VEGETATIVE PROPAGATION AND ANATOMY OF ROOT INITIATION IN *ACER SACCHARUM* 'CADDO' STEM CUTTINGS

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CHAPTER 1

SUGAR MAPLE PROPAGATION—LITERATURE REVIEW

I. Classification of Sugar Maples

A. Description

Sugar maples (*Acer saccharum* Marshall) are deciduous trees that are native to a large portion of North and South America, extending from the eastern portion of the United States and Canada to Mexico and Guatemala (van Gelderen et al., 1994). Sugar maples are slow to moderate growers, and may reach 18 to 24 m in height with a 12- to 18-m spread (Whitcomb, 1983). The trees start flowering at about 22 years of age (Godman et al., 1990), and produce inconspicuous, wind pollinated (Geburek, 1993) flowers in early spring before the leaves appear. The long-pedicelled, apetalous yellow flowers, about 64 mm long, seem to be perfect, but usually only one sex is functional within each flower (Godman et al., 1990). Both sexes are typically produced in the upper part of the crown but only staminate flowers form in the lower part (Gabriel, 1962). In some trees, certain major limbs produce only staminate and others only pistillate flowers (Godman et al., 1990). The fruit, a winged samara about 25 to 44 mm long, matures in September through October and is often devoid of sound seed (Dirr, 1998). Seed production is irregular with good or better seed crops produced every one to seven years in the United States and Canada (Godman et al., 1990).

Maple wood is fine-grained with small, evenly distributed, fairly uniform xylem vessels in the annual ring that is produced during spring and summer growth (van Gelderen

et al., 1994). Lumber harvested from sugar maples is used for flooring, shoe trees, agricultural implements, musical instruments, furniture and many other materials that need a strong, firm, fine-grained wood (Collingwood and Bush, 1979).

One of the most well-known products from sugar maples is maple syrup, collected as sap from trees in early spring when temperatures fluctuate from freezing at night to thawing in the day (Stuckel and Low, 1996). The sap is subjected to heat and filtration to produce maple syrup, with 40 gallons of sap boiled down to yield one gallon of syrup (Archibald, 1994).

Maples in temperate climates seldom suffer environmental problems, although sugar maples are susceptible to iron deficiency in alkaline soils and to so-called maple decline, seen in areas where soil has been built up and probably caused by soil compaction (van Gelderen et al., 1994). Maples are also susceptible to several fungi including those causing Verticillium wilt, and to most common plant insects. Sugar maples produce allelopathic substances against seedlings of other trees and possibly against their own seedlings (van Gelderen et al., 1994).

While sugar maples are prized for their vivid fall color ranging from yellow to orange and red, there is significant variation among members of the species (Dirr, 1998). The fall coloration is thought to be caused by high sugar (carbohydrate) production during the day and by low metabolic rates or reduced transport of the sugar during cool nights, resulting in a steady increase in the concentration of carbohydrates in the photosynthetic tissues (van Gelderen et al., 1994). As trees prepare for winter dormancy, leaf senescence begins, resulting in an export of mineral nutrients and low molecular-weight organic nitrogen compounds (Marschner, 1995). Carbohydrate backbone structures not used in nitrogen

metabolism can be used in the synthesis of anthocyanin, a purple-red pigmented flavinoid (Taiz and Zeiger, 1991). An accumulation of anthocyanins results in red leaves (Capon, 1990).

B. Taxonomy Debate

Although *A. saccharum* is generally considered a separate species of maple, some researchers continue to debate its taxonomic classification, especially its relationship to black maple (*Acer nigrum* Michx. f.). Gabriel (1973) detailed the morphological differences between black and sugar maples and their hybrids. Leaves of black and sugar maple cultivars possessed different kinds of trichomes and surface characteristics when viewed with scanning electron microscopes (Krause, 1982). On the other hand, a DNA analysis of *A. nigrum* and *A. saccharum* trees showed no genetic differences despite morphological differences (Skepner and Krane, 1998), so morphological differences may be caused by environmental differences. Skepner and Krane (1997) analyzed nucleotide sequences in chloroplast genomes of Ohio black and sugar maples and suggested the trees are very similar genetically and do not require separate taxonomic designations.

C. Climate Affects Foliar Traits

Climate is known to affect traits such as foliar drought resistance, with leaves from western sugar maples having a higher surface area, more stomata and smaller-aperture stomata than leaves from trees farther east (St. Hilaire and Graves, 1999). Some cultivars and subspecies of sugar maple are resistant to leaf scorch, a physiological stress disorder seen as a broad band of marginal desiccation, and leaf tatter, characterized as either a

physical tearing damage in young leaves caused by wind or other damaging factors, or desiccation at the tips of lobes. (Leaf scorch and leaf tatter are defined by Conley et al., 1995).

D. Caddo Sugar Maples

Dirr (1998) lists 31 cultivars and varieties of sugar maple including the Caddo maple which is considered a disjunct population of *A. saccharum* ssp. *saccharum* (Dent and Adams, 1983) native to Red Rock Canyon near Hinton in west central Oklahoma. Caddo maples are believed to be a relict of a more humid period when they could have ranged continuously to the canyons and beyond. In a dry climate like Oklahoma, the small groups of disjunct species survived only in what appear to be more favorable sites in canyon bottoms (Little, 1939; Rice, 1960). The average age of the Caddo sugar maple trees in Red Rock Canyon is 171 years (Buck, 1999).

