PART 3

THE EASTERN BLACK WALNUT TREE AND FRUIT
Carbohydrate Assimilation, Translocation, and Utilization

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GROWER RECOMMENDATIONS

Tree growth and nut production are primarily a function of carbohydrates made available through photosynthesis. Photosynthesis occurs mainly in the leaves and is controlled by the amount of light, carbon dioxide, and water available to the leaves.

Photosynthesis in walnut increases proportionally to the amount of light reaching the leaves up to one-third of full sunlight. Nut production requires use of thinning and pruning practices that develop trees with large crowns with leaves exposed to light of moderate levels of sunlight for at least part of the day. Pruning is especially important on lateral bearing cultivars to allow light penetration to the fruiting spurs. Carbon dioxide, an essential ingredient for production of photosynthates, enters the leaves primarily through the stomata. Insufficient soil moisture during summer droughts results in reduced transpiration and closure of stomata. Excessive soil moisture can lead to unhealthy roots incapable of supplying sufficient water to the leaves for transpiration. Photosynthesis and respiration are biological processes that involve plant proteins or enzymes made up of carbon, nitrogen, oxygen, and various mineral nutrients. Different parts of a walnut tree have changing demands during the year for photosynthates for respiration, growth of new plant parts, and the developing nut crop. Depending on the cultivar, heavy crop loads may adversely affect the following year’s crop by adversely affecting female flower initiation and reduced amounts of stored carbohydrates. Anthracnose, a common leaf spotting disease, utilizes carbohydrates that normally would be used by the tree for growth and nut production. Late summer fertilization in Missouri may enhance nut production by reducing severity of anthracnose or possibly altering the plant growth regulators involved in female flower initiation and development. The processes associated with nut production appear to be under strong genetic control; thus annual heavy nut production will require selection of walnut cultivars exhibiting multiple leaf layers to maximize photosynthetic production, tendencies towards lateral bearing, good resistance to anthracnose, and efficient use of photosynthates for tree growth and nut production.

TECHNICAL INFORMATION SUPPORTING RECOMMENDATIONS

Many environmental factors and cultural practices can affect the ability of walnut trees to convert atmospheric carbon dioxide (CO2) via leaf photosynthesis into carbohydrates, the subsequent translocation or distribute these carbohydrates throughout the tree, and, finally, their utilization for respiration, growth, or nut production. Little research has been done on carbohydrate assimilation, translocation, and utilization in eastern black walnut; however, what we know about these processes appears similar to what has been found for other walnut and large mast producing species. Thus, when information was not available, research information obtained by other researchers was used on carbohydrate assimilation, translocation, and utilization within English walnut (Juglans regia L.) and pecan to develop the following picture for eastern black walnut. Figure 1 is a schematic
representation of the carbon cycle for eastern black walnut as adapted from the model for English walnut developed by DeJong and Ryugo (1985).

The carbon cycle begins with the conversion of carbon dioxide and water into carbohydrates $\text{CH}_2\text{O}_n$ and oxygen through a process called photosynthesis. Green pigments (chlorophylls) capture light energy from the sun and convert it into chemical energy. This chemical energy is used to drive the reduction of carbon dioxide into simple carbohydrates that eventually become sugars. The actual process consists of a complex series of chemical reactions. As depicted in Figure 1, the leaves take up carbon dioxide and produce sugars that become part of the photosynthetic pool. Depending on the demands for energy within the plant, these photosynthates can be translocated to other plant parts or converted to starches and stored for future use. The sugars are broken down to produce the energy (growth respiration) needed to assimilate other sugar-derived chemicals into various cellular components or broken down to produce the energy needed to maintain living tissues (maintenance respiration).

The carbon dioxide used during photosynthesis comes from the air surrounding the leaves primarily through stomata located on the lower surface of the leaves. The size of the stomatal opening is regulated by two guard cells that are quite sensitive to various environmental factors. These stomatal openings are also important in controlling the transpiration rate or loss of water vapor from the leaves. If insufficient water is available to the leaves, the stomata close and cut off the supply of carbon dioxide needed for photosynthesis to occur.


Figure 1. Carbon cycle for nut-producing eastern black walnut trees.

**FACTORS INFLUENCING PHOTOSYNTHESIS, PHOTOSYNTHATE POOLS, AND RESPIRATION**

**Light intensity** - Walnut leaves produce photosynthates proportional to the amount of sunlight striking the leaf surface. Their leaves can effectively utilize up to approximately one-third of full sunlight after which increases in light do not result in additional production of photosynthates. The same photosynthetic mechanisms are activated at light intensities of five percent of full sunlight. Eastern black walnut has relatively thin crowns, so most leaves are likely to be exposed to light levels within this range for at least part of the day as the sun moves across the sky. If walnut trees are not thinned or the canopies are allowed to become too dense, the inner portions of the tree crowns become so shaded the leaves cannot produce sufficient photosynthates for maintenance respiration and die. This is especially important in high-production nut orchards using cultivars with lateral bearing characteristics. These cultivars have leafy fruiting spurs on the inside of the tree crown along the main branches. On English walnut, if the leaves on these spurs receive insufficient sunlight, the spurs eventually die. It is unclear if lateral bearing cultivars of eastern black walnut will also produce crowns sufficiently dense to lead to death of fruiting spurs.

