resident grasses should improve tree growth. Selective control can be done with over-the-top applications of post-emergent herbicides such as Fusilade (fluazifop-butyl) or Poast (sethoxydim). These herbicides are selective for grasses, although we found no reports in the literature documenting their effectiveness in hardwood plantings. Multiple applications may be needed as they are most effective if applied when grasses are 6 to 12 inches tall. Fusilade is more effective than Poast on perennial grasses; conversely, Poast is more effective than Fusilade on annual grasses such as foxtail and crabgrass. Selective control of annual grasses can also be achieved with Surflan (oryzalin) and Stomp (pendimethalin), two pre-emergent herbicides that require 1 to 2 inches of rainfall within several weeks of application for activation. These herbicides are more effective on germinating grasses than broad-leaved forbs. After tree seedlings are established, pre-emergent herbicides that are selective for germinating seeds are unlikely to harm deeper-rooted perennial plants or established tree seedlings. Selective control of the tallest and usually most competitive weeds can also be achieved using wipe-on applications of broad spectrum herbicides if care is taken to keep the herbicide from coming in contact with the walnut foliage.

MECHANICAL VEGETATION CONTROL

Mechanical vegetation control involves the cutting off at or below ground line, i.e., scalping, or the uprooting and burial of competing plants, i.e., cultivation. Mechanical control of competing ground covers tends to nearly double hardwood growth over that of trees with a ground cover of resident vegetation (Fig. 1). Growth of walnut with mechanical weed control increases on average by 117% compared to 79% for other hardwoods (217% and 179% of unmanaged control, respectively; $t' = 1.40$, 9 *df*, $p < 0.20^{ns}$). The result of no statistical difference should be considered preliminary because too few studies were found that compared growth of walnut in plantings with unmanaged control and cultivated plots.

It is generally assumed that mechanical control of competing vegetation will allow hardwood seedlings and saplings to grow at their full biological potential for the site. It is one of the more effective methods of controlling competing ground cover vegetation, especially if done when weeds are small and perennial weeds are not yet established. Exposed soils usually have higher soil temperatures which increases decomposition of organic matter and nitrogen mineralization, making more nutrients available to the trees. Cultivation also buries diseased walnut leaves that disrupt the normal dispersal of disease spores (Kessler 1988). Vegetation-free plantings also allow for better air drainage reducing the risk of spring frost injury and destruction of female flowers. Finally, bare ground treatments provide little winter cover for rabbits and mice that chew on tree seedlings and saplings.

Besides the increased potential for soil erosion, cultivation has a number of other potential disadvantages. Cultivation is relatively labor intensive because it must be repeated two to five times a year depending on the weed seeds present in the seed bank and rainfall. In years with heavy spring rainfall, cultivation on a timely basis may not be possible. Traffic from heavy equipment needed for cultivation can lead to soil compaction and impede water infiltration. Frequent and close cultivation means a higher proportion of tree stems are likely to be damaged than with other ground cover management treatments, except maybe mowing. Deep tillage also results in destruction of shallow feeder roots in the uppermost fertile layers of the topsoil. It is not uncommon to find walnut roots extending out more than twice the width of the crown. Repeated cultivation for several years decreases the amount of organic matter in the soil reducing stable soil aggregates needed for the retention of soil water and nutrients. While accelerating nitrogen mineralization, high soil temperatures in exposed soil also accelerates nitrogen loss through volatilization. Unless cleaned frequently, tillage equipment may aggravate future weed problems by spreading seed or chopped plant parts that can become established in other parts of the planting. Finally, it is unclear whether the costs incurred to cultivate a planting three or four times a year can be offset by a mere doubling of the tree growth expected in unmanaged plantings.

It is generally assumed that bare soil treatments retain significantly more soil moisture than treatments with a vegetative cover. Unless the trees are large enough to shade the planting, this may not be true. Lull and Fletcher (1962) report soil moisture depletion rates of 0.35 inches/day from surface evaporation for bare ground compared to 0.37 inches/day from evapotranspiration by herbaceous ground covers. By comparison, soil moisture depletion from woodland soils was deeper and averaged 0.42 inches/day.

There are several things that can be done to minimize the detrimental impact of cultivation. Cultivating when the ground is dry and hard and when the weather is hot is more effective and will cause less compaction than when the soil is moist. Use tractors with floatation tires to reduce compaction. Use cultivators with V-shaped sweeps or weed knives that sever weeds off near the soil line rather than deep penetrating disks or rototillers that tend to bring new weed seeds to the surface and damage tree feeder roots (Rice 2001). Specialized equipment such as the Weed Badger can be used for precision, within row tillage to reduce damage to saplings or pole-sized trees (Garrett and others 1989). Although not experimentally tested, tilling one half the planting (between alternate rows) may be as effective as annually tilling the entire tree planting (Schlesinger, personal communication).

CHEMICAL VEGETATION CONTROL

The growth response of hardwoods to herbicide applications to control competing vegetation is very similar to that for mechanical control and nearly twice that for trees in unmanaged controls (Fig. 1). Increased growth of walnut averages 95% for trees with chemical weed control compared to that in unmanaged controls and is not statistically different from increased growth of other hardwoods that averages 72% (195 and 172% of unmanaged control, respectively; $t' = 1.33$, 33 *df*, $p < 0.20^{ns}$). Tree response to chemical weed control has a large standard deviation partially because all herbicides are not equally effective and some herbicides may be slightly phytotoxic to the trees at reported experimental application rates. Hardwood plantings with repeat applications of Roundup™ (glyphosate) tend to have slightly higher growth rates than trees in treatments using pre-emergent herbicides (131% and 102% of vegetation-free control, respectively). Seifert and Woeste (2002) reported that walnut growth is greater with tillage than with spring-applied pre-emergent herbicides, especially when using Oust (sulfometuron) alone or in mixes.

Chemical vegetation control has several distinct advantages over other methods of ground cover management. It may be the least costly and least labor intensive method for reducing or eliminating ground cover competition. The application of chemicals requires fewer workers and equipment costs are less than equipment for mechanical weed control. Post-emergent herbicides are more effective for eliminating grass sod and perennial weeds than is cultivation. In many cases, a single application of a pre- and post-emergent herbicide mix will control ground cover competition for the entire growing season. Timing of herbicide applications are less critical than with cultivation. Chemical treatments usually result in higher available soil moisture and less erosion during the growing season than mechanical vegetation control.

The most significant problem associated with chemical control of ground cover vegetation is the limited and declining choice of registered herbicides that can be used (Seifert 1993). Other problems include the lack of selectivity among the currently registered herbicides and the tree damage they can cause if not applied properly (Seifert and Woeste 2002). Improper calibration, herbicide drift, or accidental spraying of saplings can result in either mortality or slowed tree growth. Wet field conditions can prevent entry into the planting when vegetation is at the appropriate stage. Conversely, lack of rainfall can keep some pre-emergent herbicides from becoming soil activated. Post-emergent herbicides generally require multiple applications per year for effective control of competing vegetation. Because soils remain relatively undisturbed, the impact of rain droplets on bare soil can lead to surface compaction thus reducing surface infiltration and increasing surface run-off and soil erosion compared to that in unmanaged plantings.

There are several approaches that can be used to minimize negative impacts of using herbicides. Resident vegetation can be allowed to develop in the spring before application of post- and pre-emergent herbicides creating a layer of dead vegetation that will shade the soil reducing surface evaporation and decreasing impact of rain drops. Because herbicides can effectively control perennial plants, spraying is not required every year assuming that annuals are less competitive than perennial vegetation. Alternatively, the area between every other tree row could be treated in alternate years retaining resident vegetation on half the planting. When more than one herbicide will control the target vegetation, the herbicide(s) that is least toxic to earthworms and other soil macrofauna and macroflora should be selected. Earthworms alone can move more soil/year than is moved with one plowing using a tractor-pulled moldboard plow (Minnich 1977). When managing for nut crops, use strip chemical control within the tree row during the growing season followed by fall mowing between rows to facilitate harvesting of nuts.

MULCHES

Ground cover management using mulches falls into two broad categories: organic carbon-based mulches and inorganic or barrier mulches. The ideal mulch will (1) be opaque or block sunlight discouraging germination and growth of weeds, (2) be porous enough to allow water to infiltrate, (3) protect the soil from sun and wind to conserve soil moisture by reducing evaporation, (4) moderate daily soil temperature fluctuations and extremes, (5) be made of biodegradable materials but still have the strength and durability to last until trees are established, (6) be easily transported to the tree planting, (7) blend into the landscape, and (8) be relatively easy to install (Windell and Haywood 1996). Other benefits of mulching include making trees more visible thereby reducing the chances for mechanical damage from mowers and other maintenance equipment.

Organic Mulches

Applying organic mulches around the base of hardwood trees is about as effective as mechanical or chemical weed control (Fig. 1). Organic mulches increase walnut growth on average by 89% compared to walnut growth in unmanaged plantings. Growth increases are similar for other hardwoods (78%) when mulched and are not statistically different from walnut (189% and 178% of unmanaged control, respectively; $t' = 0.41$, 12 *df*, $p < 0.50^{ns}$). In studies that have compared organic mulches to other vegetation-free treatments, mulching usually results in better tree growth than other bare ground treatments (Fig. 2). Based on very few studies, organic mulches may increase walnut growth by 29% over other vegetation-free treatments with similar increases for other hardwoods (26%) when mulched compared to that of other bare ground treatments (129% and 126% of vegetation-free control, respectively; $t' = 0.17$, 7 *df*, $p < 0.50^{ns}$).

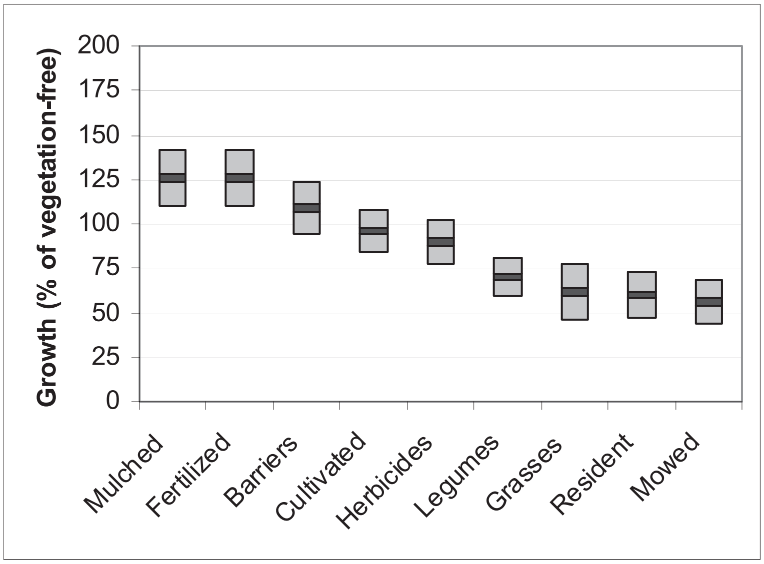


Figure 2.—Average hardwood growth for nine ground cover management approaches as a percentage of growth for black walnut and other hardwoods planted in vegetation-free control plots. Approximately two-thirds of all reported values occur within one standard deviation as shown within the shaded area.

Duryea and others (1999) indicate the physical effects of mulching are much greater than the fertilizer value associated with the release of nutrients as mulches decompose. Decomposition of organic mulches increases the organic matter content of the soil leading to better soil aggregation and increased retention of soil water and nutrients. Organic mulches also reduce the impact of raindrops, slow the flow of water over the soil surface, reduce soil erosion, and increase rates of water infiltration. Organic mulches also can increase the activity of worms, fungi, and other soil organisms resulting in increased diffusion of soil oxygen, organic matter, nutrient availability, and root growth.

Although somewhat dependent on the density and texture of the materials used, a 4-inch thick layer of an organic mulch is a good compromise—not so deep as to inhibit soil aeration but still thick enough to prevent emergence of germinating seedlings. Mulches with a good balance of carbon to nitrogen (30C:1N) are likely to increase both organic matter and mineral nitrogen in the soil. Examples of balanced C:N mulches include rotted manures and compost (20C:1N), grass clippings (20C:1N), pine needles, shredded leaves (60C:1N), chipped landscape and utility right-of-way trimmings, baled hay, and straw (80C:1N). During decomposition, mulches with high carbon to nitrogen ratios such as shredded waste paper, sawdust (500C:1N) and wood shavings (700C:1N) will compete with the trees for available soil nitrogen. When using high carbon mulches, a nitrogen fertilizer should be incorporated into the mulch or broadcast under the tree before applying the mulch.

A major problem with using organic mulches is subsidence, especially mulches with a high proportion of leaves, and the need to reapply additional mulch annually to remain effective. Without specialized equipment, mulching trees is a labor intensive operation. A cubic yard of mulch will cover approximately 20 square feet and it may require 150 cubic yards per acre to mulch hardwood plantings when applied as twenty 4-foot wide strips. Other concerns include the introduction of new weed seeds or pathogens into a planting, especially with mulches such as manure, compost, or straw. Since mulches provide cover for mice and voles, they need to be pulled away from trees during the winter when these animals do the most damage.

Inorganic or Barrier Mulches

Examples of inorganic or barrier mulches include gravel, crushed rock, or manufactured products such as plastic films and landscape fabrics. Inorganic mulches used in hardwood plantings are primarily opaque polyethylene (plastic) films and woven polypropylene (landscape) fabrics. These materials are usually placed under gravel and crushed rock to keep them from sinking into the soil. Barrier mulches can be very effective for increasing early growth in hardwood plantings (Fig. 2). When compared to other methods of maintaining vegetation-free growing space, barrier mulches increased growth of black walnut by 35% compared to a 5% increased growth of other hardwoods (135% and 105% of vegetation-free control, respectively; $t' = 1.99$, 12 *df*, $p < 0.10^{ns}$). Too few studies have been done with walnut to make statistical comparisons; however, increased growth of other hardwoods established with barrier mulches may average 66% more than for hardwoods growing in resident vegetation (Fig. 1).

Major advantages of inorganic or barrier mulches are one-time installation with minimal annual maintenance to achieve a vegetation-free zone around tree seedlings and saplings for 2 or more years. By incorporating carbon black into the polyethylene or polypropylene, the harmful effects of the sun's ultraviolet radiation can be slowed so that materials will last for 3 or more years. Woven polypropylene or landscape fabrics readily permit water infiltration; whereas, polyethylene films must be punched or fabricated with holes to permit infiltration of water. Clear and dark-colored mulches absorb and trap more solar radiation than mulched or bare soil resulting in warmer soils (3 to 10° F) and earlier root growth in the spring. Consequently, these films can result in summer soil temperatures lethal to roots and tender stems. It has lead to development of promising colored and light-reflective films that block sunlight but absorb less solar radiation (Ham and others 1993).

There are significant problems associated with use of barrier mulches besides the high cost for materials and labor. The main difficulty with plastic films and landscape fabrics is holding the material in place. Both labor costs and anchorage problems can be reduced by using tractor-drawn machines that will lay and bury edges of polyethylene films or landscape fabric on cultivated fields. Because woven polypropylene tends to be thicker, they are both more durable and more expensive than polyethylene films. Although permeable to water, infiltration rates for landscape fabrics are still relatively slow and can lead to surface runoff. In contrast, polyethylene films restrict surface evaporation, retain available soil moisture much later into the growing season, and are much slower to recharge soil moisture in the fall. Polyethylene films are readily punctured by animal hooves and sprouts of some plants, so woven polypropylene fabrics should be used in areas with high nutsedge (*Cyperus* spp.) or deer populations. Barrier mulches can lead to significant tree damage because these mulches provide an ideal habitat for rodents and screen them from predators.

Operationally, it is easiest to install barrier mulches with machines on recently cultivated sites either before or after tree seedlings are planted. Rolls 6 feet wide by 300 feet long are usually recommended, because this size allows the outside edges to be buried and still provide a 4- to 5-foot wide vegetation-free strip in which to hand-plant trees. Costs for fabric and installation are typically $0.25 to $0.40 per linear foot. Tree seedlings can also be machine-planted before laying polyethylene or polypropylene mulches. In this case, a small cut is made next to bent-over seedlings so the stem can be pulled out. The heated air under barrier mulches usually keeps most weeds from growing through the planting hole or cut slits. A shovel full of soil placed near each seedling helps to direct rain water toward the opening at the base of each tree and prevents wind from lifting the mulch. Using side-discharge mowers, clippings of resident vegetation can be blown into the tree row to cover barrier mulches during the summer, thereby, reducing the high soil temperatures encountered with dark-colored impermeable films.

COVER CROPS

Cover crops are established ground covers designed to reduce the amount of resident vegetation and delay normal succession to perennial weeds. A number of forage legumes and grasses are tolerant of light shade and could be grown in managed black walnut plantings (Lin and others 1999, Alley and others 1999). In walnut plantings managed for timber (CCF 100 to 120), light levels are approximately 40% of full sunlight (Smith 1942). In contrast, in more open walnut plantings managed for nuts (CCF 80 to 90), light levels are approximately 50% of full sunlight. Obtaining sufficient understory light can be problematic when managing cover crops in mixed hardwood plantings or natural stands. The light intensity in the understory of a mature mixed hardwood forest is generally less than 20% of full sunlight and can be as low as 1% (Dey and MacDonald 2001). At full leaf expansion, walnuts in plantings with CCF's of 100 to 120 (full site occupancy or B-level stocking) absorb approximately 60% of the incoming solar radiation. Light infiltration is curvilinear-related to CCF or residual stocking density (a function of the number of trees/acre and their average DBH) (Dey 2002). To provide light levels on the forest floor of 40% to 60% of full sunlight, may require maintaining 30% to 40% residual stocking densities and removal of approximately one-third of the overstory trees during each thinning (Schlesinger and Funk 1977, Sander 1979).

Cover crops can significantly reduce the amount of labor needed for controlling resident vegetation in new plantings with only a modest increase in costs. Other benefits include the natural accumulation of decaying plant residues on the soil surface that reduce summer soil temperatures and a rapid increase in soil organic matter by organisms that naturally till soil (Minnich 1977, Shribbs 1985). Besides increasing nutrient availability, oxygen, and water infiltration, earthworms also create soil pores that facilitate deeper root penetration by the trees. A vegetative cover either as a living mulch or decaying residues shades the soil resulting in lower soil temperatures because less solar radiation is absorbed by the soil. Wohlstenholm (1970) reported that planting legume or grass covers in pecan orchards can delay bud burst by 6 to 12 days, thereby reducing damage by late spring frosts. Cover crops also provide excellent habitat for wildlife, including rabbits, mice, and voles that frequently damage young trees.

Legume Cover Crops

The growth of hardwoods is generally better when grown with legume cover crops than with resident vegetation (Figs. 1 and 2). Legume cover crops increase walnut growth on average by 28% compared to walnut growth in unmanaged plantings. Growth increases are similar for other hardwoods (30%) and are not statistically different from walnut (128% and 130% of unmanaged control, respectively; $t' = -0.22$, > 100 *df*, $p < 0.50^{ns}$). Most studies with vegetation-free control treatments report reduced hardwood growth with legume cover crops; however, they also report legume cover crops reduce growth less than does resident vegetation. Legume cover crops reduce walnut growth by 31% compared to 44% for resident vegetation (69% and 56% of vegetation-free control, respectively; $t' = 2.36$, 35 *df*, $p < 0.05^{*}$). Responses for other hardwoods are similar where legume cover crops reduce growth by 32% compared to 37% for resident vegetation (68% and 63% of vegetation-free control, respectively; $t' = 1.24$, >100 *df*, $p < 0.20^{ns}$).

Because nitrogen tends to be the nutrient most often limiting tree growth, increased hardwood growth with legume cover crops is generally attributed to the ability of most legumes to fix atmospheric nitrogen (White and others 1981). Unless heavily fertilized, forage legumes on average obtain about 75% of their nitrogen through the fixation process. If the above ground biomass is not harvested, some of this nitrogen becomes available to the trees when plant residues decompose and organic nitrogen is converted to ammonia or nitrate nitrogen. Some mineral nitrogen is also made available to the trees through atmospheric deposition; however, the burning of fossil fuels and lightening only account for 5 to 10 pounds per acre in the central United States and 15 to 20 pounds per acre in the eastern United States. In contrast, fixation rates for forage legumes average 100 to 300 pounds per acre (Table 1). Although fixation rates are largely unknown for most native legumes,

Table 1.—Average growth and standard deviation for black walnut and other hardwoods as a percentage of tree growth in plantings without ground covers and the average shade tolerance percentile rank and reported nitrogen fixation rates for different forage legumes.

Legume Cover Crop	Tree Growth[1]	Shade Tolerance[2]	Nitrogen Fixation Rate[3]
	%	*%*	*lbs/acre*
Soybeans	99 + 26 (5)	ND	50
Crownvetch	83 + 23 (18)	78	230
Subterranean clover	81 + 66 (5)	43	90
Crimson clover	79 + 37 (18)	57	90
Hairy vetch	74 + 29 (119)	ND	120
Sweet clover	72 + 7 (4)	ND	--
Red clover	70 + 24 (91)	44	250
Sericea lespedeza	69 + 12 (22)	30	--
Kura clover	66 + 11 (9)	66	155
Birdsfoot trefoil	63 + 26 (22)	36	150
Striate lespedeza	63 + 14 (5)	ND	--
White clover	62 + 23 (43)	34	200
Resident vegetation	60 + 28 (156)	--	--
Korean lespedeza	57 + 9 (23)	12	--
Alfalfa	44 + 25 (7)	32	300

[1] Growth is percent of vegetation-free control + standard deviation (approximately 66% of reported responses) and in () number of replications extracted from the literature. Mean least significant difference is 14%.

[2] Average percentile rank under moderate and heavy shade estimated from three or more screening trials where 0% was assigned to the least and 100% was assigned to the most shade tolerant species within each light level and screening trial. ND = not tested.

[3]Average of values reported in six publications identified in vegetation management database.

several shade tolerant native legumes have been identified that could be used as living mulches in hardwood plantings and natural stands (Ponder 1994, Van Sambeek and others 2004).

Establishing legume cover crops is more expensive and requires more labor than seeding forage grasses or manipulating the resident vegetation. Legumes usually require a well prepared seedbed and may require application of lime and fertilizers to produce a stand capable of suppressing resident vegetation (Jorgenson and Craig 1983, Ingels and others 1998). Because seed of most legumes are small, they should be seeded with companion crops such as oats (*Avena sativa* L.) or barley (*Hordeum vulgare* L.) that quickly germinate providing a plant cover to minimize soil erosion and suppress potential resident vegetation (Simmons and others 1992). Stamps and others (2002) have shown that combining trees and alfalfa (*Medicago sativa* L.) will increase the number of insects that prey on other insects including those that feed on black walnut foliage. Legume ground covers have also been shown to interfere with anthracnose spore dispersal from diseased leaves or infection of new walnut leaves in the spring (Van Sambeek 2003).

Although most legumes make a better cover crop than resident vegetation, substantial variation exists among forage legumes as to their effects on hardwood growth and relative tolerance to shade (Table 1). In general, annual legumes tend to reduce tree growth less than perennial legumes. Subterranean clover (*Trifolium subterraneum* L.) and crimson clover (*Trifolium incarnatum* L.), both annual legumes, are probably excellent choices for walnut plantings; however, neither will overwinter throughout most of the black walnut range. Likewise, legumes that have decumbent stems such as crownvetch (*Coronilla varia* L.) or are vines such as hairy vetch (*Vicia villosa* Roth) are excellent choices for cover crops because they require less biomass to effectively smother other vegetation than upright legumes such as sericea lespedeza (*Lespedeza cuneata* (Dumont) G. Don). Legumes with upright stems may also require mowing after a killing frost each fall to reduce the wildfire risk. Based on few published reports, alfalfa may be the poorest choice for a cover crop in black walnut plantings.

Legume cover crops should not be harvested for several reasons. Approximately 75% of the plant biomass is in the above ground portion; thus, harvesting removes nearly all the nitrogen obtained through fixation (Watson and others 1984). If harvested, legumes may be no better than unmanaged broadleaved forbs such as rapeseed (*Brassica napus* L.) or resident vegetation. Cool-season legumes such as hairy vetch and crownvetch that produce most of their vegetative growth in the spring when available soil moisture is high are better cover crops than warm-season legumes, such as sericea lespedeza and Korean lespedeza (*Lespedeza stipulacea* Maxim.). If not harvested, most cool-season legumes set seed in the spring and remain relatively dormant throughout the summer, effectively mulching the soil surface. Harvesting stimulates new vegetative growth and a continued demand for limited available soil moisture during the summer. Lyons and others (1952) found evapotranspiration rates for annual legumes and forbs average about 4.5 inches of precipitation per ton of above ground biomass produced per acre. Within the walnut range, typical yields for forage legumes are 3 to 4 tons per cutting per acre indicating that regrowth following harvesting makes a significant demand on limited available soil moisture in the summer (Barnes and others 1995).

Grass Sods

The growth of hardwoods when grown in grass sods is similar to that of hardwoods grown with resident vegetation (Figs. 1 and 2). Growth of walnut might be reduced more by grass sods than growth of other hardwoods. In studies with unmanaged control plots, growth of walnut is 21% less than growth in control treatments compared to only a 5% reduction for other hardwoods (79 and 95% of resident vegetation or unmanaged control, respectively; $t' = -1.76$, 40 *df*, $p < 0.20^{ns}$). In studies with vegetation-free control plots, growth of walnut in grass sods is 47% less than growth in control treatments compared to only 32% reduction for other hardwoods (53 and 68% of vegetation-free control, respectively; $t' = -3.23$, > 100 *df*, $p < 0.01^{**}$). In these studies, growth of walnut in grass sods is slightly less than growth of walnut in resident vegetation (53 and 56% of vegetation-free control, respectively; $t' = -0.49$, $df = 40$, $p < 0.5^{ns}$). Similar growth differences exist for other hardwoods grown in grass sods compared to that of growth for other hardwoods in resident vegetation (68 and 63% of vegetation-free control, respectively; $t' = 1.24$, > 100 *df*, $p < 0.40^{ns}$).

The small increases or decreases in growth of walnut and other hardwoods compared to growth of trees in resident vegetation is partially due to differences among grass species (Table 2). Preliminary results suggest annual grasses such as cheat (*Bromus secalinus* L.), annual ryegrass (*Lolium multiflorum* Lam.), and cereal grains are less competitive than perennial grasses. Perennial turf-type grasses such as red fescue (*Festuca rubra* L.) and Kentucky bluegrass (*Poa pratensis* L.) also tend to be less competitive than perennial forage grasses such as orchard grass (*Dactylis glomerata* L.) and perennial ryegrass (*Lolium perenne* L.). Overall, tall fescue (*Festuca arundinacea* Schreb.) is the only grass that, based on available data, statistically reduced growth of black walnut and other hardwoods below that of resident vegetation (32% and 60% of vegetation-free control; $t' = -7.14$, > 100 *df*, $p < 0.001$***). Warm-season forage grasses should be avoided because they lack shade tolerance, vegetative growth occurs primarily when available soil moisture is low, and mowing in not recommended in the fall when nuts are to be collected in well-managed black walnut plantings (Lin and others 2000, Van Sambeek and others 2004).

Besides the reductions in potential tree growth, there are a number of other potential problems when using grass sods. Most grasses have extensive, finely branched root systems that can effectively explore greater volumes of soil than forbs and trees resulting in drier and nutrient poor soil profiles. Deep-rooted grasses like tall fescue exploit the same soil horizons as will walnut and can significantly reduce available soil nitrogen within the tree rooting zone. Van Sambeek and others (1989) reported that in stagnated walnut stands, tree growth was limited more by low available nitrate nitrogen than it was by competition for available soil moisture. Lyon and others (1952) reported average evapotranspiration rates for annual grasses of 4 inches of precipitation per ton per cutting. As with forage legumes, regrowth of grasses following cutting puts a significant demand on limited available soil moisture in the summer. Grasses that maintain their dominance through the production of phytotoxins such as tall fescue, smooth bromegrass, and broomsedge probably should also be avoided (Ponder 1986; Miller and others 1987; Rink and Van Sambeek 1985, 1987).

There are a few advantages to managing grass sods in hardwood plantings. This plant cover is probably the best to slow surface run-off and increase water infiltration, especially on steep slopes. Because equipment and labor needs for mowing are less costly than for disking or tilling, grass sods cost less to maintain than legume cover crops (Jorgenson and Craig 1983). Mowing shifts the competitive advantage toward grasses that have their growing points at the soil line and, thus, grass sods are easier to maintain than legume cover crops. A sod cover provides year-round footing for equipment to complete maintenance including mowing, spraying, pruning, or harvesting. It may be desirable to establish grass sods near the end of the rotation to concentrate on hay and nut production and allow the wide growth rings within the sapwood to convert to the more desirable heartwood if walnut trees have been managed for veneer log production. Preliminary results from shade-tolerance screening trials indicate mixes of orchard grass or Kentucky bluegrass with red clover should produce acceptable yields in closed canopy walnut plantings (Tables 1 and 2).

Managing cool-season grass and legumes offers the opportunity to implement the agroforestry practice of silvopasture management. Although forage yields are variable, several studies have shown higher

Table 2.—Average growth and standard deviation for black walnut and other hardwoods as a percentage of tree growth in plantings without ground covers and shade tolerance percentile rank for selected grass species.

Grass Sod	Tree Growth[1]	Shade Tolerance[2]
	%	%
Bromegrass	76 + 20 (10)	55
Annual cool-season	75 + 38 (8)	41
Red fescue	71 + 26 (77)	35
Bluegrass	69 + 36 (19)	58
Cereal grains	68 + 39 (15)	ND
Orchard grass	65 + 24 (84)	58
Quackgrass	64 + 19 (2)	ND
Timothy	64 + 14 (9)	37
Redtop	62 + 17 (4)	38
Resident vegetation	60 + 28 (156)	--
Perennial ryegrass	62 + 15 (8)	36
Mixed grass sod	52 + 24 (11)	--
Tall fescue	32 + 31 (93)	50

[1] Growth is percent of vegetation-free control ± standard deviation and in () number of replications extracted from the literature. Mean least significant difference is 20%.

[2] Average percentile rank under moderate and heavy shade estimated from three or more screening trials where 0% was assigned to the least and 100% was assigned to the most shade tolerant species within each light level and screening trial. ND = not tested.

protein content and increase digestibility of most forages when grown under moderate shade (Garrett and Kurtz 1983; Lin and others 1999, 2001; Huck and others 2001). Compared to open pastures with artificial shade structures, hardwood plantings alleviate heat stress better producing greater weight gains and result in more uniform grazing and waste deposits (Garrett and others 2004). Under silvopasture management, established trees must be protected from soil compaction and physical damage of roots near the soil surface through the use of rotational grazing and livestock removal during wet periods. Most silvopastoral studies have not examined effects of forage production and grazing on hardwood growth. Rotational grazing will stimulate regrowth of grasses increasing total annual biomass and expected evapotranspiration that will result in greater soil moisture depletion than may occur with grass sods that are not grazed or mowed. Although forage legumes can increase growth of hardwoods compared to grasses or resident vegetation, no studies were found that examined the growth of hardwoods with forage legume and grass mixes. We hypothesize that grasses will benefit more from the nitrogen fixed by the legumes than will the trees resulting in an improved livestock forage but even slower tree growth.

WOODY NURSE CROPS

Woody nurse crops are trees or shrubs introduced into a hardwood planting to improve growth and quality of the crop trees during the sapling and pole stages. The resulting growth of hardwoods grown with woody nurse crops is intermediate between the potential growth that occurs under treatments with no ground cover vegetation and that with cover crops or resident vegetation (Fig. 2). The increase in growth of walnut in mixed plantings averages 22% compared to growth in pure walnut plantings usually managed with a ground cover of resident vegetation. The growth increase of other hardwoods in mixed plantings tends to be greater than for walnut, but is not statistically different (122 and 138% of unmanaged control, respectively; $t' = -1.19$, 50 *df*, $p < 0.30^{ns}$). Schlesinger and Williams (1984) found that the growth of walnut with woody nurse crops is highly dependent on the planting site and which nitrogen-fixing tree species were interplanted.

The planting of other trees and shrubs that have relatively dense crowns with walnut will create a shady, cooler, more humid microenvironment more typical of that found in woodlands (Funk and others 1979, Burke and Pennington 1989). More soil moisture is made available to the trees because shade reduces surface evaporation and decreases evapotranspiration by eliminating most grasses and forbs. Mixed hardwood plantings will still result in more rapid and deeper depletion of available soil moisture than in pure walnut plantings with resident vegetation (Funk and others 1979). Lull and Fletcher (1962) reported average soil water depletion rates in the upper 20 inches of 0.099 inches/day when mulched, 0.166 inches/day with resident vegetation, and 0.197 inches/day under trees. In contrast, soil water depletion rates in the 20- to 40-inch depth averaged 0.018 inches/day when mulched, 0.039 inches/day with resident vegetation, and 0.102 inches/day under trees. A shaded microenvironment also moderates the extremes and fluctuations in soil temperature and frequently supports a wider range of beneficial soil fauna and flora than found in exposed soils. Although lateral branches still need to be pruned, creating a dense canopy results in death of most branches while still relatively small in diameter growth. Woody nurse crops may be the best alternative for ground cover management on sites not readily accessible to farm equipment such as those along meandering creeks and other riparian areas.

The major problem with using woody nurse crops in hardwood plantings is matching the growth of the woody nurse crops to the crop trees. Maximum benefits of planting woody nurse crops are usually achieved when the growth rate of the woody nurse crop is slightly greater than for the crop tree species. This can occur naturally with shrubs like autumn olive or culturally through repeated coppicing as has been demonstrated with black locust. For 2 to 4 years after establishment, mixed hardwood plantings will require some other method of ground cover management until the dense canopy of the nurse crop shades out the competing understory vegetation. Differences in sensitivity of hardwood species to herbicides may restrict which can be used to establish mixed plantings (Seifert and Woeste 2002). Compared to plantings with well managed ground covers, woody nurse crop plantings have an unkempt appearance (Burke and Pennington 1989). Pruning, thinning, and nut harvesting operations are more difficult in mixed plantings than in pure walnut plantings. Growers must make a decision balancing appearance and growth rate, but in plantings managed for timber where optimum growth and quality are the main objectives, creating a woods-like condition is probably the preferred method for ground cover management.

Currently, the species of choice as a woody nurse crop in black walnut plantings is autumn olive (*Elaeagnus umbellata* Thunb.). Unfortunately, it is an invasive exotic species and most states discourage its planting. Research is needed to identify native or less invasive shrubs or trees that produce dense canopies capable of shading out perennial resident vegetation and are capable of fixing atmospheric nitrogen (Van Sambeek and others 1985). European black alder (*Alnus glutinosa* (L.) Gaertn.), an actinorhizal tree, black locust (*Robinia pseudoacacia* L.), a nitrogen-fixing leguminous tree, and silver maple (*Acer saccharum* Marsh.) need frequent pruning and/or coppicing to keep them from overtopping walnut (Schlesinger and Williams 1984, von Althen 1989). In a recent review of the nutritional interactions in mixed tree plantings, Rothe and Binkley (2001) concluded nutritional benefits of nurse crops are mostly additive; however, when using nitrogen-fixing nurse crops, they concluded benefits can be synergistic with greater than expected yields. Evaluation of recently established hardwood plantings under the Conservation Reserve Program may yield meaningful information in the future as these plantings now require a mix of hardwoods that can include walnut.

CONCLUSIONS

The vegetation management program chosen will be influenced by many factors including management objective (timber only, nuts only, or timber and nuts), site characteristics (location, slope, hydrology), type of planting stock (seed, bare-root seedlings, or large container stock), access to equipment (tractors, cultivators, spray rigs), and, possibly most importantly, labor availability. Ultimately, the primary objective of any program is to maximize nut production or growth of quality trees while minimizing establishment and maintenance costs. The primary objective of any ground cover management system is to reduce tree competition for water and nutrients, minimize labor and equipment costs, and create a ground surface that will support equipment needs for maintenance and nut collections. After considering the advantages and problems of the various vegetation management approaches, managers of walnut or mixed hardwood plantings should be able to develop the most appropriate strategies to meet their objectives for their particular plantings.

ACKNOWLEDGMENTS

We express our appreciation to Chris Starbuck, Felix Ponder, Jr., and the Ecology and Management of Central Hardwoods Project staff for their constructive comments on database development and early drafts of this paper. A portion of this effort was supported by the U.S. Department of Agriculture, Agriculture Research Service, Dale Bumpers Small Farms Research Center, Booneville, AR.

LITERATURE CITED

Alley, J.L.; Garrett, H.E.; McGraw, R.L.; Dwyer, J.P.; Blanche, C.A. 1999. Forage legumes as living mulches for trees in agroforestry practices—preliminary results. Agroforestry Systems. 44: 289-291.

Barnes, R.F.; Miller, D.A.; Nelson, C.J. 1995. Forages. 1. An introduction to grassland agriculture. 5th ed. Ames, IA: Iowa State University Press. 516 p.

Burde, E.L., ed. 1988. Walnut notes. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 48 p. Available on line: http://ncrs.fs.fed.us/pubs/wn/walnutnotestoc.html.

Burke, R.D.; Pennington, S.G. 1989. In: Phelps, J.E., ed. The continuing quest for quality: 4th Black walnut symposium; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council: 67-83.

Davies, R.J. 1985. The importance of weed control and the use of tree shelters for establishing broadleaved trees on grass-dominated sites in England. Forestry. 58: 167-180.

Dey, D. 2002. The ecological basis for oak siliviculture in eastern North America. In: McShea, W.J.; Healy, W.M., eds. Oak forest ecosystems. Baltimore, MD: The Johns Hopkins University Press: 60-79.

Dey, D.; Conway, M.R.; Garrett, H.E.; Hinckley, T.S.; Cox, G.S. 1987. Plant-water relationships and growth of black walnut in a walnut-forage multicropping regime. Forest Science. 33(1): 70-80.

Dey, D.C.; MacDonald, G.B. 2001. Overstory manipulation. In: Wagner, R.G.; Colombo, S.J., eds. Regenerating the Canadian forest: principles and practice for Ontario. Markham, Ontario: Fitzhenry & Whiteside Ltd.: 157-175.

Duryea, M.L.; English, R.J.; Hermansen, L.A. 1999. A comparison of landscape mulches: chemical, allelopathic, and decomposition properties. Journal of Arboriculture. 25(2): 88-96.

Funk, D.T.; Schlesinger, R.C.; Ponder, F., Jr. 1979. Autumn-olive as a nurse plant for black walnut. Botanical Gazette. 140(Suppl.): S110-S114.

Garrett, H.E.; Jones, J.E.; Slusher, J.P. 1989. Integrated forestry-farming (agroforestry) with eastern black walnut—a case study. In: Phelps, J.E., ed. The continuing quest for quality: 4th Black walnut symposium; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council: 248-259.

Garrett, H.E.; Kerley, M.S.; Ladyman, K.P.; Walter, W.D.; Godsey, L.D.; Van Sambeek, J.W.; Brauer, D.K. 2004. In: Nair, P.K.R.; Rao, M.R.; Buck, L.E., eds. New vistas in agroforestry: a compendium for the 1st World Congress of agroforestry. Dordrecht, The Netherlands: Kluwer Academic Plublishers: 21-33.

Garrett, H.E.; Kurtz, W.B. 1983. Silvicultural and economic relationships of integrated forestry farming with black walnut. Agroforestry Systems. 1(3): 245-256.

Ham, J.M.; Kluitenberg, G.J.; Lamont, W.J. 1993. Optical properties of plastic mulches affect the field temperature regime. Journal of the American Society for Horticulture Science. 118: 188-193.

Hansen, N.J.; McComb, A.L. 1955. Growth, form and survival of plantation-grown broadleaf and coniferous trees in southeastern Iowa. Proceedings: Iowa Academy of Science. 62: 109-124.

Huck, M.B.; Kerley, M.S.; Garrett, H.E.; McGraw, R.L.; Van Sambeek, J.W.; Navarrete-Tindall, N.E. 2001. Effect of shade on forage quality. In: Schroeder, W.; Kort, J., eds. Proceedings, 7th Biennial conference on agroforestry in North America. Regina, Saskatchewan: Association for Temperate Agroforestry: 125-135.

Ingels, C.A.; Bugg, R.L.; McGourty, G.T.; Christensen, L.P., eds. 1998. Cover cropping in vineyards: a grower's handbook. Publ. 3338. Oakland, CA: University of California, Division of Agriculture and Natural Resources. 162 p.

Jorgenson, J.R.; Craig, J.R. 1983. Legumes in forestry: results of adaptability trials in the southeast. Res. Pap. SE-237. Research Triangle Park, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. 11 p.

Kessler, K.J., Jr. 1988. Companion plantings of black walnut with autumn olive to control walnut anthracnose. Phytopathology. 78: 1606.

Larson, M.M.; Schwarz, E.L. 1980. Allelopathic inhibition of black locust, red clover, and black alder by six common herbaceous species. Forest Science. 26(3): 511-520.

Lin, C.H.; McGraw, R.L.; George, M.F.; Garrett, H.E. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. Agroforestry Systems. 44: 109-119.

Lin, C.H.; McGraw, R.L.; George, M.F.; Garrett, H.E. 2001. Nutritive quality and morphological development under partial shade of some forage species with agroforestry potential. Agroforestry Systems. 53: 1-13.

Lull, H.W.; Fletcher, P.W. 1962. Comparative influence of hardwood trees, litter, and bare area on soil-moisture regimen. Res. Bull. 800. Columbia, MO: University of Missouri, College of Agriculture, Agricultural Experiment Station. 15 p.

Lyon, T.L.; Buckman, H.O.; Brady, N.C. 1952. The nature and properties of soils. New York, NY: Macmillian, Inc. 639 p.

Miller, L.A.; Wray, P.H.; Mize, C.W. 1987. Response of stagnated black walnut to chemical release. Northern Journal of Applied Forestry. 4: 93-95.

Minnich, J. 1977. The earthworm book: how to raise and use earthworms for your farm and garden. Emmaus, PA: Rodale Press.

Ponder, F., Jr. 1986. Effect of three weeds on the growth and mycorrhizal infection of black walnut seedlings. Canadian Journal of Botany. 64: 1888-1892.

Ponder, F., Jr. 1991. Ground cover management to increase tree crop productivity. In: Garrett, H.E., ed. The 2nd Conference on agroforestry in North America. Columbia, MO: University of Missouri: 104-115.