Caddo sugar maples are more resistant to leaf scorch and tatter than many other sugar maples (Pair, 1994). Conley et al. (1995) suggested that Caddo maples may be less susceptible to leaf tatter because of a lower percentage of spongy mesophyll cells contributing to total leaf thickness, and because the palisade cells tend to be randomly arranged rather than aligned in rows and columns as is the case with sugar maple cultivars that are more susceptible to leaf tatter. Leaves of Caddo sugar maples have a significantly lower internal surface-to-volume ratio than four cultivars of *A. saccharum* and are described as highly stress tolerant (Osterhaus and Le Duc, 1996). A low surface-to-volume ratio is one of several advantageous characteristics associated with xerophytic plants; however, other factors may help determine environmental stress tolerance since 'Wichita Mountain,'

another stress-tolerant sugar maple variety studied by Osterhaus and Le Duc (1996), had a high surface-to-volume ratio. Caddo maples have higher photosynthetic rates, stomatal conductance, transpiration rates and xylem water potentials than two cultivars of sugar maple or black maple seedlings from Iowa (Yuza et al., 1996). The higher xylem water potentials may be due to the Caddo maple's tendency to vary its stomatal conductance (Yuza et al., 1996).

Seedling Caddo maples tend to be variable in habit but more columnar than the eastern species and sometimes are quite slow-growing initially, which is thought to be caused by a high root to shoot ratio (Pair, 1995). Since they evolved in a southern climate, Caddo sugar maples begin spring growth early and the leaves tend to stay green late, with fall color beginning around 1 Nov. (Pair, 1995). Caddo maples have been recommended for high pH soils (Simpson and Hipp, 1993).

II. Sugar Maple Research

A. Seeds

Sugar maple seeds are often hollow, with fruits forming yet containing no seed inside the pericarp (Dirr, 1998). Sugar maples in Oklahoma tend to produce a seed crop only every two to three years (Steve Bieberich, personal communication). Seed propagation is not a reliable method to produce cultivars with the desirable traits exhibited by the parent trees, because the parent plants are heterogeneous and not true-breeding (Kester, 1983).

B. Budding

Vegetative propagation of sugar maple cultivars is often done by budding onto seedling understock (Dirr, 1998). Budding does not allow nurserymen to produce large numbers of clones of desirable specimens, however, because budding or grafting requires skills that laborers frequently do not possess (Bieberich, personal communication).

C. Cuttings

Since maple syrup is produced from the sap of sugar maples, researchers with the U.S. Northeast Forestry Service are interested in developing rooting techniques for cuttings from trees selected for their high sap-sugar content (Yawney and Donnelly, 1982).

Timing—Seasonal weather conditions influence the rate and pattern of tree growth, so the optimum date to take sugar maple stem cuttings varies year to year (Yawney and Donnelly, 1982) but tends to occur in June in Vermont (Yawney, 1984) and Massachusetts (Snow, 1941). In Maryland, sugar maple cuttings rooted better in June and July than in April and May (Enright, 1958). In Pennsylvania, fewer than 10% of cuttings taken in July rooted (Zaczek et al., 1997).

As a general guide, external visible characteristics of a developing sugar maple shoot that coincide with maximum rooting are when leaves have just reached full size and are bright green, the bases of petioles show signs of reddish-purple color, shoots begin to stiffen slightly and lenticels are pronounced and terminal buds are barely visible as two dark brown scales about 0.25 cm in height (Donnelly, 1977). To prevent desiccation, cuttings should be taken early in the morning and immediately placed in layers between cool, moist

sphagnum moss in a container for transport to the rooting chamber, and then stuck the same day (Yawney and Donnelly, 1982).

Cutting Size—Yawney and Donnelly (1982) found a correlation between sugar maple cutting size and rooting response, with longer cuttings rooting better. However, Snow (1941) found that rooting percentage was higher in 10 cm cuttings than in 15 cm cuttings. Since Snow's (1941) cuttings were stuck into the medium about two-thirds of their length, depth of planting may have contributed to the differences in rooting between the two lengths since the basal ends of the 15 cm cuttings were deeper than the 10 cm cuttings.

Wounding—In addition to wounding that occurs when lower leaves are stripped from the base of cuttings, researchers have commonly scraped bark from opposite sides of the basal portion of the stem to expose the cambium to auxin treatments made immediately afterward (Enright, 1958; Yawney and Donnelly, 1981).

Auxin Treatments—A variety of auxin treatments have been recommended for sugar maple cuttings, including 0.8% IBA talc (Yawney and Donnelly, 1981), 20 $g \cdot L^{-1}$ IBA (Enright, 1958) and 0.05 $g \cdot L^{-1}$ IBA for 3 h (Snow, 1941).