**Temperature** - Most plant processes, including photosynthesis and respiration, have a minimum temperature at which the process begins and an optimum temperature for maximum efficiency. Above this temperature, the processes slow until lethal temperatures are reached. For English walnut, photosynthesis has a broad optimum between 60 and 86°F after which the rate of photosynthesis rapidly declines. High temperatures are also very likely to limit the rate of photosynthesis in eastern black walnut, especially when the stomata are closed in response to limited soil moisture. Photosynthesis and respiration probably show similar response patterns to increasing temperature, although optimum temperatures for respiration are probably higher than for photosynthesis.

**Deficient water supply** - Insufficient available soil moisture causes stresses that can lead to wilting and premature defoliation under extreme conditions. Under less extreme conditions, the stomata close to decrease the rate of transpiration. When this occurs, carbon dioxide can no longer enter into the leaves through the stomata and photosynthesis decreases. If nut orchards are not going to be irrigated, then soil depth and water holding capacity become very important during site selection for the nut orchard. The water held within the rooting zone determines if adequate soil moisture is available during dry spells. In the Central Hardwood region, droughts usually occur in late summer when there is a high demand for photosynthates to fill the developing nuts. Lack of adequate soil moisture in late summer can also affect the physiological condition of the tree and suppress the initiation of female flowers necessary for the following year's crop.

**Excess water supply** - On soils subject to flooding or with shallow restrictive layers, excess soil moisture can also be a problem. Excess soil moisture during the growing season leads to decreased oxygen in the soil and death of roots needed to absorb adequate soil water during periods of high transpiration. On soils with restrictive layers in the walnut rooting zone, soil water accumulates above the restrictive layer leading to a perched water table during the dormant season. Walnut roots within the perched water table die from a lack of oxygen. If these roots are not replaced during the
growing season, it results in a reduced capacity to absorb soil moisture during the following growing season followed by stomatal closure from moisture stress and subsequent decreases in the rate of photosynthesis.

**Nutrient supply** - Maintaining proper mineral nutrition will be important for maintaining high rates of photosynthesis. Plant nutrients play important roles in the mechanisms for light trapping and in the carbon conversions associated with photosynthesis. Nitrogen is an important component of the amino acids that make up the proteins or enzymes involved in both processes. Phosphorus is an essential element in the chemical processes associated with energy transfer. Elements like potassium, magnesium, manganese, and iron are important cofactors associated with the proteins or enzymes involved with photosynthesis. Other elements like boron are important for nut production in English walnut. Research has shown that late summer application of nitrogen in black walnut plantings will enhance nut production.

**Carbon dioxide concentration** - Although atmospheric changes in carbon dioxide within walnut canopies can range from 350 to 600 ppm, these amounts are still adequate to maintain high photosynthetic rates. In fact, high carbon dioxide concentrations have been shown to increase the growth of eastern black walnut seedlings. However, stomatal closure in response to high temperatures or insufficient soil water will lead to low carbon dioxide concentrations within leaves and reduced rates of photosynthesis.

**Insects and diseases** - Anthracnose, a common leaf spotting disease, can cause significant losses in photosynthates during disease development and premature defoliation of trees. Initial infections occur in the spring and increase rapidly during wet weather. Significant amounts of photosynthates are drawn to the developing infection centers before they become visible. Heavy infection usually leads to premature defoliation during the period of female flower initiation and kernel maturation. Some walnut cultivars show good resistance to the leaf spotting disease. Cultural practices that decrease overwintering spore populations or increase nitrogen availability during the growing season have also been shown to be effective. Insects that feed on foliage reduce the leaf area and can affect the supply of photosynthates to the tree for growth and nut production.

**TRANSLOCATION AND UTILIZATION OF PHOTOSYNTHATES**

The goal of orchard management is to maximize photosynthesis and partitioning of photosynthates into a harvestable nut crop with minimal losses to pests, disease, or excessive growth. Virtually anything that is done to manage the orchard will influence some aspect of the carbon economy. The translocation of carbohydrates from the photosynthetic pool can be viewed as a series of conflicting demands made on a limited resource (Van Sambeek and Rink 1982).

Although leaves are normally thought of as a source of photosynthesize it is important to recognize that they are important sinks for photosynthates during their initiation and development in the spring. The primary sources of photosynthates at this time are stored reserves or starch in the bark and roots. Late spring killing frosts are especially damaging because the walnut tree must initiate a second set of shoots from already depleted storage reserves. It is not until leaves attain about half their maximum size that the sink-source relationships are such that a leaf produces sufficient photosynthesize to complete its development. When leaves reach two-thirds of their maximum size, they become net exporters of photosynthates.
In addition to leaf initiation and elongation, stored starch is also necessary for shoot elongation and development of male catkins and pistillate flowers. Flowers begin to appear about mid April in the south and progressively later until early June in the northern parts of the natural range of eastern black walnut. If the walnut trees produced a heavy crop of nuts the previous year, these starch reserves may only be adequate to develop weak pollen and pistillate flowers. When leaves initiated during bud burst reach full size, they become important sources of photosynthesize for the developing nuts, additional shoot elongation, and initiation of next year’s male catkins. A developing nut crop is a stronger sink for photosynthates than these other processes and can limit additional shoot and leaf initiation and elongation.