Ponder, F., Jr. 1994. Leguminous ground covers could reduce herbicide use in forestry. Res. Pap. NC-316. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 4 p.

Ponder, F., Jr. 1997. Walnut fertilization and recommendations for wood and nut production. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 128-137.

Rice, R.P., Jr. 2001. Nursery and landscape weed control manual. Fresno, CA: Thomson Publications. 370 p.

Rink, G.; Van Sambeek, J.W. 1985. Variation among black walnut seedling families in resistance to competition and allelopathy. Plant and Soil. 88: 3-10.

Rink, G.; Van Sambeek, J.W. 1987. Variation among four white ash families in response to competition and allelopathy. Forest Ecology and Management. 18: 127-134.

Rothe, A.; Binkley, D. 2001. Nutritional interactions in mixed species forests: a synthesis. Canadian Journal of Forest Research. 31: 1855-1870.

Sander, I.L. 1979. Regenerating oak with the shelterwood system. In: Holt, H.A.; Fischer, B.S., eds. The 1979 John S. Wright forestry conference. West Lafayette, IN: Purdue University: 54-60.

Schlesinger, R.C.; Funk, D.T. 1977. Manager's handbook for black walnut. Gen. Tech. Rep. NC-38. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 22 p.

Schlesinger, R.C.; Van Sambeek, J.W. 1986. Ground cover management can revitalize black walnut trees. Northern Journal of Applied Forestry. 3(2): 49-51.

Schlesinger, R.C.; Williams, R.D. 1984. Growth response of black walnut to interplanted trees. Forest Ecology and Management. 9: 235-243.

Seifert, J. 1993. A guide for weed control in black walnut plantations. Walnut Council Bulletin. 20(3/4): 10-12.

Seifert, J.R.; Woeste, K. 2002. Evaluation of four herbicides and tillage for weed control on 1-0 planted tree seedlings. Northern Journal of Applied Forestry. 19(3): 101-105.

Shribbs, J.M. 1985. The influence of twelve ground cover systems on the growth and nutrition of golden delicious apple trees. Raleigh, NC: North Carolina State University. 84 p. Ph.D. dissertation.

Simmons, S.R.; Marin, N.P.; Sheaffer, C.C.; Stuthaman, D.D.; Schiefelbein, E.L.; Haugen, T. 1992. Companion crop forage establishment: producer practices and perceptions. Journal of Production Agriculture. 5: 67-72.

Smith, R.M. 1942. Some effects of black locusts and black walnuts on southeastern Ohio pastures. Soil Science. 53: 385-398.

Stamps, W.T.; Woods, T.L.; Linit, M.J.; Garrett, H.E. 2002. Arthropod diversity in alley cropped black walnut (*Juglans nigra* L.) stands in eastern Missouri, USA. Agroforestry Systems. 56(2): 167-175.

Steel, R.G.D.; Torrie, J.H. 1960. Comparisons involving two sample means. In: Principles and procedures of statistics. New York, NY: McGraw-Hill Book Co. Inc.: 67-87.

Van Sambeek, J.W. 2003. Legume ground covers alter defoliation response of black walnut saplings to drought and anthracnose. In: Van Sambeek, J.W.; Dawson, J.O.; Ponder, F., Jr.; Loewenstein, E.F.; Fralish, J.S., eds. Proceedings, 13th Central Hardwood Forest conference. Gen. Tech. Rep. NC-234. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station: 556-565.

Van Sambeek, J.W.; Garrett, H.E.; Alley, J.L.; McGraw, R.L. 2004. Forage crops as living mulches for weed control when establishing trees in agroforestry practices. Agroforestry Systems. Submitted.

Van Sambeek, J.W.; McBride, F.D. 1991. Grass control improves early growth of black walnut more than either deep ripping or irrigation. In: Garrett, H.E., ed. The 2nd conference on agroforestry in North America. Columbia, MO: University of Missouri: 42-57.

Van Sambeek, J.W.; Ponder, F., Jr.; Rietveld, W.J. 1986. Legumes increase growth and alter foliar nutrient levels of black walnut saplings. Forest Ecology and Management. 17: 159-167.

Van Sambeek, J.W.; Schlesinger, R.C.; Ponder, F., Jr.; Rietveld, W.J. 1985. Actinorhizal species as nurse crops for black walnut. In: Proceedings, 5th Central Hardwood Forest conference. SAF Publ. 85-05. Urbana, IL: Society of American Foresters: 257-263.

Van Sambeek, J.W.; Schlesinger, R.C.; Roth, P.L.; Bocoum, I. 1989. Revitalizing slow-growth black walnut plantings. In: Rink, G.; Budlesky, C.A., eds. Proceedings, 7th Central Hardwood Forest conference. Gen. Tech. Rep. NC-132. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 108-114.

von Althen, F.W. 1989. Early height growth increased in walnut:silver maple intermixtures. In: Rink, G.; Budlesky, C.A., eds. Proceedings, 7th Central Hardwood Forest conference. Gen. Tech. Rep. NC-132. St. Paul, MN: U.S. Department of Agriculture, North Central Forest Experiment Station: 170-174.

Watson, V.H.; Hagedorn, C.; Knight, W.E.; Pearson, H.A. 1984. Shade-tolerance of grass and legume germplasm for use in the southern forest range. Journal of Range Management. 37(3): 229-232.

White, A.W., Jr.; Beady, E.R.; Tedders, W.L. 1981. Legumes for supplying nitrogen and studies on legumes in pecan orchards. Pecan South. 8(4): 24-31.

Windell, K.; Haywood, J.D. 1996. Mulch mat materials for improved tree establishment. Tech. Rep. 9624-2811-MTDC. Missoula, MT: U.S. Department of Agriculture, Forest Service, Technology and Development Program. 124 p.

Wohlstenholm, B.N. 1970. Pattern of bud break in a pecan cultivar/cultural experiment under dry land conditions. Agroplantae. 2: 61-66.

GIRDLING EASTERN BLACK WALNUT TO INCREASE HEARTWOOD WIDTH

Larry D. Godsey, W.D. "Dusty" Walter, and H.E. "Gene" Garrett[1]

ABSTRACT—Eastern black walnut (*Juglans nigra L.*) has often been planted at spacings that require pre-commercial thinning. These thinnings are deemed pre-commercial due to the small diameter of the trees and the low ratio of dark wood to light wood. As a consequence of size and wood quality, these thinnings are often an expense rather than a source of revenue. In an effort to increase the value of these thinnings it would be beneficial to increase the ratio of dark wood to light wood. One way to increase the amount of dark wood is through costly processing using steam. However, several non-scientific studies have reported that dark wood can be increased by girdling small trees and allowing them to remain on the stump for a certain period of time. This study was designed to test this idea. In a black walnut plantation scheduled for thinning, 10 trees were randomly selected and double-girdled. At that time, increment cores were taken 30.48 cm above the top girdle. These trees were allowed to remain on the stump for 21 months before they were harvested. Results of this study will describe changes in dark wood content following the girdling treatment. The results will compare light to dark wood ratios of the initial increment cores to the boards sawn from the harvested logs.

For many Missouri landowners, the idea of planting eastern black walnut (*Juglans nigra L.*) trees for the saw log or veneer market may seem like a poor investment, since it takes 60 to 80 years before a return is realized. From a financial perspective, the uncertainty and risk involved with a 60 to 80 year investment make it unfeasible. Although harvesting black walnut nuts can generate some cash flow, the prospect of selling marketable saw logs or veneer is often the deciding factor for choosing black walnut for planting.

In Missouri, markets for small diameter hardwood timber are being explored. By marketing smaller diameter trees, the investment period can be shortened considerably. Small diameter markets may work for native oaks (*Quercus* spp.); however, black walnut's appeal in the market is due to its dark colored heartwood. Small diameter black walnut logs have a large amount of light colored sapwood in relation to the darker colored heartwood (Panshin and DeZeeuw 1980). Because of this ratio of light wood to dark wood, black walnut may be an unlikely candidate for the small diameter market.

Although there are commercial methods, such as steaming (Chen and Workman 1980), that increase the amount of coloration in black walnut, these methods are often too expensive for small sawmills. The search is on for a low cost method of increasing the dark colored wood in small diameter black walnut. One method that has been rumored for many years is to kill the tree by girdling, and then leave it standing for a period of time. When the girdled tree is harvested, the light colored sapwood will have been darkened to match the color of the heartwood, thus increasing the quantity of useable wood (Chen and others 1997, 235). This study was designed to test the potential for increasing the amount of dark colored wood in black walnut through girdling.

METHODS

This study was conducted at the Sho-Neff Plantation near Stockton, Missouri. Sho-neff consists of about 194.2 ha with 123.8 ha of eastern black walnut trees of various ages planted at

[1]Economist (LDG), University of Missouri Center for Agroforestry, 203 Anheuser-Busch Natural Resources Building, Columbia, MO 65211; Senior Training Specialist (WDW), University of Missouri Center for Agroforestry; and Director (HEG), University of Missouri Center for Agroforestry. LDG is corresponding author: to contact, call (573)884-3216 or e-mail at godseyl@missouri.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

various spacings. The plantation is divided into 25 areas for research and management.

In 2001, Hammon's Products Company, owner of the Sho-Neff, conducted a thinning to remove approximately one-third of the plantation. Ten trees (Table 1) marked for thinning were chosen at random for this study from Area 16A of Sho-Neff. These trees were native seedlings, planted in 1976 at a spacing of 6 m x 12 m. Annual crops of soybeans, wheat, and milo had been grown between the rows of trees from 1977-1988. A traveling gun system irrigated the area for several years while crops were being grown.

On April 4, 2001, these 10 trees from Area 16A were double girdled with a chainsaw. Girdling started at about 25.4 cm from the ground. Approximately a 7.62 cm space was left between each girdle. An increment core was taken at approximately 30.48 cm above the top girdle at the time of girdling. The trees were left standing until January 2003. On January 24, 2003, the trees were felled, cut to 2.4 m to 3 m lengths, hauled to the Horticulture and Agroforestry Research Center at New Franklin, Missouri and placed inside a storage shed.

On July 8, 2003, a Woodmizer sawmill was used to cut the logs lengthwise. Slabs of about 2.54 cm thickness were removed from the logs until the approximate center of the log was exposed. By cutting the logs lengthwise, any color change could be examined in terms of consistency along the length of the log. Photographs of the slabs were taken for reference purposes. The slabs were numbered the same as the logs.

Table 1.—Location, reference number, and DBH for the 10 black walnut trees analyzed to measure colored wood movement after girdling and standing for 22 months.

Location	Ref. Number	DBH (cm)
Row 4 - #22	I	24.64
Row 4 - #18	II	24.89
Row 4 - #14	III	28.70
Row 2 - #16	IV	23.37
Row 2 - #18	V	27.18
Row 2 - #20	VI	25.91
Row 2 - #22	VII	27.43
Row 2 - #25	VIII	22.61
Row 3 - #19	IX	26.42
Row 3 - #24	X	26.92

In order to analyze the logs, measurements were taken from the center of the log to the edge of the dark colored wood (C-D) and then from the center of the log to the beginning of the bark (C-B). These measurements were taken using a digital caliper, and were taken at about 30.48 cm above the top girdle on the half-log that was left after the slabs were cut off. Each log was measured twice, from the center out to the left and from the center out to the right. After measuring C-D and C-B on both sides of the log, an average estimate of the relationship between dark colored wood to total wood was calculated. Averaging the measurements in two directions out from the center allowed for any elliptical distortion in the proportion of heartwood to sapwood.

The increment cores were also measured using a caliper and measured from the center to the edge of the dark wood (C-D), and from the center out to the beginning of the bark (C-B). Dark colored wood was calculated as a percent of total wood. A comparison of the C-D/C-B ratio from the increment cores was compared to the average C-D/C-B ratio from the logs to determine if any color change had occurred.

RESULTS

To analyze color movement, changes in the percentage of dark colored wood to total wood were compared from the increment borer samples and the logs. At the time of the girdling, dark colored wood and heartwood were synonymous. However, after waiting the 22 months from the time of girdling, dark colored wood could be a combination of heartwood and wood that may be dark in color due to stain or various other reasons (e.g., movement of the dark heartwood color into the lighter colored sapwood) (Bamber and Fukazawa 1985).

Table 2 shows the ratio of dark wood (C-D) to total wood (C-B) from the increment core readings taken at the time of girdling. Log VII had no reading because a portion of the increment borer sample was missing. This made it impossible to determine where the center of the log would have been. It is interesting to note the variance in the percentage of heartwood in trees that are of the same age. One of the trees had as much as 78% heartwood, while another had as little as 49%. Most of the trees had about 72% heartwood; however, the mean ratio was approximately 65% heartwood to total wood. The proportion of heartwood to total wood was inversely related to the DBH of the tree, which supports other studies on this topic (Nelson 1976). For example, the tree with 49% heartwood had the largest DBH.

Table 2.—Log number, increment borer reading taken at the time of girdling at approximately 30.48 cm above the girdle, and ratio of heartwood to total wood for each log girdled and let stand for 22 months.

Log Number	Increment Borer		% D-B3
	C-D1 (cm)	C-B2 (cm)	
I	7.8	10.9	71.56%
II	7.1	9.95	71.36%
III	6.7	13.65	49.08%
IV	7.4	9.5	77.89%
V	8.1	11.2	72.32%
VI	7.0	12.5	56.00%
VII	No reading		
VIII	7.3	10.2	71.57%
IX	6.25	10	62.50%
X	5.3	10.5	50.48%

[1]C-D: dark colored wood (heartwood) width in cm measured from center of stem to edge of dark colored wood.
[2]C-B: total width in cm measured from center of stem to inside of bark.
[3]% D-B: ratio of heartwood width to total width in cm.

Caliper measurements taken from the cut logs after the trees were allowed to stand for 22 months are shown in Table 3. Those measurements show that the percent of dark colored wood ranged from 49% to 86%. The mean ratio of dark colored wood to total wood is approximately 73%.

Table 3.—Log number, colored wood widths; and average dark wood to total wood ratios measured from the center of the log to inside the bark on both sides of the stem 22 months after girdling.

Log Number	Log Readings			
	C-D[1]	C-B[2]	% D-B[3]	Avg. %
I	8.84	10.24	86.33%	86.37%
	9.6	11.11	86.41%	
II	6.81	10.21	66.70%	67.12%
	7.45	11.03	67.54%	
III	6.61	13.8	47.90%	49.48%
	5.75	11.26	51.07%	
IV	6.73	8.68	77.53%	77.48%
	7.17	9.26	77.43%	
V	10.26	11.71	87.62%	80.67%
	9.93	13.47	73.72%	
VI	10.16	10.49	96.85%	80.77%
	7.86	12.15	64.69%	
VII	10.38	13.9	74.68%	72.52%
	8.88	12.62	70.36%	
VIII	7.01	8.96	78.24%	76.48%
	7.83	10.48	74.71%	
IX	7.94	10.73	74.00%	69.09%
	6.61	10.3	64.17%	
X	7.25	10.8	67.13%	65.80%
	7.13	11.06	64.47%	

[1]C-D: dark colored wood width in cm measured from center of stem to both edges of dark colored wood.
[2]C-B: total width in cm measured from center of stem to inside of bark on both sides of the cut log.
[3]% D-B: ratio of dark wood width to total width

DISCUSSION

The purpose of this study was to determine if girdling and leaving trees standing for a period of time would provide a cheap, effective way to increase the colored wood content in small diameter eastern black walnut trees. This idea stemmed from many discussions regarding the marketing of trees grown in agroforestry configurations that require thinning prior to the trees reaching a commercial size.

This study was an attempt to answer two main questions. First, does killing a black walnut tree by girdling and allowing it to dry on the stump increase the amount of colored wood? Second, what effect does this method have on the market value of the small diameter eastern black walnut log?

Looking at the change in dark wood to total wood ratios (Table 4) for both the increment borer samples taken at the time of girdling and the log readings taken 22 months later, it is evident that some of the logs experienced significant increases in the amount of dark wood content. Logs VI, I, and X showed the most change in dark wood to total wood ratio. Log VI had a change in colored wood of nearly 25%, whereas logs I and X had a change of approximately 15%. The remainder of the logs showed very little change in the amount of colored wood, with some logs even showing a loss in colored wood percentage. This may be attributed to shrinkage.

Seven of the nine logs that were compared showed at least a slight increase in the proportion of dark wood to total wood. However, out of the seven logs showing an increase in colored wood, none of the logs had consistent color change throughout the length of the log. In fact, the color increase in log VI was only present in an area approximately 33 cm long and only on one side of the log. This significant increase in colored wood was in the area of the

Table 4.—Comparison of dark wood to total wood ratios at the time of girdling (Increment borer) and 22 months later (Log).

Log Number	Dark Wood to Total Wood Ratio		Color Change
	Increment Borer	Log	
I	71.56%	86.37%	14.81%
II	71.36%	67.12%	-4.24%
III	49.08%	49.48%	0.40%
IV	77.89%	77.48%	-0.41%
V	72.32%	80.67%	8.35%
VI	56.00%	80.77%	24.77%
VII	No reading	72.52%	---
VIII	71.57%	76.48%	4.91%
IX	62.50%	69.09%	6.59%
X	50.48%	65.80%	15.32%

log where bark had been separated and fallen off during the early stages of the girdling treatment.

The inconsistency in the color along the length of the log leads to the second question regarding the economic potential for this type of treatment. If the color is inconsistent along the length of the log, then the log is no more valuable than it was before the treatment was applied. Likewise, the log may be less valuable because the coloration may be considered a defect.

Also, although several of the logs showed an increase in colored wood, this same increase could possibly have been experienced if the trees were allowed to grow for another 22 months. These trees were growing at a rate of approximately 1 cm per year, indicating that they could have been increasing heartwood at a rate of 1 cm per year as well. It is noted that tree growth and heartwood formation may only have a correlation coefficient of 0.56 (Bamber and Fukazawa 1985). Table 5 shows the amount of heartwood measured on the increment borer samples compared to the amount of dark wood measured in both directions from the center of the logs 22 months after girdling. An average change in dark wood content is calculated for each log.

Three of the logs averaged about a 2 cm increase in colored wood. However, looking at log VI, there was 7 cm of heartwood at the time of girdling and 22 months later the log had 7.86 cm measuring from one side of the log center and 10.16 cm measuring from the other side. It is unclear from which side of the log the increment bore sample was taken: however, the side that had 10.16 cm of dark wood was the side that had significant bark loss. The other side had only gained .86 cm in dark wood. Dividing that growth rate by nearly 2 years makes that a 0.43 cm growth in dark wood per year.

Another visible factor affecting marketability of the logs was the distinct presence of insect damage to the logs. All the logs that had significant damage to the bark had significant insect damage. Stain, or spalt, was another problem with the logs. The logs had been stored in a dry, protected building after they were felled; however, some of the logs showed dark stains.

In conclusion, based on this study, it appears that increasing dark colored wood in eastern black walnut by girdling the trees and leaving them standing does not increase the marketability of the log or the market value of the tree. Any abnormal increases in dark wood formation are

Table 5.—Average change in dark wood content from the time of girdling (Increment borer) to the time of cutting 22 months later (Log).

Log Number	Dark Wood (cm)		Change[1] (cm)	Avg Change (cm)
	Increment Borer	Log		
I	7.8	8.84	1.0	1.4
		9.6	1.8	
II	7.1	6.81	-0.3	0.0
		7.45	0.4	
III	6.7	6.61	-0.1	-0.5
		5.75	-1.0	
IV	7.4	6.73	-0.7	-0.5
		7.17	-0.2	
V	8.1	10.26	2.2	2.0
		9.93	1.8	
VI	7.00	10.16	3.2	2.0
		7.86	0.9	
VII	No Reading	10.38	--	-----
		8.88	--	
VIII	7.3	7.01	-0.3	0.1
		7.83	0.5	
IX	6.25	7.94	1.7	1.0
		6.61	0.4	
X	5.3	7.25	2.0	1.9
		7.13	1.8	

[1]Log measurement minus increment borer measurement from both sides of the stem.

counterbalanced with increased risk of insect infestation and stain formation. Steaming the logs to increase dark wood formation would be the recommended method of increasing marketable wood. More research should be directed at finding cheaper methods of steaming and/or utilization of small diameter black walnut logs including the sapwood.

ACKNOWLEDGMENTS

This work was funded through the University of Missouri Center for Agroforestry under cooperative agreement AG-02100251 with the U.S. Department of Agriculture, ARS Dale Bumpers, Small Farms Research Center, Boonville, AR. The results presented are the sole responsibility of the P.I. and/or MU and may not represent the policies or positions of the ARS.

LITERATURE CITATIONS

Bamber, R.K.; Fukazawa K. 1985. Sapwood and heartwood: a review. Forest Products Abstracts. 8(9): 265-278.

Chen, P.Y.S.; Stokke D.D.; Van Sambeek, J.W. 1997. Processing small quantities of walnut logs for lumber and craft items. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 233-235.

Chen, P.Y.S.; Workman, E.C., Jr. 1980. Effect of steaming on some physical and chemical properties of black walnut heartwood. Wood and Fiber. 11(4): 218-227.

Nelson, N.D. 1976. Gross influences on heartwood formation in black walnut and black cherry trees. Res. Pap. FPL 268. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

Panshin, A.J.; DeZeeuw C. 1980. Textbook of wood technology: structure, identification, properties, and uses of the commercial woods of the United States and Canada, 4th ed. New York, NY: McGraw-Hill Book Company.

USE OF HERBICIDE TO REDUCE STUMP-SPROUTING FOLLOWING THINNING OF AN EASTERN BLACK WALNUT AGROFORESTRY PLANTING

W.D. "Dusty" Walter, H.E. "Gene" Garrett, and Larry D. Godsey[1]

ABSTRACT—When establishing an agroforestry practice, the number of trees planted will often exceed the densities needed to achieve a final spacing or configuration. While tight spacings may facilitate certain growth parameters, such as height development, timely thinnings of plantings are required in order to maintain desirable growth rates. In managed plantations especially, the stump sprouts that often result from thinnings may be deemed undesirable for aesthetic reasons, and inhibit other management activities, such as harvesting nuts and mowing. By applying select herbicides to the stump surface following thinning operations, sprouting that often develops can be eliminated. This study was established during the thinning of an eastern black walnut (*Juglans nigra* L.) plantation, with stumps treated immediately following tree felling. Four treatments, including 3 herbicides (Garlon 3A®, Banvel® and Roundup®) and a control (no stump treatment), were applied in a randomized complete block design to 36 stumps. Treatments were applied during April of 2001, with damage to adjacent crop trees assessed later that same growing season. For 2 years following stump treatment, sprouts were counted. Garlon 3A® provided the best results with no sprouting observed over the 2 year study period.

INTRODUCTION

Of all the hardwood species, eastern black walnut (*Juglans nigra* L.) is one of the most likely to be planted in a monoculture (single species) plantation setting. As a tree with production opportunities for growing both nuts and timber of high value, it has been widely planted in agroforestry practices that seek to realize the potential of a given land area for diversified production, as well as resource stewardship. However, embodied within the goals of stewardship and productivity is the maintenance of healthy trees and forests.

When striving to promote healthy forest stands, and optimize productivity, timely thinnings become invaluable tools for forest managers. In hardwood forest stands, thinnings are usually accomplished by either mechanical means that include cutting or girdling tree stems, by chemical release using injection and/or basal bark spray treatments, or by some combination of the two that will ensure removal of select stems and prevent further regrowth. The select removal of undesirable woody plants represents a low-impact, cost-effective means of forest thinning that may also be viewed as being more environmentally friendly than general broadcast treatment methods.

Today's forestry herbicides are rigorously tested in order to meet strict standards of environmental safety and human health protection. Further, most active ingredients in forestry herbicides are below lethal-dose levels associated with many household chemicals, food additives, and nonprescription drugs (McMahon and others 1994). However, for safety and to maximize the effectiveness of the herbicide on the intended target plant, label recommendations should always be followed.

To best ensure the realization of forest management goals and optimize the use of personnel time, thinnings should be designed to reduce the competition between trees for limited site resources (light, moisture, and nutrients). Thinnings that do not eliminate resource competition do not optimize the investment of time, or the likelihood of achieving management goals associated with a thinning

[1]Senior Research Specialist (WDW), University of Missouri Center for Agroforestry, 203 ABNR, Columbia, MO; Director (HEG), School of Natural Resources, University of Missouri Center for Agroforestry, Columbia, MO; Economist (LDG), University of Missouri Center for Agroforestry, Columbia, MO. WDW is corresponding author: to contact, call (573) 884-7991.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

operation. This can occur when trees identified for removal during a thinning practice either, do not suffer loss of upper-stem growth, have upper-stem die-back but resprout from the stump, or the main stem is killed but the tree suckers from the root stock. In all cases, the competition for light may have been eliminated, yet competition for moisture and nutrients continues. This does not optimize the outcomes associated with thinning activities. Proper application of herbicides can effectively minimize the likelihood that thinned trees remain in competition for onsite resources.

This study was designed to evaluate the effectiveness of three herbicides in eliminating stump sprouting following the conventional thinning of a black walnut plantation. Following tree felling with a chainsaw, cut surface application of three herbicide formulations occurred.

Study Site

Located in Southwest Missouri, the Sho-Neff Black Walnut Farm began planting eastern black walnut in 1975. It is currently owned and managed by the Hammons Products Company of Stockton, MO. The farm totals 480 acres that are divided into 25 areas. The stump treatment study was applied in area 16B which was established in 1976. With the primary goal of nut production, trees were planted at an initial spacing of 20 x 40 feet. Agroforestry was practiced on the site in years 1 through 11, with plantings of soybean, wheat and milo in the 40-foot alleyways. A thinning was conducted in 1998-1999 to remove inferior trees, with an additional thinning occurring in 2000 to maintain growth rates on the residual trees.

METHODS

Four treatments were assessed for their effectiveness in minimizing stump sprouting following thinning. These included (1) a control with no chemical stump treatment, (2) Garlon 3A® having 44.4% Triclopyr as the active compound, (3) Banvel® having 48.2% Dicamba as the active compound and (4) Roundup® having 41% Glyphosate as the active compound. Herbicide sprays were applied full strength in order to test the maximum effect that each would have on stump sprouting and the growth of adjacent trees.

Located across 7 rows (40 feet between rows) within a black walnut plantation, 36 trees were cut in 2001, and 1 of the 4 sprouting control treatments applied. Trees were cut with a chainsaw, and application of the treatment occurred immediately following each cut. Each stump was cut low to the ground, at an approximate height of no greater than 3 inches. Herbicide was applied to the outer 2 inches of each stump surface. The herbicides were applied using an adjustable spray bottle that held 1 quart of chemical. Using a similar setting between stream and mist, the outer 2 inches of each stump was covered in chemical. At the time of thinning, the DBH (diameter at breast height, 4.5 feet above ground line) of each study tree was measured. Study trees ranged in size from 6.6 to 12.3 inches in DBH across all treatments. However, the difference in mean DBH within each treatment varied by approximately 1 inch or less (Table 1).

Using a randomized complete block design, treatments were applied to the trees marked for thinning. As many replicates as possible were applied within each tree row. Each cut-stump treatment was applied a total of nine times over seven rows.

Table 1.—Comparison of the mean number of stump sprouts during years 1 and 2 for walnut stumps treated with Garlon 3A®, Banvel® and Roundup® or left untreated (Control).

Cut-Stump Treatment	Active Ingredient	Mean DBH of Treated Stumps	Mean Number of Stump Sprouts Year 1	Mean Number of Stump Sprouts Year 2
Control	no chemical treatment	10.47	5.7A	5.7A
Garlon 3A®	44.4% Triclopyr	10.92	0B	0C
Banvel®	48.2%Dicamba	9.83	2.3B	2.8B
Rounup®	41% Glyphosate	10.56	0.2B	2.9B

** Mean number of sprouts followed with the same letter within a given year are not significantly different at alpha = 0.05 as compared by Duncan's Mutliple Range Test.*

First in July of 2001 and again in January 2003, sprouts from the stump of each tree were counted and the height of the tallest sprout measured. Statistical analyses were conducted using SAS (1999) to determine whether 1 stump treatment was superior to the others at reducing the number of stump spouts. Means of the number of sprouts occurring in year 1 and year 2 were compared using Duncan's Multiple Range Test at alpha = 0.05. By using General Linear Model (GLM) the influence of chemical and DBH of thinned trees was also assessed.

RESULTS

At the end of years 1 and 2, the number of sprouts occurring on each stump was counted. The control treatment (no herbicide applied to the cut surface) had the greatest number of sprouts in both years 1 and 2, with a range of 2-18 sprouts in year 1 and 2-10 sprouts in year 2. The stump with the most spouts occurred within the control treatment. A 9.3-inch DBH stump produced 18 sprouts in year 1. By year 2, the most sprouts counted on a single stump was 10, and they also occurred on a control stump. By comparison, Garlon 3A® had the fewest sprouts. In both years, zero-sprouts were observed across all stumps.

In year 1 no significant differences were found between the three treatments receiving herbicides, but all three were significantly different from the control (no herbicide) (Table 1). However, by year 2 treatment differences, as measured by the number of sprouts, had changed. The mean number of sprouts per stump increased in both the Banvel® and Roundup® treatments resulting in Garlon 3A® having significantly fewer sprouts than all other treatments (Table 1).

In measuring the influence of chemical treatment and DBH on the propensity of a treated stump to sprout, chemical treatment exerted greater significance. The GLM (General Linear Model) of stump sprouting based on chemical treatment and DBH had an R-square of 0.52, indicating that all variance is not accounted for by the two independent variables. However, within the model, the chemical treatment was significant at a 0.99% confidence level.

DISCUSSION

Properly applied herbicide's can be an effective tool for maximizing the benefits from a thinning operation. Numerous studies have examined the use of chemicals for their effectiveness when applied as cut-surface, injection and basal sprays. Thomas and others (1988) used several cut-surface treatments on sugar maple stumps in an effort to eliminate sprouting. He identified a change over time in the number of sprouts, with stumps that initially appeared dead (without sprouts), developing sprouts during the second year. Tordon RTU® and Garlon 3A® were identified as maintaining good control of sprouts for 2 years in the study (Thomas and others 1988).

Other studies, including those by Miller (1993) and Van Sambeek and others (1995), have identified the effectiveness of injection and basal spray treatments as measured by crown reduction and/or tree mortality. These studies have identified differences in effectiveness based on species, and diameter within a species. Their studies tested a variety of chemicals and chemical rates, with varied success. Pannill (1997) expressed dissatisfaction with the results of the hack-and-squirt method when applying Roundup®. He identified an associated mortality of 75% of treated trees.

Additionally, in plantings of like species trees, there should always be a concern with flashback when applying herbicides to thin trees in plantations. Flashback is the unintended negative impact of chemical application on trees adjacent to those treated during the thinning process. This occurs when a chemical translocates from the stem into the root system and via root grafting, moves into an adjacent tree. Often flashback is first evidence in the dieback or yellowing of the foliage of adjacent trees. Our study identified flashback in two separate cases. One resulted from a stump treated with Banvel® and was evidenced by approximately 20% crown dieback of the adjacent tree. The second occurred with Roundup® and caused 30-40% crown dieback. Both trees recovered, but when a thinning is designed to enhance growth and development of released crop trees, any flashback is undesirable. Only the control and Garlon 3A® treatments did not result in any occurrences of flashback.

Our study demonstrates significant differences between herbicides in controlling walnut stump sprouting. Furthermore, it is clear from our results that 1 year is an insufficient time frame within which to evaluate the true effects of herbicides. While all three herbicides tested were found to significantly reduce sprouting after 1 year from that observed when no herbicide was applied, no significant differences were found between the herbicides. However, as a result of the recovery of stumps treated with Banvel® and Roundup®, both had significantly more sprouts after 2 years than were observed on stumps treated with Garlon 3A®.

Clearly, Garlon 3A® was the superior herbicide tested in this trial and a minimum of 2 years following the application of the chemical were required to make a valid comparison.

LITERATURE CITED

McMahon, C.K.; Miller, J.H.; Thomas, D.F. 1994. The role of low impact herbicide treatments in ecosystem management. In: Foley, Louise H., comp. Silviculture: from the cradle of forestry to ecosystem management, proceedings of the National Silviculture workshop; 1993 November 1-4; Hendersonville, NC. Gen. Tech. Rep. SE-88. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station: 242-251.

Miller, J.H. 1993. Tree injection of sweetgum (*Liquidambar styraciflua* L.) with Imazapyr, Triclopyr, and Picloram plus 2,4-D. In: Weed science in harmony with the environment: 46th annual meeting of the Southern Weed Science Society; 1993 January 18-20; Charlotte, NC. Champaign, IL: Southern Weed Science Society: 194.

Pannill, P. 1997. Thinning by injection. Walnut Council Bulletin. 24(2): 12.

SAS Institute Inc. 1999. SAS/STAT OnlineDoc, Version 8. Cary, NC: SAS Institute Inc.

Thomas, M.W.; Garrett, H.E.; Pallardy, S.G. 1988. Susceptibility of sugar maple and oak species to chemical control in Missouri's oak-hickory forests. In: Kaufman, J.I., Westerdayl, H.E., eds. Chemical vegetation management; proceedings of a symposium organized by the Plant Growth Regulator Society of America; San Antonio, Texas; August 1, 1988.

Van Sambeek, J.W.; Kia, D.A.; Shenaut, D.B. 1995. Chemical release of pole-sized trees in a central hardwood clearcut. In: Proceedings, 10th Central Hardwood Forest conference; 1995 March 5-8; Morgantown, WV. Gen. Tech. Rep. NE-197. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 152-157.

Diseases of Intensively Managed Eastern Black Walnut

Manfred E. Mielke and Michael E. Ostry[1]

ABSTRACT—Eastern black walnut has few serious disease problems in its natural woodland setting. However, trees in plantations are subjected to various cultural activities that can create stand conditions that increase pathogen populations and abiotic injuries that often interfere with landowner's objectives.

Native diseases are largely a preventable threat to stands of eastern black walnut (*Juglans nigra*). Eastern black walnut grows naturally in small groups or scattered individuals on a variety of sites associated with assorted tree species of the central hardwood zone. It grows best on well-drained bottomland soils and in coves from the Midwest to the Appalachians. However, when large numbers of eastern black walnut are grown together and managed in plantations, poor site conditions, stress caused by weed competition, soil compaction, injuries from equipment, improper pruning, and wildlife, and adverse microclimatic conditions can predispose trees to fungal pathogens.

NURSERIES

Nurseries epitomize intensive management. The high density of seedlings in production beds and related microclimate conditions leads directly to potential high incidences of foliage and stem diseases and the buildup of inoculum in soil and seed borne pathogens. These conditions and pathogens can result in seedling mortality, weakened seedlings, or the inadvertent planting of infected nursery stock, that in turn can lead to plantation failure.

Root Diseases

Several root diseases have been reported that either kill nursery-grown seedlings outright or cause seedling losses in storage, or during shipping and handling. Disease incidence depends primarily on high soil moisture and the presence of pathogens in the nursery beds or adjacent areas. The fungi *Phytophthora citricola*, *P. cactorum*, *Fusarium episphaera*, *Cylindrocarpon radicicola*, *C. candidum*, *Pythium vexans*, and *Cylindrocladium* sp. have all caused seedling mortality (Berry 1973, Green and Ploetz 1979, Roth and Griffen 1979). Fumigation or treatment using soil chemicals can have short-term benefits, but inevitably beds are re-infested from nearby affected areas. Reducing standing moisture by growing seedlings in well-drained beds may minimize losses (Berry 1973, Kessler 1982).

Seed Borne Pathogens

Recently in Canada, eastern black walnut and butternut (*J. cinerea*) seed that were apparently infected by the butternut canker fungus (*Sirococcus clavigignenti-juglandacearum*) were sown in nursery beds resulting in cankers developing on the seedlings of both walnut and butternut. The entire crop of both species was destroyed to prevent long distance spread of inoculum by shipping the infected planting stock. The butternut canker fungus has been reported on mature eastern black walnut in natural stands, but the fungus has not caused as severe damage as on butternut (Ostry and others 1997).

PLANTATIONS

Walnut trees growing in plantations and the application of various cultural activities used in managing these trees can create stand conditions that increase pathogen populations and abiotic injuries that are often not present in natural stands so disease incidence and severity is usually greater in plantations.

[1]Plant Pathologist (MEM) USDA FS, 1992 Folwell Ave., St. Paul, MN 55108 and Research Plant Pathologist (MEO) USDA FS, 1992 Folwell Ave., St. Paul, MN 55108. MEM is the corresponding author: to contact, call 651-649-5267 or email at mmielke@fs.fed.us

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Stem Diseases

Most canker fungi need some type of opening in the bark in order to invade and infect susceptible tissues of trees. Wounds result from a variety of management activities, by wildlife and from several abiotic causes. Preventing wounds can minimize serious damage caused by many fungi. A few examples of walnut stem diseases; their cause and ways to minimize tree damage are discussed in the next sections.

Wounds Caused by People

Any open wound caused by equipment and improperly timed or poorly made pruning cuts can lead to infection by *Fusarium* spp., the cause of the annual walnut canker, or infection by the perennial target canker fungus *Nectria galligena* (Berry 1973, Cummings and Kuntz 1985, Cummings and others 1993). Pruning should be done properly (removing branches outside the branch collar area taking care to not tear bark below the collar) during the late winter, although anytime during the leaf-off period is acceptable (Bedker and others 1995).

Slow tree growth caused by grass competition results in inadequate or delayed natural pruning and may prevent rapid closing of pruning wounds. Herbicides will control weeds but soil compaction and mechanical wounds to trees associated with chemical applications may occur. Care should be taken to avoid operating equipment in the field during wet weather or in early spring when soils may be saturated to avoid soil compaction and root damage. Obviously, bumping trees with equipment and otherwise scraping off bark should always be avoided.

Wounds Caused by Wildlife

Damage to tree bark caused by deer rubbing their antlers and pecking of the bark by birds can cause tree damage directly as well as lead to infection by canker organisms. There are myriad products that claim to protect trees from deer damage. Various chemicals or physical barriers such as tree shelters can yield variable results. Experiment with a variety of materials to see what works best in your area.

There is little that can be done to protect trees from being fed on by the yellow-bellied sapsucker. It is a protected songbird and in large numbers can cause widespread damage. A possible approach is to leave trees in stands that have been attacked previously since the birds seem to be attracted to previously attacked trees, thereby minimizing damage to remaining trees.

Wounds Caused by Weather Extremes

In the northern extent of its range, and elsewhere in cold air drainages during severe winters, freeze damage causes dieback and mortality of the above ground portions of black walnut, often resulting in infection by *Fusarium* spp. causing annual cankers (Cummings and others 1993, Hayes 1995). This fungus is soil-borne and gains entrance through breaks in the bark anytime during the growing season.

In most cases, little can be done to protect trees from breakage caused by wind, snow and ice. However, timely corrective pruning can help trees survive and retain acceptable crown architecture while protecting them from breakage and subsequent entry by decay fungi.

Decay organisms will eventually infect aging trees, and trees with old branch stubs, large open wounds, and cankers. Eastern black walnut is host to several heart rot fungi. The two most common are *Phellinus igniarius* and *Laetiporus sulphureous* (Berry 1973). Maintaining tree vigor and preventing wounds is the best way to avoid damage by decay fungi.

FOLIAGE DISEASES

Foliage diseases are the most serious diseases of plantation eastern black walnut. Factors that lead to infection and increased disease incidence and severity are high humidity (> 98%), free moisture on leaves (rain, dew, fog, or from irrigation), low light intensity, and temperatures around 21° C. There are four common leaf diseases of black walnut, Microstroma white mold, bull's-eye leaf spot, Mycosphaerella leaf spot, and walnut anthracnose. The most common and most serious of these is walnut anthracnose (Weber and others 1980). All but Microstroma white mold can cause premature leaf loss resulting in reduced growth, increased susceptibility to other diseases, and reduced quantity and quality of nuts.

All of these fungal diseases have a similar biology. Primary infections occur in the spring, May through early June, from ascospores emerging from fruiting bodies on over-wintered leaves on the ground. Once primary infection occurs, lesions appear on leaves within a couple of weeks. Subsequent secondary infections occur on leaves throughout the summer by wind-dispersed spores (conidia) produced in these lesions. Production of spores increases gradually during July and reaches maximum numbers in August. The infected, fallen leaves serve as the reservoir for next year's inoculum, completing the annual life cycles of these fungi.

Microstroma White Mold

Microstroma white mold (or downy leaf spot), caused by *Microstroma juglandis*, is more unsightly than damaging. This disease does not kill the leaf and is not known to cause defoliation. The effect to the tree is minimal, probably resulting in a minor reduction in photosynthesis. Symptoms are a yellowish discoloration on the upper surface of the leaf, and a whitish growth on the underside of the leaf, often concentrated along the veins.

Bull's-Eye Leaf Spot

Bull's eye leaf spot (or zonate leaf spot); caused by *Grovesinia pyramidalis* (asexual state = *Cristulariella moricola*) causes leaf spots and premature defoliation. Maple, hickory and many common weeds are also infected. Symptoms are unique in that the dark lesions on leaves are rounded and have concentric white rings, giving the spot a target-shaped appearance, hence the name bull's-eye leaf spot.

Mycosphaerella Leaf Spot

Mycosphaerella leaf spot, caused by *Mycosphearella juglandis* (asexual state = *Cylindrosporium juglandis*) causes leaf spots that are angular, reaching a maximum size as large as 4 mm in diameter. By midsummer, lesions from secondary infections result in affected trees becoming chlorotic. Coalescing lesions produce a vein pattern or a leaf scorch symptom. By late summer, especially in dry weather, severely diseased leaves fall.

WALNUT ANTHRACNOSE

The most serious and widespread leaf disease of black walnut is walnut anthracnose, caused by *Gnomonia leptostyla* (asexual state = *Marssonina juglandis*). The disease results in leaf spots ranging in size from a few mm to 1.25 cm in diameter. Dark spots first appear on the leaf blades and petioles in the spring. The leaves infected early in the year are most important for photosynthesis during the critical period of nut formation and severe disease can cause reduced yield and nut crop failure. The fungus may infect the nuts directly, causing nutmeats to shrivel and darken. The older leaves in the lower and interior portions of the tree crown are most severely affected, show the most chlorosis and are the first to fall prematurely.

Adjacent trees in plantations may exhibit varying levels of disease, indicating existence of natural resistance to anthracnose. This resistance is highly heritable, with provenances from the relatively arid western edge of the natural range of black walnut (Kansas and Oklahoma) being most susceptible. This is likely due to the limited natural selection for anthracnose resistance in this region since disease incidence is much lower under arid conditions.

When evaluating differences among cultivars in susceptibility to anthracnose one must take into account the nut load (William Reid, personal communication). This is because non-fruiting shoots have more terminal leaves and these leaves have fewer anthracnose lesions because they matured later in the season after the primary infection period for the fungus had passed. Thus, a tree producing a large quantity of nuts will look more susceptible to anthracnose than a tree producing few or no nuts.

Control of Leaf Diseases

Most of the research on control and management of foliar diseases of black walnut has been directed toward walnut anthracnose, although many of the strategies available to growers probably would be effective against all the walnut leaf diseases.

Cultural management is the best approach to minimizing the impact of leaf diseases, particularly in plantations. First, if possible, plant only seedlings known to be resistant to disease. Presently, growers are at the mercy of nut collectors when buying seedlings. It is incumbent on nurseries to only sow nuts from known sources, preferably from resistant trees within the region where seedlings ultimately will be planted. Avoid shipping seedlings from the western area of the natural range of black walnut to other regions. In established plantations, growers should observe which seedlings exhibit symptoms and select against the most susceptible individuals during thinning operations.

Most cultural practices should be directed toward reducing free moisture on leaves. Dense stands are susceptible to disease because of increased humidity, caused by reduced wind flow through these stands, resulting in free moisture on leaf surfaces for extended periods of time. Thinning trees improves drying of leaf surfaces and reduces leaf shading between trees thereby reducing incidence and severity of disease (Tisserat 1985). Ponds, lakes and streams release heat gradually on cool nights, so establishing plantations near bodies of water, particularly on the lee side, lessens dew formation on leaves. Avoid establishing plantations in cold seeps or small openings since this leads to increased dew formation. If possible, avoid overhead irrigation if moisture on leaves will be maintained for long periods of time.

Weeds can contribute to high relative humidity around seedlings, increasing leaf disease, so growers should control weeds either through cultivation or the use of herbicides. Cultivation after leaf fall has the added benefit of incorporating infected leaves into the soil, thereby promoting their decomposition and reducing pathogen populations.

Depending on the crop, intercropping may also contribute to increased humidity. Growers will have to weigh the risk of increased potential of leaf diseases versus the benefits of intercropping. Interplanting autumn olive, hairy vetch, crown vetch, or serecia lespedza may reduce infection by increasing total foliar nitrogen and by preventing primary infections due to increased decomposition of fallen black walnut leaves thereby lowering ascospore production.

April and June applications of nitrogen may reduce infection as well as increase growth of trees. However, according to W. Reid, high rates of nitrogen stimulate non-fruiting shoots to grow late into the summer well after the infection period. These leaves give the tree a healthy appearance and mask the defoliation of older leaves. Soil application of ammonium sulfate, ammonium nitrate, and urea all are effective, although foliar application of urea is not. The addition of phosphorus and potassium has been shown to diminish the benefit of nitrogen fertilizer.

Direct control using fungicides can be effective, although the availability of suitable materials is in question. Since the status of all agricultural chemicals is always under review, contact your local extension agent for the latest information. Infection can occur anytime during the growing season so timing of applications presents a problem. A practical approach is to use two applications: the first during the last week in May to prevent the primary infection by ascospores, and the second during the first week of July to inhibit secondary infection by conidia.

Overall, native insects and pathogens should not pose a significant threat to black walnut management. Planting quality seedlings from geographically suited seed sources, and taking care to avoid injurious management practices will result in healthy growing stock. Currently no known exotic pests threaten black walnut. Given the high amounts of global trade, and the frequent introduction of forest pests, plantings should be monitored closely since they can serve as an early detection network for potentially invasive and damaging pests.

LITERATURE CITED

Bedker, P.J.; O'Brien, J.G.; Mielke, M.E. 1995. How to prune trees. NA-FR-01-95. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Area, State and Private Forestry. 12 p.

Berry, F.H. 1973. Diseases. In: Black walnut as a crop: 2nd Black walnut symposium; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep. NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 88-90.

Cummings, J.E.; Kuntz, J.E. 1985. Stem canker on black walnut caused by *Fusarium sporotrichioides*. Phytopathology 75:1279.

Cummings Carlson, J.; Mielke, M.E.; Appleby, J.E.; Hatcher, R.; Hayes, E.M.; Luley, C.J.; O'Brien, J.G.; Rugg, D.J. 1993. Survey of black walnut canker in plantations in five central states. Northern Journal of Applied Forestry. 10: 10-13.

Green, R.J.; Ploetz, R.C. 1979. Root rot of black walnut seedlings caused by *Phytophthora citricola*. In: Walnut insects and diseases workshop proceedings; 1978 June 13-14; Carbondale, IL. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 5-10.

Hayes, E.M. 1995. Freeze injury to black walnut plantations in Minnesota during the winter of 1993-94. 86th Annual Report of the Northern Nut Growers Association: 21-27.

Kessler, K.J.; 1982. Control of black walnut root rot diseases in nurseries. Res. Pap. NC-228. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 8 p.

Ostry, M.E.; Katovich, S.; Anderson, R.L. 1997. First report of *Sirococcus clavigignenti-juglandacearum* on black walnut. Plant Disease. 81: 830.

Roth, D.A.; G.J. Griffen. 1979. Cylindrocladium root rot of black walnut seedlings: root rot of black walnut seedlings caused by *Phytophthora citricola*. In: Walnut insects and diseases workshop proceedings; 1978 June 13-14; Carbondale, IL. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 11-21.

Tisserat, N.A. 1985. Foliage and stem diseases of black walnut. In: 76th Annual Report of the Northern Nut Growers Association: 121-130.

Weber, B.C.; Anderson, R.L., Hoffard, W.H. 1980. How to diagnose black walnut damage. Gen. Tech. Rep. NC-57. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 20 p.

Spread of Butternut Canker in North America, Host Range, Evidence of Resistance Within Butternut Populations and Conservation Genetics

M.E. Ostry and K. Woeste[1]

Abstract—Butternut canker is killing trees throughout the range of butternut in North America and is threatening the viability of many populations in several areas. Although butternut is the primary host, other *Juglans* species and some hardwood species also are potential hosts. Evidence is building that genetic resistance within butternut populations may be exploited for conservation and restoration of the species.

INTRODUCTION

Butternut (*Juglans cinerea* L.) is being killed throughout its range by a canker caused by the fungus *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, and Kuntz, described as a new species in 1979 (Nair and others 1979). Although there are no reports of this fungus outside of North America, it is thought to be an exotic pathogen (Furnier and others 1999). Spores of the fungus develop under infected bark in sticky masses and are dispersed by rainsplash and wind during the growing season.

Butternut is valued for many uses and is important for wildlife and forest diversity, however, its infrequent occurrence within forest stands and its relatively small kernel and hard shell have, in part, limited its commercial importance as a timber or nut species (Ostry and Pijut 2000). As local supplies of healthy butternut trees become scarce the value of the wood has increased.

Butternut was listed under Category 2 on the list of Endangered and Threatened Plants under the Federal Endangered Species Act of 1973, however, this category has been eliminated and currently butternut has no official listing status. The first state to enact a measure to conserve butternut was Minnesota where in 1992 a moratorium on the harvest of healthy butternut on State lands was enacted. Butternut remains a “species of concern” or a “sensitive species” in many states and is a Regional Forester Sensitive Species in the Eastern Region on 13 of the 16 National Forests. In Canada, butternut was listed endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in November 2003.

SPREAD OF THE PATHOGEN

The first reported occurrence of butternut canker was from southwestern Wisconsin where all but two butternut trees in a 40-acre woodlot were diseased (WI Conserv. Dept. 1967). A survey of butternut in 36 Wisconsin counties in 1976 revealed that 31 and 9% of the trees were diseased and dead, respectively. In contrast, in a 1992 resurvey of 32 Wisconsin counties 92 and 27% of the trees were diseased and dead, respectively (Carlson and Guthmiller 1993).

A survey for butternut canker in the eastern United States revealed that the disease was present in at least 14 of the 16 states surveyed (Anderson and LaMadeleine 1978). In that report the authors mention the disease had essentially eliminated many populations of butternut in North and South Carolina. Early reports of butternut decline throughout the northeastern United States were attributed to the fungus *Melanconis juglandis* Ellis & Everhart Graves (Ostry 1997b) that causes branch dieback but not stem cankers. Although cankers are obvious, unless close examinations

[1]Research Plant Pathologist and Research Plant Molecular Geneticist, North Central Research Station, USDA Forest Service, 1561 Lindig Avenue, St. Paul, MN 55108 (MEO) and Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Forestry Building, 195 Marsteller St. West Lafayette, IN 47907 (KW). MEO is corresponding author: to contact call (651) 649-5113 or e-mail at mostry@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

were made of trees, these reports may have mistakenly attributed tree decline to *M. juglandis* and butternut canker may have been present much earlier than reported.

In Canada, butternut canker was first detected and confirmed from Ontario and Quebec in 1991 (Davis and others 1992) and in New Brunswick in 1997 where it was thought to have been present for at least 7 years (Harrison and others 1998).

The most recent U.S. Department of Agriculture, Forest Service, Forest Inventory and Analysis survey data examined for butternut (NCRS, FIA Web site, October 2003) revealed that overall in seven Midwestern states the number of butternut trees in all size classes decreased by 23%, however, the number of trees recorded increased in three of the states. The states with a decrease in the number of trees and the inventory interval from which the data were collected are as follows: Michigan, 89% (1993-2001); Illinois, 87% (1998-2002); Wisconsin, 44% (1996-2001); and Iowa, 40% (1990-2001). An increase in the number of butternut trees was recorded in Minnesota, 55% (1990-2002); Indiana, 41% (1998-2002); and Missouri, 25% (1989-2002). This increase in number of trees was predominantly in the smallest size class (1.0-2.9 inch); the number of trees in all other size classes revealed decreases ranging from 13% (11.0-12.9 inch) to 100% (21.0+).

Butternut and eastern black walnut (*J. nigra* L) seed are known to harbor *S. clavigignenti-juglandacearum* (Innes 1997). There is no evidence that it can be spread on Japanese walnut (*J. ailanthifolia* Carr.) seed or seedlings but this walnut species has been widely planted throughout the eastern United States (Bixby 1919). One can only speculate whether the fungus could have been inadvertently introduced into the United States from Asia on seed. There also is evidence that several insect species are closely associated with healthy and diseased butternut and because some of these insects were shown to be contaminated with the fungus they may act as vectors of the pathogen although the exact mode of spread is unknown (Katovich and Ostry 1998, Halik and Bergdahl 2002). Birds also may come into contact with the sticky spores and spread them from diseased to healthy trees within and between forest stands.

NATURAL AND EXPERIMENTAL HOST RANGE

Butternut is the only species that is killed by this canker disease. However, other *Juglans* species and hybrids are diseased to varying degrees. Orchard and others (1982) inoculated 10- to 20-year-old trees of several *Juglans* species and found that Japanese walnut, heartnut (*J. ailanthifolia* var. *cordiformis* (Maxim.) Rehd.) and hybrids between them and butternut expressed greater resistance than either eastern black walnut or Persian walnut (*J. regia* L.) with the latter developing the most severe disease symptoms.

In Canada and the United States tree species other than butternut have been affected by butternut canker over the past several years. It was reported eastern black walnut and butternut seedlings were naturally infected by *S. clavigignenti-juglandacearum* in a nursery in Quebec (Innes 1997). Stem cankers were confirmed on a 48 cm diameter eastern black walnut in North Carolina, and branch cankers were detected on 20-year-old eastern black walnut trees in Minnesota (Ostry and others 1997). Branch cankers were also found on a 25-year-old heartnut in Iowa (Ostry 1997a). These reports indicate that this fungus may be a potential threat to walnut plantations.

Nearly the entire U.S. Persian walnut crop is produced in California (Beede and Hasey 1998). *S. clavigignenti-juglandacearum* is not known to be present in California and a quarantine on importing *Juglans* species from the eastern U.S. was put in place, but it is unknown what impact, if any, this pathogen may have on walnut cultivation should it become established there. Grafted plants of several *Juglans* species and hybrids have been artificially inoculated in the greenhouse, including several accessions from the National Clonal Germplasm Repository in Davis, California. Three of the leading cultivars grown, 'Hartley', 'Chandler', and 'Payne' (Beede and Hasey 1998) were among the most susceptible Persian walnut selections tested (Ostry and Moore, unpublished data) indicating caution should be exercised to avoid the movement of the pathogen into California. Interestingly, a "Paradox' hybrid (*J. hindsii* x *J. regia*), a hybrid commonly used as rootstocks for Persian walnut (Beede and Hasey 1998), was highly resistant.

Using artificial inoculations of greenhouse seedlings, several other hardwood species have been shown to be susceptible and may be able to harbor the fungus (Ostry 1997b). Species in *Carya*, a genus in the walnut family (*Juglandaceae*) that were demonstrated to be susceptible include pecan (*C. illinoensis* [Wangenh.] K. Koch) and shagbark hickory (*C. ovata* [Mill.] K. Koch). Although not causing large cankers, the fungus was recovered beyond the inoculation point from northern red (*Quercus rubra* L.), black (*Q. velutin*a Lam.), and white oak (*Q. alba* L.) and black cherry (*Prunus serotina* Ehrh.). Bitternut hickory (*C. cordiformis* (Wangenh.) K. Koch) also has been shown to support the growth of the pathogen in greenhouse tests (Ostry and Moore, unpublished data). These preliminary results indicate that species of genera other than *Juglans* may serve as a reservoir of the pathogen within forest stands.

EVIDENCE OF RESISTANCE

In many areas throughout its range, healthy butternut have been found growing adjacent to trees infected and killed by the disease. Some of the trees we have monitored have remained healthy for over 12 years despite the severe disease on neighboring trees, minimizing the likelihood that disease escape is responsible for trees being symptom-free. Although these relatively rare trees may be disease resistant, we do not have experimental data as yet to demonstrate the existence of effective resistance.

Our current evidence of resistance mechanisms is circumstantial based on examining butternut over the years in search of trees that may have disease resistance. During our examinations we have detected two bark phenotypes on trees of the same size and relative age. One is a dark colored bark with deep bark fissures resembling the bark of eastern black walnut. The other is a light gray bark color with shallow bark fissures. These bark types and various intermediate types have been found on adjacent trees in many woodlots in Minnesota and Wisconsin.

Often the dark/deep bark phenotype is associated with healthy trees and the light/shallow bark with diseased trees (Ostry and others 2003). Part of our research is directed at determining if bark phenotype and disease severity are genetically based traits that may help elucidate the mechanism of host resistance and potentially be used in conservation and genetic improvement of the species.

Disease resistance screening was initiated in one of the five grafted butternut clonal archives in 2003 (Ostry and others 2003). Three trees 7-11 years old from each of 22 accessions propagated from diseased and healthy source trees and unselected 9-year-old butternut trees were wound inoculated each month from April through October with two isolates of *S. clavigignenti-juglandacearum*. The objective was to mimic natural infection in the field to compare time of inoculation and host responses of selected grafted lines of butternut with putative disease resistance to grafted clones of butternut that are known to be highly susceptible.

Although it is too early for reporting definitive results from this screening trial, indications are that infection resulted from all inoculation dates and several selected butternut lines have limited canker development compared to unselected or diseased source trees (Ostry and Moore, unpublished data). As with inoculations of plants in the greenhouse, screening trees in the field this way may allow us to separate groups of highly resistant selections from those that are highly susceptible.

The potential for plant pathogens to overcome host resistance can be high, especially with pathogens associated with long-lived trees. Agriculture is in a constant race with plant pathogens to develop and incorporate new genetic resistance into important crops as pathogens evolve and overcome them. Pathogens with a high evolutionary potential are more likely to overcome resistance compared to pathogens with a low evolutionary potential and knowledge of the genetic structure of a pathogen may be useful in predicting its future evolutionary potential (McDonald and Linde 2002). Pathogens having both a sexual and asexual reproduction system, high genotype flow, large effective population size and a high mutation rate will have the greatest potential to overcome host resistance.

Evaluating the potential for *S. clavigignenti-juglandacearum* to overcome resistance in butternut within the framework outlined above results in guarded optimism that resistance may be long-lasting. First, a sexual state of *S. clavigignenti-juglandacearum* is not known to be present, therefore recombination via outcrossing resulting in new gene combinations that could overcome resistance is not likely. DNA fingerprinting (Furnier and others 1999) revealed limited genotype diversity supporting this theory.

Second, gene flow, exchange of either alleles (genes) or individual clones (genotypes) among populations is more limited with *S. clavigignenti-juglandacearum* than many other tree pathogens because it lacks an efficient long-range airborne spore stage. However, the sticky spores may be moved considerable distances by insects or birds countering this. Another mode enabling pathogens to move beyond their natural dispersal range is through human transport of infected plants or plant parts and *S. clavigignenti-juglandacearum* can be seedborne and also moved on logs and scionwood. Thus, although lacking an efficient airborne state, *S. clavigignenti-juglandacearum* can still be dispersed long distances.

Another source of genetic variation in pathogens is mutation resulting in new strains that could overcome host resistance genes. However, these mutations are more likely to occur and be selected for in pathogens that exist in large populations in individual plants, such as with bacteria and viruses. Butternut canker is not systemic and small populations of the fungus exist within relatively few diseased trees in any given area. Thus, the potential for a mutant strain of *S. clavigignenti-juglandacearum* to multiply, spread to a susceptible host, infect, successfully colonize and then reproduce on that host is probably not very high.

In summary, considering what we know about the genetics of *S. clavigignenti-juglandacearum*, there is realistically a low to moderate risk that it will evolve strains capable of overcoming disease resistance in butternut populations that may exist today or will be developed in the future.

BUTTERNUT CONSERVATION GENETICS

The decline in butternut populations at local, regional and national levels raises questions about whether the long-term genetic viability of the species has been compromised. Stated another way, how much genetic variability did butternut have historically, how much remains, and is there enough for butternut to fulfill its ecological functions, resist disease and adapt to environmental change (Yang and Yeh 1992)? At present, there are few answers.

Available data indicate that butternut has considerably lower genetic diversity (as measured by percent polymorphic loci and number of alleles per locus) than similar species based on allozyme and RFLP marker systems (Morin and others 2000, Fjellstrom and Parfitt 1994). Morin and others (2000) reported that less than 20% of the loci they evaluated in butternut were polymorphic, with 1.3 alleles per allozyme locus or fewer. By comparison, eastern black walnut had 42 – 88% polymorphic loci and about 2.9 alleles per locus. The preliminary findings of Morin and others (2000) indicate that butternut may be slightly more genetically diverse in the US than in Canada, but they were unable to determine the cause or causes of the lower-than expected genetic diversity of butternut.

Microsatellite DNA polymorphisms (SSRs) are rapidly becoming the marker system of choice for population genetic studies, and several of the SSRs originally identified in black walnut (Woeste and others 2002) are also polymorphic in butternut (Woeste, unpublished data). Nuclear SSRs used in tandem with chloroplast markers can potentially be used to confirm if butternut has been through a genetic bottleneck and to predict whether the bottleneck was recent or ancient. SSRs are also an excellent tool for evaluating regional and local genetic diversity of butternut. Comparative studies of allele sizes of SSRs that can be amplified in butternut, eastern black walnut, and Japanese walnut might also be useful for identifying hybrids.

As previously mentioned, many of the apparently canker resistant butternut trees we have examined are characterized by deeply fissured, darkly colored bark. This phenotype, not typically associated with butternut, is similar to the bark of black walnut. The origins of this dark-barked phenotype are unknown. It is possible that dark-barked trees are an ecotype of butternut that was previously unnoticed, either because it was rare or because dark-barked butternuts were mistaken for walnuts by casual observers. Because of the phenotypic similarity between dark-barked butternuts and black walnut, we investigated whether the ITS region of some of the dark-barked trees indicated hybrid origins. Published literature was clear that the (*J. nigra* x *J. cinerea*) hybrid was not possible (Funk 1970), but unsubstantiated claims of the existence of such hybrids infrequently arise. For example, trees catalogued as *J. nigra* x *J. cinerea* hybrids were maintained at the Tree Improvement Center (TIC) of the Carbondale work unit of the North Central Research Station, and seeds of putative *J. nigra* x *J. cinerea* have been sold by nurseries. The trees in the TIC were grown from seeds provided by Michigan State University researchers in the late 1950s. We have concluded from preliminary analyses of the dark-barked butternuts and putative *J. nigra* x *J. cinerea* hybrids that some of the dark-barked butternuts are true butternuts (non-hybrids) and that at least one of the many putative *J. nigra* x *J. cinerea* hybrids at the TIC may in fact be such a hybrid.

Several unknowns confound these results, but first among them is that we do not know if we are able to accurately differentiate among *J. cinerea* x *J. ailanthifolia*, *J. nigra* x *J. cinerea* and all possible three-species hybrids such as (*J. nigra* x *J. ailanthifolia*) x *J. cinerea*, in part because there are not as yet any positive controls for the experiment. Furthermore, it is not known if hybridization that may have occurred two or three generations ago can be detected accurately using internal transcribed spacer (ITS) sequences, although the phenotypic impact of such hybridization may still be present. In other words, dark-barked butternuts with greater canker resistance may be butternuts; they may be the product of a rare, natural hybridization between eastern black walnut and butternut that occurred a few generations ago; or they may be something else. Simple analysis of ITS regions only may not be able to demonstrate or rule out any of the possibilities. Butternut may have hybridized with eastern black walnut in the recent past, the more distant past, or both. Detailed analysis of the chloroplast sequences of both species and the putative hybrids may provide some insight into this question. Since chloroplasts are strictly maternally inherited in *Juglans*, the presence in dark-barked trees of eastern black walnut chloroplast sequences would indicate a hybrid between eastern black walnut and butternut in which butternut was the male.

It should be noted that hybridization between butternut and eastern black walnut seems ecologically unlikely since interspecific hybridization usually happens in the contact zones of spatially separated species, and the ranges of eastern black walnut and butternut overlap across almost the entire northeast quarter of the US (Funk 1970).

Butternut hybridizes with Persian walnut to produce *J.* x *quadrangualata* (Carr.) Rehd., with Japanese walnut to produce *J.* x *bixbyi* Rehd and with heartnut to produce "buartnut". The striking vigor of the F1 hybrid between butternut and Japanese walnut is one phenotype that can be used to distinguish these hybrids, also called buarts or butterjaps, from butternuts. Field observations indicate that buarts are more common in old, abandoned farmyards, on pasture edges, and in the yards of houses in small, rural towns. The leaves of buarts may be greener and more persistent than those of butternut, not abscising until well into October; whereas butternut leaves typically turn yellow and abscise in early to mid-September. There are reports that butternut also hybridizes with little walnut (*J. microcarpa* Berland) and Manchurian walnut (*J. mandshurica* Maxim.).

To identify and characterize hybrids it will be essential to develop markers that clearly differentiate among *Juglans* species, specifically eastern black walnut, butternut, and Japanese walnut. A full study of the variability of the nuclear, chloroplast and mitochondrial genomes of all three species will probably be necessary before any marker system can be used to detect and identify the parents of hybrids with a high level of confidence. One method for finding species specific DNA sequence signatures is the analysis of DNA sequences from highly conserved genes. It may be possible to identify species-specific polymorphisms in the introns or nearby non-coding regions from six or seven genes, and these could be used to detect backcrosses or even three-species hybrids. Unfortunately, there is almost no DNA sequence data available in public databases for butternut and Japanese walnut, and very little for eastern black walnut. All methods for evaluating species diversity and hybridity have drawbacks and blind spots, and as such a combination of morphological and molecular techniques seems likely to produce the most reliable results.

At present, the best tool for discriminating *Juglans* species and their hybrids is the sequence polymorphism found within the ITS regions of the nuclear ribosomal DNA. The ITS region is present in most genomes as thousands of copies of tandem repeats at one or many loci (Baldwin and others 1995). Because the sequence undergoes rapid, concerted evolution leading to a high level of intergenic uniformity, the ITS region has proven generally useful in studies of angiosperm taxonomy (Baldwin and others 1995) and it has been used specifically to decipher the species identities and hybrid origins of *Juglans* rootstocks used in the California walnut nursery industry (Potter and others 2002). We have identified primers that amplify the ITS region of eastern black walnut, butternut, and Japanese walnut. By digesting the PCR products with restriction enzymes, DNA fragments of diagnostic sizes are produced for each species. This marker system is imperfectly co-dominant because there can be within genome length variation in the ITS (Sytsma and Schaal 1990 and our preliminary results from eastern black walnut), because the ITS regions of hybrids may homogenize to a single sequence at different rates, and because there may not be sufficient polymorphism within the ITS to determine which species among several possible are represented in a hybrid and at what percentage.

Taxonomists and dendrologists have traditionally used morphology to distinguish species and their hybrids. This approach is complicated in the genus *Juglans* because there are few readily discernable traits that distinguish the species. A careful evaluation of trichomes or other more subtle features may yet prove valuable in the identification of *Juglans* species and first generation hybrids. One potential problem with any approach to distinguishing hybrids is that Japanese walnut has been widely propagated in the US for over 150 years (Crane and Reed 1937). Some features that we now associate with butternut may have been introduced by gene flow from Japanese walnut to butternut a generation ago. Similarly, Persian walnut, a species that can hybridize with eastern black walnut, butternut, and Japanese walnut, has been propagated in the US since colonial times. In areas where butternut populations have undergone a severe decline, one may be justifiably skeptical concerning the identification of the remaining 'butternuts', especially if these trees express some morphological or genetic variability that is uncommon elsewhere. Do remnant trees represent the best opportunity to capture rare local diversity, or are they Trojan horses carrying genes, including perhaps genes for resistance to butternut canker, from other species? The study of herbarium sheets of butternut collected before 1860 may be one way to evaluate the morphological diversity of butternut before there was any potential impact by hybridization with other *Juglans* species.

CONCLUSIONS

Since the detection of butternut canker in 1967 researchers have clarified several aspects of the disease, including the description of the causal agent, its biology, a partial host range, and they have documented limited examples of potentially disease resistant trees. However, many gaps remain in our knowledge including the origin of the pathogen, the level of genetic diversity in butternut across its range, and silvicultural techniques to retain butternut in our forests and to restore the species where it has been eliminated by the disease.

Butternut canker and its spread raises fundamental issues with respect to the productivity and health of the central hardwoods landscape. Exotics invade native landscapes on several levels: physically, they occupy the space where endemics once grew, modifying the environment there and often making it less hospitable to native flora and fauna; they invade by introducing pests and they invade at the genetic level by hybridizing with endemic flora or other introduced species. The genetic invasion is often unseen and difficult to monitor unless (or until) the hybrids themselves become invasive weeds. More sensitive genetic and phenotypic marker systems are needed to monitor the genetic invasion of the exotics such as Japanese walnut into the central hardwoods region. Butternut has been the most affected by Japanese walnut. But over the long-term the possibility exists that black walnut could also be adversely affected by exotic invasion at the genetic level.

Butternut is rapidly being lost in our forests from a variety of causes in addition to butternut canker. Genetic diversity in species such as butternut is needed for its long-term survival, future adaptation and evolution. There is an urgent need to conserve genetic diversity among butternut populations before valuable populations are lost.

ACKNOWLEDGMENTS

We thank the many individuals in various state and federal agencies and the numerous private landowners who continue to support our research efforts. We especially thank M. Moore and Bo Li for technical support in the conduct of laboratory, greenhouse and field research.

LITERATURE CITED

Anderson, R.L.; LaMadeleine, L.A. 1978. The distribution of butternut decline in the Eastern United States. For. Serv. Rep. S-3-78. Washington, DC: U.S. Department of Agriculture, Forest Service. 5 p.

Baldwin, B.G.; Sanderson, M.J.; Porter, J.M.; Wojciechowski, M.F.; Campbell, C.S.; Donoghue, M.J. 1995. The ITS region of nuclear ribosomal DNA a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden. 82: 247-277.

Beede, R.H.; Hasey, J.K. 1998. The history of the walnut in California. In: Ramos, D.E., ed. Walnut production manual. Publ. 3373. University of California, Division of Agriculture and Natural Resources: 8-15.

Bixby, W.G. 1919. The butternut and Japan walnut. American Nut Journal. 10: 76-83.

Carlson, J.C.; Guthmiller, M. 1993. Incidence and severity of butternut canker in Wisconsin in 1976 and 1992. Phytopathology. 83: 1352. Abstract.

Crane, H.L.; Reed, C.A. 1937. Nut breeding. In: Yearbook of agriculture. U.S. Department of Agriculture, 75th Congress, 1st Session, House document No. 28. 827-889.

Davis, C.N.; Myren, D.T.; Czerwinski, E.J. 1992. First report of butternut canker in Ontario. Plant Disease. 76: 972.

Funk, D. 1970. Genetics of black walnut. Res. Pap. WO-10. Washington, DC: U.S. Department of Agriculture, Forest Service. 13 p.

Fjellstrom, R.G.; Parfitt, D.E. 1994 Walnut (*Juglans* spp.) genetic diversity determined by restriction fragment length polymorphisms. Genome. 37: 690-700.

Furnier, G.R.; Stolz, A.M.; Raka, M.M.; Ostry, M.E. 1999. Genetic evidence that butternut canker was recently introduced into North America. Canadian Journal of Botany. 77: 783-785.

Halik, S.; Bergdahl, D.R. 2002. Potential beetle vectors of *Sirococcus clavigignenti-juglandacearum* on butternut. Plant Disease. 86: 521-527.

Harrison, K.J.; Hurley, J.E.; Ostry, M.E. 1998. First report of butternut canker caused by *Sirococcus clavigignenti-juglandacearum* in New Brunswick, Canada. Plant Disease. 82: 1282.

Innes, L. 1997. *Sirococcus clavigignenti-juglandacearum* on butternut and black walnut fruit. In: Foliage, shoot and stem diseases of trees: proceedings of the IUFRO WP 7.02.02 meeting. Quebec City, Quebec, Canada: Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre: 129-132.

Katovich, S.A.; Ostry, M.E. 1998. Insects associated with butternut and butternut canker in Minnesota and Wisconsin. The Great Lakes Entomologist. 31: 97-108.

McDonald, B.A.; Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology. 40: 349-379.

Morin, R.; Beaulieu, J.; Deslauriers, M.; Daoust, G.; Bousquet, J. 2000. Low genetic diversity at allozyme loci in *Juglans cinerea*. Canadian Journal of Botany. 78: 1238-1243.

Nair, V.M.G.; Kostichka, C.J.; Kuntz, J.E. 1979. *Sirococcus clavigignenti-juglandacearum*: an undescribed species causing canker on butternut. Mycologia. 71 :641-646.

Orchard, L.P.; Kuntz, J.E.; Kessler, K.J., Jr. 1982. Reactions of *Juglans* species to butternut canker and implications for disease resistance. In: Black walnut for the future: 3rd walnut symposium; 1981 August 10-14; West Lafayette, IN. Gen Tech. Rep. NC-73. St. Paul, MN: U.S. Deparment of Agriculture, Forest Service, North Central Forest Experiment Station: 27-31.

Ostry, M.E. 1997a. *Sirococcus clavigignenti-juglandacearum* on heartnut (*Juglans ailanthifolia* var. *cordiformis*). Plant Disease. 81: 1461.

Ostry, M.E. 1997b. Butternut canker in North America 1967-1997. In: Foliage, shoot and stem diseases of trees: Proceedings of the IUFRO WP 7.02.02 meeting. Quebec City, Quebec, Canada: Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre: 121-128.

Ostry, M.E.; Katovich, S.; Anderson, R.L. 1997. First report of *Sirococcus clavigignenti-juglandacearum* on black walnut. Plant Disease. 81: 830.

Ostry, M.E.; Pijut, P.M. 2000. Butternut: an underused resource in North America. HortTechnology. 10: 302-306.

Ostry, M.E.; Ellingson, B.; Seekins, D.; Ruckheim, W. 2003. The need for silvicultural practices and collection of butternut germplasm for species conservation. In: Van Sambeek, J.W.; Dawson, J.O.; Ponder, F., Jr.; Loewenstein, E.F.; Fralish, J.S., eds. Proceedings, 13th Central Hardwood Forest conference; 2002 April 1-3; Urbana, IL. Gen. Tech. Rep. NC-234. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station: 551-555.

Potter, D.; Gao, F.; Baggett, S.; McKenna, J.R.; McGranahan, G.H. 2002. Defining the source of Paradox: DNA sequence markers for North American walnut (*Juglans L.*) species and hybrids. Scientia Horticulturae. 94: 157-170.

Sytsma K.J.; Schaal B.A. 1990. Ribosomal DNA variation within and among individuals of *Lisianthius* (Gentianaceae) populations. Plant Systematics and Evolution. 170: 97-106.

Woeste, K.; Burns, R.; Rhodes, O.E., Jr.; Michler, C. 2002. Thirty polymorphic nuclear microsatellite loci from black walnut. The Journal of Heredity. 93(1): 58-60.

Yang, R.-C.; Yeh, F.C. 1992. Genetic consequences of *in situ* and *ex situ* conservation of forest trees. The Forestry Chronicle. 68: 720-728.

INSECTS ATTACKING BLACK WALNUT IN THE MIDWESTERN UNITED STATES

Steven Katovich[1]

ABSTRACT—Black walnut has only a handful of insects that would be considered significant pests. Of the leaf feeders, the walnut caterpillar is the most likely to cause significant defoliation and damage to trees. However, severe infestations are infrequent and tend to be restricted to small geographic areas. Two other commonly encountered defoliators are the yellow necked caterpillar and the fall webworm. The black walnut curculio is the major nut pest on black walnut. The walnut shoot moth attacks buds and shoots of black walnut. Destruction of the terminal bud or shoot on young trees can cause excessive branch forking that can change the shape of a tree. Walnut does have several wood boring insects that will invade the main trunk and larger branches. Most of them infest trees that are in poor health. Many can also invade freshly cut logs. An ambrosia beetle, *Xylosandrus germanus,* can attack apparently healthy trees and attacks are often associated with the pathogen *Fusarium.* Management practices are provided that should minimize insect caused impacts

INTRODUCTION

Black walnut has an array of insects that feed on its leaves and developing nuts, and tunnel into buds, shoots, twigs, and through bark into the wood. However, only a few insects would be considered significant pests. Perhaps the most important are those that tunnel into developing shoots and buds on younger trees. The destruction of the terminal bud or shoot on a young tree can cause excessive branch forking that can change the shape of a tree and reduce its timber value. There are also insects that can infest the main stem of living trees though generally this occurs on weakened or damaged trees. Freshly cut logs can also be invaded. These wood boring insects can introduce a number of fungi and other pathogens, and their tunnels can cause degrade in the value of wood products. Leaf feeding insects are not uncommon and outbreaks do occur. But, most outbreaks tend to be short-lived on walnut and widespread tree mortality is not often reported. Nut production can be reduced by weevil attacks on developing nuts and insects that infest the husk.

SPECIFIC DAMAGING INSECTS

Weber and others (1980) developed a publication on diagnosing the most common damaging agents on black walnut. This publication is no longer in print, but can be accessed via the internet at: http://www.na.fs.fed.us/spfo/pubs/howtos/ht_walnut/cover.htm.

Information on identification of many walnut insects can also be obtained by using Johnson and Lyon (1988) and USDA (1985). Information on wood boring insects attacking walnut is available in Solomon (1995).

Insects Damaging Foliage

Loss of leaf tissue is referred to as defoliation. The impact that insect caused defoliation has on a tree can include growth loss, mortality, and increased susceptibility to other insects and pathogens (Kulman 1971). But, leaf feeding and leaf loss may not always be a problem, in some situations it may even be beneficial by stimulating growth through increased nutrient cycling or by acting as a thinning agent (Schowalter and others 1986). Therefore, leaf feeders do not always need to be controlled. Control activities should be limited to protecting young trees and trees weakened by previous defoliation or drought stress. Though not well documented in black walnut, reduced tree vigor can make trees more susceptible to wood boring insects and some pathogens. In most situations, trees that are

[1]Forest Entomologist, USDA Forest Service, Forest Health Protection, 1992 Folwell Avenue, St. Paul, MN 55108. To contact, call 651-649-5264, or e-mail at skatovich@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

healthy or vigorous will survive defoliation events and probably do not need to be protected.

A number of defoliators of black walnut are listed in USDA (1985) and Johnson and Lyon (1988). The most commonly encountered defoliators on black walnut are the walnut caterpillar, *Datana integerrima*, Grote & Robinson, yellow necked caterpillar, *Datana ministra* (Drury), and the fall webworm, *Hyphantria cunea* (Drury). These are all moth species. They are discussed in more detail below. Two other moth species that can on occasion be locally abundant include the variable oakleaf caterpillar, *Heterocampa manteo* (Doubleday), and the pecan leaf casebearer, *Acrobasis juglandis* (LeBaron).

In addition, there are several very large caterpillars that feed on walnut, the adult moths are some of our largest native insects. They include the walnut sphinx, *Cressonia juglandis* (J.E. Smith), hickory horned devil, *Lophocampa caryae* (Harris), and luna moth, *Actias luna* (L.). These species are rarely damaging and in most cases they do not need to be controlled.

A few beetle adults and sawfly larvae eat black walnut leaves, though none are considered major pests. June beetles, *Phyllophaga* sp., and the Japanese beetle, *Popillia japonica* Newman, can defoliate trees. June beetles often feed at night and large groups tend to congregate on a single tree. Widespread damage is unusual. The butternut woollyworm, *Eriocampa juglandis* (Fitch), is a sawfly that feeds on *Juglans*. Larvae feed in groups and can defoliate individual branches or small trees. The butternut woollyworm can be conspicuous because of white “woolly” tufts that cover the larval body.

Some leaf damage can occur from aphid, lace bug and mite feeding that can cause leaves to become distorted, discolored or to senesce and drop. Aphids and lace bugs are small insects that use their straw-like mouthparts to suck sap. Mites also remove sap and can scrape away leaf tissue. The walnut lace bug, *Corythuca juglandis* (Fitch), is a common species found on black walnut leaves. Both adults and immatures feed on the undersurface of the leaf creating small necrotic spots. The velvet gall mite, *Eriophyes caulis* Keifer, forms a twisted velvety red growth on the stem of leaflets. This is conspicuous, but relatively harmless.

The walnut caterpillar is the most likely to cause significant defoliation and damage to trees (Farris and others 1982). Trees of all sizes can be attacked though damage is often greatest on isolated individual trees. Severe infestations are infrequent and tend to be restricted to small geographic areas. It generally requires 2-3 consecutive years of heavy defoliation before trees begin to die and most outbreaks last no longer than 2 years. Tree mortality has not been widely reported following walnut caterpillar outbreaks. The number of generations varies from one in northern portions of the U.S. to three in southern regions. A number of natural enemies appear to be important in maintaining walnut caterpillar populations at reasonable levels (Farris and others 1982). On small trees hand removal of larvae can be helpful, especially since caterpillars tend to congregate when young. Insecticide treatments can be successful, but are rarely warranted. The yellow necked caterpillar is a close relative of the walnut caterpillar and outbreaks should be treated in a similar manner to walnut caterpillar.

Fall webworm outbreaks occur in late summer. The caterpillars form large webs that can enclose branches and even entire trees. The large webs can bring attention to the presence of this insect. However, late season defoliation (September) does not often impact trees as much as early season defoliation (May and June) and damage. Growth loss or branch and tree mortality from this insect is rarely reported.

Insects Damaging Nuts

There are insects that specifically feed on developing nuts. A weevil, the black walnut curculio, *Conotrachelus retentus* (Say), is the major insect pest on black walnut in the eastern U.S. In addition, the codling moth, *Cydia pomonella* (L.) and two closely related fly species, the walnut husk maggot, *Rhagoletis suavis* (Loew) and walnut husk fly, *R. completa* Cresson, can infest the husk that covers nuts. Codling moth larvae can attack at an early stage in nut development leading to premature nut drop. On mature nuts, the husks can be infested with codling moth larvae or the maggot stage of the husk flies. This can result in husks turning black and shriveling making nut extraction difficult.

The adult black walnut curculio is a relatively small weevil, about 1/5 inch long. Larvae feed in developing nuts on black walnut causing nuts to drop prematurely (Blair and Kearby 1979). This is referred to as the “June drop” of walnuts (Weber and others 1980). Egg laying occurs on developing nuts shortly after flowers are fertilized. Larvae tunnel into developing nuts and within a few weeks infested nuts drop from the tree. On the ground, larvae remain in the nuts for about 2 weeks after which they chew an exit hole in the husk and enter the soil. In the soil they pupate into adults. The adults emerge in mid to later summer and spend the remainder of the year feeding on black walnut foliage. When leaf drop occurs, adults move into the soil where they spend the winter.

A 2-year study done in Missouri, reported 51 percent of the annual nut production dropped early as a result of black walnut curculio infestations (Blair and Kearby 1979). In a 10-year study on the impact of the black walnut curculio in southwestern Missouri, Linit and Stamps (personal communication) reported that curculio-caused nut loss ranged from virtually none to 26% of the available nuts. For years in which the final harvest nut crop was known, loss of nuts from weevil oviposition was high in years with small nut crops and low in years with large nut crops. This suggests weevil populations may remain relatively stable and destroy a constant number of nuts from year to year.

A sanitation program based on rapid removal and destruction of nuts that have fallen in early summer should reduce the local populations of weevils. However, this must be done before weevils migrate to the soil. Sanitation can also help reduce codling moth and husk fly populations. Early summer insecticide applications can be targeted at these insects. A pheromone attractant is available for adult codling moths that can help in the proper timing of any insecticide application. Baits are also available for attracting the husk flies.

Insects Damaging Buds and Shoots

Bud and shoot damage can appear rather innocuous. However, the destruction of buds or shoots, especially the terminal bud or shoot on young trees, can cause excessive branch forking that can change the shape of a tree and reduce its timber value. The lack of a single dominant straight stem can be a major impediment to growing well-formed trees.

There are several insects that can damage shoots and buds, but only one appears to be a significant pest. The walnut shoot moth, *Acrobasis demotella* Grote, is commonly found attacking buds and shoots of black walnut (Kearby 1979, Martinat and Wallner 1980). On occasion, a related species, the pecan leaf casebearer, *A. juglandis* (Le Baron), may be problematic. It does not feed in shoots but can kill significant numbers of buds. The butternut curculio, *Conotrachelus juglandis* LeConte, a weevil, can infest black walnut shoots though it prefers butternut (Solomon 1995).