During May and June, the fertilized nuts go through a period of rapid expansion. At the same time, the trees are putting on rapid height and diameter growth. During July, photosynthesize demands remain high as nuts go through a period of nut expansion and shell hardening for the next month. Summer droughts during these three months will reduce photosynthesis and availability of photosynthates to the developing nuts resulting in small nuts.

In August, the embryo grows into the nut cavity and absorbs the endosperm. During September, fats are deposited as the kernel develops and matures. This is also the period when walnut forms pistillate flower initials in the terminal buds. Because photosynthesize sinks closest to the leaves tend to be the strongest, foliar leaf diseases like anthracnose can utilize a significant amount of the photosynthesize produced. Insufficient photosynthates in the late summer and fall can result in shriveled kernels and development of weak pistillate flowers. Once the nuts are mature and until the leaves are lost to disease or a killing frost, photosynthates are translocated to storage tissues for utilization the following spring. The mature nuts usually drop a few days after the leaves fall in October.

Recent studies in southern Missouri have shown mid-August fertilization with chemical weed control can significantly enhance nut production the following year. Because late summer fertilization can delay dormancy, recommendations still need to be developed for the timing of fertilization in the northern part of the walnut range to prevent winter damage. Several explanations have been suggested as to why later summer fertilization increases nut production. Increased nitrogen within the branch tips may alter the plant growth regulators responsible for the fall initiation of female flowers with the dormant buds. It may be the added nitrogen suppresses foliar diseases so that leaves are retained for a longer period and increase the stored reserves. It is generally recognized that increasing the length of leaf retention following nut maturation will decrease the alternate bearing tendency. The choice of ground covers can also greatly influence the retention of leaves into the fall and the amount of stored reserves that are deposited in the bark and roots.

Some studies suggest photosynthetic efficiency, translocation, and nut production appears to be under strong genetic control. In one study, Hammons Products Company determined the individual tree production on more than 12,000 trees during a seven-year period. During this time, around 40 trees produced no nuts while three trees produced more than 300 pounds of nuts annually. In general the heavy bearing trees are not significantly smaller than adjacent trees that have produced few nuts. The heavy bearing trees have lateral bearing characteristics with numerous fruiting spurs. These spurs normally produce eight to ten leaves which may dramatically increase the total leaf area and the amount of photosynthates needed for high nut production.
LITERATURE CITED


Floral Biology And Pollination Of Eastern Black Walnut

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GROWER’S RECOMMENDATION

Controlled pollination in black walnut is primarily used to supply nuts for breeding purposes, not for the mass production of nuts for food or reforestation. While not absolutely foolproof, controlled pollination ensure that both parents of the progeny are known. With open pollination (pollen spread through the air by the wind), only the mother tree on which the nut is found is known. Nut yields per pollination bag are not very high; the average yield is usually less than one. Of course, this value depends on how many flowers are initially in the bag. Open pollinated flowers, also, have poor survival after pollination. Linit and Necibi (1995) found that only 30 percent of walnut flowers matured into nuts under open pollinated conditions of agroforestry.

The following paragraphs give a brief description for how to make a controlled pollination. This is not meant to be the last word in learning the technique, but rather an attempt to arouse your curiosity and start you thinking about making a cross of your own. Ok, how do you make a controlled pollination?

• The first step is to select the trees that you will use as female and male parents.
• Identify the pistillate flowers (females) and the staminate flowers (pollen catkins) before the pollen starts to shed. Controlled pollination is not reliable once pollen is in the air.
• Place a pollination bag over the female flowers while the pollen catkins are still green, making sure to remove any catkins on last year’s branch that may accidentally be enclosed in the bag. You don’t want to contaminate the flowers with selfed pollen. Pollination bags with clear plastic windows can be purchased at supply houses. Wrap the branch with “polyfill” where the mouth of the bag will make contact. This will provide a good barrier to prevent stray pollen from entering the bag. Wrap the bag and polyfill tightly to the branch with a “pulltight” to prevent the bag from moving or opening.
• Collect pollen catkins from the desired male tree when they are “plump” and yellow, but before they begin to shed their pollen. Put all the catkins into a brown paper (lunch) bag and close the bag to prevent pollen from leaving. Make sure that you write the tree’s identity on the bag.
• Let the catkins dry in a warm location for a day or two, so that the pollen is released when the closed bag is gently tapped. Make sure other bags containing catkins are kept closed when you open the bag. Open the bag SLOWLY and gently pour the contents of the bag into a small-hole screen sieve that is setting on a clean sheet of white paper. The pollen will pass through the screen and most of the catkin material will remain. Pour the pollen into a small glass vial and cover. Attach a #22 disposable needle to a disposable hypodermic syringe that is labeled with the pollen source. Then pull the plunger from the syringe and pour some pollen into the barrel. Carefully insert the plunger into the barrel so that is sealed. Store temporarily in a cool place so that the pollen doesn’t overheat. After each bag is processed, wipe the area that you are working
in with a 70 percent ethyl alcohol solution to kill the residual pollen. Do this step inside where there are no drafts.