Females of the walnut shoot moth are present in mid-summer and lay eggs on the underside of walnut leaflets. Larvae soon emerge and begin feeding on leaf tissue. When foliage begins to change color larvae migrate to twigs and construct small silken shelters called hibernacula, where they spend the winter. In the early spring, larvae emerge and begin to bore into and hollow-out buds. As leaves begin to emerge and new shoots expand, larvae tunnel into the pith of developing shoots. Entrance holes are covered with silk and frass. Tunnels can reach 18 inches in length, shoots die above the tunnel. By early summer larvae finish feeding, leave the shoots, drop to the ground, and burrow into the litter layer and pupate inside an earthen cell.

Following damage to terminal buds or shoots, McKeague and Simmons (1979) recommended corrective pruning. They defined corrective pruning as the removal of new shoots developing along a branch, leaving one to become the new terminal leader. This should be done in early summer when bud damage is apparent and shoot growth has not been completed. A benefit of pruning is that it helps correct for both frost and insect caused injury. Frost is a common damaging agent to buds.

Insects Damaging Twigs and Small Branches

In contrast to new shoot growth discussed in the section above, a few insects can damage older twigs and small branches. Injury to twigs and small branches would rarely impact tree form or overall tree health. The most common injuries are oviposition wounds from cicadas, planthoppers, and treehoppers, all members of the insect order Homoptera. Females slit the bark on twigs and small branches and imbed eggs within the slits. These wounds can kill twigs and branches.

Twig and branch dieback can also occur due to infestations of scale insects although they are not widely reported on black walnut. Marshall (1989) did list the oystershell scale, *Lepidosaphes ulmi* (L.) as a common walnut pest.

Insects Damaging the Trunk and Large Branches

Walnut does have several wood boring insects that will invade the main trunk and larger branches (Solomon 1995). Most are beetles, a few are moth larvae. Only a few species are considered significant pests and most of these do not kill trees but cause problems by providing sites where disease organisms can become established or by causing defects in wood products through tunnels or associated staining. As mentioned, these insects rarely kill trees but most of them are associated with trees that are declining, dying or recently killed, including cut logs. Only *Xylosandrus germanus* (Blandford), an ambrosia beetle, has been reported attacking apparently healthy trees (Weber and McPherson 1984).

Ambrosia beetles are relatively small beetles that burrow through the bark and into the sapwood and feed both as an adult and larvae on fungi that they cultivate in their tunnels. These fungi often impart a dark blue or black stain along the tunnels. This staining can cause a degrade in sawn lumber. Black walnut has several species of ambrosia beetles that can infest the trunk and larger branches (Table 1). Most of these attack trees that have existing damage, are growing slowly or have recently died or been harvested. As mentioned earlier, *Xylosandrus germanus* can attack apparently healthy trees and attacks by this beetle have been found associated with the fungus *Fusarium* (Weber and McPherson 1985). *Xylosandrus germanus* was introduced into North America and now occurs throughout the Midwest. Young trees up to 8 years old are most often attacked. The adults bore small entrance holes, about 1/32 inch in diameter. Attacked trees often have top dieback and profuse sprouting from the base of the tree.

Pinhole or wormhole type damage, diagnostic of ambrosia beetles, can also be caused by the sapwood timberworm, *Hylecoetus lugubris* Say. This beetle often infests green saw logs as well as weakened and dying trees. In addition to pinholes that penetrate into the sapwood, there are also many small laterally oriented galleries across the surface of the sapwood.

Larger beetles that infest black walnut in the eastern U.S. include the flat-headed apple tree borer, *Chrysobothris femorata* (Oliv.), the pole borer, *Parandra brunnea* (Fabricius), and the ash and privet borer *Tylonotus bimaculatus* Haldeman. In almost all cases, these insects infest wounded, weakened or dying trees. Externally, infestations can be found via entry wounds that bleed sap or have extruding wood fibers or sawdust.

Recommended control measures for wood-boring insects revolve around maintaining tree vigor. Most of these insects are incapable of successfully invading healthy, vigorous trees. Therefore, management practices such as weed control and thinning can be beneficial. Sanitation, or the removal of infested material can also be useful in reducing local populations of wood boring insects. In addition, limiting wounds on the main stem should reduce attacks from some of these insects. Wounds caused by mowing or logging equipment can be attractive to many wood-boring insects. Pruning wounds tend to heal quickly and are probably not overly attractive. Pruning in the fall and winter would further eliminate the risk of attracting these insects.

Freshly cut logs should be removed rapidly from the woods to avoid attracting wood boring insects. In addition, harvesting activities should be done during colder periods of the year, thus reducing the likelihood of insect activity.

Table 1.—Ambrosia beetles reported attacking the trunk and larger branches of walnut by Solomon (1995).

Name	Importance
Black stem borer, *Xylosandrus germanus*	Considered a significant walnut pest, attack sites are often associated with *Fusarium* cankers. Can attack vigorous trees, especially young walnut trees in plantations. Also attacks dying and diseased trees. Sap may ooze from pinhole sized entrance holes. Fine dust-like frass is often present. Infested trees often suffer top dieback.
Lesser shothole borer, *Xyleborinus saxeseni*	Not considered a major walnut pest. Infests dying or dead material, can attack fresh-cut logs causing a stained pinhole defect. White frass on bark.
Pear blight beetle, *Xyleborus dispar*	Not considered a major walnut pest. More common on fruit trees. Infests injured or dying trees.
Cosmopolitan ambrosia beetle, *Xyleborus ferrugineus*	Not considered a major walnut pest, has a host range of more than 180 species worldwide. Most common attacking stumps and logs on the ground. White frass on bark.
Oak timber beetle, *Xyleborus xylographus*	Not considered a major walnut pest, prefers oaks. Beetles favor lower stumps and lower portions of dead and dying trees. Often attacks around wound sites. White frass on bark.

MANAGEMENT RECOMMENDATIONS

Management of black walnut is largely driven by the desire to develop well-formed trees that have a straight stem. Management activities may be needed to protect young trees from insects that kill the terminal buds or shoots, specifically the walnut shoot moth. Unfortunately, limited direct control options are available for this insect.

1. Corrective pruning can develop a single terminal bud after insect attack or frost injury.

2. Younger trees will often self-correct or straighten if neighboring trees provide competition for light. Therefore, maintain well-stocked stands.

Wood-boring insects tend to infest trees weakened by poor growing conditions or injuries to the main stem. In addition, repeated defoliation by insects or leaf diseases can weaken trees. Therefore, management tactics should target maintaining healthy, vigorous trees. Further, trees should not be damaged during mowing or logging activities.

1. Proper weed control can allow for rapid early growth developing healthy trees.

2. Thinning older stands to develop trees with large vigorous crowns can maintain tree health.

Insecticide applications targeted at defoliating insects are rarely warranted. Most healthy trees should withstand a single defoliation event, especially if it occurs in late summer. But, repeated defoliation events may need to be more actively managed. In addition, young trees and trees stressed by drought events may also require protection.

1. Monitor local populations of insects such as the walnut caterpillar. Seek the advice of a professional forest entomologist if outbreak populations persist for more than 1 year.

Newly harvested logs should be removed from the woods as quickly as possible, especially during the warmer months of the year.

LITERATURE CITED

Blair, L.M.; Kearby, W.H. 1979. The black walnut curculio and its impact on nut production. In: Kessler, K.J.; Weber, B.C., coords. Walnut insects and diseases workshop proceedings; 1978 June 13-14; Carbondale, IL. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 51-54.

Farris, M.E.; Appleby, J.E.; Weber, B.C. 1982. Walnut caterpillar. For. Insect and Dis. Leafl. 41. Washington, DC: U.S. Department of Agriculture, Forest Service. 6 p.

Johnson, W.T.; Lyon, H.H. 1988. Insects that feed on trees and shrubs. Ithaca, NY: Cornell University Press. 556 p.

Kearby, W.H. 1979. The life history of Acrobasis demotella and its role in seedling and sapling stem deformities in Missouri. In: Kessler, K.J.; Weber, B.C., coords. Walnut insects and diseases workshop proceedings; 1978 June 13-14; Carbondale, IL. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 55-57.

Kulman, H. 1971. Effects of insect defoliation on growth and mortality of trees. Annual Review of Entomology. 16: 289-324.

Marshall, P.T. 1989. Insects that affect growth and quality of black walnut. In: Phelps, J.E., ed. The continuing quest for quality: 4th Black walnut sympoisum; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council: 193-202.

Martinat, P.J.; Wallner, W.E. 1980. Notes on the biology and damge of two *Acrobasis species* (Lepidoptera: Pyralidae) on black walnut in Michigan. Great Lakes Entomologist. 13: 41-48.

McKeague, M.; Simmons, G. 1979. Effects of insecticidal and cultural methods to reduce *Acrobasis* spp. and frost injury on black walnut. In: Kessler, K.J.; Weber, B.C., coords. Walnut insects and diseases workshop proceedings; 1978 June 13-14; Carbondale, IL. Gen. Tech. Rep. NC-52. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 44-50.

Schowalter, T.D.; Hargrove, W.W.; Crossley, D.A. 1986. Herbivory in forested ecosystems. Annual Review of Entomology. 31: 177-196.

Solomon, J.D. 1995. Guide to insect borers of North American broadleaf trees and shrubs. Agric. Handb. 706. Washington, DC: U.S. Department of Agriculture, Forest Service. 735 p.

USDA. 1985. Insects of eastern forests. Misc. Publ. 1426. Washington, DC: U.S. Department of Agriculture, Forest Service. 608 p.

Weber, B.C.; Anderson, R.L.; Foffard, W.H. 1980. How to diagnose black walnut damage. Gen. Tech. Rep. NC-57. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 20 p.

Weber, B.C.; McPherson, J.E. 1984. Attack on black walnut trees by the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Forest Science. 30: 864-870.

Weber, B.C.; McPherson, J.E. 1985. Relation between attack by *Xylosandrus germanus* (Coleoptera:Scolytidae) and disease symptoms in black walnut. Canadian Entomologist. 117: 1275-1277.

Control of Deer Damage with Chemical Repellents in Regenerating Hardwood Stands

Brian J. MacGo-wan, Larry Severeid, and Fred Skemp, Jr.[1]

ABSTRACT—Wildlife damage can be a major problem in natural tree regeneration or tree plantings. In the North Central Hardwoods region, white-tailed deer (*Odocoileus virginianus*) are a significant cause of damage to hardwood seedlings. We evaluated the use of a combination of chemical repellents (Hinder®, Tree Guard®, chicken eggs, and Plantskydd®) in restoring a poor hardwood stand to higher-valued, native trees in Hixon Forest, La Crosse, Wisconsin. Chemically treated oak (*Quercus spp.*) and black cherry (*Prunus serotina*) seedlings had less terminal browse damage ($P < 0.005$) during the growing season than untreated seedlings. Results during the winter will also be presented. Preliminary results support further study of an integrated use of chemical repellents in regenerating hardwood stands.

In the Central Hardwoods Region, white-tailed deer are arguably the most important species causing damage to forest resources (Conover and Decker 1991). Their social and economic importance as a game species (USFWS 2001), reproductive capacity (McCullough 1997), and wide distribution (Fig. 1) are all contributing factors.

Deer can impact natural tree regeneration (Tilghman 1989, Trumbull and others 1989, Jones and others 1993, Cornett and others 2000), tree plantings (Beringer and others 1994), nurseries (Conover and Kania 1995), or orchards (Mower and others 1997, Lemieux and others 2000b). Repeated browsing of terminal shoots distorts growth and suppresses tree seedling height and longer rotations and mortality can result (Nolte and Dykzeul 2002). Conover and others (1995) estimated total losses from wildlife damage to the timber industry in the U.S. to be $3.4 million annually. Annual economic losses due to wildlife damage to all forest resources in Oregon were estimated at $333 million (Nolte and Dykzeul 2002). With no animal damage management, the total predicted reduction in value for Oregon forests alone was estimated at $8.3 billion. These figures underscore the importance for understanding the types and amounts of damage caused by wildlife, the factors that influence the severity of damage, and scientifically-based methods to reduce wildlife damage.

Chemical repellents have been evaluated in deterring deer damage in pen (Andelt and others 1992, Lutz and Swanson 1997, Witmer and others 1997, Wagner and Nolte 2000) and field (Conover 1984, Swihart and Conover 1990, Fargione and Richmond 1995, Witmer and others 1997, Lemieux and others 2000b) trials. However, few studies tested the efficacy of repellents in an integrated approach to minimizing deer damage. Switching among repellents has the potential to maximize the strengths of each repellent. Also, strategies using multiple techniques have a better chance of success than those that incorporate a single technique (Conover 2002:375). Jordon and Richmond (1992) found that the use of repellents increased the effectiveness of fencing alone. In our study, we evaluated the combined use of Hinder®, Tree Guard®, chicken eggs, and Plantskydd® to minimize deer damage to tree seedlings in a natural stand and present preliminary results in this paper. We also summarize and discuss research on available options to control deer damage in commercial tree plantings and natural hardwood regeneration.

[1]Extension Wildlife Specialist (BJM), Department of Forestry and Natural Resources, Purdue University, 1250 North Franklin Avenue, P.O. Box 265, Brookville, IN 47012; (LS), 2524 Zephyr Circle, La Crosse, WI 54601; and (FS), 2210 Park Drive, La Crosse, WI 54601; BJM is corresponding author: to contact, call (765) 646-3538 or e-mail at bmacgowan@fnr.purdue.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

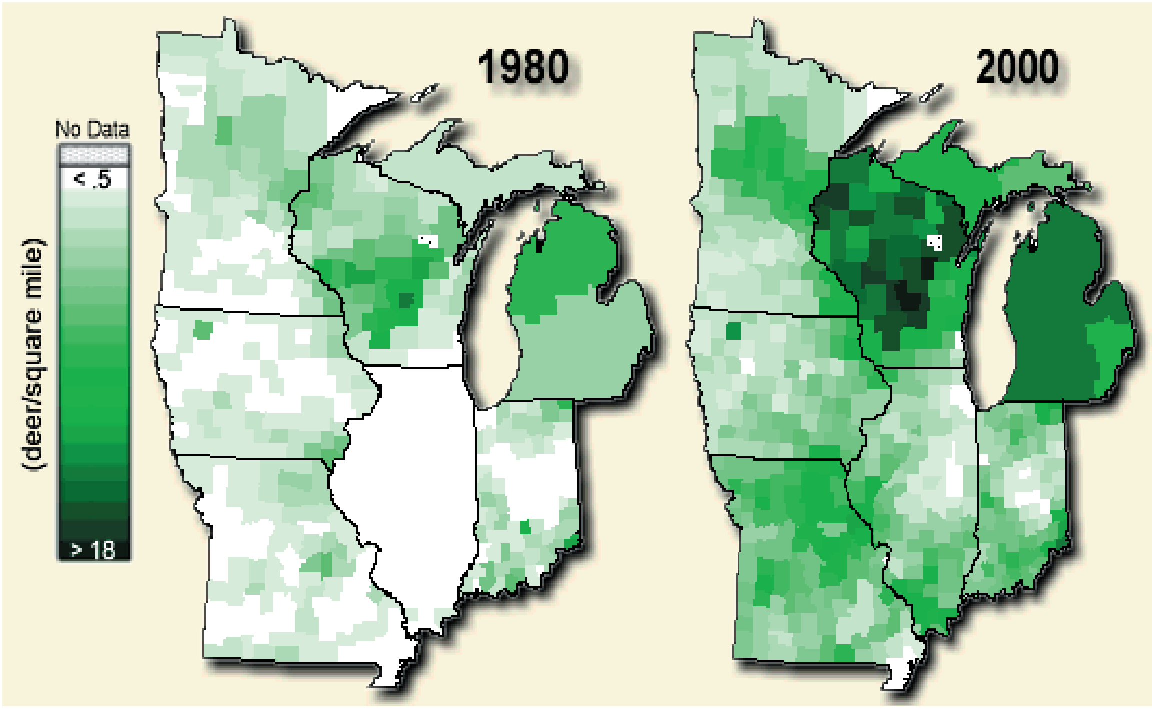


Figure 1.—Total deer harvested per square mile (region for Michigan) in the Midwest in 1980 and 2000. The total number of whitetail deer harvested increased by nearly 250 percent during this time period. (USDA Forest Service, North Central Region)

METHODS

The Hixon Forest is a 291 ha woodland located within the city of La Crosse, Wisconsin. The forest has been uncut for the past 90 years and is dominated by a mixture of timber oaks (*Quercus* spp.), hickory (*Carya* spp.), bigtooth aspen (*Populus grandidentata*), black walnut (*Juglans nigra*), black cherry (*Prunus serotina*), and basswood (*Tilia americana*). Because of it is located within the city limits of La Crosse, deer densities and browse pressure are presumably high, although we did not measure this.

We were interested in regenerating shade intolerant species that would be available for education and research. We chose an area accessible to the public that was dominated by black locust (*Robinia pseudoacacia*), an invasive tree not native to the region. In winter of 2002, a 0.6 ha opening was cleared in an initial attempt to evaluate the feasibility of regenerating more economically valuable tree species using a combination of chemical repellents and herbicide treatment. All trees and shrubs were cut except a few trees which were left for aesthetics at the request of the park. Dominate species removed included black locust, bigtooth aspen, birch (*Betula* spp.), box elder (*Acer negundo*), and buckthorn (*Rhamnus* spp.). The opening was bordered on the west by a maintained mowed area (approx. 1 acre).

The clearing was divided into one treatment plot (approx 0.5 ha) and two control plots (each approx. 0.05 ha). We maintained several access trails within the treatment block. Control plot A was planted with tree seedlings, and both controls had no weed control or repellent applications.

During the spring of 2003 in the treatment block, control plot A, and the perimeter of control plot B we planted 1,157 2-year-old seedlings of white oak (*Quercus alba*), swamp white oak (*Quercus bicolor*), red oak (*Quercus rubra*), shagbark hickory (*Carya ovata*), black cherry, white ash (*Fraxinus americana*), black walnut, butternut (*Juglans cinerea*), and white pine (*Pinus strobus*) in a 6 ft by 7 ft spacing. Each seedling was mulched with woodchips and marked with a wood stake. Seedlings were planted in clusters of 7-9 seedlings of the same species. In the treatment plot, we sprayed a glyphosate herbicide (Round-Up®) at the labeled rate in an approximately 50 cm radius band around each seedling. Thirty-four oaks, walnuts, and butternut were replanted in the fall of 2003 to replace early seedling mortality.

Prior to planting the treatment plot, we brushed Tree Guard® (a Bitrex formulation, [0.2% denatonium benzoate]) onto each terminal bud. When the seedlings began to break bud post planting, we sprayed the terminals with a premixed formulation of Plantskydd® (87% edible animal protein), a taste and smell deterrent. Beginning in May, using a hand-held sprayer we applied a mixture of 200 ml Hinder® (0.66% ammonium soaps of higher fatty acids), six blenderized chicken eggs, and 3.78 L water at approximately monthly (4-6 weeks) intervals until the seedlings went dormant in October. Each application took approximately 1.5 man-hours. After all seedlings were dormant, we reapplied Tree Guard® with a paint brush on each terminal bud. A second application was applied in February 2004.

In the treatment plot, competing vegetation was controlled once it began to shade planted seedlings. Vegetation was removed by hand-pulling or with the use of brush cutters. A glyphosate herbicide (Round-Up®) at the labeled rate was applied with a wick applicator or hand-held sprayer onto all buckthorn and honeysuckle bushes, and stump sprouts of any species.

In all plots, we tallied tree survival during the first year of growth. In the fall of 2003 and spring of 2004, we inspected the terminal of each tree for browse damage. Any sign of damage was counted as being browsed and recorded for each species or species group. We present data from the treatment and control plot A.

RESULTS

Until November 2003, 9.4% of the seedlings died (n = 112). Seedling survival was similar in both groups. The hickory seedlings had the lowest survival rate (68%) and accounted for most of the seedlings that failed the first growing season. Only black walnut seedlings (86%) had a survival rate below 90%.

Summer browse damage was confined mainly to black cherry and white ash, and occasionally oaks (Table 1). Percent browsed was much higher in the control plot for oaks (x^2 = 13.1, df = 1, P = 0.0003) and black cherry (x^2 = 8.2, df = 1, P = 0.004). Percent browsed for shagbark hickory and white ash in the treatment plots were 1.7% and 11.9%, respectively. Neither of these species showed browse damage in the control plot.

Preliminary inspection this winter indicates that most of the white pine seedlings have browse damage while other species had an increased damage rate during this period. This data has yet to be collected and will be presented during our oral presentation.

DISCUSSION

Our data suggest that a combination of chemical repellents and herbicide treatments has the potential to provide satisfactory results in controlling deer browsing of tree seedlings in the first year post harvest. However, conclusions should be drawn with caution since our results are preliminary and sufficient replication was lacking. The ultimate measure of success will be the number of tree seedlings that survive to a height beyond the reach of a deer.

Repellents are more appropriate for short-term problems (Conover 2002) and are typically reserved for smaller areas because it is cost-prohibitive. For

Table 1.—Percent of tree seedlings with terminal bud browsed by white-tailed deer during 2003 growing season.

	Control Plot A			Treatment		
Species	Total Trees	Number of Trees Browsed	Percent Browsed	Total Trees	Number of Trees Browsed	Percent Browsed
Oak spp.	29	7	24.1	253	14	5.5
White pine	18	0	0.0	115	0	0.0
White ash	13	0	0.0	67	8	11.9
Butternut	15	0	0.0	98	0	0.0
Black walnut	16	0	0.0	137	0	0.0
Black cherry	16	7	43.8	166	40	24.1
Shagbark hickory	15	0	0.0	58	1	1.7
Total	122	14	11.5	894	63	7.0

our study, an annual cycle of repeated applications of chemical repellents on the treatment plot (0.6 ha) was approximately $71.00. Many studies site cost as a downside to using chemical repellents. However, our costs were relatively inexpensive, although cost of labor would significantly increase our costs. Costs would also be higher for remote sites.

In a review of the current literature, El Hani and Conover (1997) did not find a repellent that consistently reduced deer damage > 50% in field trials. Despite the wide variance in study design, location, deer and plant species, trial duration, criteria of success, and time of year, they found BGR® was the most effective repellent. Predator urine, although not yet labeled by the EPA for use as repellents, showed promise. We used a combination of repellents that had mixed to favorable results in previous studies. In studies reviewed by El Hani and Conover (1997), Hinder® was ranked as effective (n = 3), intermediate (n = 2), or slightly effective (n = 1); chicken eggs was effective (n = 2). In a test of 20 repellents and 2 delivery systems, Wagner and Nolte (2001) found only BGR® and Plantskydd® reduced browse damage to western red cedar by penned black-tailed deer. In their study, Hinder® protected seedlings up to only 4 weeks and Tree Guard® up to 12 weeks. In a trial of Hinder®, Tree Guard®, Miller Hot Sauce®, Deer Away®, chicken eggs, and predator urine, Lutz and Swanson (1997) found Tree Guard® to be the most ineffective while the others had mixed results. Our study involved the use of several repellents with repeated applications (7) per year.

We cannot differentiate if one, two, or all three repellents were effective. Our study site was located in an area with presumably high deer populations typical of urban areas not open to hunting. Deer browse pressure is at its highest during the winter when alternative forage is limited. Chemical repellents have been found to loose efficacy when presented to hungry cervids (Andelt and others 1991, 1992). Efficacy of our methods may be reduced during the winter and may approach results of other studies (i.e., < 50%).

Methods other than chemical repellents have been used to minimize deer damage in natural stands and commercial nurseries. Combining one or more of these methods in conjunction with chemical repellents may reduce deer browsing than any method used alone. A summary of common techniques and their results is presented below.

Cultural Methods

Tree seedlings are vulnerable to deer browsing up to about 1.8 m (Craven and Hyngstrom 1994). One strategy to minimize deer damage in regenerating stands is to decrease the length of time terminal shoots are available to deer or physical prevent access to tree seedlings. Cultural methods to reduce deer damage to tree seedlings in harvested stands include leaving slash piles (Bergquist and Örlander 1998) or management of the surrounding vegetation. Gourley and others (1990) found that vegetation control helped minimize the effects of deer browsing by reducing competition for the tree seedlings, allowing for increased growth rates. However, surrounding vegetation may also protect seedlings by concealing them or by providing an alternate food source (Campbell and Evans 1978). Diversionary foods may deter small herbivore damage to tree seedlings (Sullivan and others 2001).

The size of forest opening may influence the amount of deer damage. Akins and Michael (1997) found that percent browsed for all tree species groups was generally lower in 0.8 ha clearcuts compared to 0.2 ha clearcuts. Our openings approached the lower end of their study. Further research needs to done to test the effectiveness of our methods or the amount of repellents and treatments on larger openings. If larger openings have lower browse pressure, chemical repellents can potentially be more cost efficient on a per acre basis, although total costs may still be prohibitive.

Fencing

Electric fences can reduce deer damage (Craven and Hyngstrom 1988, Jordan and Richmond 1992). Fencing has the advantage of being a long-term solution. Cost varies by fence type, but all are relatively expensive compared to other techniques (Table 2). Fences require regular maintenance, are effective on areas 16 ha or less, and success is maximized if installed prior to planting (Craven and Hyngstrom 1994). A temporary electric fence provides inexpensive protection when the goal is to minimize browse damage to terminal buds of trees until they have outgrown the reach of deer.

Lethal Control

Have been used successfully in urban areas and lands previously protected from deer harvest. A shotgun-archery hunt reduced a residential deer herd by 92% in 6 days (Kilpatrick and others 2002). Success would likely be lower in more rural areas with more abundant habitat and would depend in part on surrounding hunting pressure and hunter skill. Lethal control is often sited as the most cost-effective method of controlling wildlife populations (Conover 2002). Most states have a program where

Table 2.—Cost comparison of different fencing types (from Craven and Hyngstrom 1994, Bender 1998).

Fence Type	Cost Per Linear Foot	Cost Per Acre	Items Included in Cost	Year
8-ft. mesh / woven wire fence	$1.05 $2-$4	$1222	Materials	1990 1994
	$4-$6		Materials & installation	
High tensile electric fence (5-ft.)	$0.89-$2.03		Materials & installation	1998
		$30/year	Maintenance	1998
High tensile electric fence (6.5-ft.)	$2.15-$2.70	$368	Materials & installation	1992
High-tensile fence (8-ft.)	$0.75-$1.50		Materials	1994
Polytape electric fence	$0.50-$1.20		Materials	1997
	$0.11		Materials	1994
Slanted seven-wire fence	$0.75-$2.00		Materials	1994

deer can be taken out of season with a permit when damage exceeds a set economic threshold.

Methods used to control deer damage in natural stands are commonly utilized in tree plantations and nurseries (Lemieux and others 2000a). Damage caused by deer is difficult to control in nurseries because their home range likely will include the nursery and the surrounding area. Because of their small home ranges, damage caused by smaller herbivores are less influenced more by physical characteristics of a plantation rather than surrounding habitat (Pietrzykowski and others 2003). Annual variation of deer browsing in Connecticut nurseries was not associated with local deer densities, the availability of native browse, or winter weather conditions (Conover and Kania 1995). Physical size of nurseries, the degree bordered by woodlots, or level of remoteness has not been found to influence observed levels of deer browsing, but the size of adjacent woodlots and the combined area of woodlots within 2 km has (Conover 1989).

In conclusion, our preliminary results support further study of an integrated use of herbicides and chemical repellents in regenerating natural hardwood stands. Their success will likely depend on use of other methods, local deer browse pressure, surrounding habitat, and weather and other stochastic variation. Further research is needed to identify influencing factors that explain the level of deer browsing in different landscapes at different temporal scales. As development pressure increases and forestland ownership continues to become increasingly fragmented, economic pressures to regenerate nature stands of high-valued timber may necessitate more intensive and costly control of deer damage and other limitations to regeneration.

LITERATURE CITED

Andelt, W.F.; Burnam, K.P.; Manning, J.A. 1991. Relative effectiveness of repellents for reducing mule deer damage. Journal of Wildlife Management. 55: 341-347.

Andelt, W.F.; Baker, D.L.; Burnam, K.P. 1992. Relative preference of captive cow elk for repellent-treated diets. Journal of Wildlife Management. 56: 164-173.

Anderson, R.C.; Loucks, O. L. 1979. White-tailed deer (*Odocoileus virginianus*) influence the structure and composition of *Tsuga canadensis* forests. Journal of Applied Ecology. 16: 355-361.

Akins, J.W.; Michael, E.D. 1997. Impact of clearcut size on white-tailed deer use and tree regeneration. Proceedings, 6th Eastern Damage Control Conference. 6: 185-195.

Bender, B.A. 1998. Reducing deer damage to forest crops. Michigan Agricultural Experiment Station Publication. East Lansing, MI: Michigan State University.

Bergquist, J.; Örlander, G. 1998. Browsing damage by roe deer on Norway spruce seedlings planted on clearcuts of different ages: 2. Effects of seedling vigor. Forest Ecology and Management. 105: 295-302.

Beringer, J.; Hansen, L.P.; Heinen, R.A.; Giessman, N.F. 1994. Use of dogs to reduce damage by deer to a white pine plantation. Wildlife Society Bulletin. 22: 627-632.

Campbell, D.L.; Evans, J. 1978. Establishing native forbs to reduce black-tailed deer browsing damage to Douglas-fir. In: Proceedings, 8th Vertebrate pest control conference. 8: 145-151.

Conover, M.R. 1984. Effectiveness of repellents in reducing deer damage in nurseries. Wildlife Society Bulletin. 12: 399-404.

Conover, M.R. 1989. Relationship between characteristics of nurseries and deer browsing. Wildlife Society Bulletin 17: 414-418.

Conover, M.R. 2002. Resolving human-wildlife conflicts: the science of wildlife damage management. Boca Raton, FL: Lewis Publishers. 418 p.

Conover, M.R.; Decker, D.J. 1991. Wildlife damage to crops: perceptions of agricultural and wildlife professionals in 1957 and 1987. Wildlife Society Bulletin 19: 46-52.

Conover, M.R.; Kania, G.S. 1995. Annual variation in white-tailed deer damage in commercial nurseries. Agriculture, Ecosystems and Environment. 55: 213-217.

Cornett, M.W.; Frelich, L.E.; Puettmann, K.J.; Reich, P.B. 2000. Conservation implications of browsing by *Odocoileus virginianus* in remnant upland *Thuja occidentalis* forests. Biological Conservation. 93: 359–369.

Craven, S.R.; Hyngstrom, S.E. 1988. Electric fences and commercial repellents for reducing deer damage in cornfields. Wildlife Society Bulletin. 16: 291-296.

Craven, S.R.; Hygnstrom, S.E. 1994. Deer. In: Hygnstrom, S.E.; Timm, R.M.; Larson, G.E., eds. Prevention and control of wildlife damage. Lincoln, NE: University of Nebraska Cooperative Extension: D25-D40

El Hani, A.; Conover, M.R. 1997. Comparative analysis of deer repellents. In: Mason, J.R., ed. Repellents in wildlife management. Proceedings, 2nd National wildlife research center special symposium; 1995 August 8-10; Denver, CO. [Denver, CO]: U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center: 147-155.

Fargione, M.J.; Richmond, M.E. 1995. Advancing deer repellent performance: fine-tuning Hinder® applications and potential uses for uses in insecticidal soaps. In: Proceedings, 6th Eastern Wildlife Damage Control Conference; Ashville, NC. 6: 137-144.

Gourley, M.; Vomocil, M.; Newton, M. 1990. Forest weeding reduces the effect of deer browsing on Douglas fir. Forest Ecology and Management. 36: 177-185

Jones, S.B.; DeCalesta, D.; Chunko, S.E. 1993. Whitetails are changing our woodlands. American Forests. 99: 20-25, 53-54.

Jordan, D.M., Jr.; Richmond, M.E. 1992. Effectiveness of a vertical 3-wire electric fence modified with attractants or repellents as a deer exclosure. Proceedings, 5th Eastern Wildlife Damage Control Conference. 5: 44-47.

Kilpatrick, H.J.; LaBonte, A.M.; Seymour, J.T. 2002. A shotgun-archery deer hunt in a residential community: evaluation of hunt strategies and effectiveness. Wildlife Society Bulletin. 30: 478-486.

Lemieux, N.; Maynard, B.K.; Johnson, W.A. 2000*a*. A regional survey of deer damage throughout Northeast nurseries and orchards. Journal of Environmental Horticulture. 18: 1-4.

Lemieux, N.; Maynard, B.K.; Johnson, W.A. 2000*b*. Evaluation of commercial deer repellents on ornamentals in nurseries. Journal of Environmental Horticulture. 18: 5-8.

Lutz, J.A.; Swanson, B.T. 1997. Reducing deer damage to woody and herbaceous plants. In: Mason, J.R., ed. Repellents in wildlife management, proceedings, 2nd National wildlife research center special symposium; 1995 August 8-10; Denver, CO. [Denver, CO]: U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center: 231-240.

McCullough, D.R. 1997. Irruptive behavior in ungulates. In: McShea, W.J.; Underwood, H.B.; Rappole, J.H., eds. The science of overabundance: deer ecology and population management. Washington DC: Smithsonian Institute Press: 69-98.

Mower, K.J.; Townsend, T.W.; Tyznik, W.J. 1997. White-tailed deer damage to experimental apple orchards in Ohio. Wildlife Society Bulletin. 25: 37-343.

Nolte, D.L.; Dykzeul, M. 2002. Wildlife impacts on forest resources. In: Clark, L.; Hone, J.; Shivik, J.A.; Watkins, R.A.; Vercauteren, K.C.; Yoder, J.K., eds. Human conflicts with wildlife: economic considerations. Proceedings, 3rd National wildlife research center special symposium; 2000 August 1-3; Fort Collins, CO. [Fort Collins, CO]: U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center: 163-168.

Pietrzykowski, E.; McArthur. C.; Fitzgerald, H.; Goodwin, A.N. 2003. Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. Journal of Applied Ecology. 40: 458-469.

Sullivan, T.P.; Sullivan, D.S; Hogue, E.J. 2001. Influence of diversionary foods on vole (*Microtus montanus* and *Microtus longicaudus*) populations and feeding damage to coniferous tree seedlings. Crop Protection. 20: 103-112.

Swihart, R.K.; Conover, M.R. 1990. Reducing deer damage to yews and apple trees: testing Big Game Repellent®, Ro-pel®, and soap as repellents. Wildlife Society Bulletin. 18: 156-162.

Tilghman, N.G. 1989. Impacts of white-tailed deer on forest regeneration in northwestern Pennsylvania. Journal of Wildlife Management. 53: 524-532.

Trumbull, V.L.; Zielinski, E.J.; Aharrah, E.C. 1989. The impact of deer browsing on the Allegheny forest type. Northern Journal of Applied Forestry. 6: 162-165.

USFWS. 2001. National survey of fishing, hunting, and wildlife-associated recreation. Washington, DC: U. S. Department of Interior, Fish and Wildlife Service.

Wagner, K.K.; Nolte, D.L. 2000. Evaluation of Hot Sauce® as a repellent for forest mammals. Wildlife Society Bulletin. 28: 76-83.

Wagner, K.K.; Nolte, D.L. 2001. Comparison of active ingredients and delivery systems in deer repellents. Wildlife Society Bulletin. 29: 322-330.

Whitmer, G.W.; Sayler, R.D.; Pipas, M.J. 1997. Repellent trials to reduce reforestation damage by pocket gophers, deer, and elk. In: Mason, J.R., ed. Repellents in wildlife management, proceedings, 2nd National wildlife research center special symposium; 1995 August 8-10; Denver, CO. [Denver, CO]: U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center: 321-332.

A New Tree Improvement Programme for Black Walnut in the United Kingdom

Karen Russell and Gabriel E. Hemery[1]

ABSTRACT—Black walnut is an introduced species in the United Kingdom (UK) and economically one of the more productive broadleaved species. Currently it is not widely planted in the UK as there is insufficient knowledge about the species among foresters and very little, if any, improved material is available. A research programme was initiated in 2001 to address both these issues. A range-wide seed collection from plus trees 20 US provenances and 10 European provenances and 2 European populations has been undertaken with a view to establishing two provenance/progeny trials. A collection of trees grafted from 63 individual plus trees has also been planted for assessment of form, vigour and disease resistance.

INTRODUCTION

Black walnut (*Juglans nigra* L.) was introduced to Britain from North America in the early 17th Century (Bean 1981) and was widely planted for its valuable timber. The species can produce high quality timber on a relatively short rotation of about 60 years and is consistently in great demand by end-users. During 2001 alone, UK imports of this valuable timber rose by 51% (Buckley 2002). Currently, the species is often disregarded by UK foresters because of its reputation as being site-demanding, usually of poor form and vulnerable to frost. No research has effectively addressed these problems in the UK.

The Forestry Commission established a series of black walnut provenance experiments between 1986 and 1987; a limited range of material from four provenances (Wisconsin, Southern Illinois, Ohio and Vermont) was tested (Kerr 1993). Insufficient seed was available for testing all material across four low-quality UK trial sites. A more comprehensive selection of material, selecting desirable, straight-stemmed and lightly-branched trees suitable to the climatic conditions of the UK should encourage a renewed interest in the species.

In 2001, Horticulture Research International (HRI) and the Northmoor Trust (NMT) initiated a new project for the improvement of black walnut in the UK to provide information and material. This is a collaborative project under the auspices of the British and Irish Hardwoods Improvement Programme (BIHIP) with sponsorship from the National Forest Company, the Department for Environment, Food and Rural Affairs (Defra) and the Forestry Commission.

Seed material was collected across the current natural US and introduced European ranges of the species and is being tested in a long-term provenance/progeny research programme. Field trials containing European and North American seedlots will be established during winter 2003 and winter 2004 with the aim of identifying superior black walnut trees that can act as seed trees or be cloned for timber production. A clonal collection of superior 'plus' trees has been established at HRI. This paper summarizes progress made to the autumn of 2003 and outlines expected outcomes from the research programme.

FIELD TRIAL METHODS

Selection of Seed Sources

Research institutes in 27 European countries were contacted to establish the importance of black walnut in their country and their interest and willingness to participate in the black walnut

[1]Woodland & Forestry Specialist (KR), Horticulture Research International, East Malling, Kent, ME19 6BJ, UK; and Forestry Research Manager (GEH), Northmoor Trust, Little Wittenham, Oxfordshire, OX14 4RA, UK. KR and GEH are corresponding authors, to contact call ++44 (0)1732 843833 or email Karen.Russell@hri.ac.uk or ++44 (0)1865 407792 or e-mail Gabriel.Hemery@NorthmoorTrust.co.uk. website: www.NorthmoorTrust.co.uk.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.; Coggeshall, M.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

improvement programme. Institutes that wished to participate then identified suitable stands and trees for seed collection. In the UK, individual landowners were targeted through the forestry press (Hemery and Russell 2002). Within the programme, provenance is defined *as a seed collection unit composed of a community of potentially interbreeding trees in an area subject to uniform environmental conditions* (adapted from Turnbull and Griffin 1986). In countries with scattered individual trees the term ‘population’ is used. Progeny refers to half-sib families.

During July 2001, the authors attended the annual meeting of the Walnut Council in Wisconsin, USA, and visited staff of the Hardwood Tree Improvement and Regeneration Centre (HTIRC) at Purdue University to encourage the search for North American provenances (Hemery 2002). HTIRC agreed to coordinate the identification and collection of seedlots from across the natural range of black walnut in the USA.

Seed Collection

Thirty-two provenances were targeted, 20 from the United States and 12 (including two populations) from Europe, each ideally comprising 20 half-sib progenies, or otherwise as bulked provenances/populations. Twenty viable seeds from each of 20 mother trees of good or outstanding phenotypic character (Fig. 1) were required per provenance. The target number of seeds required per mother tree was 30 assuming a 60% germination rate (Brinkman 1974), giving approximately 600 seeds or approximately 7 kg of seed (cleaned weight) per provenance.

A minimum distance of 100 m was specified between mother trees within provenances and a wide geographic distribution among provenances within each country was requested of collaborators in each country. The identity of each mother tree was maintained for each. seedlot. The phenotypic characters of each mother tree was assessed and their environmental location recorded. Seeds were collected at seed fall during both 2002 and 2003. Where black walnut trees were scarce, a lower number of progenies or a bulked population collection was accepted.

The European seedlots were sent to HRI-East Malling where the seed was cleaned and sorted before distribution to a commercial nursery. The North American seedlots were cleaned and dispatched from HTIRC directly to Alba Trees plc, the UK nursery. After a minimum of 120 days of stratification, the seeds were sown into root-trainers of 400 cc volume and 17 cm depth and germinated in a protected environment (polytunnel). One-year-old seedlings will be used in the establishment of the field trials.

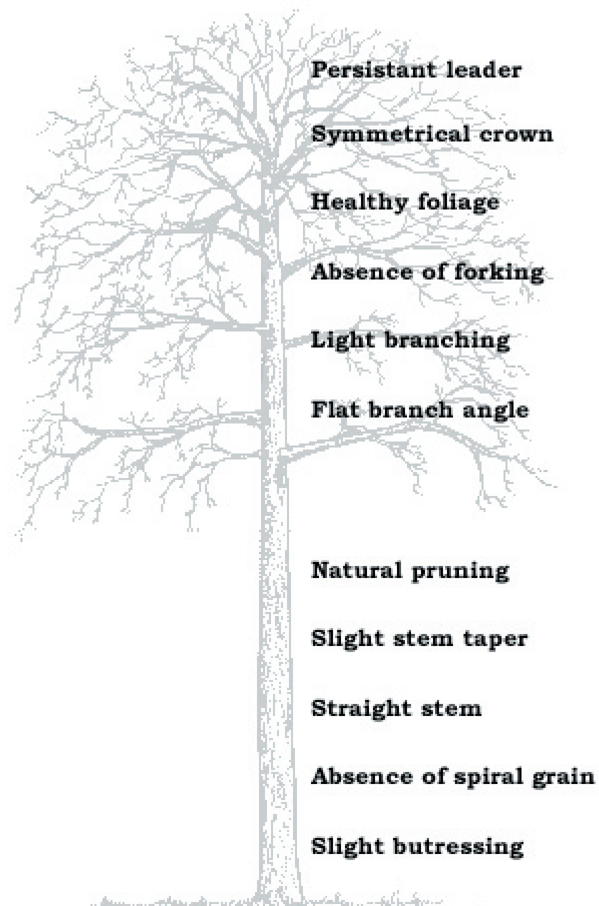


Figure 1.—The ideal black walnut plus-tree or ‘ideotype’.

Experimental Design

The main purpose of the field trials is to assess the suitability of provenances for timber production under UK conditions, with particular emphasis on selection for good form and late flushing. The trials will also provide a resource for genetic diversity studies. The trees will be planted out in combined provenance/progeny experiments at two sites during 2003 and 2004. Seedlots were received during 2 years, therefore, one UK progeny will be established at each field trial in both years as a control population.

Field trials will be established at two sites in central and southern England (Fig. 2). The central England site is at the National Forest at Lount in Derbyshire (52° 36’ N/1° 25 W, soils pH 7.0, altitude 120 asl, annual precipitation 600 mm). The southern England site is at the Northmoor

Figure 2.—Distribution of European black walnut provenances sampled for UK trials.

Trust's Forest Research Centre in Oxfordshire. (51° 38' N/1° 12' W, soils pH 6.2 to 7.7, altitude 50 m asl, annual precipitation 570 mm). The proposed design combines both provenance and progeny assessments in a single experiment. Provenances will be distributed in non-contiguous multiple-tree plots. Progenies will be distributed as single-tree plots within a randomised complete block design, the replicate (block) size being dependent on the final number of progenies available (usually $\sqrt{n}$ progenies). The 30 provenances will provide 411 progenies for evaluation at each site, the remainder will consist of bulked population collections.

Plantation Design

Trees will be spaced at 2 x 2 m (2,500 trees ha^{-1}) and protected by 0.75 m tree shelters. The non-contiguous provenance plots will allow thinning whilst minimizing progeny losses. A study using molecular markers of the genetic diversity of genotypes within the trials is planned early in the life of the programme to minimise the impact of thinning.

RESULTS

Out of a total of 16 European countries who expressed interest in the research programme, seven provided seedlots. Four countries provided seedlots (number in brackets) during 2002: Austria (2), Czech Republic (3), Slovak Republic (2) and the UK (1). The seedlots from the UK were typically collected from individual trees, often distributed in parkland and only rarely in woodland and are termed a population.

In the autumn of 2003, seedlots were provided by France (1), Italy (2), Serbia and Montenegro (2), and additional seedlots were collected in the UK (1). The seedlot from France was collected from a seed orchard as was one seedlot from Italy. The second Italian seedlot was collected within a stand. The seedlots from Serbia and Montenegro and the UK (2002 and 2003) were from scattered individual trees and therefore treated as populations. A total of 10 European provenances and two populations have been collected (Fig. 2).

Seedlots were collected from 20 provenances across 13 States in eastern North America (Fig. 3): Alabama (1), Illinois (2), Indiana (3), Iowa (1), Kentucky (2), Maryland (2), Minnesota (1), North Carolina (1), Ohio (1), Pennsylvania (1), Tennessee (1), Wisconsin (3), and Missouri (1). The majority of the American seedlots were collected from trees within stands and the number of trees per stand from which seed was collected varied from 10 to 30.

Over 1,600 seedlings were raised in 2003 for establishment in the first phase of the provenance trials in winter 2003/04. The germination rates for the Austria and Czech Republic provenances were similar at 74% and 72% respectively; however for the UK population only 28% germinated as seed from some trees failed to germinate. One of the provenances from the Slovak Republic failed to germinate whereas the other had a germination rate of 39%. Some 20,000 seeds were stratified in winter 2003 and sown in spring 2004. The resulting seedlings will be planted out in the second phase of the provenance trial in winter 2004/05.

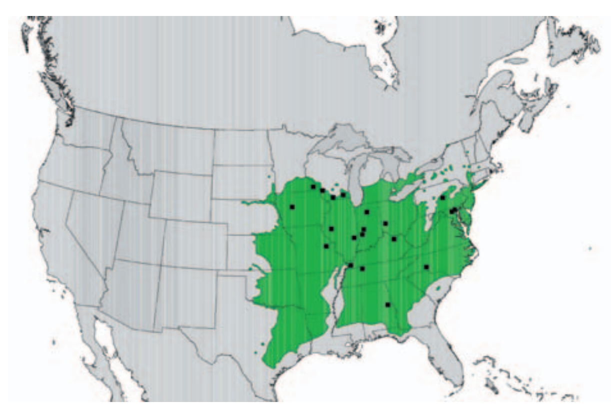


Figure 3.—Distribution of North American black walnut provenances sampled for UK trials.

CLONAL COLLECTIONS

Between 1997 and 2003, graftwood from superior black walnuts was supplied by INRA Bordeaux, France, and HTIRC, Indiana, USA, and collected by HRI from outstanding mature trees in the UK. The graftwood was propagated at HRI-East Malling on seedling rootstocks of common walnut (*Juglans regia* L.) using a 'hot pipe' technique developed for the propagation of difficult species (Toogood 1999). A total of 63 superior trees were successfully propagated (26 US, 25 UK, 12 Mainland Europe) and planted in the walnut collection at Bradbourne House, East Malling (Russell 2002) which also includes accessions of common walnut (fruit and timber selections) and interspecific timber selections. The accessions are being assessed for form, vigour, budbreak, and resistance to pests and diseases, including anthracnose.

FUTURE WORK

The provenance/progeny trials will be assessed for survival, flushing, vigour (height and diameter), form, and occurrence of anthracnose. Data will be analyzed and superior progenies and individuals will be selected to form the basis of the next stage of the improvement programme after 10 years. It is anticipated that clonal trials of plus trees and/or of seedling selections will be established in the future. In addition, it is hoped that a collaborative research programme on the genetic diversity of these collections can be initiated with HTIRC with the objectives of assessing the extent of diversity in black walnut represented in the programme and potentially providing information on origin of the European material.

ACKNOWLEDGMENTS

The programme has been supported in the UK by Jaguar Cars, The National Forest Company, Woodland Heritage, the East Malling Trust for Horticultural Research, the Northmoor Trust for Countryside Conservation, the Department for Environment, Food and Rural Affairs and the Forestry Commission. Our thanks go to the research institutes and individuals in Austria, Czech Republic, France, Italy, Slovak Republic, Serbia and Montenegro, and North America, particularly the HTIRC, who have provided seedlots and/or graftwood to date and to Alba Trees plc for raising the seedlings.

LITERATURE CITED

Bean, W.J. 1981. Trees and shrubs hardy in the British Isles. 8th ed. Revised, Vol. II D-M. London: John Murray. 476 p.

Buckley, M. 2002. Revivalist movement. Timber Trades Journal, 9th February: 14.

Brinkman, K.A. 1974. *Juglans* L., walnut. In: Seeds of woody plants in the United States. Agric. Handb. 450. Washington, DC: U.S. Department of Agriculture, Forest Service: 454-459.

Hemery, G.E. 2002. Walnut forestry tour of the mid-western United States. Woodland Heritage: 20-22.

Hemery, G.E.; Russell, K. 2002. Filling walnut's black hole. Forestry and British Timber. December: 22-23.

Kerr, G. 1993. Establishment and provenance of walnut in Britain. Forestry. 66: 381-393.

Russell, K. 2002. The Walnut collection at HRI-East Malling 2002. The Walnut Club Newsletter. Issue 3 (July 2002): 13-15.

Toogood, A., ed. 1999. The Royal Horticultural Society - propagating plants. London: Dorling Kindersley. 109 p.

Turnball, J.W.; Griffin, A.R. 1986. The concept of provenance and its relationship to infraspecific classification in forest trees. In: Styles, B.T., ed. Infraspecific classification of wild and cultivated plants. Oxford: Clarendon Press: 157-189.

GENETIC AND SILVICULTURAL RESEARCH PROMOTING COMMON WALNUT (*JUGLANS REGIA*) FOR TIMBER PRODUCTION IN THE UNITED KINGDOM

Gabriel E. Hemery[1]

ABSTRACT—A combination of genetic and silvicultural research is required to improve the viability of common walnut for timber production in the UK. A summary of a research programme, initiated in 1996, is provided. Establishment of walnut plantations using tree shelters indicated positive benefits using 0.75 m shelters but larger shelters (1.20 m) caused early flushing and increased dieback. Direct seeding trials indicated that pre-treatment of seed with gibberellins was highly effective in reducing the need for lengthy pre-treatments and promoted 87% germination. Artificial application of nitrogen fertiliser on newly established walnut trees indicated that applications up to 500 kg ha^{-1} had no effect on survival or early growth. Assessments of a range-wide collection of walnut, including provenances from Kyrgyzstan, point to high variability in flushing and growth between provenances and progenies 4 years after establishment. Genotype by site interaction within the three provenance trials is evident. Adoption of new, to the UK, silvicultural systems incorporating tree and shrub nurses indicated promising benefits from using the nitrogen-fixing nurse species autumn olive and Italian alder.

Common walnut (*Juglans regia* L.) is an ancient introduced species to the United Kingdom but today there are probably fewer trees than at any time since the late sixteenth or early seventeenth centuries. Interest in walnut as a timber waned with the increasing availability of tropical hardwoods from the early nineteenth century onwards and it has been infrequently planted in the UK since that time, particularly as a forest tree. Supplies of hardwoods such as mahogany are now, nearly 200 years later, becoming scarce. There is also an increasing awareness by European consumers that the use of tropical timbers may contribute to deforestation, resulting in a reluctance to buy them. There are therefore good reasons to believe that valuable, decorative, temperate hardwoods are likely to be in much greater demand as tropical supplies decline.

Common walnut is perhaps the finest of these valuable species, and is seen as a tree that could regain the place it had centuries ago, as the provider of high quality timber on relatively short rotations. The wood is used for making quality furniture and producing highly figured veneers, usually from burrs, which are used for cabinet-making and decorative panels. At present however, common walnut is often overlooked by foresters due to its reputation as being a species which is site demanding, usually of poor form and which suffers badly from the effects of frost.

The tree phenotypes seen in the UK today are often of poor form for timber production because their likely origin is from European trees originally selected for nut production. Phenotypes for timber or nut production are generally viewed as incompatible because good phenotypes for timber (e.g., long and straight stemmed, finely branched) have deliberately been selected against. Short-boled, spreading and branched trees were sought for high nut productivity and ease of harvesting. Additionally, some phenotypes in Britain may originate from ancient introductions, taken from environments unsuitable for widespread introduction to the British climate.

To encourage a revival of interest in walnut in the UK and to promote best practise, a number of activities were necessary:

[1]Forestry Research Manager (GEH), Northmoor Trust, Little Wittenham, Oxfordshire, OX14 4RA, UK. Call ++44 (0)1865 407792 or e-mail Gabriel.Hemery@NorthmoorTrust.co.uk. website: www.NorthmoorTrust.co.uk.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.; Coggeshall, M.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

1. research directed towards improving initial establishment;

2. desirable, straight stemmed and finely branched trees needed to be selected to suit the climatic conditions of the UK;

3. the collected genotypes needed to be tested in a range of conditions in the UK;

4. novel plantation designs needed to be tested to improve silvicultural conditions;

5. research programmes and promotion of walnut growing needed to be coordinated.

PLANTATION ESTABLISHMENT

Tree Shelters

Background and Method

Tree shelters are widely used in the UK as a means to protect establishing trees from pests and additionally to provide protection from herbicides in combination with micro-climatic benefits. Costs for both shelter and supporting stake vary according to size but for an average 0.75 m shelter/stake costs approximately $1.6 at current prices, and are often more cost effective than fences when the trees are spaced widely. An experiment was established in 1996 testing the effects of two common sizes of shelter (0.75 and 1.2 m) and a control (no shelter) on the establishment and early growth of common walnut, planted as bare-rooted transplants (Hemery and Savill 2001). Observations were made on height and stem diameter growth, flushing (leaf burst) and dieback over a 4-year period.

Results and Discussion

Treeshelters were beneficial for both tree height increment and stem diameter growth however, the 1.2 m tall shelters promoted earlier flushing than the other shelter treatments, resulting in a risk of increased frost damage (Hemery and Savill 2001). Additionally, a substantial degree of stem dieback occurred during one dormant season in the 1.2 m shelters when 46% of tree height was lost, whereas there were no significant differences for dieback between those trees without shelters and those in 0.75 m shelters (20% and 15%, respectively). Hemery and Savill (2001) therefore recommended medium sized (0.60 or 0.75 m) shelters where possible for the successful establishment of walnut. Medium-sized tree shelters were therefore adopted for most of the experiments described here and are widely adopted by landowners planting walnut in the UK.

Direct Seeding

Background and Method

Walnut plantations can be costly to establish both in terms of plant cost (typically equivalent to $1.6 or more per 60 – 90 cm transplant in the UK) and labour due to the large taproot. Walnut also has a reputation as being sensitive to transplant shock and intolerant of damage to the taproot (Popov 1981). Direct seeding was proposed as an alternative method to establish a walnut plantation although methods of ensuring adequate germination and growth needed to be determined.

The effectiveness of four different seed preparation techniques in relation to walnut establishment by direct seeding was tested in a trial sown in spring 2001. The trial was a randomized complete block design with five nine-tree replicates per treatment. In addition to a control (no pre-treatment), seeds were stratified according to recommended practise (Dirr and Heuser 1987) where seeds were placed in damp peat (sedge peat -neutral pH) and stored between 0 and 4° C for 4 - 6 weeks. Another treatment included water-soaked seeds where these were partially opened at the basal joint with a blunt knife and soaked for 48 hours in water, immediately preceding sowing. The final treatment was GA3 soaked seeds, where seeds were partially opened at the basal joint with a blunt knife but soaked for 48 hours in a solution of gibberellic acid (GA_3 – Sigma 7645) at 48 ppm[2], immediately preceding sowing. Gibberellins were successfully used by Hemery (2000) in nursery trials of walnut seed germination.

The seeds were sown by placing them at twice their width below ground and then covered by sedge peat. A tree shelter was placed over each sown seed. Germination and tree height growth data were assessed and analyzed using Genstat (Lawes Agricultural Trust, Rothampstead Experimental Station, UK).

Results and Discussion

There were no significant differences for germination between different pre-treatments. The lowest mean germination rate was for control treatments at 76% and highest for GA3-soaked seed at 87%. Overall 80% of seeds germinated. In the first year following germination, trees that had grown from stratified and GA3-soaked seeds grew more ($p < 0.05$) than those water-soaked and control treated. By year 2 and onwards however,

[2] Example calculations: 45 seeds of mean volume 14.5 cm^3 each = 652 cm^3 total volume whilst approximate bucket volume = 2356 cm^3. Remaining bucket volume (2356-652) = 1704 cm^3 (1.7 litres). GA_3 required at 100 ppm = 0.1g l-1 = 0.17g for 1.7 litres of water.

there were no differences for height between the treatments.

The high germination rates achieved by the use of gibberellins indicate a highly practicable way of pre-treating seeds for direct sowing but further research is needed to study methods that may improve germination in comparison to traditional methods. This research does indicate the efficiency of establishing walnut by direct seeding, particularly in terms of the cost of a seed, which is typically only 2% the cost of a transplant. Although not tested in an experimental framework, it is interesting to note that the 3-year-old seedlings in the trial averaged 98 cm tall compared to 'control' common walnut trees in a neighboring trial (described below) whose mean height was 166 cm after the same three growing seasons, being planted at 46 cm tall.

Nitrogen Fertiliser

Background and Method

Visual observation of many young walnut plantations on ex-farmland in the UK, where leaves often turned yellow and growth slowed 5 to 8 years after establishment, indicated nitrogen (N) deficiencies. Foliar analysis subsequently confirmed critical levels of N in these cases 2.0% - 2.5% element N in dry weight (Bonneau 1995).

A trial neighboring the direct seeding experiment was established in 2001 to test the effects of applying artificial nitrogen on the establishment and early growth of walnut transplants, both common and black (*Juglans nigra*). The trial was planted in ground that had been fallow for 7 years preceding establishment of the trial. It was laid out as a full factorial incorporating the two walnut species and six nitrogen treatments in a randomized complete block experiment with two replicates. In addition to a control (no N applied) there were five N application treatments covering the range of recommended rates of N in addition to low and high extremes (100, 200, 300, 400 and 500 kg N ha^{-1}). Savill and others (1997) stated that the normal range would be 150-250 kg of element N ha^{-1}. The two walnut selections were common walnut (provenance RA464) and black walnut, both sourced from France. The N was applied once at planting, in the form of ammonium nitrate (NH_4NO_3) in which N was present at 34.5%, and again at the start of the third growing year. Survival and tree height data were assessed and analyzed using Genstat.

Results and Discussion

After both growing seasons following application of nitrogen (years 1 and 3) there were no significant differences for height growth between the different nitrogen treatments. Over the three growing seasons the common walnut grew more in height ($p > 0.05$) than black walnut but was this was not attributable to the nitrogen treatments, the interaction being non-significant. Trees subjected to the highest dose of nitrogen (500 kg N ha^{-1}) grew less (80 cm) than all other treatments (range 96 – 106 cm) but these differences were non-significant. Extreme high doses of nitrogen would be expected to have a negative effect on growth but the results of this experiment indicate than nitrogen can be applied at nearly double the recommended rate without adversely effecting growth. More importantly it indicates that applying nitrogen may be a costly exercise, both in terms of unnecessary monetary expense and environmental pollution, and therefore not recommended.

SELECTION OF GENOTYPES

Natural Range of Common Walnut

The natural range of common walnut is confined to the Asian continent extending across 21 modern political boundaries: from Turkey, Azerbaijan, Armenia, Russia, Georgia, and Iraq (Kurdistan) in the west, across the northern lands of Iran and Afghanistan and the heart of central Asia in the independent states of Kazakhstan, Turkmenistan, Uzbekistan, Kyrgyzstan, Tajikistan and their giant neighbor China in the Xinjiang Autonomous Region, formerly 'Chinese Turkestan', extending further south in a narrowing range nestling in the mountains of Pakistan, northern India and Nepal, and finally reaching its eastern extent in Bangladesh, Myanmar (Burma), Bhutan and southern China (Nekrassowa 1927, Schmucker 1942, Berg 1950, Browicz 1976, Davis 1982, Puri and others 1983, Komarov 1985). Jalas and Suominen (1976), and Tutin and others (1993), reported that common walnut might be native to Greece and elsewhere in the Balkan Peninsula.

Seed Collections

In provenance selection, the movement of seed from a source of origin that is very different to the introduced location can be problematic if high altitude or high latitude sources are moved to low altitudes or low latitudes, or indeed the reverse (Zobel and Talbert 1984). However, high altitude sources from low latitudes can often be introduced successfully to low altitude locations at high latitudes (Zobel and Talbert 1984). The prime area for concentrating sampling was Kyrgyzstan which, although situated 10° south of the UK, it is situated at the northern limit of the natural range of the species (Schmucker 1942, McGranahan and Leslie 1991). Therefore the seed sources from this mountainous country, where the average elevation of collections made were 1,460 m above sea level (a.s.l.), provided a possible source for successful introduction due to the altitude difference between the two locations (UK trial sites ranged from 15 to 245 m a.s.l.).

The genetic resources of forest trees are usually located in primeval or ancient forests (Frankel and others 1995). Kyrgyzstan, containing 25,600 ha of natural walnut forest (Musuraliev 1998), was therefore the main focus for seed collecting with the dual purposes of sampling genetic variation (Hemery and Popov 1998) and identifying superior genotypes for timber production.

Eleven provenance collections, incorporating 253 half-sib progenies, were sampled from Kyrgyzstan in September 1997 during a 3-week expedition (Hemery 1998). On average, 23 trees were sampled from each provenance location, each containing a wide variety of tree phenotypes, ranging in height from 7 to 34 m and in DBH from 13 to 128 cm (Hemery 2000). A wide array of tree characteristics were assessed including height to first branch, branch angle, crown diameter, stem straightness, presence/absence of burrs and many seed parameters. Provenance sites were surveyed for accurate location, altitude, aspect, slope, soil type, forest species diversity and basal area.

A further 13 provenance collections (and 122 progenies) were amassed through collaboration with research institutes and individuals across the natural and introduced ranges of the species covering 12 countries. Overall 18 provenances contained progeny-level data.

PROVENANCE AND PROGENY PERFORMANCE

Method

The 375 common walnut genotypes were established across three provenance/progeny trials in southern England during winter 1998. The three provenance trials are arranged as randomized complete block designs with unbalanced multiple-tree plots. The largest of these (Oxfordshire) was a combined provenance and progeny trial containing 18 provenances and 199 half-sib progenies. The progeny trial in a randomized incomplete block design with single-tree plots. Tree heights and stem diameters were measured annually and flushing (leaf-burst) assessed using a scoring system developed for this research, on two days in spring 1999, and analyzed using the Kruskal-Wallis non-parametric test. Survival percentages were arcsin transformed for analyses. All statistical analyses were conducted on plot means using Genstat.

Results and Discussion

Across all three field trial sites survival has been impressive at 98.4% after four growing seasons. Within the 18 provenances with progeny-level data, mortality was only observed within the Kyrgyz provenances (mean 97.4% survival).

At the end of the fourth growing season (2002) mean tree height was 122 cm but variable between sites ($p < 0.001$) and provenances ($p < 0.001$). Provenance x site interaction was also present ($p < 0.001$) with all main 18 provenances being tallest at the Somerset site and shortest at the Gloucestershire site (Fig. 1). Notably, provenance T1 (Trabzon region, Turkey) was twice the height ($p < 0.001$) at Somerset than at Gloucestershire.

Flushing was highly variable for provenances ($p < 0.001$) on Julian day 85 (March 26th 1999), between the earliest flushing provenances E1 (Spain), T1 and T2 (both Turkey) and the latest flushing provenance J1 (Tajikistan). Within the Kyrgyz provenances (K1-11) there was no significant variation for flushing at day 85. On day 97 (April 7th 1999) provenances K1 and J1 were the only provenances ($p < 0.001$) not to have flushed.

This long-term research programme continues with the ultimate aim of identifying genotypes suitable for producing timber in the UK. Crown closure is predicted by year 15 (2013) by which time thinning will become necessary. There is therefore a window of opportunity in the intervening years in which to conduct thorough research, both phenotypic characterisation and genetic variation studies, before some genotypes will be lost due to silvicultural needs within the trials.

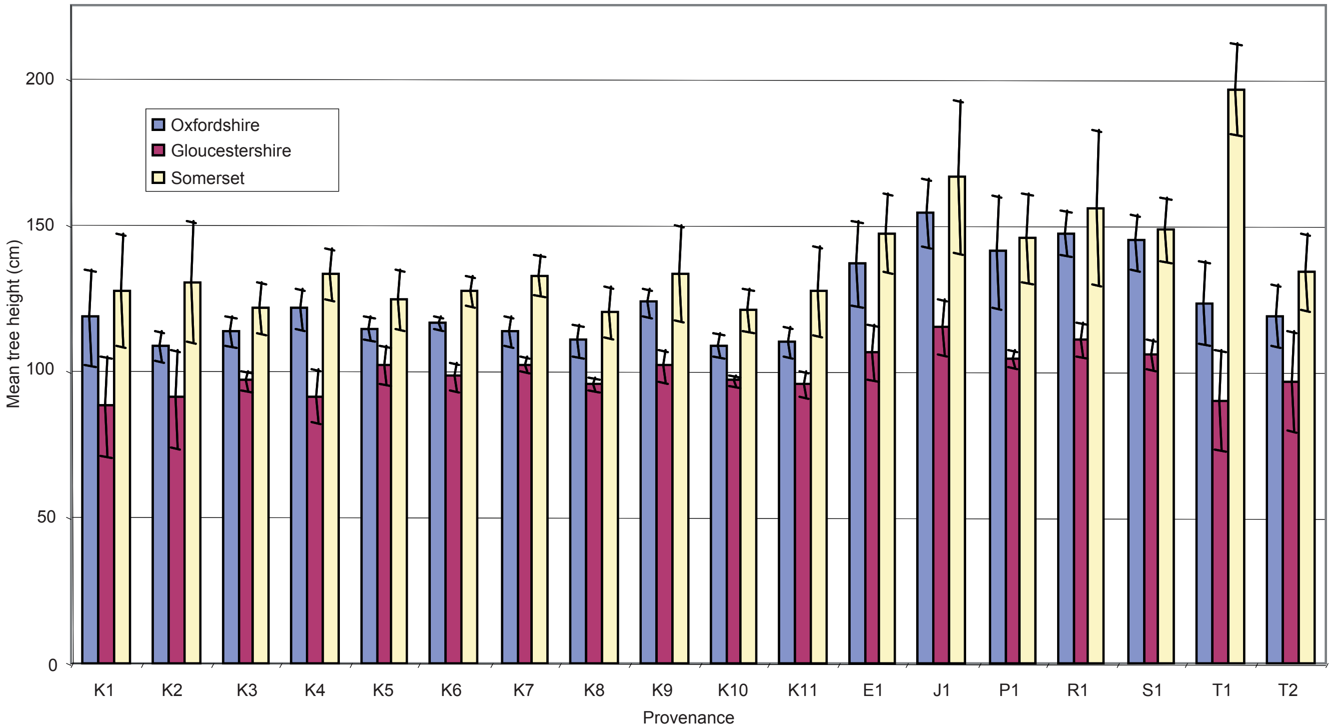


Figure 1.—Mean tree heights for provenances 1 – 18 in 2002, based on plot means within each of the field trial sites; Oxfordshire, Gloucestershire and Somerset. Provenance K stands for Kyrgyzstan (1 – 3 Ak-Terek, 4 Sharap, 5 Yaradar, 6 Shaidan, 7 Kyzyl-Ungur, 8 Katar-Yangak, 9 Kyok-Sarau, 10 Kyr-Sai, 11 Ters-Kolt), E1 Catalonia - Spain, J1 Tajikistan, P1 Karaj - Iran, R1 Romania, S1 Slovakia, T for Turkey (1 Trabzon, 2 Anatolia). Error bars show 95% confidence limits.

PLANTATION DESIGN

Walnuts in Britain are usually grown in pure stands or as individual trees, rather than in mixed plantations. Research conducted in the USA and Italy has indicated that walnuts can benefit from being planted in mixed stands, particularly with nitrogen-fixing species. Advantages may include increased growth rates and improvement of tree form, combined with protection from frost damage and reduced weed competition.

Background and Method

Common walnut is a light-demanding species and has the largest crown diameter, in relation to any given stem diameter, of the main timber-producing species used in British forestry (Hemery 2000). Traditionally in Europe it is usually recommended that common walnut is planted at very wide spacing, due to its intolerance to shading, unless the accompanying nurse species is very carefully maintained (MacDonald and others 1957, Evans 1984). Becquey (1997) recommended planting at 10 to 12 m for the common walnut but importantly, emphasized the importance of the genotype at such low densities.

There is however, increasing evidence that walnut may benefit when planted with suitable nurse or companion trees and shrubs. In the USA, Schlesinger and Williams (1984) tested black walnut in mixtures with black locust (*Robinia pseudoacacia*), autumn-olive (*Elaeagnus umbellata*) and alder (*Alnus glutinosa*). These species were chosen for their nitrogen-fixing (N-fixing) capabilities because many hardwood trees, including black walnut, have shown improved growth when grown with N-fixing species (Finn 1953). Autumn olive is a fast growing, multi-stemmed shrub reaching 6 to 9 m in height and wide-spreading (3.6 to 5.5 m), which is tolerant of exposure and will grow in most soil types in the UK (Bean 1950), and the most hardy of the Genus (Crawford 1998). Schlesinger and Williams (1984) demonstrated that walnut height growth improved with certain nurse species. Walnuts interplanted with autumn olive resulted in height gains of 56 to 351% over non-nursed walnuts. Campbell and Dawson (1989) calculated projections of growth that showed average DBH of 28 cm in 31 years for walnut inter-planted with the autumn olive. They projected that 40 years of growth would be required for the walnut to achieve 28 cm DBH with an alder nurse, or 80 years for those planted without a nurse.

In central Italy, mixed plantations with black walnut established in 1985, have also demonstrated impressive growth (Buresti and Frattegiani 1994). The first plantations with common walnut were established more recently, using nurses of cherry (*Prunus avium*), oleaster (*Elaeagnus angustifolia*), Italian alder (*Alnus cordata*) and robinia (Buresti 1995). Six year results indicated that walnut increased in height by 48% and stem diameter by 36% when planted with cherry (non N-fixing) compared to those walnuts with no nurse (Buresti 1995). However, walnuts with N-fixing nurses were 76% taller and average DBH was 42% greater than for pure-grown walnuts (Buresti 1995).

Research was initiated in 2000 with the aim of investigating planting mixtures that promote the growth of common walnut in UK, in terms of stem quality and vigor, leading to a reduction in rotation time (Hemery 2001). Several field trials were established in southern England, three with common walnut during spring 2000, and a large replicated trial with common, black and hybrid walnuts in 2002, to test the effects of growing these species with a number of companion species.

The trials include all combinations of the nurses (17): tree nurses only (4), tree + shrub nurses (12) and a walnut-only treatment (1). The trials were a randomized complete block (RCB) design, comprising 17 plots in each block (replicate), and two blocks per site. The walnuts were planted in a triangular pattern at approximately 5 x 5 m. At this spacing, based on a predicted crown diameter: stem diameter relationship for walnut (Hemery 2000), and a stem diameter increment of 1 cm year^{-1}, the onset of crown competition between walnuts would begin 15 to 20 years after planting. The tree nurse was planted at similar spacing between the walnuts in an alternative triangular pattern. Shrub nurses, where included, were planted in alternate rows, effectively surrounding the walnuts.

Results and Discussion

At the end of the second growing season walnut survival was high at 99.85%, with mortality only recorded at one site. No differences were recorded for height between sites or treatments at the end of the second of third growing seasons. At this early stage no effect from the nurse treatments would be expected although the observed impressive growth of the autumn olive and alder is predicted to have a positive effect on the walnut from the fifth growing season onwards.

DISCUSSION

The research programme described aims to build on the wealth of experience already gathered across the world in walnut growing for timber production. Walnut forestry in the UK is undergoing a revival of interest fuelled by the research programmes initiated in the country. Variation in performance has been demonstrated within existing widely planted genotypes and between those sampled within the novel research programme including Kyrgyz provenances. Several key common misconceptions relating to the difficulty of establishing walnut successfully have been laid to rest by this research, not least by the excellent survival reported in all the walnut trials described above. Phenology remains an important research area for the future, where the importance of selecting late-flushing phenotypes is paramount for successful walnut establishment, given the unpredictable spring climate in the UK. Future research must also address predicted climate change that might favour the silviculture of walnut species in the UK. Strategic consideration needs to be directed towards supplying the predicted increased demand for tested and quality-assured walnut planting stock for use in the UK. It is hoped that the combined silvicultural and genetic improvement research programmes will continue to encourage and foster a revival of interest in growing walnut in the UK.

ACKNOWLEDGMENTS

Woodland Heritage for supporting the establishment of the walnut nitrogen and companion planting trials and for sponsoring several overseas walnut field trips. Collaborative partners within the British & Irish Hardwoods Improvement Programme and overseas, including Istituto Sperimentale per la Selvicoltura (Italy) and Institute of Forest and Walnut Breeding (Kyrgyzstan). Direct support from The Royal Forestry Society, The National Forest Company, Nicholson Nurseries and landowners hosting external trials. Finally the trustees and patrons of the Northmoor Trust for their continued support.

LITERATURE CITED

Bean, W.J. 1950. Trees and shrubs hardy in the British Isles, 7th ed., Vol. 1. London: John Murray.

Becquey, J., ed. 1997. Les noyers a bois, 3rd ed. Paris: Institut pour le developpement forestier

Berg, L.S. 1950. Natural regions of the U.S.S.R. New York, NY: Macmillan Company.

Bonneau. (1995). Fertilisation des forêts dans les pays tempérés. Nancy Cedex, France: ENGREF.

Browicz, K. 1976. Juglandaceae. In: Flora Iranica. Rechinger, K.H., ed. Austria: Akademische Druck-u Verlagsanstalt. 121: 1-5.

Buresti, E. 1995. Walnut trees in mixed stands with shrubs and trees. In: European development of walnut trees for wood and fruit production as an alternative and extensive system to agricultural crops. Workshop proceedings of E.U. AIR/walnut project; March 27-30; Thessaloniki, Greece: 72-81.

Buresti, E.; Frattegiani, M. 1994. First results of mixed plantation with high quality timber broadleaves and N-fixing trees: mixed stands. In: Proceedings from IUFRO sysmposium, 1994 April 25-29. Portugal: Louse/Coimbra.

Campbell, G.E.; Dawson, J.O. 1989. Growth, yield, and value projections for black walnut interplantings with black alder and autumn olive. Northern Journal of Applied Forestry. 6: 129-132.

Crawford, M. 1998. Nitrogen-fixing plants for temperate climates, 2nd ed. Devon: Agroforestry Research Trust.

Davis, P.H., ed. 1982. Flora of Turkey and the east Aegean islands. Vol. 7. Edinburgh: Edinburgh University Press. 654 p.

Dirr, M.A.; Heuser, C.W. 1987. The reference manual of woody plant propagation: from seed to tissue culture. Georgia: Varsity Press Inc.

Evans, J. 1984. Silviculture of broadleaved woodland. For. Comm. Bull. 62. London: H.M.S.O.

Finn, R.F. 1953. Foliar nitrogen and growth of certain mixed pure forest plantings. Journal of Forestry. 51: 31-33.

Frankel, O.H.; Brown, A.H.D.; Burdon, J.J. 1995. The conservation of plant biodiversity. Cambridge: Cambridge University Press.

Hemery, G.E. 1998. Walnut (*Juglans regia*) seed-collecting expedition to Kyrgyzstan in Central Asia. Quarterly Journal of Forestry. 92: 153-157.

Hemery, G.E. 2000. *Juglans regia* L.: genetic variation and provenance performance. Oxford: Department of Plant Sciences, University of Oxford. 176 p. D.Phil. thesis.

Hemery, G.E. 2001. Growing walnut in mixed stands. Quarterly Journal of Forestry. 95: 31-36.

Hemery, G.E.; Popov, S.I. 1998. The walnut (*Juglans regia* L.) forests of Kyrgyzstan and their importance as a genetic resource. Commonwealth Forestry Review. 77: 272-276.

Hemery, G.E.; Savill, P.S. 2001. The use of treeshelters and application of stumping in the establishment of walnut *Juglans regia*. Forestry. 74: 479-489.

Jalas, J.; Suominen, J., ed. 1976. Atlas Florae Europaeae, Vol. 3. The committee for mapping the flora of Europe, Helsinki.

Komarov, V.L. ed. 1985. Flora of the U.S.S.R, Vol. 5. Germany: Koeltz Scientific Books: 197-199.

MacDonald, J.; Wood, R.F.; Edwards, M.V.; Aldhous, J.R. 1957. Exotic forest trees in Great Britain. For. Comm. Bull. 30. London: H.M.S.O.

McGranahan, G.; Leslie, C. 1991. Walnuts (*Juglans* L.). In: Genetic resources of temperate fruit and nut crops. Moore, J.N.; Ballington, J.R., eds.Wageningen: International Society for Horticultural Science: 907-951.

Musuraliev, T.M. 1998. The current condition of walnut-fruit forests of the Kyrgyz Republic. In: Blaser, J.; Carter, J.; Gilmour, D. Gland, eds. Biodiversity and sustainable use of Kyrgyzstan's walnut-fruit forests. Switzerland and Cambridge, UK and INTERCOOPERATION, Bern, Switzerland: IUCN: 3-18.

Nekrassowa, V.L. 1927. The genus *Juglans* in Turkestan. Bulletin of Applied Botany, of Genetics and Plant-Breeding. 18: 303-360.

Popov, S. 1981. Morphological peculiarities and dynamics of the growth of the European walnut root system depending on the methods of planting and growing of the plantations. Goroskoistspanska Nauka. 18: 25-33.

Puri, G.S.; Meher-Homjii; Gupta, R.K.; Puri, S. 1983. Forest ecology, Vol. 1: Phytogeography and forest conservation. New Delhi: Oxford and IBH Publishing Co.

Savill, P.; Evans, J.; Auclair, D.; Falck, J. 1997. Plantation silviculture in Europe. Oxford: Oxford University Press.

Schlesinger, R.C.; Williams, R.D. 1984. Growth response of black walnut to interplanted trees. Forest Ecology and Management. 9: 235-243.

Schmucker, T. 1942. The tree species of the northern temperate zone and their distribution. Berlin: Silvae Orbis 4. Berlin-Wannsee.

Tutin, T.G.; Burges, N.A.; Chater, A.O.; Edmondson, J.R.; Heywood, V.H.; Moore, D.M.; Valentine, D.H.; Walters, S.M.; Webb, D.A. 1993. Juglandaceae. In: Moore, D.M., ed. Flora Europaea, 2nd ed. Vol. 1. Cambridge: Cambridge University Press.

Zobel, B.; Talbert, J. 1984. Applied forest tree improvement. New York, NY: John Wiley and Sons.

WALNUT TISSUE CULTURE: RESEARCH AND FIELD APPLICATIONS

Jose M. Lopez[1]

ABSTRACT—Vitrotech Biotecnologia Vegetal began researching propagating *Juglans regia* (English walnut) and various *Juglans* hybrids by tissue culture in 1993 and has operated on a commercial scale since 1996. Since this time, more than one and a half million walnuts of different species have been propagated and field planted. Tissue cultured walnuts on their own roots have proven to be more vigorous and productive than conventionally propagated walnuts. Also, tissue culture is an efficient tool to help breeders select and test possible superior genotypes.

WALNUT: A DIFFICULT TREE TO PROPAGATE

Most of the species of the genus *Juglans* are difficult to propagate by conventional vegetative propagation using rooted cuttings, budding, and grafting (Coggeshall and Beineke 1997). Walnut trees are propagated by commercial nurseries by grafting onto seedling rootstocks both for *J. regia* (English walnut) nut production as well as clonal selections of *J. nigra* (black walnut for timber production. Variability with different conditions (climatic, type of plant material, and technique) also affects the success of budding and grafting. Results may be quite variable from year to year. Budding and grafting are still widely used and there are a large number of commercial nurseries around the world that are propagating and selling walnuts with this procedure.

Cuttings have been tried many times with variable success (Coggeshall and Beineke 1997). In fact, the most important limitation to commercial propagation has been the variability of the results. McKenna (1997) got good results with rooted cuttings, but the trees were not able to be commercially deployed. Some investigators indicate that viability of propagated cuttings has been worse than seedlings.

Walnut has proven to be a genus that is also hard to propagate by tissue culture (Rodriquez and others 1989, Preece and others 1989). Attempts have been made by many different researchers (Driver and Kuniyuki 1984; McGranahan and Leslie 1988; Rodríguez and others 1989; Gruselle and Boxus 1990; Gautman and Chalupa 1990; McGranahan 1992; Jay-Allemand and others 1993; Bourrain and Navatel 1994, 1995). The difficulties have been related to: internal contamination of plant material and susceptibility of explants to damage caused by disinfectant substances, oxidation of the explants, maladaptability to the culture media, poor proliferation, poor elongation and rooting rates, difficult acclimatization (stage IV) of the rooted propagules and complications when growing small plantlets in the nursery. Despite this, Vitrotech Biotecnologia Vegetal began the research in this field in 1993 and walnut commercial propagation by tissue culture in 1996. Since then this company has propagated around one and a half million trees of different species of this genus.

WALNUT PROPAGATION: WHAT TO GET AND HOW?

Commercial tree propagation usually starts with the best method to get the maximum profit from the plant material. This consists of grafting the selected clones/varieties/cultivars onto selected clonal rootstocks using scions that have desirable traits for yield, fruit or timber quality, resistance to diseases and/or adverse climatic conditions. The investigator can also use rootstocks selected for good soil adaptability, vigour or resistance to soil pathogens. In the case of walnuts, this has not been available, because the lack of a propagation method for rootstocks, or when selections have been made, no scientific tests could be performed because no replicates of the selected clone were available.

[1]General Manager (JML), Vitrotech Biotecnologia Vegetal, N-340 Km 631, 30840 Alhama de Murcia (Spain). JML is corresponding autor; to contact call +34 968 633231 or e-mail at jose.m.lopez@vitrotech.es

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

When Vitrotech started commercial propagation of *J. regia* by tissue culture, we found that no rootstocks were available for their propagation, or the ones accessible were not tested thoroughly. Under such circumstances we decided to try the propagation of the varieties on their own roots. We started with walnut tissue cultures obtained from other researchers around the world (UC Davis, Burchell Nursery, INRA, and CTIFL) and propagated some varieties/clones on their own roots.

Most of the initial production of our company was based on English walnut nut cultivars, though some clones of *J. hindsii,* paradox (*J. hindsii* x *J. regia*), and other hybrids (*J. major* x *J. regia, J. nigra* x *J. regia*) were also propagated. Throughout Europe and most of the world (except California) *J. regia* cultivars were grafted onto *J. regia* seedlings. Our company thought that a tissue cultured walnut tree on its own roots should not have less productivity than a grafted tree. In addition, most of the walnut cultivars come from mother trees grown on their own roots (seedlings), that could show us that those cultivars can be good root systems (or, at least, not worse than the average of seedlings).

AGRONOMIC FEATURES OF MICROPROPAGATED WALNUTS

We were surprised to find that the performance of self-rooted walnut cultivars was better than expected. In most cases, they were superior to performance with grafted trees. Prunet and Ginibre (2000) described that self-rooted *J. regia* 'Lara' were more vigorous than trees of the same cultivar grafted onto seedling rootstocks of *J. regia* and *J. nigra* x *regia* hybrids, as well as clonal *J. regia* rootstocks that had been selected for their excellent vigour and propagated by tissue culture.

Hasey and others (2001) found that self-rooted *J. regia* 'Chandler' was much more vigorous than Chandler grafted onto Paradox rootstock (Table 1). This was a not expected, as Paradox hybrid is more vigorous than conventional *J. regia* rootstocks of which Chandler is considered as a medium-vigor cultivar (Table 1). Cumulative yield over 5 years on self-rooted Chandler reached close to three times that of conventional grafted Chandler onto Paradox (Table 2). In addition, self-rooted Chandler trees were supporting a higher nematode population on their roots than grafted trees (causing nematode root lesions) and some of them died back. Hasey and others (1999) mentioned that it was striking to observe the higher vigor of those self-rooted Chandler, even with a high nematode population on their roots. They explained that a similar situation was found when vigorous Paradox rootstocks showed more tolerance to nematodes than *J. hindsii* rootstocks. One more interesting observation was that several grafted Chandler onto Paradox exhibited crown galls (*Agrobacterium tumefaciens*), while none of the self-rooted Chandler did.