- Examine the female flowers through the window in the bag. When the stigmas of the flowers are expanded, pinkish in color, and appear moist, it is time to apply the pollen into the bag. Insert the needle of the syringe into the bag and squirt pollen toward the each flower. It doesn’t take much pollen to make an effective pollination. Remove the needle from the bag and put a piece of tape across the hole. The tape has to be resistant to moisture so it doesn’t fall off. Write the pollen source on the bag so that you know the identity of the male parent.

- In several days, examine the female flowers on the trees to see if they are no longer receptive. The stigmas should be dry and starting to turn brown. At that point you can remove the pollination bags. Cut the pulltight with wire cutters, gently open the mouth of the bag, and remove the bag and polyfill. Count the number of female flowers that were in the bag and put it in your record book. Place an aluminum identity tag on the branch where the polyfill was located. You will know that any nuts beyond the tag were the result of your pollination. This tag, see below for example, should have the female tree number (9825), the number of that specific branch (01), and the identity of the male parent (8686). When the surviving nuts are removed from the tree, the tag should accompany them; it is their identity and lineage.

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9825-01 \times 8686
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The formulation of a cultural program for eastern black walnut (Juglans nigra L.) that does not primarily depend upon information gathered from the English walnut (J. regia L.) industry in California is a slowly evolving process. This is not to say that the scientific and institutional wisdom from that industry is not valid, it just may not always be applicable. Members of that California industry have made large investments of resources to attain and fine-tune that wisdom; we have not yet arrived at that point of making large investments or of accumulating as much wisdom. To that end, this paper will comment on our state of knowledge concerning flowering biology and pollination of black walnut. Accurate information is necessary if we are to consistently produce and manage a flower crop and bring it to maturity as a nut crop. Readers are referred to a previous paper on this topic to assess where we have been (Cecich 1989). The objective of this paper is to discuss how far we have come in the last 10 years and how far we have yet to go. Definitions of some terminology are provided at the end of this chapter.

A REVIEW OF EASTERN BLACK WALNUT FLOWERING

We see flowers only as they emerge from the bud and get pollinated; then we see the fruits as they develop and eventually mature. However, flowering is a very complex process and most of it occurs at the microscopic level. Table 1, an outline of a typical classification scheme for studying flowering biology, demonstrates this complexity. There are three major components to the flowering process in walnut: 1) Initiation of primordia, 2) Differentiation or development of the staminate and pistillate inflorescences and their flower primordia, and 3) Emergence of the flowers, followed by receptivity of the stigmas and shedding of pollen.
Initiation

Initiation refers to the process by which chemical, genetic, and abiotic factors interact during a critical time period to cause a meristem, or a cell within it, to commit itself to become a flower or flower part. This is not the same as differentiation wherein the structural manifestation of the initiation process occurs; e.g., the appearance and development of an inflorescence. Most of what we know about flower initiation is based on research with annual plants (Bernier 1988, Bowman et al. 1989, Shannon and Ry Meeks-Wagner 1990, Smyth et al. 1990). However, woody plants behave differently than annuals. They have long juvenile periods during which they don’t flower, even though the proper environmental stimuli may be present. Because woody plants must grow year after year and maintain a certain crown structure, their flowers are normally in an auxiliary position, not terminal as they usually are in annuals.

Differentiation

Differentiation of reproductive structures extends from the time of inflorescence initiation in a bud (possibly early summer in black walnut) to the time of pollen shed for staminate flowers and post-emergence for pistillate flowers about one year later. This time period does not include the events following fertilization of the ovule; that period is allocated to fruit or nut development. During that year, discrete stages of flower development can be observed with a microscope, most occurring in the last 2-3 months before flower emergence. Environmental factors such as ice storms, insects, and drought can have an impact on differentiation of plant tissues. So we ask: Can the success or failure of flower development during that year be attributed to these factors? Except for deep freezes in late spring that kill the swelling buds or foliage, there is no definitive proof that weather affects differentiation of the flowers. When pistillate flower production of a good producer is low in any given spring, was flower differentiation in that tree disrupted by genetic, physiological, or environmental factors during critical times; or were the pistillate flower primordia ever initiated? There is not likely to be one simple answer to this question. Linit and Necibi (1995) determined that about 30 percent of the pistillate flowers on J. nigra grown under agroforestry conditions matured into nuts. However, of the 70 percent of the flowers that aborted, curculio weevils (Conotrachelus retentus (Say)) were estimated to account for 8-17 percent of the total flowers. The largest component of flower loss (about 50 percent) occurs before the loss related to curculios.