With our own experience, we have found that self-rooted walnuts of different species and cultivars have shown outstanding vigor, even with very difficult growing conditions (Fig. 1). On a farm located in Murcia province, Spain, soil pH ranged from 8.2 to 9.3. Active calcium content reaches between 23% and 30%. The soil was extremely rocky, and soil preparation was very poor. Climatic conditions were good (no late or early frost,

Table 1.—Trunk circumference (cm) and trunk cross sectional area measured at 60 cm from 1998-2001 for trees planted in 1991 (Hasey and others 2001).

	Circumference (cm)				Trunk Cross Section Area			
Treatment	1998	1999	2000	2001	1998	1999	2000	2001
Own-rooted	64.3 a	68.1 a	71.4 a	74.6 a	340 a	381 a	419 a	457 a
On Paradox	41.4 b	45.1 b	49.4 b	51.7 b	146 b	173 b	208 b	227 b

Means followed by the same letter in a column are not significantly different (LSD P ≤ 0.05)

Table 2.—Yield in kgs/tree from 1995-2001 (Hasey and others 2001).

Treatment	1995	1996	1997	1998	1999	2000	2001
Own-rooted	10.8 a	23.4 a	43.2 a	29.1 a	41.9 a	33.2 a	46.4 a
On Paradox	4.2 b	7.9 b	14.2 b	10.1 b	19.8 b	19.7 b	31.6 a

Means followed by the same letter in a column are not significantly different (LSD P ≤ 0.05)

Figure 1.—Summer 2001 photograph of a self-rooted walnut tree (*Juglans regia*) planted on a farm located in Murcia (Spain) in 1997.

maximum temperatures around 35-40° C and minimum around -5° C), with a long vegetative season (from mid-March to the end of October). Water quality was excellent. The height of the plants planted on the farm ranged from 4 to 20 cm (Fig. 2). The main planting was done between February and April, 1997. At the second year, trees of some of the more vigorous cultivars (*J. regia* 'Sunland' and 'Serr') reached more than 3.5 m. Table 3 shows average diameters per cultivar found in September 2001. Fruit bearing was also very precocious. Some trees produced their first female flowers during their second leaf in the field. Female flower production increased since then, and is still increasing. However catkin production was very delayed compared to conventional grafted trees. First catkins were seen at the sixth leaf, depending on the cultivar (Frutos 2003). Yield has not been recorded yet, as all the original trees on this farm, including pollinators, were self rooted, so most of the possible production has been lost because the lack of pollen.

A further and clear advantage of the use of self-rooted cultivars over grafted trees onto seedling rootstocks is the higher homogeneity of the plantations due to using clonal rootstocks of the same cultivar. Some of the stronger defenders of the use of rootstocks could say that by using self-rooted cultivars an unknown clonal root system is being utilized, which could be very risky. However, our evidence shows that more than 20 different clones on their own roots have shown a better performance than the original mother tree, and no further problems have been found regarding adaptability to different soils than those already present on

Figure 2.—Typical in-leaf tissue-cultured walnut plants when planted at an irrigated farm located in Murcia (Spain).

Table 3.—Trunk diameters (cm) of the different treatments ± SE. Different letters in the same row indicate differences at 95% level (Lopez 2001).

Age/cultivar	Sunland	Serr	Chandler	Sorrento	Vina
2nd leaf	10.94±0.50a	9.88±0.28a	---	8.01±0.25b	7.01±0.26b
3rd leaf	18.68±0.33a	17.88±0.20a	13.85±0.22b	12.67±0.47b	---

the different species/clones. In some cases this adaptability has been clearly better when using self-rooted trees with many different clones.

What is the reason for such improved behavior? Several possible explanations can be considered:

1. Root architecture of self-rooted walnut trees is completely different than walnut seedlings. Instead of the usual main taproot, self-rooted trees exhibit a fibrous root structure. We have counted more than 20 primary roots with all of them coming directly from the crown (Fig. 3). Anchorage is very good and no guides are used with most of our trees. Such root structure may provide for excellent water and nutrient absorption. At the Murcian farm mentioned before, we observed that, in the same conditions, grafted or seedling trees showed leaf chlorosis (due to the limiting soil conditions relative to pH and calcium content) much easier than self-rooted trees. Also, vigor of self-rooted trees was much higher than the vigor of grafted trees.

2. The lack of a graft union seems also to improve the growth of the trees. Olson and Walton (2001) found that self-rooted Chandler (SRCh) walnut trees are more vigorous than SRCh scions grafted onto SRCh rootstocks with similar root architecture. In fact, SRCh were the most vigorous of any of the treatments when Chandler scions were grafted to Paradox, *J. regia* seedlings, or clonal Paradox. Thus, it was concluded that graft unions limit the vigor of the walnut trees. Prunet and Ginibre (1999) also reported that self-rooted Lara trees were more vigorous than Lara grafted onto very vigorous clonal *J. regia* rootstocks propagated by tissue culture. These differences cannot be explained by the root architecture (similar on the treatments), but by the presence/absence of the graft union.

3. Homogeneity of the plant material in terms of genetics. Root systems have a strong influence on the vigor of the tree. Vigorous rootstocks are commonly used to increase productivity or to decrease it (dwarfing rootstocks). This feature is especially important when dealing with trees, as most of the research in breeding with walnuts has been looking for very vigorous scions that must be grafted on seedling rootstocks of unknown and heterogeneous growth capabilities. Once selected scions are shown to be very vigorous and productive on their own roots, it can be assumed that micropropagated selections of walnuts must result in more homogeneous and vigorous trees than the same selections grafted onto seedling rootstocks.

Figure 3.—Root architecture of a 3-year-old walnut tree (*J. regia* cv. *Vina*).

4. Disease tolerance/resistance. No evidence has shown that tissue cultured walnuts of any clone/cultivar are resistant to any soil disease. As stated before, Hasey and others (2001) said that nematode root lesion had affected SRCh trees less than expected, and they assumed that was due to their superior vigor. The same authors found, while in the same block, some grafted trees on Paradox rootstock were affected by crown gall and not one of the self-rooted trees showed this disease. The reason was that soil diseases on grafted trees grown in a nursery row were much more difficult to control than when the trees are grown in pots (case of the tissue cultured self-rooted). Also, digging usually causes injuries to the roots that can be the entry points for such pathogens.

OUTLOOK OF THE USE OF TISSUE CULTURED WALNUTS

Commercial walnut propagation by tissue culture has been successfully achieved by our company. This does not mean that all the walnut plant material is ready to be propagated.

Walnut tissue culture requires research to be performed on almost every new clone/variety to be propagated. Differences with tissue culture behavior between species are greater than with other vegetative propagation methods. *J. nigra* has shown to have very different requirements than *J. regia*, and its performance *in vitro* is more difficult. This is just one illustration, but shows the trend of the entire genus.

Other limitations to walnut mass propagation by tissue culture are the need of more and longer tissue culture stages, which involves larger facilities and more staff to produce the same amount of plants than for other species. The great influence on the process of supposedly less important factors, which with walnuts can limit the production to levels sometimes below what can be considered to be commercially viable, also affect production costs.

In the case of *J. regia* cultivars used for nut production, the use of self-rooted cultivars is of high interest particularly on those situations where no specific rootstock needs are required. These are mostly on sites where *J. regia* seedlings are used as rootstock. There is some advantage in price, but the main benefits are found with the performance of the trees.

When a specific rootstock is needed because the presence of particular soil condition, it is desirable to use clonal material coming from superior selections. Under those circumstances, performance of the trees will be better than those grafted onto seedling rootstocks. Tissue culture is also a determinant tool to make a good rootstock selection, because there is a reliable method for the necessary propagation of the original selected trees in order to get enough repetitions to perform field tests in different conditions. This is the case of most of the California walnut orchards that commonly require the use of Paradox rootstocks because they are more tolerant than *J. regia* to *Phytophtora* species, nematodes, and water logging. Paradox rootstocks in California are also more vigorous when compared to other species of the genus, but in this case, the results of the trials performed to compare self-rooted trees with trees grafted on Paradox have shown that self-rooted trees are more vigorous.

The use of clonal rootstock is justified by a specific requirement (mostly related with adaptability to soil conditions or tolerance/resistance to soil diseases), and when such requirement cannot be obtained by the use of self-rooted trees. The cost of a grafted tree onto a clonal rootstock propagated by tissue culture will be clearly higher than the one of a self-rooted tree. If the grafted tree is not providing a commercial advantage then there is no reason to use more expensive walnut trees.

The use of tissue-cultured walnuts for timber production seems to be as favorable as for nut production, or probably more, because vigor is usually a more desirable feature for timber production. Selected clones can improve both productivity and timber quality, and can allow the utilization of plant material adapted to specific soil or climatic conditions, or resistant/tolerance to either soil or air borne pathogens. Uniformity of plantations will be greater, and phytosanitary treatments can be applied more effectively. In the case of self-rooted trees, the lack of graft union may avoid the presence of defects to the wood that could reduce the price of the timber, although selected clonal rootstocks can be used if necessary. Particularly, *J. nigra*, native to the eastern USA, where soil diseases are not a problem, and most of the clonal selections are based on the features of the scion, propagation of the same by tissue culture to produce self-rooted clonal trees is a feasible possibility when looking for increased productivity, shortened rotations and higher quality timber. In the same way as for nut producing trees, when clonal rootstocks provide desirable features, tissue culture is the only propagation method available for them.

LITERATURE CITED

Bourrain, L.; Navatel, J.C. 1994. Micropropagation of the walnut tree *Juglans regia* L. Part 1: *In vitro* production. Infos-Ctifl. 98: 40-46.

Bourrain, L.; Navatel, J.C. 1995. Micropropagation of the walnut tree *Juglans regia* L. Part 2: Acclimatization in the greenhouse and nursery performance. Infos-Ctifl. 112: 28-33.

Coggeshall, M.V.; Beineke, W.F. 1997. Black walnut vegetative propagation: the challenge continues. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut: 5th Black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 70-77.

Driver, J.A.; Kuniyuki, A.H. 1984. In vitro propagation of Paradox walnut rootstock. HortScience. 19(4): 507-509.

Frutos Tomas, D. 2003. Diferenciacion floral en vergel de los cultivares de nogal (*Juglans regia*, L.) enraizados in vitro. Actas de Horticultura nº 39. X Congreso Nacional de Ciencias Horticolas, Pontevedra, España: 271-273.

Gautam, R.; Chauhan, J.S. 1990. A physiological analysis of rooting in cuttings of juvenile walnut (*Juglans regia* L.). Acta Horticulturae. 284: 33-41.

Gruselle, R.; Boxus, P. 1990. Walnut micropropagation. Acta Horticulturae. 284: 45-52.

Hasey, J.; Westerdahl, B.; Micke, W.; Ramos, D.; Yeager, J. 2001. Yeild performance of own-rooted 'chandler' walnut versus 'chandler' walnut on paradox rootstock. In: Germain, E.; Calvi, D., eds. Proceedings, 4th International walnut symposium; Bordeaux, France. Acta Horticulturae. 544: 482-489.

Hasey J.; Westerdahl, B.B.; Lampinen, B.D. 1999. Long-term performance of own rooted 'chandler' vs 'chandler' on paradox rootstock: the walnut research reports annual proceedings. Sacramento, CA: The Walnut Marketing Board of California: 87-92.

Hasey J.; Westerdahl, B.B.; Lampinen, B.D. 2001 Long-term performance of own rooted 'chandler' vs 'chandler' on paradox rootstock: the walnut research reports annual proceedings. Sacramento, CA: The Walnut Marketing Board of California. 87-92.

Jay-Allemand, C.; Peng S.; Capelli, P.; Cornu, D. 1993. Micropropagation of hybrid walnut trees: some factors involved in rooting. Acta Horticulturae. 311: 117-124.

Lopez, J.M. 2001. Field behaviour of self-rooted walnut trees of different cultivars produced by tissue culture and planted in Murcia (Spain). In: Germain, E.; Calvi, D., eds., Proceedings, 4th International walnut symposium. Bordeaux, France: Acta Horticulturae. 544: 543-546.

McKenna J.; Sutter, E. 1997. Root formation in walnut hybrid rootstoocks (*Juglans hindsii* X *J. regia*). In: Altman, A.; Waisel, Y., eds. Biology of root formation and development. Basic Life Sciences. 65: 85-90.

McGranahan, G. 1992. Micropropagation of persian walnut (*Juglans regia* L.). In: Bajaj, Y.P.S., ed. Biotechnology in agriculture and forestry, high-tech and micropropagation II. Berlin, Heidelberg: Springer-Verlag. 5(18): 136-150.

McGranahan, G.; Leslie, C.A. 1988. *In vitro* propagation of mature persian walnut cultivars. HortScience. 23(1): 220.

Olson, B.; McKenna, J.; McGranahan, G.; Walton, J.; Bertagna, N. 2001. Walnut rootstock trial 2001. The walnut research reports annual proceedings, Sacramento, CA: The Walnut Marketing Board of California: 99-100.

Preece, J.E.; Van Sambeek, J.W.; Huetteman, C.A.; Gaffney, G.R. 1989. Biotechnology: *In vitro* studies with walnut (*Juglans*) species. In: Phelps, J.E., ed. The continuing quest for quality: 4th Black walnut symposium; 1989 July 30-August 2; Carbondale, IL. Indianapolis, IN: Walnut Council: 159-180.

Prunet, J.P.; Ginibre T. 2000. Comportement agronomique des nouveaux portegreffes CTIFL *Juglans regia*. Travaux de Recherche et d'expérimentation. Station Expérimentale de Creysse.

Rodríguez R. Revilla A.; Albuerne M.; Pérez C. 1989. Walnut (*Juglans* spp.). In: Bajaj, Y.P.S., ed. Biotechnology in agriculture and forestry. Berlin, Heidelberg: Springer-Verlag: Trees II. 5: 99-126.

Current Research in Spain on Walnut for Wood Production

Neus Aletà[1]

ABSTRACT—The Department of Mediterranean Trees at the Institut de Recerca i Tecnología Agroalimentàries (IRTA) in Spain initiated a research program in 1993 to examine the variability among walnut species for wood production and to establish orchards with improved selections. The main objective of the programme is to obtain superior Persian walnut (*Juglans regia* L.) selections, half-sib progenies or clones, for wood production. Another important goal is to use controlled crosses among different walnut species to produce new hybrids, especially Persian x black (*J. nigra* L.) for wood production adapted to Spain's Mediterranean climate.

Fossil evidence indicates that Persian walnut (*J. regia*) was present in Europe as long ago as the end of the Pliocene and later widely spread by the Greeks and Romans through the Mediterranean basin, including the Iberian Peninsula (Manchester 1989, Rivera and others 1997, Leslie and McGranahan 1998). Wild walnut trees in large numbers grew throughout Spain for centuries. During the 1940s and 1950s, the dire social situation in Spain after the civil war led to the removal and sale of many outstanding trees. There was little regeneration, and as a consequence the number of wild walnut trees in the countryside decreased drastically (Ministerio de Agricultura Pesca y Alimentación 2001).

In Western Europe, the demand for quality timber is leading to farmers to plant deciduous hardwoods, particularly *Juglans* spp. This trend started few years ago in France, Italy, and some countries of central Europe. However, in Spain the interest in timber production is recent; new plantations in fields previously designated for horticulture appeared only 10 years ago. In the 1990s, the EU started programs for reforestation and plantations for timber production. Several large enterprises decided to invest in the production of quality wood using intensive management practices, and many farmers started to show an interest in shifting from horticultural orchards to woodlands.

Farmers and companies wondered what they should plant. No Persian walnut timber selections were available. Biotypes or land-races such as 'Lozeronne' or 'Charente in France or 'Bleggiana', 'Feltrina' or 'Sorrento' in Italy were all that was available. On the other hand, the French hybrid progenies, 'Mj209xRa' and 'Ng23xRa' (*J.* x *intermedia*) were outstanding for their vigor and growth habit (Aletà and others 2003, Fady and others 2003). In Spain, farmers decided to plant these hybrids since the climate in Spain is suitable for timber production in intensively managed orchards. At the end of 2003, nearly 5,000 acres of *Juglans*, mainly hybrids, were growing in Spain under intensive management.

Starting in 1993, the Institut de Recerca I Tecnologia Agroalimentàries (IRTA) initiated a research programs concentration on the selection of walnut for wood production and development of improved practices for woodland management. The main aims of the IRTA programme are:

- Evaluate variability within remaining Persian walnut populations.
- Selection of Persian walnut progenies for wood production.
- Selection of genitors for breeding new hybrid progenies.
- Clonal selection.
- Evaluation of training systems for wood production.
- Development of irrigation schedules to optimize supply of water.
- Design mixed planting systems for timber production.

[1]Research Genetist (NA), Mas Bové Center, Departament of Mediterranean Trees, Institut de Recerca i Tecnología Agroalimentàries, P.O. Box 415, 43280 Reus (Spain). NA is corresponding author; to contact, call (34) 977 328424 or e-mail neus.aleta@irta.es

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

VARIABILITY WITHIN SPAIN'S WALNUT POPULATIONS

Morphological and isoenzymatic variability are being studied in different populations of wild Persian walnut. Fifty individuals from nine populations have been analyzed using six enzymatic systems. Morphological characteristics of trees in field/germplasm collections are also being evaluated on representative clones that originated in different parts of Spain.

In 2003, we collected seed from Persian walnut from throughout Spain. In 2004, seed from more than 50 wild populations will be sowed in three progeny tests planted in different environments near Galicia (northwest of Spain), Zaragoza (north central of Spain), Granada (southeast of Spain) and Catalonia (northeast of Spain). Progeny testing will allow for the preservation of germplasm and the study of the variability present in existing Persian walnut populations, especially variability in traits important for timber production.

We already have several publications on the variability within wild clones/populations of Persian walnut (Aletà and Ninot 1995, Malvolti and others 1998, Ninot and Aletà 2003). Our recent work shows large genetic differences exist between the northern populations and southern populations in Spain.

SELECTING FOR WOOD PRODUCTION

Many surveys in different areas of Spain have been made since 1982 looking for wild Persian walnut trees with exceptional traits for wood production (Germain and others 1997). Selected trees have been grafted and established in field collection to produce seeds. Seed is collected and planted in the nursery for 2 consecutive years. Progenies with seedling collar diameters less than 12 mm and survival less than 60% are rejected. Progeny tests of the juvenile growth of more than 40 half-sib families in the nursery have resulted in the identification of 28 families for further field-testing. Sixteen of these families are already under evaluation for further selection (Aletà and others 2004).

Seedlings of the best progenies are being planted in field progeny tests with different ecologies. In addition to evaluating wood production, progenies are tested for resistance to the soil diseases including Armillaria root and crown rot (*A. mellea*) and *Phytophthora*.

From superior half-sib progenies that have been identified using data from nursery and field trials, genitor clones are grafted. Grafts are established in field to be used as seed producing trees. In parallel studies, seed clonal orchards have been prepared using some pre-selected individuals to obtain controlled seeds of Persian walnut for timber production. Currently this material comes from five pre-selected IRTA-clones: 'MBT-122', 'MBT-218', 'MBT-231', MBSB-13' and 'MBPo-6'.

BREEDING NEW HYBRID PROGENIES

To increase the variability in our breeding germplasm, based mainly on Persian walnut, we have introduced black walnut (*J. nigra* L.), Argentine walnut (*J. australis* Grisebach), California black walnut (*J. hindsii* Jeps.), and Arizona walnut (*J. major* Torr.) Heller) materials in field collections. A genitor is evaluated both by the percentage of successful crosses and the growth characteristics of its progenies. For several years we have been doing controlled crosses between Persian walnut clones and some black walnut trees. The aim is to produce Persian x black walnut hybrids suitable for woodland plantations. In 2004, we will be evaluating 15 full-sib progenies from controlled crosses. The programme of controlled crosses is going to continue for 3 more years to increase the number of individuals per progeny.

CLONAL SELECTION

The decision as to which individual to clone is based on the characteristics of both the mother plant and the environment where the tree grows. Trees must show a suitable growth habit and high vigour plus some resistance to soil diseases or to drought to be cloned. We are testing some selected clones, together with pre-selected progenies, for Armillaria root and crown rot soil disease. Outstanding trees are micropropagated and studied in field trials together with commercial progenies, 'Lozeronne' provenance as *J. regia* reference or 'Mj209xRa' and 'Ng23xRa' as references for hybrid materials. In field trials clones will be evaluated for at least 10 years. At present an IRTA clone, IRTA X-80, is being made available commercially as micropropagated plants. IRTA X-80 is a hybrid walnut of unknown origin having excellent growth and stem form that is well adapted to areas with a long growing season.

TRAINING SYSTEMS FOR WOOD PRODUCTION

Persian walnut and *Juglans* x *intermedia* are not forest species and training in juvenile orchard

stage must be done to get timber quality.
Two training systems are under evaluation involving these to species: ‘dynamic pruning’ and ‘systematic pruning’. Trees grown under ‘dynamic training’ are pruned, either in summer or in winter or in both seasons, looking for a clear leader dominance, eliminating all branches which compete with the leader; lateral shoots measuring more than 1 inch in basal diameter are cut. In contrast, ‘systematic training’ involves removing all lateral shoots during the summer so that there are no branches on the log below 3 m height. Under this kind of pruning trees need a high tutor. All results are still preliminary but it has been observed that half-sib progeny of ‘Ng23xRa’, are well adapted to ‘dynamic training’. This system allows reaching logs taller than using ‘systematic pruning’ in which secondary growth is faster too. However, ‘dynamic training’ could cause bigger wood internal defects.

IRRIGATION PROGRAM DEVELOPMENT

In the Mediterranean basin, walnut trees need to be irrigated to produce regular trunk growth. We have calculated water demand based on potential evapotranspiration corrected by Persian walnut crop coefficients (Kc) established for fruit production. To optomize water use in relation to tree growth, two schedules of water supply using drip irrigation, in Persian walnut and ‘Ng23xRa’, are being evaluated. The first irrigation programme irrigates during the vegetative period and for the second programme, the hydric support finishes when first growth stops. Diameter contractions during the vegetative period are measured continuously by dendrometers placed on the trunk. Until the fourth growing season, there are no differences in juvenile growth between the two irrigation calendars.

MIXED PLANTING SYSTEMS FOR TIMBER PRODUCTION

Starting in 2000, the effects of inter-row planting of shrubs to naturally train walnut for timber production are under evaluation. Progeny of the hybrid ‘Ng23xRa’ was established, at 6 m x 6 m spacing, with European hazel (*Corylus avellana* L.), at 2 m from the walnut trees, and autumn olive (*Eleagnus umbellata* Thunb.), at 1 m from the walnut trees. After three growing seasons, some natural stem training and reductions in branching with European hazel have been already observed.

LITERATURE CITED

Aletà, N.; Ninot, A. 1995. Field evaluation of *J. regia* selected clones from seedling populations of Mediterranean and Atlantic Spanish coasts. Acta Horticulturae. 442: 63-67.

Aletà, N.; Ninot, A.; Voltes, J. 2003. Caracterización del comportamiento Agroforestal de 12 genotipos de nogal (*Juglans* sp) en dos localidades de Cataluña. Investigación Agraria: Sistemas de Recursos Forestales. 12(1): 39-50.

Aletà, N.; Ninot, A.; Voltes, J. 2004. Retrospective evaluation of parental selection in *Juglans regia* L. using a mixed model analysis. Sylvae Genetica. (in press).

Fady, B.; Fulvio, D.; Aletà, N.; Becquey, J.; Díaz, R.; Fernández, J.; Jay-Allemand, C.; Lefèvre, F.; Ninot, A.; Panetsos, K.; Paris, P.; Pisanelli, A.; Rumph, H. 2003. Walnut demonstrates strong genetic variability for adaptive and wood quality traits in a network of juvenile field tests across Europe. New Forest. 25: 211-225.

Germain, E.; Aletà, N.; Rouskas, D.; Gomes-Pereira, J.; Monastra, F.; Limongelli, F.; Ninot, A.; Zakinthinos, G. 1997. Prospections réalisées dans les populations de semis de noyer d’Espagne, de Grèce, d’Italie et du Portugal: caractérisation des populations et description des présélections issues de ces prospections. CIHEAM-CE/DG, ed. Options Mediterranéennes. Série B: Études et recherches. 6(16): 9-40.

Leslie, C.; Granahan, G.H. 1998. The origin of the walnut. In: Ramos, D.E., ed. Walnut production manual. Publ. 3373. Oakland, CA: University of California: 3-7.

Malvolti, M.E.; Aletà, N.; Ninot, A.; Spada, M. 1998. Walnut (*J. regia*) genetic resources in Europe. Nucis FAO-Newsletter. 7: 20-23.

Manchester, S.R. 1989. Early history of the *Juglandaceae*. Plant Systematics and Evolution. 162: 231-250.

Ministerio de Agricultura Pesca y Alimentación, 2001. Anuario de estadística agroalimentaria. Subdirección General de Estadística. Madrid, Spain.

Ninot, A.; Aletà, N. 2003. Identification and genetic relationship of Persian walnut genotypes using isozyme markers. Journal of the American Pomological Society. 57(3): 106-114.

Rivera, D.; Obón de Castro, C.; Ríos, S.; Selma, C.; Méndez, F.; Verde, A.; Cano, F. 1997. Juglandáceas. In: Las variedades tradicionales de frutales de la cuenca del río Segura. Catálogo Etnobotánico. Ed. Univ. de Murcia- Spain: 44-55.

Beyond the Wild Nut: Moving Toward Profitable Black Walnut Nut Crops

Brian Hammons, Felix Ponder, Jr., and John Rickman[1]

ABSTRACT—Currently, about 2 million pounds of black walnut nutmeats are consumed annually, requiring about 26 million pounds of wild in-shell nuts (hulled, wet weight). Walnuts from wild trees are variable in quality, yield, and moisture, reducing the amount of good, salable nutmeats produced. Consequently, the price that can be paid to the harvester/producer is limited. Improved varieties of black walnut trees differ from wild trees in that they are typically planted in orchards, produce nuts more consistently and the nuts have higher percent kernel yield and quality. Thus, the price on such improved nuts can be higher. The black walnut industry (Hammons Products Company) has developed quality guidelines whereby growers of improved varieties can receive more for nuts with higher nutmeat yields and desirable characteristics. High yield of good quality nutmeats is the key to profitable nut crops.

There is an increasing need for a greater, more stable supply of black walnuts to support long-term consumption growth. One way to help increase the supply is through the use of more nuts from trees of improved varieties. Virtually all the available supply of black walnuts for the commercial market is now harvested from wild trees in a few states. The wild nuts are variable in quality, yield, and moisture, which reduces the amount of good, salable nutmeats produced. The average yield, based on wet drying weight, is only about 7.5% (Hammons 2001). This low yield, plus increasing hauling costs and freight costs, keeps the purchase price low at the buying stations. Also, because yields on nuts from eastern states are much less than nuts from the central states, the purchase price there is even lower.

The price for black walnuts must be higher in order to encourage growers to invest in orchards and harvest more nuts. In addition to volume, production of high quality black walnut nuts also will require a much higher price. The following sections review evaluations of “Quality” and then discuss one mechanism for determining a higher price based upon high quality.

QUALITY

Over the years, many hours of work have gone into evaluating black walnut nutmeat quality. In 1977, the Nebraska Nut Growers Association decided to hold a contest to find the largest black walnut nut (Bish 1999). However, soon, it was decided that big was not always better. In that test, the largest nut, Rowher variety, weighed 24.3 g, but yielded only 27.3% nutmeat compared to nuts of the Emma K variety, which weighed 17.2 g and had a yield of 35.9% nutmeat (Table 1). By comparison, kernel percent from wild trees averaged only 23%. Nut quality evaluation of the species must be an ongoing process to select high-producing, quality varieties that require fewer resources to produce.

There is evidence to suggest that the performance of nut varieties may be affected by climate or geographic area (Hanson 1999, Rink and others 1994). Earlier, Bey (1973) demonstrated that the growth performance of black walnut was affected by geographic origin in provenance tests. In test plantings, black walnut trees originating well to the south consistently outgrew those of local origin. The geographic region for nut varieties may be even narrower than for timber trees (Hanson 1999).

[1]President (BH), Hammons Products Company, P.O. Box 140, Stockton, MO 65758; Research Soil Scientist (FPJr), USDA Forest Service, North Central Research Station 208 Foster Hall, Lincoln University, Jefferson City, MO 65102; and Plant Manager (JR), Hammons Products Company, P.O. Box 140, Stockton, MO 65758; FPJr is the corresponding author: to contact, call 573-681-5575 or 573-875-5341, ext. 234 or e-mail at fponder@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Table 1.—Black walnut nut characteristics for variety and plantation grown wild trees.

Tree Type	Nut Weight	Kernel Weight	Percent Kernel
Variety[1]	*g*	*g*	
Emma K	17.181	6.17	35.91
McGinnis	17.83	5.66	31.24
Surprise	20.22	6.61	32.71
Sauber	14.70	4.93	33.55
Hay #1	20.90	6.77	32.10
Sparks 147	16.90	5.77	33.42
Rowher	24.26	6.37	27.34
Sparks 127	15.01	5.11	33.69
Myers	15.52	5.03	32.89
Krause	18.36	4.77	28.00
Wild[2]			
Area 17	12.44	2.86	22.14
Area 22	14.14	2.66	23.95

[1]*Data for variety trees adapted from Bish (1999).*

[2]*Each value is the mean from 100 nuts. Wild trees are on Hammons Products Company SHONEF Plantation, Stockton, MO.*

For nut varieties, this region should be bounded at about 50 miles north and south and about 100 miles east and west of a variety's viable location. This does not mean trees will not perform well outside the area. It does mean that if one is going to invest in nuts for commercial purposes, one should be conservative on large-scale selections. Trees should be tested by planting small tests of varieties outside the geographic region and expand after proven performance. It also appears that if a variety is viable within a region, the source of scion wood has little or no impact on that cultivar's viability (Hanson 1999).

Sustainable, commercial black walnut nut production in orchards will be impossible without substantial yearly inputs of nitrogen (N) and perhaps other nutrients. Although specific recommendations are still in the developmental stage, all nut tree literature supports the need for N for producing trees. In general, to satisfy N needs for nut production, a split application of 90 to 100 lbs of actual N per acre should be applied with about two-thirds applied in March to early April and the remainder in early August. Phosphorus and potassium (K) should be applied based on soil and analyses. Both P and K, when needed, do not exceed a rate of 60 to 100 pounds of actual ingredient and should be applied in the spring. In cases of severe deficiency larger quantities can be applied. To conserve moisture and improve nutrient uptake when both nutrients and moisture are adequate, mulching trees may be applicable.

Most producers plant more than one variety, primarily for adequate pollination, because varieties behave differently to natural environmental occurrences and to cultural treatments. Aside from nut maturity (early, mid season, and late), planting several varieties will likely minimize risks of weather insects, and disease; and for growers with limited labor and equipment, spread out the harvest workload. Spreading out the commercial harvest would allow more nuts to be harvested before juices produced by decaying husk darken nutmeats and degrades nut quality.

With all this information for the grower, "Quality" for black walnuts is ultimately defined by the consumer (and therefore the industry). It is a nutmeat that is light brown to tan in color, is full-meated, with a rich, naturally pungent flavor. It must also be firm, but not too crunchy in texture, and relatively large in size, over 0.25 inches (~6.3 mm). To provide these nutmeats, the industry needs in-shell nuts that produce a high percentage of high quality kernels. And the growers will need a higher price.

PRICING MODEL

If growers can produce and deliver nuts with higher nutmeat yields that meet the desired characteristics of quality and flavor, Hammons Products Company, currently the only commercial processor of eastern black walnuts, can pay producers a much higher price based upon a hand-tested sample of each delivery. For example, a load of nuts of a single variety, hulled and in-shell, and dry, delivered to the shelling plant, with a hand test yield of 28%, could be worth at least $0.42 per pound. In some years, such nuts with particularly good flavor and color could be worth between $0.50 and $0.60. Nuts with 30% nutmeats could bring $0.45, or perhaps over $0.60 in some years. The seller gets the best price when varieties and orchard locations are kept separate because poor quality nuts will affect the overall price of the load. Also, if varieties are commingled, larger testing samples may be needed to accurately measure average yield, and the price may be less. Other key variables affecting growers' profit will be moisture, harvesting, hulling, drying, and freight costs.

Guidelines developed by Hammons Products Company provide a mechanism for industry and growers to use in planning and trading eastern black walnuts from improved varieties (Table 2). These guidelines show that the price for nuts increases as nutmeat yield increases. This will result in more dollars to the grower for better quality nuts. The price quoted per percent yield may vary upward in some years, as noted above.

It is important to note that in order to sell improved varieties of nuts using these guidelines the amount of hulled nut delivered should weigh at least 1,000 pounds and be clean and dry (Table 2). (Smaller deliveries have been handled in order to test the concept, but ideally in the future purchases would be in full truckload lots of 35,000 – 40,000 pounds.) An adjustment in nut weight is necessary to compensate for the moisture that must be removed before processing (Table 3). In this example, the in-shell delivered weight was reduced by 72 pounds (8.0% moisture – 4.5% moisture = 3.5% moisture x 1.25% of weight for each 1% above 4.5 = 4.375% x 1635 pounds = 71.53 or 72 lbs). In order to hull and clean the nuts, the buyer may provide that service and charge separately, or the buyer may lease the hulling machine to the seller. The buyer, a third party, or a grower cooperative may also provide drying service. If the buyer provides hulling or drying, a fee for that could be stated separately and deducted.

Price per pound of delivered nuts is based upon nutmeat grade and yield. The price per percent yield is based on a buyer's assessment of crop availability, market conditions, and nutmeat desirability. For example, in Table 3, the quoted price of $0.018 per 1% yield of fancy kernel, with $0.0075% yield of standard kernel is not static. Thus, if a delivery tested 30% average hand-test yield, all fancy grade, and if all nuts were cleaned with moisture less than 4.5%, the grower could be paid $0.45 per pound. If the nut quality is particularly desirable, especially in flavor, aroma, and color, the quoted price could be higher, perhaps $0.02 or more per 1% yield resulting in $0.60 per pound or more. Compared to $509.54 that was paid to the seller in the example in Table 3 for nuts from trees of an improved variety, the seller would have been paid only $164.00 at $0.10 per pound for the same amount (1,635 lbs) of nuts from wild trees.

Equipment modifications for processing nuts from trees of improved varieties are made necessary because of shell thickness. Generally, nuts from trees of improved varieties crack more easily. Also, processing time could be reduced because nuts of improved varieties tend to have fewer small kernel parts.

The vision for the future of the black walnut nut industry includes new ways of buying nuts and paying much higher prices for improved varieties harvested from managed orchards. It is reasonable to anticipate that harvesting, hulling and drying nuts will be done differently as more managed plantations come into production. Most importantly for the grower is, how much will nuts be worth and how can the price be higher? Our long-term goal is to assure that both industry and consumers have a growing supply of black walnuts at a reasonable cost. Increasing the purchase price of improved varieties of orchard grown black walnuts will help the species to become a more popular and viable agricultural crop.

Table 2.—Purchase guidelines for improved varieties of eastern black walnuts.

Delivery—Minimum delivery quantity of 1,000 pounds from one orchard or contiguous orchards planted predominately in one variety. All nuts are delivered clean and dry.

Sampling—Approximately one pound (25-30 nuts) per 1,000 pounds will be drawn from different points within the load (in bags or bulk, maximum of ten samples). After mixing all samples together, two one-pound samples will be tested.

Testing—Each sample will be hand-cracked using an appropriate cracker before removing and separating all nutmeats into 3 grades.

1. Fancy - light brown to tan in color, full-meated;
2. Standard – dark brown to nearly black, but full-meated;
3. Reject – dark, shriveled, light weight material that will not produce salable nutmeats.

All nutmeats from the sample will then be tested for moisture with the results reported on the sample evaluation.

Moisture—Kernel moisture must be no greater than 4.5% based on oven or validated meter test. For nuts delivered with greater moisture, an adjustment of 1.25% of the delivery weight will deducted for each 1% moisture over 4.5% in calculating delivery weight for payment.

Payment—The sample evaluation will report the percentage of total in-shell weight for each grade and yield, which will be the basis for payment. Moisture is considered in adjusting the purchase weight and not the price.

Table 3.—An example of Hammons Products Company black walnut test report.

NAME OF GROWER/PRODUCER ____________

DATE OF DELIVERY 12/14/02 DATE OF TEST 1/03/03

NUT VARIETY Emma K

POUNDS IN-SHELL DELIVERED 1635

MOISTURE ADJUSTMENT (less 1.25% of wt. for each 1% above 4.5 - 72 lbs

POUNDS IN-SHELL DELIVERED, net of adjustment 1563

SAMPLE ANALYSIS, from 2 samples of about 1 pound each

	In-shell weight	Fancy kernel		Choice kernel	
	g	Weight (g)	Percent	Weight (g)	Percent
Sample #1	388.0	80.9	20.9	1.9	0.49
Sample #2	481.4	70.1	14.6	12.5	2.60
TOTAL	869.4	151.0	17.4	14.4	1.66
Moisture test (%)	8.0				

PRICE CALCULATION:

1. Price quoted for Fancy Kernel $0.018 X Percent Fancy 17.4 = 0.313

2. Price quoted for Choice Kernel $0.0075 X Percent Choice 1.66 = 0.013

3. Total price per pound $0.326

4. Total Pounds Delivered, net of moisture adjustment 1563

5. TOTAL PURCHASE PRICE TO BE PAID $ 509.54

Tester ____________ Accepted by ____________

LITERATURE CITED

Bey, C.F. 1973. Growth of black walnut trees in eight midwestern states—a provenance test. Res. Pap. NC-91. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 12 p.

Bish, C. 1999. Twenty years of nut evaluation. Annual Report Northern Nut Growers Association. 90: 67-83.

Hanson, B.L. 1999. Commercial black walnut orchard in Iowa (Ben's black walnuts). Annual Report Northern Nut Growers Association. 90: 119-126.

Hammons, B. 2001. A vision for the black walnut industry: more $$$$ with better nuts. Annual Report Northern Nut Growers Association. 92: 57-59.

Rink, G.; Kung, F.H.; Cutter, B.E. 1994. Selection effectiveness for black walnut nuts and timber. Annual Report Northern Nut Growers Association. 85: 44-52.

Factors Affecting the Quality of Walnut Lumber and Veneer

Daniel L. Cassens[1]

ABSTRACT—Walnut is a unique species in both its timber and wood characteristics. Although market conditions vary it is generally considered a valuable species. Because of these factors, setting quality (value) levels for both lumber and veneer can be involved. Lumber grades are quantitative thus straight forward once the system is understood. Determining quality in veneer is much more subjective. This paper will discuss important factors related to quality in both lumber and veneer. Landowners, foresters, researchers, and others concerned with the production of walnut timber for lumber and veneer should consider these factors before proceeding with their work.

American black walnut (*Juglans nigra* L.) has been and continues to be a premier species for both the lumber and face veneer industries. The heartwood color is unique and once finished properly it produces an unsurpassed and unique brilliance. The wood is easily worked with hand and power tools and it is strong for its weight. As a result, it was one of our most valuable woods until the early 1970s. At that time, its value peaked (Hoover 2003), its popularity declined probably due to its cost and availability and consumers became attracted in other species. Since 1997 however, its popularity and thus value has been slowly increasing.

LUMBER

Commercial sawmills use the National Hardwood Lumber Association (2003) grading rules for hardwood lumber to set quality and price levels for walnut lumber. These rules are quantitative. There is one general set of rules referred to as standard. Then, there are adjustments made for the characteristics and situations unique to each species. As an example, plain sawn oak is graded standard with one exception for mineral stain. For walnut, several adjustments are made to the standard rule. These adjustments allow some lumber to be moved into the higher grades which would not otherwise qualify under the standard rules. Grade for grade, the quality of walnut lumber is less than that for those species graded with the standard rule.

Factors which determine a board's grade are its width and length, size of clear cuttings which can be placed between carefully defined defects, number of clear cuttings and the percent of the board in clear face cuttings (Cassens 2001). The grade separations for walnut are FAS, FIF, Selects, No. 1 Common, No. 2A Common, No. 2B Common and No. 3 Common. The white sapwood is considered a defect unless the lumber is steamed to darken it. Steaming walnut is a standard procedure for any commercial mill producing walnut. The most significant differences between the walnut rules and the standard rule are as follows:

1. The minimum clear cutting sizes for FAS walnut are 4" x 3' and 3" x 6' compared to 4" x 5' and 3" x 7' for the standard rule.

2. The minimum board width is 5" compared to 6' for the standard rule.

3. The FAS walnut grade will admit 6' and 7' long pieces as compared to 8' and longer for the standard rule. The short pieces are graded on a defect basis. Certain defects are carefully defined and then limited by the number present and their location. The requirements for the size and number of clear cuttings and the percent of clear area are dropped for this short lumber.

4. An extra cutting is allowed for certain sized boards in the FAS grade.

[1]Purdue University, Department of Forestry and Natural Resources, 175 Marsteller Street, West Lafayette, IN 47907-2033.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

5. The FIF grade will accept 6' and 7' lumber as compared to 8' for the standard rule.

6. For FIF lumber the reverse side can be sound as defined in sound cutting or not below No. 1 Common. Sound cuttings admit small defect, holes and bird peck. The standard rule requires the reverse side of FIF lumber to grade No. 1 Common. The standard rule requires that the cuttings on the No. 1 Common side be clear face cuttings so no defects are allowed.

7. For the Selects grade the cutting sizes are reduced as for the FAS grade.

8. For No. 1 Common and No. 2A Common walnut there is no limit as to the number of cuttings allowed. The standard rule limits the number of cuttings allowed.

9. For No. 1 Common pieces with just 1' of surface measure must yield 12 cutting units as compared to being clear in the standard grade.

10. The revenue side of the cuttings in No. 1 Common and No. 2A Common only need to be sound as compared to clear in the standard grade.

11. The minimum size of cutting for No. 2A Common is 2 inches or wider containing 72 square inches as compared to 3" by 2' for the standard grade.

In summary, a load of any particular grade of walnut (except No. 3 Common) will appear to be substantially reduced from that of a species such as red oak which is graded standard. Over time, the rules for walnut have been eroded in an attempt to better utilize the available resource. However, within the last couple of years there has been an effort to remove the short lumber (6' and 7') from the upper grades. This effort which originated from a few producers was unsuccessful. However, buyers can still specify that they will not accept this material. The seller will probably ask for a premium. Anyone interested in grading walnut should refer to the National Hardwood Lumber Association (2003) grading rules and also obtain training from a qualified instructor.

VENEER

There are several methods used to produce veneer (Cassens, 2003). Today, the most common and simplest procedure is to saw two faces on a debarked log. The log is then cut in half with each half being called a flitch. The flitches are heated to soften them. After cleaning, the flitches are sliced into veneer ranging from about 1/32 to 1/48 inches in thickness (Fig. 1). Each sheet of veneer is kept in order and the stack of sliced veneer is also called a flitch. The veneer is then dried and clipped. After drying, and if the veneer is destined for the domestic market, three sample sheets are removed (Fig. 2). These sample sheets are examined by a potential customer to determine if he wants to purchase the entire flitch. Veneer destined for the export market has all of the waney edges clipped off and all major defects removed. The veneer from several trees is usually packed down on pallets. Veneer from the same flitch is kept together. The buyer will usually look at all of the veneer.

Figure 1.—A veneer slicing operation showing the flitch or half log (upper right) being moved downward against a knife. The resulting veneer is being conveyed forward and stacked in order.

Figure 2.—Three sample sheets from a very high quality walnut flitch showing no defects, a centered cathedral pattern, and excellent color. The third sheet (right) taken from near the center of the tree is beginning to show some discoloration in the center.

The cost and time required to process a log into veneer is much more than simply sawing it for lumber. Thus, buyers must be particular in what they accept as veneer quality logs. If the log does not meet expectations the log and manufacturing cost can easily exceed the value of the veneer.

The use of American black walnut for face veneer is a significant and high valued use for the species. There are several factors which affect the quality and thus value of walnut trees or veneer logs. Many of these factors are difficult to determine in standing trees and to determine value, buyers must depend on their previous experience in the immediate area as well as the condition and the history of the entire stand. Once a tree is cut the log ends can be observed and a much better evaluation can be made. Value will ultimately be determined by the buyer's judgment of the trees and market conditions at the time. Some companies will have individuals who specialize in standing timber and others who deal with cut logs.

First, any bark surface irregularities such as overgrown branch stubs, insect damage, old mechanical damage, etc., will likely disqualify the log as high valued potential veneer. It is generally assumed that no surface indicators of interior defects are present in the butt section of a quality veneer tree or log.

Veneer or logs should be straight and well rounded. Bow and crook in a log creates an aesthetic problem by causing the cathedral pattern in flat sliced veneer to run in and out of the sheet. Tension wood is frequently present in leaning trees and buckle can occur when the veneer is dried (Fig. 3). Logs which are not well rounded or have an off-center pith also result in veneer with less than desirable grain pattern and are also likely to result in buckle.

Growth rates and thus ring width should be uniform across the entire cross section of the log. Thus, thinning to encourage faster growth of potential veneer trees may not be desirable. Growth rates of six to nine rings per inch are usually acceptable. Fast growth or very slow growth rates are not preferred. The industry uses the word "texture" to define growth rate. Soft texture refers to a slow growth rate while hard texture refers to a fast growth rate.

Figure 3.—Buckle in walnut veneer. Note the label.

Veneer quality trees should be healthy, well formed trees on good well-drained timber sites. A past history of grazing and or fire will reduce the quality and value of any potential veneer tree.

Most hardwood species grow over a wide geographic range. As such, climatic conditions, soil types, elevations, insect and disease potential, and other factors vary. Within the geographic range of each species there are certain specific areas where buyers feel the highest quality trees come from. Buyers will indicate that high quality trees can come from other regions as well but the probability is much reduced.

Color and uniformity of color in walnut veneer is an extremely important factor in determining value and it is a very difficult factor for the novice to judge. When trees are first cut or a fresh cut is made on the end of a log the heartwood should have a mint green color which changes to a uniform mousy brown color. Cooking schedules for walnut are extended over those of other species with the intent of darkening the color and the flitches are allowed to set several hours after slicing. This waiting period allows the mint green color to change to the preferred brown color before drying.

Uniformity of color is also critical (Figs. 2, 4). Like most other species, walnut can develop streaks of light and dark colors. The wood can also develop a dark splotchy appearance and this condition is sometimes referred to as "muddy".

Bird peck, also called worm in the face veneer industry, is another very important defect in walnut (Fig. 5). Yellow-bellied sapsuckers probably cause most of the damage. It is generally believed that the bird pecks a hole to cause the flow of sap. Sap wet trees in the spring are easily spotted. Insects are attracted to the sap and the bird then feeds on the insects. The peck marks often circle the tree and birds tend to return to the same trees. If the cambium is penetrated, a small hole often with flagging will be found in the veneer. The peck mark is plugged or occluded with bark by the tree and after some time, it can become very difficult to spot. Like many smaller defects, once detected, the buyer will generally assume there are several more present which cannot be seen.

Figure 4.—Two sample sheets from a walnut flitch showing numerous problems. Knots and irregular grain patterns are present throughout. The color is not uniform and the sapwood is excessive.

Pin knots (Fig. 6) like bird peck can be hard to recognize in standing walnut trees, especially when only a few are present. These defects are the result of suppressed dormant buds which persist for many years as a bud trace or pin knot. As the name implies, the buds may not actually break through the bark, so in some instances they cannot be easily detected. Sometimes, due to a stimulus such as thinning and light, the bud may sprout. The sprout may develop into a small limb that often dies, but normally the bud trace continues to form. Pin knots are best observed on the end of the log after the tree is cut or where the bark has peeled loose and they appear as sharp spikes. On flat-sliced veneer, they appear as pin knots, but on quartered surfaces they appear as a streak or "spike" across the sheet of veneer. Purchasers of veneered panels will often specify no pin knots or limit the actual number of pin knots per square foot of veneer in the finished panels.

Fast growth trees also tend to have a wide sapwood zone (Fig. 7). The sapwood is the light colored wood to the outside of the darker heartwood. Sapwood is usually discarded in high quality walnut veneer. Deeply furrowed bark which is not patchy tends to be normal to faster growth and in some situations it may have a wide ring of sapwood. Figure 8 shows the bark on a normal growth and slow growth tree. Also, slow growth trees may not have the preferred color for walnut.

Figure 5.—"Worm" or bird peck on the end of a walnut log.

FIGURED WOOD AND BURLS

Walnut tends to have "flash" or figure in the wood. Small amounts of figure are not desirable (Fig. 9) for commercial applications. End consumers tend to ask questions when one piece of furniture or paneling has some figure and the next piece does not. Also, office furniture is often bought in units and additional pieces purchased at a later date. For this application, it is particularly important that the grain and color characteristics be consistent.

On the other hand, intense figure in particularly large flitches can command a premium. Figure 10 shows an example of a heavy figured flitch which will produce a beautiful and unique end product. When the flitches are large the value increases because they can now be used as a matched and continuous pattern for paneling in large rooms.

Walnut burls are another unique item. Substantial burl wood was used for decorative Victorian walnut furniture manufactured from the 1870s to about 1900. These burls were taken from old growth American black walnut. Black walnut burls are essentially nonexistent today. Figure 11 shows an example of a sheet of veneer from a small one. Most of the walnut burls used today are taken from old California nut orchards where the English walnut is grafted onto the American walnut root stock and a burl develops. These burls are often sold by the pound and at least some of the veneer is used in the automobile industry.

Crowns and stump or root wood is also veneered for its figure. Large old growth walnut trees developed large swellings at the ground level. This material was also sliced into veneer and produced some very figured and decorative patterns. Figure 12 shows a sample of crown wood veneer produced in the 1940s.

Walnut typically has a large tap root and it too has been sliced for veneer because of the figure it produces.

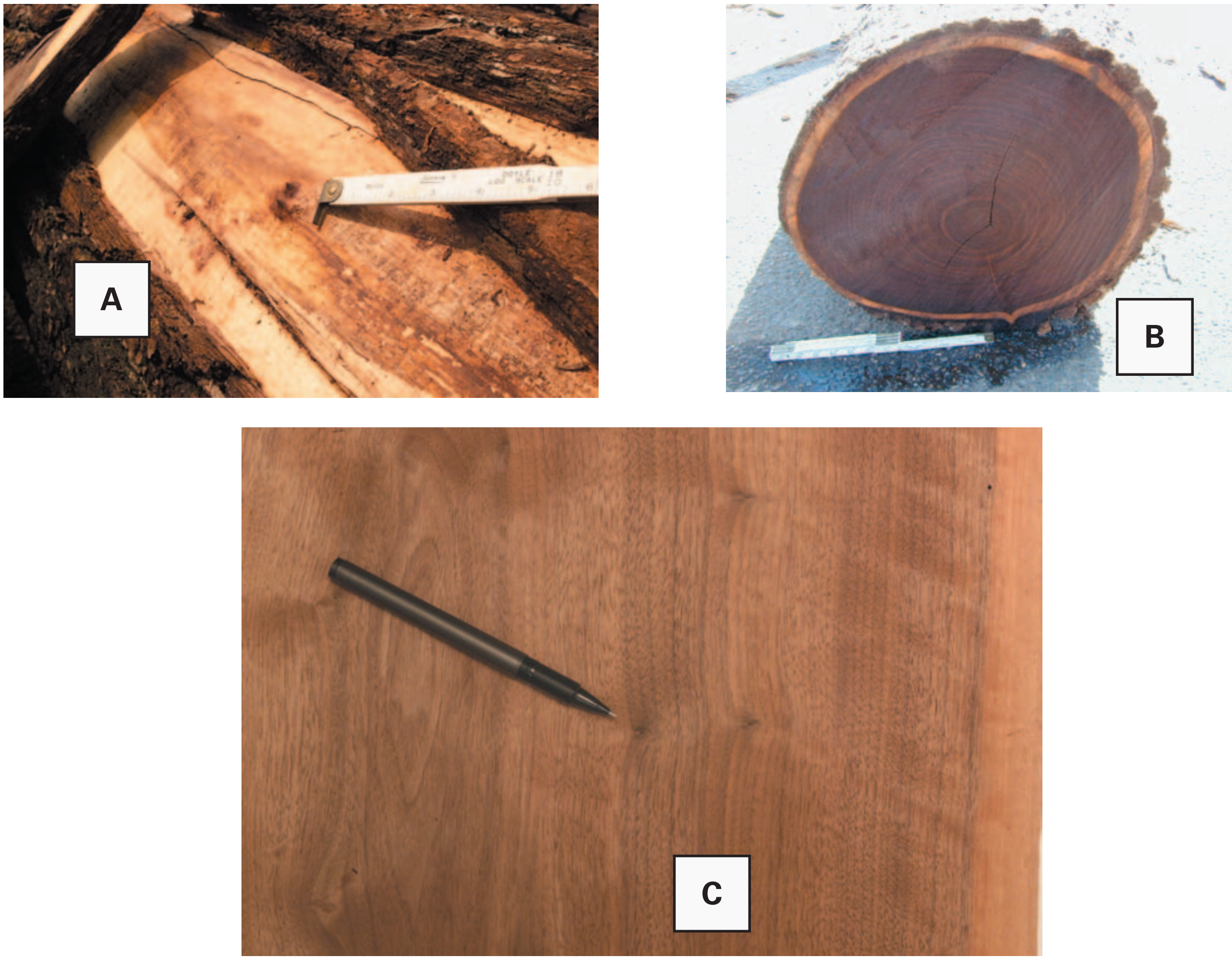


Figure 6.—Pin knots in walnut. (A) shows an easily seen pin knot on a debarked surface of a log. These pin knots are hard or impossible to detect unless the bark is removed or the log is cut (B) from the tree. They will appear as a spot on flat sliced veneer (C) and as light streaks on a quartered surface (B).

Figure 7.—Wide (left) and narrow sapwood on two walnut logs. Sapwood is considered a defect in walnut veneer.

Figure 8.—Bark on normal and slow growth walnut trees.

Figure 9.—Slight flash or figure in walnut veneer. This level of figure is not desirable. Numerous pin knots can also be seen.

Figure 10.—Heavy and very desirable figure in a flitch of walnut veneer.

Figure 11.—Veneer from a low quality and small natural black walnut burl.

Figure 12.—Figured wood from a walnut stump.

SUMMARY

Walnut is one of our most unique and valued species. In regards to planting, natural regeneration, growth characteristics and genetics, it is probably the most studied temperate hardwood species. Wood quality characteristics at least for veneer, are not generally well understood outside of the veneer industry. Only a limited number of walnut trees have the quality characteristics which command exceptional value. These characteristics need to be studied and better understood.

LITERATURE CITED

Cassens, D.L. 2001. Quality control in lumber purchasing: hardwood lumber grades. FNR-130, West Lafayette, IN: Purdue University, Department of Forestry and Natural Resources. 8 p.

Cassens, D.L. 2003. Factors determining the suitability of trees and logs for the face veneer industry. Purdue University Extension Publication in process.

Hoover, W. 2003. Personal communication on the prices of Indiana walnut saw logs adjusted for inflation.

National Hardwood Lumber Association. 2003. Rules for the measurement and inspection of hardwood and cypress. Box 34518, Memphis, TN 38184-0518. 136 p.

THE BLACK WALNUT RESOURCE IN THE UNITED STATES

Stephen R. Shifley[1]

ABSTRACT—Between 1989 and 1999 the total volume of black walnut (*Juglans nigra* L.) in the United States increased from 1.6 to 2.4 billion cubic feet. Saw log volume (International ¼-inch scale) increased from 4.3 to 7.2 billion board feet. Increases occurred in most states; however, in Michigan, Ohio, Virginia, Pennsylvania, and Kansas black walnut volumes decreased or remained nearly constant and/or mean tree quality appeared to decrease. National forest inventory data is available online and can readily be queried to summarize and map characteristics of black walnut and other species by individual states, groups of counties, or other geographic areas. The majority of black walnut trees occur in natural stands in association with other fine hardwoods. On average black walnut comprises about 11% of the total volume in stands where it occurs. Thus, opportunities to apply silvicultural treatments to increase walnut volume or value in natural stands usually also provide the option to do the same for other associated hardwood species.

Black walnut (*Juglans nigra* L.) occurs naturally throughout the eastern United States (Williams 1999). Black walnut trees typically occur as scattered individuals or in small clusters; rarely do they comprise a majority of the stand basal area or volume in natural stands. Walnut growth and value increment are closely related to site quality, and, natural stands with high quality walnut trees often contain other valuable hardwoods such as northern red oak (*Q. rubra* L.), white oak (*Quercus alba* L.), white ash (*Fraxinus americana* L.), black cherry (*Prunus serotina* Ehrh), or yellow-poplar (*Liriodendron tulipifera* L.).

The vast majority of black walnut occurs in natural stands. Walnut plantations only cover approximately 13,800 acres in the United States, and plantations (walnut and mixed hardwood plantations) currently include only about 1% of all black walnut cubic foot volume in the United States. In natural stands, management (including harvesting) of black walnut is often carried out as part of prescription applied to a mixture of hardwood tree species (Slusher 1997).

Black walnut is highly valued for lumber and veneer. This fact, coupled with walnut's relative rarity, has resulted in an ongoing interest in inventory data detailing the location, volume, size, and quality of black walnut trees and how those attributes have changed over time (e.g., Quigley and Lindmark 1966, Blythe 1973, Cooper and others 1973, Schmidt and Kingsley 1997).

This paper provides a contemporary overview and update of the status of the black walnut resource. In addition to summarizing current conditions, it presents some large-scale trends in the walnut resource over the last 10 years based on the latest data available from the USDA Forest Service's Forest Inventory and Analysis (FIA) program (Miles 2001, Miles and others 2001). The latest available inventory information (mean inventory year of 1999) is compared to information reported for inventory year 1989 by Schmidt and Kingsley (1997) at the Fifth Black Walnut Symposium (Van Sambeek 1997). The latest inventory data is further analyzed to estimate the relative abundance of hardwood species that frequently occur in association with black walnut.

METHODS

Forest inventory data for black walnut and associated species were derived from data available on the USDA Forest Service FIA Web site (http://www.ncrs2.fs.fed.us/4801/FIADB/index.htm). Most data queries and initial summaries were conducted using the FIA data base (Miles and others 2001)

[1]Research Forester (SRS), North Central Research Station, USDA Forest Service, 202 Anheuser-Busch Natural Resources Building, University of Missouri, Columbia, MO 65211-7260. SRS is corresponding author: To contact, call (573) 875-5341 ext 232 or e-mail at sshifley@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

coupled with the on-line FIA Mapmaker software (Miles 2001). Additional summaries and analyses were performed using spreadsheet and statistical software.

Except where otherwise noted, results are for timberland. Timberland is forest land that is capable of producing at least 20 cubic feet per acre per year of industrial wood crops and that is not administratively removed from timber utilization (e.g., through park, wilderness, or preserve status). In the eastern United States, 94% of forest land is classified as timberland (Smith and others 2002). Non-timberlands, (e.g., wooded pastures and wooded strips) do, in aggregate, contain significant quantities of black walnut that contribute to timber supply, wildlife values and other benefits. Schmidt and Kingsley (1997) indicate that black walnut on non-timberland has been estimated to range from 5 to 25% of that on timberland.

FIA inventory methods are undergoing a change from periodic statewide inventories at roughly 10-year intervals to on-going annual inventories that survey approximately 20% of a state's timberland each year (McRoberts 2000). Under the annual inventory system it requires approximately 5 years to complete a state inventory that is equivalent to the prior periodic inventories; thereafter the annual inventory system continuously provides current inventory information based on the most recent 5 years of inventory data. Change in some forest attributes can be estimated from as little as 1 to 4 years of annual inventory data, although the precision of estimates decreases with decreasing sample size.

States in the eastern United States are in differing stages of transition from the periodic to the annual inventory methods. The mean year of inventory and type of inventory data analyzed used in this paper are reported in Table 1. The mean inventory year for all states combined was computed as a weighted mean based on black walnut volume per state. The mean inventory year for the most current available data is 1999. This amounts to a mean remeasurement interval of 10 years from results reported by Schmidt and Kingsley (1997) for the black walnut resource in 1989. As noted in Table 1, there was no new inventory data for Kentucky, Louisiana, Mississippi, New York, North Carolina, or Oklahoma since Schmidt and Kingsley's (1997) report.

For reporting purposes some information on associated trees species was grouped into the select white oak group (primarily white oak; bur oak, *Quercus macrocarpa* Michx.; and chinkapin oak, *Quercus muehlenbergii* Engelm.), the select red oak group (primarily northern red oak; and cherrybark oak, *Quercus falcate* var. *pagodifolia* Ell.), the other

Table 1.—Year of most recent inventory, type of inventory, and remeasurement interval used for analysis. Periodic inventories were conducted prior to 2001 and are complete inventories of all timberland in a state. Annual inventories measure approximately 20% of a state's timberland each year on a continuous basis with each year's selection of plots systematically spread across the state. Five years of annual inventory are typically needed to complete a full state inventory cycle; inventories based on fewer than 5 years of data are unbiased but have a higher variance than a full state inventory cycle. The number of annual measurements is shown in parentheses for the annual inventories used in this analysis. The remeasurement interval indicates the number of years between these most current inventories and those reported by Schmidt and Kingsley (1997) which are used to evaluate change. NA indicates that there is no newer information than that reported by Schmidt and Kingsley (1997).

State	Inventory Type	Remeasurement Interval (yrs)
Alabama	2000 periodic	10
Arkansas	2001 annual (2)	13
Delaware	1999 periodic	13
Georgia	1997 periodic	8
Illinois	1998 periodic	13
Indiana	2002 annual (4)	14
Iowa	2001 annual (3)	10
Kansas	2002 annual (2)	20
Kentucky	1988 periodic	NA
Louisiana	1991 periodic	NA
Maryland	1999 periodic	13
Michigan	2001 annual (2)	8
Minnesota	2002 annual (4)	10
Mississippi	1994 periodic	NA
Missouri	2002 annual (4)	11
Nebraska	2002 annual (2)	8
New Jersey	1999 periodic	12
New York	1993 periodic	NA
North Carolina	1990 periodic	NA
Ohio	2001 annual (1)	10
Oklahoma	1993 periodic	NA
Pennsylvania	2002 annual (3)	12
South Carolina	2002 annual (5)	7
Tennessee	1999 periodic	10
Texas	2002 annual (3)	9
Virginia	2001 annual (5)	8
West Virginia	2002 periodic	13
Wisconsin	2001 annual (2)	5
Mean	1999	10

red oak group (primarily black oak, *Quercus velutina* Lam.; pin oak, *Quercus palustris* Muenchh.; and scarlet oak, *Quercus coccinea* Muenchh.), the ash group (*Fraxinus* spp.), the hickory group (*Carya* spp.) and the hard maple group (primarily sugar maple, *Acer saccharum* Marsh.) (Miles and others 2001).

Trees were classified into four grade classes with grade 1 representing the best quality. Tree grade definitions varied among states, but were most often based upon grading rules by Hanks (1976) and/or by Rast and others (1973). In general, tree grade was determined from the best 12 feet of the lowest 16-foot log or the best 12-foot upper section of the tree if the butt log did not meet minimum grade requirements (e.g., due to rot or defects). Typical minimum DBH limits for grades 1, 2, and 3 were 16 inches, 13 inches, and 11 inches, respectively. Typical minimum scaling diameters inside bark for grades 1, 2, and 3 were 13 inches, 11 inches, and 8 inches, respectively. Additional grading criteria took into account the number of surface defects on logs, and deductions for cull, crook, and/or sweep. The best source of detailed information on grading rules is the appendix of FIA inventory reports for individual states of interest (e.g., Schmidt and others 2000). The most up-to-date source of information on inventory reports is the FIA webpage (http://www.fia.fs.fed.us/).

RESULTS

From 1989 to 1999 the total number of black walnut trees 5 inches DBH and larger increased 23% (162 to 200 million trees) (Fig. 1A). On a percentage basis the greatest increase was in walnut trees > 21 inches DBH; that diameter class increased by 132% (1 million trees) during the inventory interval. However, that increase was on a relatively small initial number of trees. In absolute numbers of trees, the greatest increase (14 million trees) occurred in the 11-inch DBH class (Fig. 1B).

Black walnut volume increased in the 10 states with the greatest current walnut cubic foot volume (Fig. 2). Iowa, with a 13% annual rate of increase (cubic foot basis), had the largest net gain. Virginia and Michigan saw net annual decreases of 3% and 7%, respectively, in black walnut cubic foot volume. Rankings for board foot volume increase and percent volume increase differed slightly from those for cubic volume, but Iowa still had the greatest increase (15% annually) while Virginia and Michigan both declined. The composite category representing “other states” also showed large annual increases in black walnut volume: 10% annual increase for cubic foot volume and 9% annual increase in board foot volume over a 6-year remeasurement interval (Fig. 2). The combined total black walnut cubic foot volume for all states increased from 1.6 to 2.4 billion cubic feet (50% or approximately 4% annually) over the 10-year remeasurement interval (Table 1). Total board foot volume (International ¼-inch scale) increased from 4.3 to 7.2 billion board feet (67% or approximately 6% annually) over the same interval.

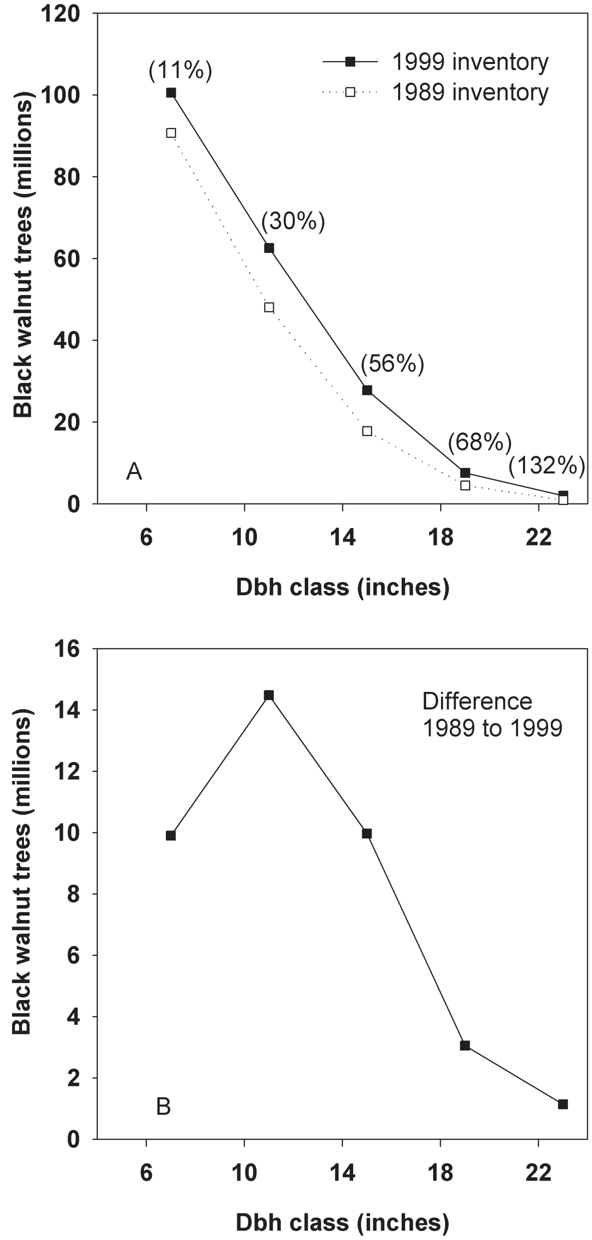


Figure 1.—(A) Number of black walnut trees by DBH class for past (1989) and current (1999) inventories. Inventory dates for individual states are listed in Table 1. Past inventory values are based on Schmidt and Kingsley (1997). Percent increase is shown for individual diameter classes. (B) Net increase by diameter class.

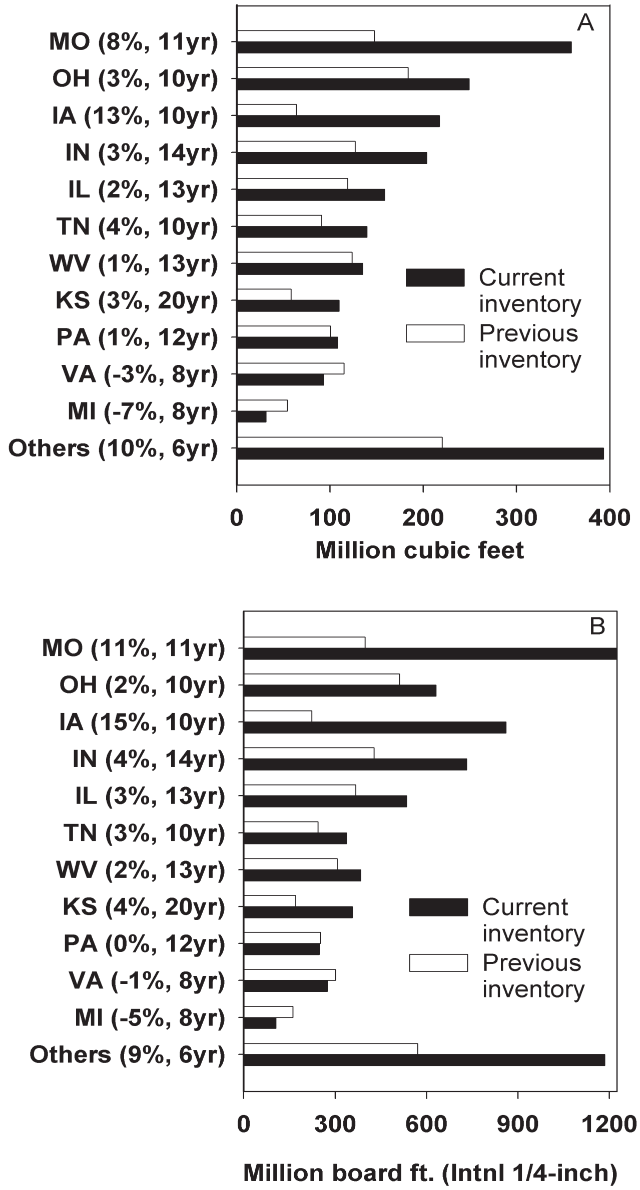


Figure 2.—Volume of black walnut growing stock on timberland. Current values are for the inventory years listed in Table 1; previous values are based on Schmidt and Kingsley (1997). Periodic increase per state is indicated by the difference between bars. State labels show the annual percent change in volume and the remeasurement interval for each state; NA indicates that no remeasured data were available. States are ordered from greatest to least current volume (cubic foot basis). Total cubic foot volume increased from 1.6 to 2.4 billion cubic feet over the period. Total board foot volume (International ¼-inch scale) increased from 4.3 to 7.2 billion board feet (International ¼-inch) over the period.

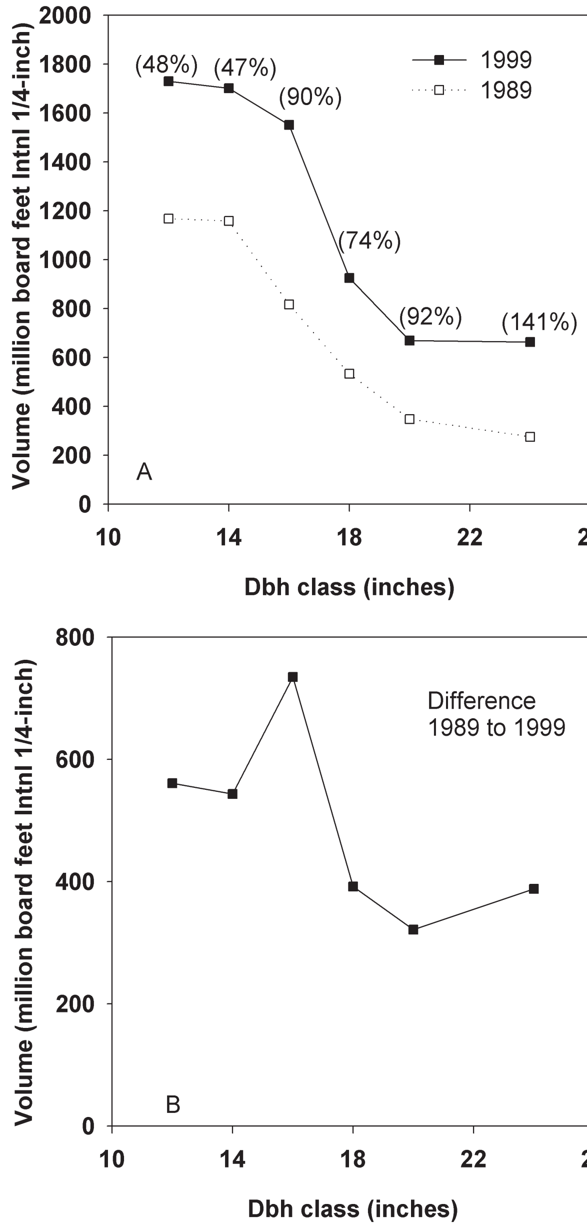


Figure 3.—(A) Black walnut board foot volume by diameter class for past (1989) and current (1999) inventories. Inventory dates for individual states are listed in Table 1. Past inventory values are based on Schmidt and Kingsley (1997). Percent increase is shown for individual diameter classes. (B) Net increase by diameter class over the remeasurement interval of 10 years. Volume increased in all diameter classes with the greatest increases in the 16-inch DBH class.

Across the range of black walnut, the board foot volume of growing stock on timberland increased in all tree diameter classes (Fig. 3). As was observed for the total number of trees, the greatest percentage increase in black walnut volume occurred in the larger diameter classes. Over the 10-year remeasurement period volume increased by more than 90% for black walnut trees > 19 inches DBH. However, on an absolute basis the most volume (735 million board feet) accrued in the 16-inch DBH class.

For all states combined, 16% of black walnut volume was classified as tree grade 1 (best) (Table 2). Tree grades 2 and 3 each included 35% of black walnut board foot volume, and tree grade 4 included the remaining 14%. Michigan and Iowa were on the high end of the quality range for black walnut; they had at least 20% of black walnut volume in tree grade 1. In Michigan, Iowa, Tennessee, Pennsylvania, Indiana, Illinois, and the "Other" group of states at least 50% of black walnut volume was in tree grades 1 and 2. Since the previous measurement, the proportion of volume in tree grades 1 or 2 declined in Ohio, and Kansas. In most other states the proportion of volume in tree grades 3 or 4 decreased while volume in tree grades 1 and 2 increased.

On inventory plots where black walnut occurred, it comprised an average of 11% of the total cubic foot volume (Fig. 4). Iowa and Kansas were at the high end of the range with black walnut comprising 23 and 18%, respectively, of the total volume on sites where walnut occurred. Tennessee was at the low end of the range with an average of 7% black walnut volume on sites where walnut occurred. Across all states black walnut was most commonly found growing in association with select white oaks (10%), yellow-poplar (9%), hickory (9%), other red oaks (8%), ash (7%), select red oaks (5%), and hard maple (4%). Other species comprised the remaining 35% of the volume on plots where black walnut occurred. In most states black walnut comprised the plurality of the volume by species or species groups (Fig. 4) in plots where it occurred. In Missouri and Illinois the total volume of the select white oak group exceed the total walnut volume on plots where walnut occurred. Similarly the total volume of yellow-poplar volume exceeded black walnut volume on plots in Kentucky, Tennessee, West Virginia, and the group of "other" states.

DISCUSSION

In the 10 years since the last comprehensive inventory and summary of the black walnut resource in the eastern United States (Schmidt and Kingsley 1997), the number and volume of black walnut trees has increased (Figs. 1, 3). This continues a trend that goes back to at least 1966 (Quigley and Lindmark 1966). Thus, across the region there is little reason to be concerned that the black walnut is being depleted at current rates of

Table 2.—Black walnut sawtimber volume by state and tree grade, 1999. Percent change from previous inventory as reported by Schmidt and Kingsley (1997) is shown in italics. State entries are ordered from best (lowest) to worst (highest) average tree grade. Tree grade definitions differ among some states as described in the text. Statistics showing change over time within a given state are based on a consistent set of tree grade definitions.

State	Remeas. Interval	Grade 1		Grade 2		Grade 3		Grade 4	
		Current	Change	Current	Change	Current	Change	Current	Change
MI	8	28	*8*	41	*-5*	31	*0*	0	*-3*
IL	13	16	*3*	48	*10*	33	*-8*	3	*-5*
IA	10	20	*8*	42	*8*	29	*-21*	9	*5*
Others		17	*7*	42	*15*	32	*-21*	9	*-1*
PA	12	18	*15*	36	*11*	34	*-12*	11	*-15*
TN	10	19	*14*	31	*13*	42	*-21*	8	*-6*
IN	14	17	*6*	36	*6*	27	*-23*	19	*10*
MO	11	15	*10*	31	*12*	38	*-7*	15	*-16*
WV	13	15	*2*	32	*1*	34	*-4*	19	*1*
KS	20	5	*-19*	41	*4*	44	*5*	10	*10*
VA	8	8	*4*	32	*2*	41	*-14*	19	*8*
OH	10	16	*7*	15	*-7*	42	*-21*	28	*21*
All states	10	**16**	*6*	**35**	*7*	**35**	*-16*	**14**	*3*

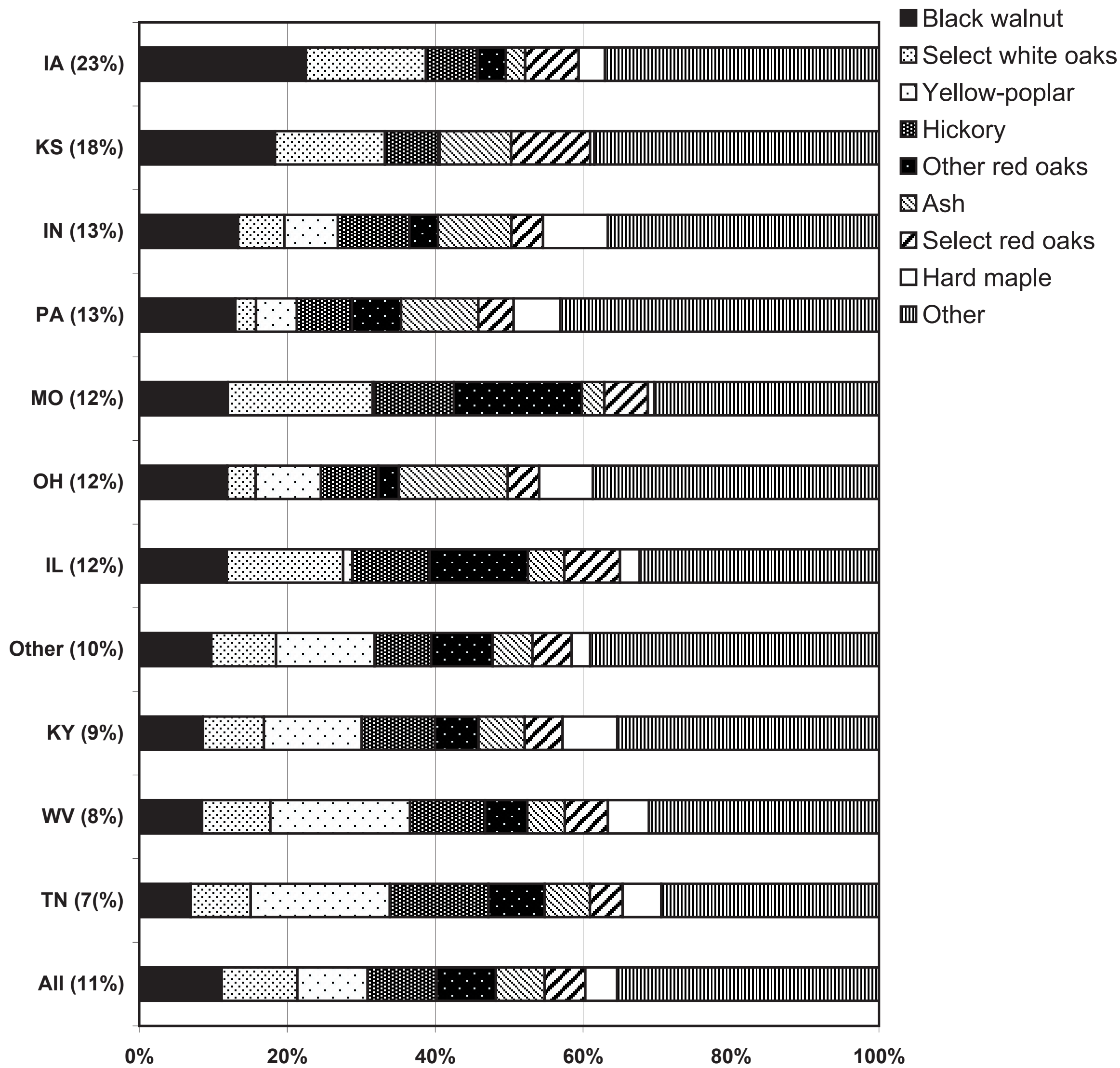


Figure 4.—Proportion of volume by species or species groups for inventory plots that contain black walnut. Proportions are based on total cubic feet of growing stock volume on timberland. The total proportion of black walnut volume is listed with the state name. Across all states, black walnut comprises 11% of the volume where it occurs.

harvest and natural mortality. However, the walnut resources merit continued scrutiny in Michigan, Virginia, and Pennsylvania where walnut volume is flat or decreasing. The shift to annual (continuous) FIA inventories (McRoberts 2000) provides an excellent mechanism to monitor the black walnut resource on a continuing basis.

The composite reverse-J-shaped diameter distribution for black walnut trees in the region (Fig. 1A) is expected for any species that has experienced decades of successful reproduction and growth in the absence of excessive depletion by insects, disease, harvesting or other disturbance factors. What is puzzling about this pattern is that the greatest absolute increase in walnut abundance is in the 11-inch DBH class and not in smaller (7-inch) DBH class (Fig. 1B). This could be a natural consequence of an abundance of well-stocked, maturing forests that provide fewer opportunities for walnut regeneration than in the past. Over the long term this pattern bears watching regionally and locally to ascertain whether or not fewer black walnut trees will be moving into the 11-inch DBH class in coming decades. In the short term the fact that the largest percentage increases in the number and volume of black walnut trees has occurred in the largest diameter classes bodes well for those interested in an abundance of large, black walnut trees for products or other values.

Percentage increases in board foot volume were high in the larger diameter classes (Fig. 3), but the absolute increase in board foot volume peaked in the 16-inch diameter class (735 million board feet). The overall increase in walnut volume was substantial (nearly 3 billion board feet over the remeasurement interval), but states did not share equally in the increase. Although most states registered net increases in black walnut board foot volume (2% to 15% annually) (Fig. 2), volume in Pennsylvania remained virtually unchanged and decreased in Virginia (-1%) and Michigan (-5%). This highlights the fact that broad regional trends tell only part of the story. Local patterns may differ from regional trends due to a whole host of factors.

The availability of web-based tools for summarizing FIA data makes it relatively easy to explore trends for individual states, ecoregions, groups of counties, or even circular areas around a specific point (e.g., the procurement zone around a sawmill) (Miles 2001, Miles and others 2001). Data presented in Table 2 and Figures 1-4 were summarized from retrievals made from the FIA web-based retrieval system. Individuals with interest in a smaller geographic region can summarize similar statistics specific to that area. Web-based mapping tools provide opportunities to display spatial patterns of resource data for counties or larger areas. A web browser and internet access are all that is required to access these data. Spreadsheet software is usually a helpful addition for summarizing and graphing results.

Black walnut lumber and veneer value is greatly affected by log grade. FIA inventories use tree grade as an indicator of quality and value. The proportion of black walnut volume in the two best quality classes increased in most states. This can partially be attributed to the overall increase in the mean diameter of black walnut trees in the sawlog size class during the remeasurement interval. Other factors being equal, tree grade increases when trees increase in scaling diameter to exceed specific merchantability limits (e.g., 8, 11, or 13 inches). Other factors related to tree quality may also be involved in the observed increase in tree grade, but it is difficult to evaluate them separately. As described in the Methods section, grading rules differed among some states. However, the percent changes in tree grade for an individual state (Table 2) for are based on a consistent set of grade definitions.

Ohio, Virginia, Michigan, and Kansas stand out as states that have (1) modest or negative total volume change, (2) decreases in the proportion of volume in tree grades 1 and 2, and (3) increases in the proportion of volume in tree grades 3 and 4. Those states are worthy of closer monitoring as new inventories are completed in coming years.