Genetic control over nut production (not flower production) in walnut has been demonstrated by a number of investigators (Funk 1970). Fecundity can be increased by selecting high-yielding clones and putting them into a grafted orchard (Farmer 1981). However, in a given year, seed production among clones may vary according to the percentage of pistillate flowers that were fertilized; while year-to-year differences may be associated with the number of flowers available for pollination. You can not simply select for nut production, because nut production depends on the number of flowers that survive. Selection must also take into account flower production -- male and female. The best nut-producing selection in the world is no good if there is no pollen to complete the journey from the pistillate flower to the nut.

Perfect flowers, those in which the male (staminate) and female (pistillate) flower parts exist within the integrity of a single flower, do not occur in walnut. Walnut trees are wind-pollinated and classified as monoecious; male and female flowers are on the same tree, but separated from each other. However, the male flowers on a given tree do not normally shed pollen when the female flowers on that tree are receptive. This condition is called dichogamy. If the male flowers shed their
pollen before the females are receptive, that type of dichogamy is known as protandry. If the pollen is shed after the female is receptive, it is classified as protogyny. It is believed that dichogamy has evolved to reduce or prevent inbreeding or self-pollination in a tree. Therefore, to increase the probability of cross-pollination, plantings should include cultivars or selections in which pollen shed and female receptivity occur at the same time. This alignment can be independent of whether one uses protandrous or protogynous selections of black walnut.

**Emergence, Receptivity, and Shedding**

Emergence of the staminate inflorescences (catkins) and shedding of pollen increase or hasten with rising temperatures and associated lower relative humidity. Rainy weather has the opposite effect and reduces pollen dispersal. In oaks, pollen dispersal occurs when relative humidity remains below about 50 percent for 3-4 hours (Sharp and Chisman 1961, Wolgast 1972). We don't have that detailed information for black walnut, but it can be readily gathered. For instance, monitoring the weather at an orchard site during the expected pollination period means that fewer variables are left to guesswork, making it easier to predict the success or failure of the nut crop.

Probably the most important factor controlling the emergence of pistillate flowers and their receptivity is temperature, through its direct or indirect effects on branch and leaf elongation. However, until we begin to work with clonal propagules to reduce genetic variation, an evaluation of flowering, fruit set, and nut yield of the currently planted walnut wild types in relation to low temperatures will remain inconclusive. Low temperatures in the spring may not affect flowering unless there is a hard enough freeze to damage shoots and leaves.

Floral sex ratios can change with site conditions. Variation in temperature along a site gradient can influence the physiological basis for sex allocation. In a study of oaks (Aizen and Kenigsten 1990), only stems at the top of the slope had mature fruits, apparently related to the increase in pollen availability at the top of the slope (higher air temperatures and lower relative humidity). Therefore, where we plant our trees can have a dramatic effect on nut production. If a planting site is chosen because of its deep, well-drained soil, but it happens to be in a frost pocket, the trees will not be happy!

**NEW INFORMATION ABOUT FLOWERS**

A significant contribution to our understanding of floral biology in eastern black walnut occurred with the publication by Schaffer et al. (1996). Their objective was to obtain information on the development of the pistillate (female) flower of the cultivar Ogden from early stages of differentiation to fertilization. Examining how many topics in Table 1 and Table 2 they observed shows a quick test of their success. Various perspectives of the pistillate flower structure are shown in Figures 1-4 (Schaffer et al., 1996).

Bract development, the first sign of a differentiating flower, was observed by Schaffer et al. (1996) in terminal buds collected in late February. Flowers at the same developmental stage were found in over wintering buds of protandrous cultivars of English walnut (Polito and Li 1985). By mid-April, sepal and pistil development was visible in cv. Ogden. Sepals and bracts will eventually form the husk of the walnut fruit. The pistil differentiated into the stigmas, styles, and ovary. The ovary, within which ovule formation occurs, is at the base of the pistil. The outer portion of the ovule
differentiates into the integument while the central portion becomes the nucellus. Simultaneously, the integument elongates to surround the nucellus, which produces the embryo sac.

A small aperture remains open at the tip of the integument through which the pollen tube can reach the embryo sac to fertilize the egg. As the integument and embryo sac differentiate, the stigma and style tissues also elongate and enlarge. Although numerous pollen grains were observed on the stigmatic lobes in early May, no pollen tubes were seen. One study of J. regia indicates that fertilization occurs 2-5 days after pollination (Nast 1935). Collections of cv. Ogden made during the week of May 18 included fertilized flowers containing endosperm tissue. By this time, the cv. Ogden stigmas were fully expanded and exhibited dry, necrotic areas. Stigmas that are dry or necrotic cannot function as a transport medium for pollen tubes (Masters 1974, Polito 1985). Although these pistillate flowers emerged in late April and their stigmas were fully expanded in early May, further internal differentiation of flowers continued after they emerged.

Up until now, this discussion has been related to pistillate flower development. What about staminate flowers found along the length of the staminate inflorescence or catkin? The paper by Schaffer et al. (1996) does not supply much new information about these structures. The male flowers begin to differentiate early in the summer of the year before blooming (Funk 1970) and are found in cone-shaped buds that are apparent by early autumn along the previous year’s branches. These “male” buds tend to be found at the distal end (furthest from the base) of the branches (Figures 5, 6). Pollen-related studies in trees tend to lag behind those of pistillate flowers, at least until there is recognition that pollination is critical, or even limiting, to the entire process of seed production. However, because the pollen is dispersed by wind, the pollen source for the pistillate flowers remains difficult to control.

**Prediction of Flowering**

Can we predict the size or presence of a walnut flower crop 6 months or a year in the future? At the moment, the answer is probably not. If we need an estimated 2 weeks before the expected date of flower emergence, then the information is easily obtained by dissecting terminal buds and searching for the pistillate flowers. An estimated 2 months ahead of the flowering date will require a little more creativity. For instance, we could make the assumption that, once a flower is initiated, the pathway for its development will be continuous and successful. This "all-or-none" hypothesis can be tested by sampling a population of buds in late winter and counting the inflorescence primordia with a dissecting microscope. In early spring, sample branches from the same tree(s) could be put into bottle culture indoors, forcing the buds to flush so that flowers could be observed. The number of flowers and inflorescences could be observed, and these values could be compared to flower numbers from intact branches on the source tree later in the spring. A significantly lower flower count at anthesis could indicate that either the differentiation process was disrupted or the sampling was inadequate.

Research on annual plants has shown that many genes regulate flower development (Bowman et al. 1989, Shannon and Ry Meeks-Wagner 1991, Smyth et al. 1990). Today, it is not unreasonable to assume that genes with the same construct as in annuals, or genes with similar structure, control the development of various flower components in trees. Therefore, an alternative hypothesis, and one that is probably more realistic, is that there are many independent steps to successful flower emergence, beginning with inflorescence initiation. At each step there is some potential for a proportion of the flowers to abort. Testing for the expression of specific gene activity requires both
traditional breeding protocols and current molecular technologies, such as DNA hybridization, RAPD (Randomly Amplified Polymorphic DNA), and PCR (Polymeric Chain Reaction). Unfortunately, a constraint with breeding trees is the many years required for the progeny to flower, i.e., we must wait for the juvenile-mature phase change to occur. It is generally accepted that the change from a juvenile to a mature state in forest trees occurs at the time of first flowering (Zimmerman 1972, Poethig 1990).

Current molecular technologies that use vegetative tissues to identify the expression of specific genes may overcome some of the time constraints. For instance, Weigel and Nilsson (1995) isolated a gene that regulates early flowering (precocious flowering) from an annual plant and then inserted the gene into a Populus tree via an Agrobacterium transformation procedure. Weigel and Nilsson produced transgenic plants of Arabidopsis in which the flower-meristem-identity gene LEAFY (LFY) was constitutively expressed. They demonstrated that LFY encodes for a developmental switch that can convert all lateral shoot primordia into solitary flowers so that flowers are produced precociously. The effects on the main shoot are modulated by day length, suggesting that meristems must acquire some competence to respond to LFY activity. Activity of the APETALA1 (AP1) gene can also turn vegetative meristems into flowers (Mandel and Yanofsky 1995). The normally indeterminate shoot apex of Arabidopsis becomes a floral meristem and forms a terminal flower in the AP1 transgenic plants. The AP1 gene alone can convert inflorescence shoots into flowers, even though AP1 is not normally absolutely required to specify floral meristem identity. Thus, both the LFY and AP1 genes can convert vegetative meristems into flowers. Mutations in the AP1 gene attenuate the phenotype of LFY-transgenic plants, but not the reverse combination, suggesting to Mandel and Yanofsky (1995) that AP1 acts “downstream” of LFY to specify meristem identity, and that one of the meristem-identity roles of LFY may be to activate AP1. Ectopic AP1 activity significantly reduces the time to flowering and reduces the delay to flowering of long-day plants in short-day conditions. The authors could not conclude if the early flowering phenotype was a direct consequence of AP1 activity at the shoot apical meristem, or was an indirect result of altered plant growth and metabolism caused by altered activity of “downstream” genes regulated by AP1. For those of us in the tree “flowering business,” the discovery and use of these flowering genes are the most exciting pieces of information to appear for many years.

How do you get a tree to pass more quickly from the juvenile to the mature phase? A common strategy is to grow seedlings in an environment that greatly increases growth rate. Some of the cultural methods include elevated temperatures, long photo periods, adequate water, and fertilization. Size per se, especially within a family, is positively correlated with precocious flowering (Cecich et al. 1994); that is, the tallest seedlings in a family (those siblings produced by the same mother tree) are most likely to flower before their shorter siblings. There is no guarantee of this, only the increased chance that they will flower earlier than the short seedlings. The inheritance of precocious flowering has been demonstrated in many forest trees (Chalupka and Cecich 1997). Because precocious flowering is an inherited trait and is positively correlated with height, selection pressure can be applied to increase the frequency of progenies that flower early by selecting only the tallest 25 or 50 percent of the seedlings. If precocious flowering walnut trees can be identified and selected, it should be possible to attain future generations or lines relatively quickly.