Black walnut rarely occurs in pure or nearly pure stands. However it often occurs on good sites in association with other valuable hardwoods. Across all plots where black walnut occurred, it comprised an average of 11% of the board foot volume—more than any other individual species. Select white oaks, yellow-poplar and hickories had slightly less volume, followed by other red oak, ash, select red oaks, and hard maples. Other species comprised the remainder, an average of 35% of the saw log volume. Consequently, black walnut growing in natural stands usually provides the opportunity to simultaneously manage a suite of hardwood species with the potential to produce high-valued timber and often to produce abundant mast as well.

There was considerable variation among states in the relative abundance of black walnut sawtimber volume on plots where black walnut occurred. For individual states black walnut had the plurality of board foot volume in only five: Iowa (23%), Kansas (18%), Indiana (13%), Pennsylvania (13%), and Ohio (12%). In Tennessee, West Virginia, and the group of "other" states the volume of yellow-poplar exceeded walnut volume on those plots where walnut occurred. For Illinois and Missouri the volume of select white oaks exceeded the volume of walnut on plots where walnut occurred. Thus, black walnut management opportunities are most often found in mixed hardwood stands where general hardwood management guides (e.g., Sander 1977, Clark and Hutchinson 1998, Perkey and others 1993, Johnson and others 2002) apply. For best results with black walnut, however, hardwood management guides should be modified with guidelines specific to black walnut (e.g., Schlesinger 1977, Burde 1988, Van Sambeek 1997).

CONCLUSIONS

The number, volume, and general quality of black walnut trees in the eastern Unites States has increased since conditions were last reported by Schmidt and Kingsley (1997) at the Fifth Black Walnut Symposium. These increases continue a trend dating to 1966 (Quigley and Lindmark 1966). Across the region increases in black walnut abundance were observed for a wide range of diameter classes. The number of black walnut trees between 5 and 9 inches DBH (the smallest DBH class examined) increased, but at a rate less than or equal to some larger diameter classes. This pattern should be monitored as new inventory information becomes available.

All states did not fair equally in the generally rosy outlook for the black walnut resource. Michigan, Ohio, Virginia, Pennsylvania, and Kansas stand out as states where black walnut volume is low or declining and/or where overall tree quality appeared to decrease. This too should be monitored in subsequent inventories.

Some excellent tools are available to query FIA databases for specific information about forest resources in individual states, groups of counties, or mill procurement zones. These web-based tools can be accessed at http://www.ncrs2.fs.fed.us/4801/FIADB/index.htm

The vast majority of black walnut volume is found in natural stands in association with other hardwoods, many of which are valuable or potentially so. In stands where black walnut occurs, it is often the species with the greatest volume per acre, but on average it comprises only about 11% of the total volume. Thus, opportunities to increase walnut volume or value generally provide opportunities to do the same for other hardwood species.

ACKNOWLEDGMENTS

Pat Miles and Gary Brand (North Central Research Station, St. Paul, MN) provided technical assistance necessary for some of the more complex retrievals from the FIA on-line databases. Jerry Van Sambeek (North Central Research Station, Columbia, MO) and Hank Stelzer (Department of Forestry, University of Missouri, Columbia) provided helpful comments on an earlier version of this manuscript. I thank them all.

LITERATURE CITED

Blythe, J.E. 1973. Timber demand and use. In: Black walnut as a crop; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 7-9. http://ncrs.fs.fed.us/pubs/gtr/gtr_nc004.pdf.

Burde, E.L., ed. 1988. Walnut notes. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. loose-leaf. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=1748.

Clark, F.B.; Hutchinson, J.G., eds. 1989. Central hardwood notes. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. loose-leaf. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=1437.

Cooper, G.A.; Landt, E.F.; Lindmark, R.D.; Steward, H.A. 1973. Changing resource and utilization. In: Black walnut as a crop; 1973 August 14-15; Carbondale, IL. Gen. Tech. Rep NC-4. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 10-16. http://ncrs.fs.fed.us/pubs/gtr/gtr_nc004.pdf.

Hanks, L.F. 1976. Hardwood tree grades for factory lumber. Res. Pap. NE-333. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 81 p. http://www.fs.fed.us/ne/newtown_square/publications/research_papers/pdfs/scanned/rp333.pdf.

Johnson, P.S.; Shifley, S.R.; Rogers, R. 2002. The ecology and silviculture of oaks. Wallingford, Oxon, UK: CABI Publishing. 503 p.

McRoberts, R.E. 2000. Background for AFIS, the annual forest inventory system. In: McRoberts, R.E.; Reams, G.A.; Van Deusen, P.C., eds. Proceedings, 1st annual forest inventory and analysis symposium; 1999 November 2-3; San Antonio, TX. Gen. Tech. Rep. NC-213. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station: 1-4. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=277.

Miles, P.D.; Brand, G.J.; Alerich, C.L.; Bednar, L.F.; Woudenberg, S.W.; Glover, J.F.; Ezell, E.N. 2001. The forest inventory and analysis database description and users manual version 1.0. Gen. Tech. Rep. NC-218. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 130 p. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=840.

Miles, P.D. 2001. Forest Inventory Mapmaker users guide. Gen. Tech. Rep. NC-221. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 52 p. http://www.ncrs.fs.fed.us/pubs/gtr/gtr_nc221.pdf.

Perkey, A.W.; Wilkins, B.L.; Smith, H.C. 1993. Crop tree management in eastern hardwoods. Forest resources management publication NA-TP-19-93. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Northeastern Area, State and Private Forestry. 104 p. http://www.fs.fed.us/na/morgantown/frm/perkey/ctm/ctm_index.html.

Quigley, K.L.; Lindmark, R.D. 1966. Timber resources. In: Black walnut culture (First black walnut symposium). St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 6-12.

Rast, E.D.; Sonderman, D.L.; Gammon, G.L. 1973. A guide to hardwood log grading. Gen. Tech. Rep. NE-1. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 31 p. http://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/scanned/gtr1.pdf

Sander, I.L. 1977. Manager's handbook for oaks in the north central States. Gen. Tech. Rep. NC-37. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 35 p. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=102.

Schlesinger, R.C.; Funk, D.T. 1977. Manager's handbook for black walnut. Gen. Tech. Rep. NC-38. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 22 p. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=103

Schmidt, T.L; Kingsley, N.H. 1997. Status of black walnut in the United States. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut; proceedings, 5th black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 9-22. hwttp://ncrs.fs.fed.us/pubs/viewpub.asp?key=255.

Schmidt, T.L.; Hansen, M.H.; Solomakos, J.A. 2000. Indiana's forests in 1998. Resour. Bull. NC–196. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 139 p. http://ncrs.fs.fed.us/pubs/rb/rb_nc196.pdf.

Smith, W.B.; Vissage, J.S.; Darr, D.R.; Sheffield, R.M. 2002. Forest resources of the United States, 1997. Gen. Tech. Rep. NC-219. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 190 p. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=845.

Slusher, J.P. 1997. Principles of managing for black walnut in mixed hardwood stands. In: Van Sambeek, J.W., ed. Knowledge for the future of black walnut; proceedings, 5th black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 148-154. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=255.

Van Sambeek, J.W., ed. 1997. Knowledge for the future of black walnut; proceedings, 5th black walnut symposium; 1996 July 28-31; Springfield, MO. Gen. Tech. Rep. NC-191. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 256 p. http://ncrs.fs.fed.us/pubs/viewpub.asp?key=255.

Williams, R.D. 1999. *Juglans nigra* L.—Black walnut. In: Burns, R.M.; Honkala, B.M., tech. coords. Silvics of North America, Volume 2, Hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 391-399. http://www.na.fs.fed.us/spfo/pubs/silvics_manual/table_of_contents.htm.

PERFORMANCE OF BLACK WALNUT IN THE YELLOW RIVER WATERSHED OF THE PEOPLE'S REPUBLIC OF CHINA

J. W. Van Sambeek, Sheng-Ke Xi, William A. Gustafson, and Mark V. Coggeshall[1]

The introduction and evaluation of black walnut (*Juglans nigra* L.) into the People's Republic of China is a relatively recent development (Xi and others 1999). Small isolated black walnut plantings established by missionaries from the United States can be found throughout much of China; however, few written records document the growth of the surviving black walnut trees. In the early 1980s, the Chinese Academy of Forestry in Beijing and the University of Nebraska initiated a cooperative venture with the Friendship Nursery in Henan Province to exchange walnut and pecan germplasm. Wang (1996) estimates this venture resulted in more than 40 acres of black walnut plantings as nine demonstration plantings, nut cultivar trials, or scionbank orchards.

In March 1995, the Forestry Research Institute of China, the University of Nebraska, the Northern Nut Growers Association, and the Forestry Bureau of Luoning County called together nursery specialists and research horticulturists from China and the United States to evaluate the suitability of introducing black walnut and its economic potential for plantation culture in the semi-arid regions of China. Evaluation of existing plantings indicated black walnut is well adapted to the lower and middle regions of the Yellow River watershed.

Wang (1996) report expected annual growth of 4 to 6 feet for height and 0.6 to 1.0 inches in trunk diameter. He also reported walnut to be tolerant of droughts and deeply rooted with taproot length exceeding height. Because of the rapid growth and high value for the wood, the economic value of black walnut was estimated at 10-fold that for Chinese poplar (*Populus* spp.) and paulownia (*Paulownia tomentosa* (Thunb.) Sieb. & Zucc.). One conference recommendation suggested that black walnut should gradually replace both poplar and paulownia when reforesting farming areas in the Yellow River watershed. Under the Natural Forest Conservation Program initiated in 1998, China plans to convert 15 million acres of farmland to plantation forests within 10 years for soil erosion control in the Yangtze and Yellow River watersheds (Zhang and others 2000). Recently, Xi (1999) published guidelines in Chinese for the introduction, establishment and management of black walnut.

In the fall of 1996, a 10-person delegation from the Chinese Academy of Forestry and the Henan Bureau of Forestry journeyed to Nebraska, Kansas, Missouri, and Illinois to collect nuts from timber-type black walnut trees. Their objective was to establish multiple progeny tests and possibly identify the most appropriate areas in the United States for future seed purchases. Nuts were collected from plus-trees growing at the following five locations: the Pleasant Valley seed orchard (PVSO) located in Alexander County, IL and maintained by the Shawnee National Forest; the Tree Improvement Center arboretum (TICA) located in Jackson County, IL and maintained by Southern Illinois University; a grafted orchard (HGO) maintained by Leander Hay and located in Saline County, MO; Tuttle Creek Experimental Area provenance test (KPT) located in Riley County, KS and maintained by the Kansas State University; and several natural stands located near the Blue Bird Nursery (BBN) in Colfax County, NE. When possible, the source of the maternal germplasm was determined from plantation records or cultivar histories to identify county and state of origin, plant hardiness zone (Cathey 1990), and normal annual total precipitation and mean length of frost-free period (Hicks 1998). Plant hardiness zones are 4a for BBN, 5b for KPT and HGO, 6a for TICA, and 6b for PVSO.

Nuts from 37 individual trees (half-sib families) were cleaned and floated at the University of Nebraska. Sound seed was flown to Beijing, stratified for 3 months, pre-germinated, and planted into containers in a greenhouse. In-leaf seedlings of

[1]Research Plant Physiologist (JWVS), North Central Research Station, USDA Forest Service, Columbia, MO 65211-7260; Horticulture Specialist (S-KX), State Administration for Foreign Experts, Beijing, People's Republic of China; Horticulturist (WAG), University of Nebraska, Lincoln, NE 68583; and Tree Improvement Specialist (MVC), Center for Agroforestry, University of Missouri, 10 Research Drive, New Franklin, MO 65274. JWVS is corresponding author: to contact, call 573-875-5341 or e-mail at jvansambeek@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

most families were transported and planted at sites in plant hardiness zones 6 (Beijing provenance), 7 (Shanxi provenance), and 8 (Henan provenance) (Widrlechner 1997).

The Beijing progeny test (40.5 N, 116.5 E) is located on a first terrace site near the Badaling station of the Great Wall (Fig. 1). This planting site has loam soils and subject to periodic flooding. The Shanxi progeny test is part of the XiaoYi Nursery in the TaiYuan Basin (Fig. 1). This site has deep, slightly alkaline, dark brown soils and a mean annual precipitation of 18 to 20 inches with a 160- to 180-day growing season. The Henan progeny test (34.8 N, 113.5 E) is part of the Friendship Nursery near Zhengzhou (Fig. 1). This planting site has a deep sandy loam with a 60-foot deep water table, mean annual precipitation of 23 to 24 inches falling primarily in the fall, and a 220-day growing season.

In-leaf seedlings of 30 or more families were planted in June 1997 in each progeny test using a randomized block design with row plots within each of four blocks. Planting design used four-tree plots at Henan and Shanxi tests and five-tree plots at Beijing test. Tree spacing is nominally 10 feet apart within row and 12 to 15 feet between rows. Within row vegetation was controlled for 4 years by hand cultivation. Survival, stem height (H) and

Figure 1.—Black walnut progeny tests were established within the Yellow River watershed near Beijing, in Shanxi provenance near Taiyuan, and in Henan Provenance near Zhengzhou. For comparison, maps of China and North America are aligned along 18 and 54 degrees north latitude.

trunk diameter (D) have been determined annually. Stem volume was estimated as D^2H. For each planting site, the performance index of each family was calculated as the average of the individual family percentile rank for stem height, diameter, and volume. Percentile rank within plantings was calculated as ((family mean—mean of poorest family)/(mean of best family—mean of poorest family) for each variable.

After four growing seasons, survival of the walnut seedlings has exceeded 95% at both the Henan and Shanxi plantings. Survival among families at the Beijing progeny test range from 20% to 95%. Mortality is the result of a summer flood in 1997 when 26% of the seedlings died and then failure to overwinter when an additional 17% of the seedlings died. In the spring of 1999, the ambrosia beetle (*Xylosandrus germanus*) attacked many of the surviving saplings causing extensive stem dieback and resprouting but little additional mortality.

The Henan progeny test has had the most rapid tree growth with average tree heights ranging from 16 to 23 feet among families after four growing seasons (Table 1). Because of the rapid height growth, the trees are exceedingly thin with average trunk diameters of only 1.1 to 1.5 inches. In the fall 2000, many of the trees showed premature defoliation in response to walnut anthracnose (*Gnomonia leptostyla*). Although anthracnose has relatively little impact on growth of walnut in the Central Hardwood region (Van Sambeek 2002), the longer growing season in China will likely result in a higher incidence of premature defoliation and a greater impact on tree growth. In addition, future growth in the Henan progeny test is likely to be slowed following the removal of all lateral branches during August 2000 and used as scionwood in the nursery.

The Beijing progeny test had the most variable height growth ranged from 14 to 24 feet among families (Table 1). Part of the variation is in response to the ambrosia beetle that caused extensive stem dieback and subsequent basal sprouting in the spring 1999. Weber and McPherson (1984) indicate this insect was introduced from eastern Asia into the United States. One growing season after being attacked, most trees had produced basal sprouts that were nearly as tall as the pre-attack tree heights. Weber (1981) reported similar basal sprouting and height growth on walnut saplings attacked by the ambrosia beetle in the Central Hardwood region. The walnut saplings in the Beijing progeny test are also exceedingly thin with average trunk diameters of only 0.7 to 1.3 inches.

Table 1.—Black walnut progeny collection number and name; site of nut collection; origin of maternal germplasm by state, plant hardiness zone, annual rainfall, and frost-free days; performance index (mean of the percentile rankings[1]); and mean height of 4-year-old saplings at three locations in Yellow River watershed of central China.

Progeny		Harvest Site	Maternal Germplasm Variables				Perf. Index	Walnut Age 4 Height		
No.	Name[2]		Origin	P.H. Zone	Rain-Fall	Frost-Free		Henan	Shanxi	Beijing
					-in-	-days-	-%-	-ft-	-ft-	-ft-
16	NC-5869 AA-34	TICA	IL	6a	41	200	0.90	22.2	14.4	-----
27	NC-6134 IB-07	TICA	TN	7a	52	180	0.80	20.4	16.6	-----
20	NC-6616 B#1	PVSO	IL	6b	45	210	0.73	23.2	14.4	-----
23	Native Hay	HGO	MO	5b	40	180	0.72	20.9	13.5	23.0
4	NC-6600 B#4	PVSO	TN	7a	50	200	0.71	21.7	-----	23.3
29	NC-6603 B#3	PVSO	TN	7a	50	210	0.68	20.4	13.3	-----
8	NC-6138 PB-09	TICA	IA	5a	32	170	0.67	22.1	16.3	17.4
13	Carb #1	BBN	NE	4b	30	160	0.65	19.2	16.2	21.0
15	Cheeks #2 B#3	HGO	unk	unk	unk	unk	0.61	19.5	13.3	24.3
21	NC-5872 CC-20	TICA	MO	6a	45	190	0.59	21.8	13.3	-----
24	Big Tree Clark	BBN	NE	4b	30	160	0.56	21.9	12.9	17.7
9	Kwik Krop	HGO	KS	6a	40	200	0.54	19.9	13.0	18.4
33	NC-6609 B#1	PVSO	KY	6b	48	200	0.54	-----	14.4	18.4
22	NC-6622 B#2	PVSO	TN	6b	50	190	0.52	19.4	14.0	-----
3	NC-5869 AA-30	TICA	IL	6a	41	200	0.52	-----	12.3	21.3
6	KS-2707 #1	KPT	MO	6a	44	200	0.51	21.2	13.4	20.3
12	NC-5875 EE-20	TICA	MI	5b	34	150	0.49	20.7	14.0	19.7
28	Walter Jackson	HGO	KS	5b	37	190	0.47	17.7	15.2	-----
1	KS-2707 #3	KPT	MO	6a	44	200	0.46	17.4	14.2	20.3
36	Clarkson #3-2	BBN	NE	4b	30	160	0.45	22.7	-----	19.0
11	Mixed seedlot	HGO	MO	5b	40	180	0.36	19.3	14.0	18.0
7	NC-6160 KK-13	TICA	IN	5a	38	170	0.36	20.5	13.3	14.4
18	NC-6164 FA-07	TICA	IN	5a	38	170	0.34	16.9	14.3	-----
2	NC-6176 MB-09	TICA	VA	7a	40	180	0.33	20.6	11.2	17.2
10	NC-6163 OA-09	TICA	IN	5a	38	170	0.31	16.0	13.1	20.0
26	NC-6134 IB-05	TICA	TN	7a	52	180	0.25	17.7	14.0	-----
25	Carb #2	BBN	NE	4b	30	160	0.24	-----	12.7	15.4
17	NC-5875 EE-20	TICA	MI	5b	34	150	0.24	18.0	12.9	-----
37	Clarkson #2	BBN	NE	4b	30	160	0.23	17.6	12.5	16.4
5	Clarkson #3-1	BBN	NE	4b	30	160	0.11	16.3	11.7	17.0

The Shanxi progeny test had the slowest height growth and tree height among families averaged between 11 and 17 feet (Table 1). As found in the other two progeny test, the trees in the Shanxi progeny test were also exceeding thin for their height with average trunk diameters of 0.7 to 1.5 inches.

No families were consistently the best or poorest performers across all three plantings (Table 1); however, computing a performance index from percentile ranks did allow us to rank the families across the three plantings. The performance index of 0.90 for progeny #16 indicates it was not always the top performer for height, diameter, and volume; however, it does suggest it was one of the best performers in the progeny tests that it was included in. Likewise, the performance index of 0.11 for progeny #5 indicates it performed poorly but was not consistently the poorest performer.

All five locations (PVSO, TICA, HGO, KPT, and BBN) from which nuts were collected produced families with performance indices above and below the mean. All locations, except for the BBN, were established with seed or scionwood from the walnut range, thus, the source of the paternal genes (pollen parent) is largely unknown. We determined the original source of the maternal genes for most of the open-pollinated families. Presumably by analyzing climatic variables from which maternal genes originated and the family performance index, we could identify the most appropriate provenances within the Central Hardwood region for future seed collections.

Maternal genes originated from 10 states and four plant hardiness zones (Table 1). Families originating from Tennessee (plant hardiness zones 6b and 7a) frequently have higher performance indices than families from Nebraska (plant hardiness zone 4b). Statistically, there is a significant linear relationship between the plant hardiness zone from which the maternal genes originated and the mean performance index of the half-sib progeny ($R^2 = 0.161$, $p = 0.031$, 27 df). This relationship may be curvilinear because the performance index for families originating from plant hardiness zones 6a and 6b have the greatest percentage of families with above average performance (Fig. 2).

There is a highly significant linear relationship between mean annual rainfall from the region where the maternal genes originated and the mean performance index of the half-sib progeny ($R^2 = 0.3011$, $p = 0.002$, 27 df). Seven of the eight families originating from areas in the United States with greater than 40 inches of annual rainfall had above average performance indices (Fig. 3). This was unexpected considering precipitation averages less than 25 inches annually at all three sites in China.

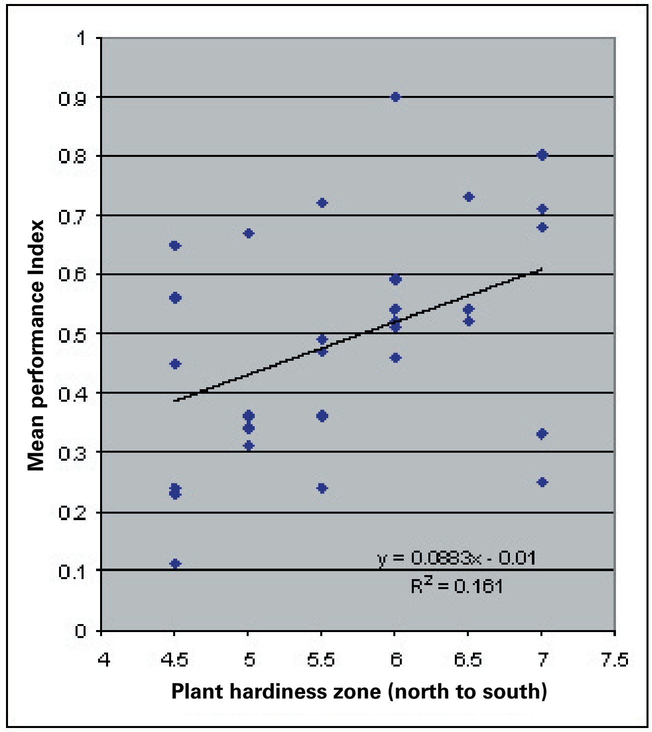


Figure 2.—Performance index for open-pollinated families in Yellow River watershed and plant hardiness zone origin of maternal genes.

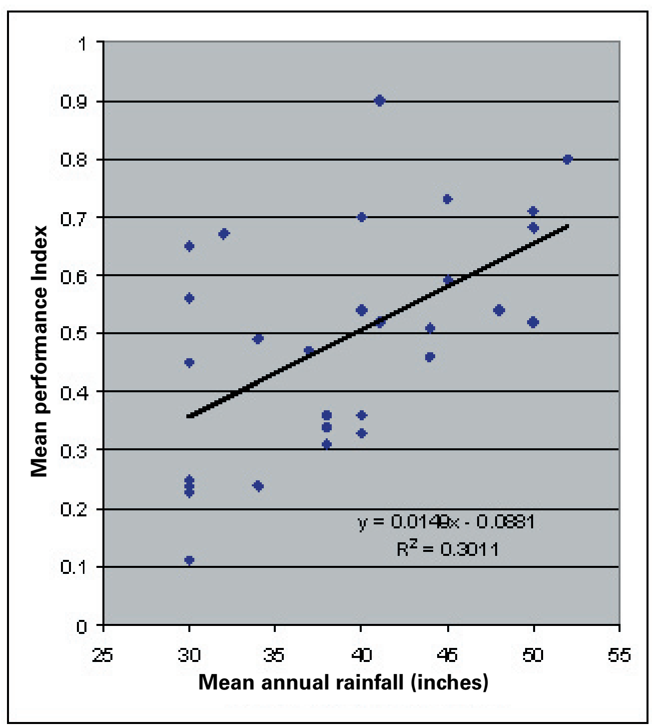


Figure 3.—Performance index for open-pollinated families in Yellow River watershed and mean annual rainfall for region from which maternal genes originated.

There is also a highly significant linear relationship between mean number of frost-free days in region from which maternal genes originated and the mean performance index of the half-sib progeny (R^2 = 0.2868, p = 0.003, 27 df). Nearly all families where maternal genes originated from regions with 190 or more frost-free days during the growing season had above average performance indices (Fig. 4).

In conclusion, we found families throughout the Central Hardwood region that performed well in the Yellow River watershed of China. We also found that seed collections from certain geographic regions were more likely to yield highly successful families. Preliminary guidelines suggest seed should originate from areas within plant hardiness zones 6a and 6b that have a mean annual rainfall in excess of 40 inches with a growing season of 190 or more frost-free days.

ACKNOWLEDGMENTS

The authors express their appreciation to Cyril Bish, Leander Hays, Wayne Geyer, Todd Morrisey, and Harlan Hamernik for assistance in seed collections and Lindsey Van Sambeek for preparing illustrations.

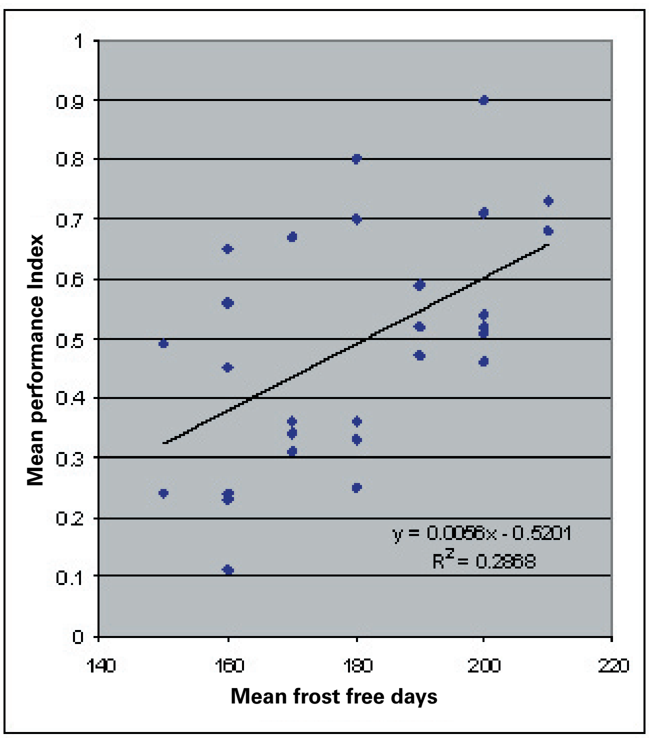


Figure 4.—Performance index of open-pollinated families in Yellow River watershed and length of growing season where maternal genes originated.

LITERATURE CITED

Cathey, H.A. 1990. USDA plant hardiness zone map. Misc. Publ. 1475. Washington, DC: U.S. Department of Agriculture, Agriculture Research Service.

Hicks, R.R., Jr. 1998. The Central Hardwood Region. In: Ecology and management of Central Hardwood Forests. New York, NY: John Wiley & Sons, Inc.: 1-73.

Van Sambeek, J.W. 2002. Legume ground covers alter defoliation response of black walnut saplings to drought and anthracnose. In: Van Sambeek, J.W.; Dawson, J.O.; Ponder, F., Jr.; Loewenstein, E.F.; Fralish, J.S., eds. Proceedings, 13th Central Hardwood Forest conference. Gen. Tech. Rep. NC-234. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station: 556-564.

Xi, S.K. 1999. Introduction and cultivation of the American eastern black walnut. Beijing, China: Chinese Academy of Forestry, Institute of Forestry. 100 p.

Xi, S.K.; Wang, Z.L.; Dong, P.; Dong, F.-X. 1999. American black walnut importation and extension. Unnumbered report. Beijing, China: Black Walnut Project Group, Chinese Academy of Forestry. 18 p.

Wang, Z.L. 1996. The introduction and extension of American black walnut. 11 minute videotape. Forestry Bureau of Luoning County, Henan Province, People's Republic of China.

Weber, B.C. 1981. Ambrosia beetles in your black walnut plantation – how serious are they? Annual Report of the Northern Nut Growers Association. 72: 68-74.

Weber, B.C.; McPherson, J.E. 1984. Attack on black walnut trees by the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Forest Science. 30: 864-870.

Widrlechner, M.P. 1997. Hardiness zones in China. Unnumbered map. Ames, IA: U.S. Department of Agriculture, North Central Region Plant Introduction Center, Iowa State University. 1 p.

Zhang, P.C.; Shao, G.-F.; Zha, G.; Le Master, D.C.; Parker, G.R.; Dunning, J.B., Jr.; Li, Q.-L. 2000. China's forest policy for the 21st Century. BioScience. 288: 2135-2136.

Finding a Walnut in a Haystack: How Molecular Biology Can Help Rediscover Lost "Elite" Black Walnuts

Rodney L. Robichaud, James McKenna, and Keith Woeste[1]

The tree improvement program at Purdue University established seed orchards from grafted "elite" source trees (ortets) found in the wild. Several hundred candidate elite trees were sampled from many locations over the past 40 years. Several dozen researchers were involved in the process. It is sometimes desirable or necessary to resample the ortets, but due to either vague or missing records, the exact location of these trees may not be known. We have employed microsatellite DNA markers to unambiguously identify five ortets being tested in the black walnut improvement program. Identification of ortets was accomplished by fingerprinting candidate trees using six highly polymorphic microsatellites and comparing their genotypes to the ramets growing in a black walnut seed orchard.

---

[1]PhD candidate (RLR), Department of Forestry and Natural Resources, Purdue University, 1159 Forestry Building, West Lafayette, IN 47907-1159; Operational Tree Breeder (JM), USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, Purdue University, 1159 Forestry Building, West Lafayette, IN 47907-1159; and Research Molecular Geneticist (KW), USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, Purdue University, 1159 Forestry Building, West Lafayette, IN 47907-1159. RLR is corresponding author: to contact, call (765) 496-7685 or e-mail at rrobichaud@fnr.purdue.edu

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Relationships Between Tree Size and Nut Production for Eastern Black Walnuts

David Brauer, Adrian Ares, and Andrew Thomas[1]

ABSTRACT—Eastern black walnut (*Juglans nigra* L.) is an important tree species for agroforestry and forestry practices. However, the relationships between tree size and nut production are poorly understood. We are reporting results from two studies. In thefirst study, we examined data reported by Zarger (1946) on average trunk diameter (DBH) and annual nut production from 1941 to 1946 for over 150 trees. The R-square values for equations relating nut yield to tree DBH ranged from a low of 0.04 to a high of 0.18 when data from individual years were analyzed. Averaging nut yields over 2 consecutive years did not improve R-square values. However, the R-square value increased to 0.33 when nut yield data from 4 or 6 consecutive years were averaged and then regressed against average DBH.

In the second set of studies, nut yields per tree in 2002 and 2003 were determined for 14 plots with 10 to 50 trees per plot. R-square values of 0.25 and 0.34 were obtained for the regression equations between nut yield and tree DBH for data collected in 2002 and 2003, respectively. The R-square value increased to 0.45 when the 2-year averages for DBH and nut yield were regressed. The R-square value was greater for the regression equation generated from native tree stands then for improved cultivars (0.57 versus 0.27). These results indicate that data from at least 2 consecutive years are needed to assess a tree's nut yield potential and that the relationship between DBH and nut yield may differ between native and improved cultivars.

LITERATURE CITED

Zarger, T.C. 1946. Yield and nut quality of the common black walnut in the Tennessee Valley. Annual Report of the Northern Nut Growers Association. 37: 118-124.

[1]Research Agronomist (DB) and Research Forester (AA), Dale Bumpers Small Farms Research Center, Agriculture Research Service, U.S. Department of Agriculture, Booneville, AR 72927-9214; and Research Associate (AT), Southwest Research Center, University of Missouri-Columbia, Mt. Vernon, MO. DB is corresponding author: to contact, call (479) 675-3834 or e-mail at dbrauer@spa.ars.usda.gov.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen.

Sugar-bushing Black Walnut

G.G. Naughton, W.A. Geyer, and D.L. Chambers[1]

Native Americans enriched their diet of wild game, nuts, and berries with the sugary products from the native maples (*Acer* spp.) in eastern United States. As Europeans settled the land, they also tapped the maple trees eventually improving on the methods of collection. Today, the syrup enjoyed topping our flapjacks is made mostly from the sap of sugar maple (*Acer saccharum* Marsh.); however, most other maples can also be used. Various reports have suggested that sap from other hardwoods could also be used including black walnut (*Juglans nigra* L.). The objective of our study was to determine if sap could be collected from pole-sized black walnut trees and boiled down to make syrup.

Twenty black walnut trees were tapped beginning in middle February and the sap was collected 5 weeks. Trees were approximately 23 years old and 25 to 30 cm in DBH. Trees were tapped about 1 m above the ground on the east side. A carpenter's brace with a No. 10 bit was used to drill the holes. Holes were drilled deep enough to fully penetrate the sapwood. We used polyvinyl double-male hose splicing inserts for spiles. Plastic 3.8 liter milk jugs were used as the primary collectors. A small hole was cut with a knife at the top of the handle and the spile was inserted into the hole. The caps were left on the jugs to minimize contamination. Sap was collected daily when it flowed freely. Later we coordinated the collection with cold nights and warm days. A standard brewer's hydrometer (Balling scale) was used to estimate the sugar content in percentage by weight. Then the sap was mixed together for refrigerated storage until processing. When a sufficient quantity was collected, it was boiled down to 25% of the original volume. Later, a small electric hotplate and a 2 liter cast-iron saucepan were used to concentrate the sap.

The sap quantity varied by individual trees and days. All trees produced sap, but with great variation. Although most trees produced fewer than 2.0 L, the highest producer had 13.21 L and 0.43 L for the lowest tree. The average sugar content was 2.08% with a 1:60 sugar:sap ratio. Sap production was highly correlated with the thickness of the sapwood. We thought that the finished walnut syrup was dark coffee-brown in color, was sweet to the taste, and had no hint of any typical 'walnut flavor'.

Subsequently, we submitted the syrup to the Sensory Center at Kansas State University for evaluation where over 100 consumers taste-tested our product on pancakes. Comparisons were made to Log Cabin syrup, pure maple syrup, and our black walnut product. The Log Cabin product was preferred over the walnut syrup because it was sweeter and had a greater aftertaste. The walnut syrup was diluted to 85% and 50% with little change in appearance. Additional tests with five highly trained tasters indicated that the 50% dilution was more desirable having lower color intensity and no woody, nutty or musty/earthy flavor.

---

[1]Retired Professor (GGN), Professor (WAG), Forestry Department and Professor (DLG), Human Nutrition, Kansas State University, Manhattan, KS 66506. WAG is corresponding author: to contact call (785) 532-1409 or e-mail at wgeyer@oznet.ksu.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Arthropod Communities in Monocropped and Black Walnut-Intercropped Forages

W. Terrell Stamps, Terry L. Woods, Robert L. McGraw, and Marc J. Linit[1]

ABSTRACT—Alley cropping holds promise for increasing insect diversity and reducing pest problems by improving natural enemy complexes and adding competition to pest species. In a series of experiments, we have examined the effects of forages on growth, nut production, and arthropod communities of alley cropped eastern black walnut (*Juglans nigra* L.). An initial experiment compared alley cropped and monocropped smooth bromegrass (*Bromus inermis* Leyss.), alfalfa (*Medicago sativa* L.), and vegetation-free alleys. There were no differences in tree growth among alleyway treatments. The first season's nut yield was greater from trees with vegetation-free alleyways; otherwise nut production did not differ among the treatments. Arthropods were more numerous and diverse in alley cropped alfalfa than in alley cropped bromegrass or in the vegetation-free controls. Arthropod diversity in the tree canopies did not differ among treatments. Alley cropped forages supported a more diverse and even arthropod fauna than did adjacent monocropped forages.

A second, larger, experiment comparing experimental plots of traditionally-grown alfalfa and plots of alfalfa intercropped between rows of black walnut trees has yielded similar results. Herbivore numbers were significantly lower and beneficial arthropod numbers were significantly higher in the more structurally complex alley cropped alfalfa plots than in the monoculture alfalfa plots. On the other hand, alfalfa yields were very poor for alley cropped alfalfa, although fiber values for alley cropped alfalfa indicated higher quality forage. We are addressing the yield problem in a new study incorporating wider alley ways in the hopes of improving alfalfa yields while retaining the insect community benefits of an agroforestry practice.

---

[1]Postdoctoral fellow (WTS), Research Specialist (TLW), and Professor of Entomology (MJL), Department of Entomology, 1-87 Agriculture Bldg.; and Associate Professor of Agronomy (RLM), Department of Agronomy, 210 Waters Hall, University of Missouri, Columbia, MO 65211. WTS is corresponding author: to contact, call (573) 882-0193 or e-mail at stampst@missouri.edu.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

CLIMATIC FACTORS AND BLACK WALNUT NUT PRODUCTION

Neal Sullivan, Felix Ponder, Jr., and Viniece Jennings[1]

Black walnut (*Juglans nigra* L.) nut yield varies both temporally and spatially. That is, production varies among years and for any given year, and production varies across the range for the species. Unpredictable annual yields restrict both market expansion and management of black walnut for nut production. New knowledge on the bearing habits of wild and plantation grown trees is needed. We have begun to examine the relationship between climatic variables and walnut production over a 10-year period for trees grown in the wild. Hammons Products Company, located in Stockton, Missouri, provided walnut production data (metric tons per station per year) from their network of buying stations for the years between 1986 and 1996. This network ranges across 18 states from Kansas east to New York and Tennessee to southern Wisconsin. Because buying stations may have only been open for part of the study period, the data was summarized by dividing the study area into regions defined by latitude and longitude (1° by 1°) and summing walnut production for the region. This data was converted to an index of production for each region-year combination where the index represents the annual production as a proportion of the mean production for that region. In this way, differences among regions in walnut collection intensity are standardized and differences among years are emphasized. Climate data summaries for the 94 regions in which buying stations occurred were extracted from National Climate Data Center records. The climate variables of interest were: early frost for the year preceding nut production; freezing temperatures during the expected pollination period (EPP) for the region; duration of cool temperatures during EPP; relative humidity during the EPP; and cumulative growing season rainfall. Regression analysis is being used to determine if these climatic variables can be used to predict walnut production and the reliability of these predictions.

---

[1]Forest Ecologist (NS), North Central Research Station, 202 ABNR Bldg. University of MO, Columbia, MO 65211, Research Soil Scientist (FPJr), North Central Research Station, 208 Foster Hall, Lincoln University, Jefferson City, MO 65102, and 1890 Scholar (VJ), Delaware State University, Dept. of Agriculture & Natural Resources, 1200 North Dupont Hwy., Dover, DE 19901. NS is corresponding author: to contact, call (573) 875-5341, Ext. 235 or e-mail at nsullivan@fs.fed.us.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

How Much Regeneration is Needed to Minimize Genetic Erosion via Timber Harvest?

Jeffrey C. Glaubitz, Rodney Robichaud, Keith Woeste, and Olin E. Rhodes, Jr.[1]

There is an urgent need for a coordinated and systematic approach to the *in situ* conservation of the genetic resources of commercially important forest tree species in the Central Hardwoods. Effective *in situ* management of genetic resources would benefit from clear guidelines for how many adult trees can be harvested with minimal impact on allelic diversity, given the amount of regeneration that is expected to survive to reproductive maturity. We are constructing a computer simulation model for this purpose, and present preliminary results based upon replicate harvests of a virtual forest stand consisting of 200 adult trees. Our model explores how much regeneration is needed so that there is no more than a 10% risk of retaining less than 90% of the original allelic diversity. In the absence of regeneration, up to 55% of the adult trees can be harvested without exceeding the 10% risk level. At higher harvest intensities, locally-derived regeneration is needed to replace the alleles removed from the adult population. When all 200 adult trees are harvested, the 10% risk level is not exceeded if there are at least 116 regenerants, provided that these are derived from pre-harvest random mating among the adults. In the presence of substantial pollen flow from a genetically differentiated outside pollen source (e.g., 10-20% pollen flow), the minimum amount of regeneration needed is reduced. This indicates that outside pollen can be more efficient, relative to pollen from within the stand, at replacing alleles lost from the adult population. This higher efficiency of pollen from the outside pollen source likely stems from a subset of alleles that are rare in the pre-harvest adult population—and thus prone to loss—yet present at higher frequencies in the outside pollen source. We hope that ongoing refinements of our current model will make it a useful tool to aid the effective management of the genetic resources of valuable Central Hardwood tree species such as black walnut (*Juglans nigra* L.).

---

[1]Geneticist (JG), Hardwood Tree Improvement & Regeneration Center, Dept. of Forestry & Natural Resources, Purdue University, West Lafayette, IN 47907; Molecular Geneticist (RR), Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907; Molecular Geneticist (KW), USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907; Wildlife Geneticist (OR), Dept. of Forestry & Natural Resources, Purdue University, West Lafayette, IN 47907.

Citation for proceedings: Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds. 2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Michler, C.H.; Pijut, P.M.; Van Sambeek, J.W.; Coggeshall, M.V.; Seifert, J.; Woeste, K.; Overton, R.; Ponder, F., Jr., eds.

2004. Black walnut in a new century, proceedings of the 6th Walnut Council research symposium; 2004 July 25-28; Lafayette, IN. Gen. Tech. Rep. NC-243. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Research Station. 188 p.

Presents papers and abstracts relating to genetic improvement, nursery production, plantation establishment, natural stand management, pest management, agroforestry and economics of black walnut and related *Juglans* species.

KEY WORDS: *Juglans*, genetic improvement, plantation culture, agroforestry.

Send requests for publications to:

USDA Forest Service
North Central Research Station
Distribution Center
One Gifford Pinchot Drive
Madison, WI 53705

OR

Walnut Council
Wright Forestry Center
1011 N 725 W
West Lafayette, IN 47906-9431
Tel: [765] 583-3501

Mission Statement

We believe the good life has its roots in clean air, sparkling water, rich soil, healthy economies and a diverse living landscape. Maintaining the good life for generations to come begins with everyday choices about natural resources. The North Central Research Station provides the knowledge and the tools to help people make informed choices. That's how the science we do enhances the quality of people's lives.

For further information contact:

North Central
Research Station
USDA Forest Service
1992 Folwell Avenue
St. Paul, MN 55108

Or visit our web site:

www.ncrs.fs.fed.us

Black Walnut in a New Century

GTR-NC-243