Precocious flowering was noted by Mr. Wayne Lovelace at the Forrest-Keeling Nursery in Elsberry, MO, as he developed a protocol to remove the taproots of oak seedlings for easier handling in the nursery. One unexpected product of the protocol, known as the root production method (RPM’), is that the juvenile phase of the seedlings is greatly reduced; the plants flower in 2-3 years from seed
instead of in 15-20 years. The RPM protocol is currently being tested on black walnut, but results are not yet available.

Supplemental Mass Pollination (SMP) has been used for several years in the southeast United States (Bridgewater et al. 1987). Since the annual requirement of millions of genetically improved seeds cannot readily be produced with controlled pollination bags, this alternative is being tested. The essence of the technique is to spray large amounts of select pollen at the orchard trees with a mobile pump at critical flower developmental stages; that is, when the ovulate strobili (“female flowers”) are receptive. In theory, contamination from airborne, non-select pollen may be acceptable because it should account for only a small percentage of the pollen reaching the females. Therefore, a large percentage of the potential genetic gain due to the pollen source should be attained. However, some of the continuing research of Bridgewater et al. indicates that the SMP pollen may be less competitive than airborne pollen in causing fertilization.

Can this approach work with walnut trees? Possibly. However, there will always be a need to evaluate some of the implications noted with the pine seed orchards. For instance, certain Juglans regia cultivars abort significant numbers of their flowers. The problem, known as PFA (pistillate flower abscission), seems to be caused by high pollen loads on the stigmas (McGranahan et al. 1994). Flowers receiving high pollen loads abscise from the tree before the unpollinated flowers. A similar response with poor nut set in black walnut (Beineke and Masters 1976) was attributed to too much pollen in the pollination bags. There is not yet a definitive answer to whether too much pollen on black walnut stigmas is a cause of flower abortion. A test whereby incremental increases in pollen are put onto flowers in pollination bags could easily be done on clonal material, so as to reduce tree-to-tree variation.

Until recently, attempts to repeatedly induce flowering in hardwoods have failed. This included the application of mineral fertilizers, which seem to have no direct effect on flowering, although there may be an indirect effect from a correlated increase in crown size or vigor. However, Jones et al. (1995) applied mineral fertilization (granular NPK, 13-13-13) in late summer for 5 consecutive years. During those years, there was a 48-percent increase in nut production on trees receiving the fertilizer compared to the control trees that were not fertilized. Spring fertilization had no statistically significant effect on nut production. In a follow-up study, Gray (1997) found that low levels of ammonium or nitrate N, applied in spring or fall, had a positive, but not significant, effect on the number of pistillate flowers found. Although the fertilized trees produced 2.3 to 3.4 times as many females as the controls, there was too much tree-to-tree variation to detect any statistical difference. The need to control variation is an important argument for using clonal propagules in future walnut orchards or plantings.

Another potential source of good news for the black walnut industry is being nurtured in Indiana. The USDA Forest Service - North Central Research Station and Purdue University, in conjunction with several other organizations, are developing a hardwood biotechnology research and technology transfer program. Black walnut will be one of the species studied. Precocious flowering will be one of the initial research problems because of its requirement for completely testing the products of genetic engineering. There will be an active technology transfer component of this program, funded by the Forest Service’s State and Private Forestry division, so that new results are made available to the states as quickly as possible.
Where do we go from here? Tables 1 and 2 show briefly where we have been and what remains to be done. For instance, all we can discuss with confidence about staminate flowers is that they occur on 1-year-old branches and we have observed pollen dispersal when the relative humidity was low on warm days. We have made progress in understanding pistillate flower development (Schaffer et al. 1996), but we still don’t know when the pistillate flower is initiated. The efficacy of flower-inducing techniques will revolve around that one piece of information. The paper by Schaffer et al. (1996) provided some data related to embryology, the earliest stages of seed development. The differentiation of the walnut seed remains to be explored. Although we have made progress in the last 10 years, there is still much research to be done to answer the questions about flowering.

**TABLE 1. A classification scheme for studying the floral biology of black walnut.**

<table>
<thead>
<tr>
<th>TO DO#</th>
<th>1989</th>
<th>1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAMINATE FLOWER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiation of inflorescence</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Differentiation of inflorescence</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Initiation of floral meristems on the inflorescence</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Differentiation of floral meristems</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Bracts, perianth, filament, pollen sacs, pollen grains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergence of staminate flowers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elongation of inflorescence (Catkin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispersal of pollen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination of pollen</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Pollen tube growth</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>II.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PISTILLATE FLOWER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiation of inflorescence (Peduncle)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Differentiation of inflorescence (peduncle)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Initiation of floral meristem</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Differentiation of floral meristem</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Bracts, involucre, sepals, pistil, stigma, style, ovary, ovule</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Emergence of pistillate flowers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continued differentiation of flower parts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptivity of stigmas</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

TO DO# indicates the processes that had not documented in 1989 and have not been documented as of 1998. The progress in the last 10 years has been in understanding the differentiation of the pistillate flower before and after emergence.
Table 2. The anatomical and structural components of eastern black walnut seed development.

TO DO*
1989  1998

III. EMBRYOLOGY

Nucellus *
Integument *
Megaspore mother cell *
Meiosis *
Embryo sac ontogeny *
Fertilization *
Endosperm ontogeny *
Embryo ontogeny *

III. SEED DEVELOPMENT

Cotyledon Enlargement *
Starch deposition *
Lipid deposition *
Protein deposition*I  *
Embryo Axis Development *
Apical meristem *
Stele *
Root meristem *
Husk and Shell Development *

TO DO* indicates the processes that had not been documented in 1989 and have not been documented as of 1998. The progress in the last 10 years has been in understanding the differentiation of the pistillate flower before and after emergence.

LITERATURE CITED


**DEFINITIONS**

Primordium: Undifferentiated tissue, usually found in a bud, that will develop into a specific tissue or organ.

Inflorescence: A structure on which one or more flowers are formed.

Axillary: The position that a flower or bud occupies in the acute angle formed by a leaf petiole and the stem.

Terminal: The position located at the end of a branch.

Pistillate Flower: A reproductive structure in which the egg develops.

Staminate Flower: A reproductive structure that gives rise to pollen and the male gamete.

Fecundity: The quality of having attained the ability to sexually reproduce.

Precocious Flowering: An exceptional flowering event that occurs much earlier than the average age for that species.
Missouri Eastern Black Walnut Breeding Program

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School of Natural Resources, University of Missouri
Columbia, Missouri

INTRODUCTION

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

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

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

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

CULTIVAR DEVELOPMENT

Increased yields of a high quality product result from a combination of improved cultural practices and the development of superior cultivars. Cultivar development is a process of engineering design motivated by a need to address production problems through the creation of better adapted germplasm. Implicit in this process is the importance of a precise definition of the target environment. The environment includes not only climatic and edaphic features, but also the end-product use, size of the industry, degree of capital investment, and intensity of management.
The major issues for defining the target environment of a black walnut breeding program center on grower’s objectives. The relative importance of nut and timber production must be clearly established and a strategy for achieving these objectives must be delineated. For example, if growers want both nut and timber production they could either 1) dedicate separate acreage to nut or timber trees, 2) intercrop nut and timber trees in alternate rows, 3) high graft superior nut producing cultivars onto superior timber producing understock, or 4) grow trees that are superior for both timber and nut production. Cultural practices are strategy dependent and are an important component of the environment. Therefore, strategy needs to be specified before selection for improved genotypes can proceed. Also, the timber industry must be consulted about their receptivity to purchasing trees grown under each of the strategies.

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

**Germplasm Evaluation**

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

**Trait definition** - A breeding program(s) progress is inversely related to the number of independent traits being improved (i.e., for n uncorrelated traits under selection genetic gain will be only n^0.5 as great as the gain for a single trait under selection, assuming equivalent genetic parameters). Selection pressure is diluted by the inclusion of each additional trait. Therefore, it is imperative that a breeding program clearly prioritize, and periodically review, those traits being improved. Although the number of traits to which selection pressure is applied must be minimized, many other traits need to be monitored as they also directly impact product quality. Important horticultural traits are: anthracnose resistance, vigor, consistency of nut yield, percent lateral fruitfulness, nut shape, and nut quality (shell thickness, percent crack out and ease of crackability, kernel weight, kernel plumpness, kernel color, flavor and kernel oil content). Timber quality is also a composite of several traits--straightness of bole and specific gravity being the most important traits. Phenological traits are critical to crop productivity. These include leafing date, first pollen shed, last pollen shed, peak female bloom, and harvest date. Ideally, data should be collected on all of these traits for each individual tree in a breeding program. Practically, compromises need to be made because resources are limited.
Multi-location cultivar trial - Baseline data on existing cultivars are needed to provide recommendations to growers and determine limitations of existing germplasm for particular environments. These data should be from a cultivar trial using grafted rootstock of cultivars grown in common orchards and replicated across diverse environments. The primary goal of the variety trial is to determine the extent of genotype stability. Secondly, the cultivar trial should determine the optimal genotype and cultural practice for the various geographic regions of Missouri. Breeding strategies based upon these data will permit a more efficient targeting of production deficiencies amenable to genetic improvement. Sites chosen for the variety trial should meet the following criteria: they should sample the diversity of environments confronting the Missouri grower, be relevant to the maximum number of growers, and be placed in accessible locations (collection of phenological data is especially sensitive to the last criterion).

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

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

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

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

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

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

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

**Selection and Hybridization Program**

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

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

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

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

**ANCILLARY RESEARCH**

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

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

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

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

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

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

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

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

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

Genetic correlations and heritabilities of black walnut traits - Reliable estimates of genetic parameters require establishing breeding populations of sufficient size and family structure. These estimates are used to predict genetic gain from selection programs, define selection indices, and determine if a program should be infused with new germplasm.
**Kernal color measurement** - Nut quality and value is directly related to kernal color. Developing an objective, precise, and automated protocol for kernal color measurement based on spectrophotometry will facilitate nut grading by processors. It also could lead to the development of a differential pay scale to growers based on the nut quality of their crop. A breeding program could use this technique to investigate cultivar post-harvest color stability.

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

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