Tree Variability—Rooting response of three sugar maples in Vermont varied widely, but cuttings from individual trees tended to be consistent in their rooting ability from year to year (Yawney and Donnelly, 1982). Individual trees also varied widely in their response to hormone treatments, with low auxin concentrations stimulating rooting in cuttings from two trees but inhibiting rooting in a third tree (Donnelly, 1971). When cuttings were taken based on stem morphology, a high percentage of cuttings from four sugar maples rooted well at the stage considered optimum, but cuttings from a fifth tree rooted equally well when they were quite immature (Donnelly, 1977).

Yawney and Donnelly (1982) hypothesized that the differing sugar maple responses to hormone treatments could be caused by inherent differences in endogenous auxin concentrations; however, Greenwood et al. (1976) were unable to detect any correlation between endogenous auxin concentration of sugar maple cuttings and rooting ability.

D. Other Methods

Some novel vegetative propagation methods have been investigated for sugar maples. Only 15% of stem cuttings from sugar maple shoots forced into early budbreak in a greenhouse rooted (Henry and Preece, 1997). Cuttings of 'Legacy' sugar maples also rooted poorly (less than 10% rooting overall) and slowly (104 to 112 d to form roots) when placed under shade treatments of 83%, 91% or 97% of ambient sunlight (Zaczek et al., 1997).

Rooting percentage of sugar maples treated with 0.05 $g \cdot L^{-1}$ IBA for 3 h followed by 2% or 5% sucrose for 72 h ranged from 33% to 60% (Snow, 1941). The sucrose treatments did not increase rooting beyond the optimum seen with auxin treatments alone, but apparent toxic effects of 3-hour IBA treatments at concentrations of 0.1 $g \cdot L^{-1}$, 0.2 $g \cdot L^{-1}$ and 0.4 $g \cdot L^{-1}$ were slightly reduced with the sucrose treatments, possibly because some of the auxin moved out of the cuttings and back into the sucrose solution during the 72-h treatment (Snow, 1941).

Sugar maples have been known to form roots when layered but do not reproduce vegetatively from roots (Fayle, 1996).

III. Cutting Propagation—Factors That Influence Rooting

Factors that influence production of adventitious roots on cuttings are successful timing and stem morphology, the balance of root promoters/inhibitors, carbohydrate and nutritional levels, and physical barriers (Dirr and Heuser, 1987). Other factors include the rooting environment and biological age of stock plants (Hartmann et al., 1997).

A. Timing and morphology

Successful timing is among factors that influence production of adventitious roots on cuttings (Dirr and Heuser, 1987). Many cuttings taken from trees will root successfully only during a short time period during the year. Species with a narrow window of rootability include Japanese maple (*Acer palmatum* Thunb.), birch (*Betula nigra* L. and *Betula pendula* Roth), ash (*Fraxinus* L.), lacebark elm (*Ulmus parvifolia* Jacq.), smoke tree (*Cotinus coggygria* Scop.) and redbud (*Cercis canadensis* L.) (Barnes and Lewandowski, 1991), Chinese pistache (*Pistacia chinensis* Bunge.) (Dunn, 1995), apple (*Malus* Mill.) (Burd and Dirr, 1977), pecan (*Carya illinoensis* Wang. K. Koch) (Smith and Chiu, 1980), pin oak (*Quercus palustris* Munchh.) and littleleaf linden (*Tilia cordata* Mill.) (Chapman and Hoover, 1981), native *Rhododendron* L. (Nienhuys, 1980), lilac (*Syringa* L.) (Wedge, 1977; Mezitt, 1978) and myrtle (*Myrtus communis* L.) (Pokorny and Dunavent, 1984).

Numerous studies have investigated the ideal timing for successful production of adventitious roots on cuttings. With mature apple, May and early June cuttings produced the highest rooting percentages (Burd and Dirr, 1977). Juvenile pecan cuttings rooted best when taken in February, June and August, but adult cuttings rooted best from 15 Aug. cuttings (Smith and Chiu, 1980). Pin oak cuttings rooted best in early July, and littleleaf linden rooted

better with cuttings taken in late June (Chapman and Hoover, 1981). Difficult-to-root *Chionanthus retusus* Lindl. cuttings rooted in high percentages during a one-week period in May (Stoutemyer, 1942).

In addition to timing based on calendar days, researchers have identified morphological stages that result in optimum rooting (Donnelly, 1977; Dunn et al., 1996; Howard, 1987; Kraus, 1953). Morphologically, stems of woody plants may be divided into three growth stages: softwood, semi-hardwood and hardwood (Hartmann et al., 1997). Softwood cuttings are taken from current season's growth before extensive lignification has occurred (MacDonald, 1986). Other characteristics associated with softwood cuttings include stems that bruise easily, tendency to wilt readily, gradation in leaf size from terminal to base, and a requirement for high humidity (mist) to ensure rooting (Barnes and Lewandowski, 1991). Semi-hardwood cuttings can be subdivided into two groups, soft and firm, based on the degree of lignification (MacDonald, 1986). With soft semi-hardwood cuttings, the shoot is still growing but the lower region of the stem is becoming lignified, whereas with firm semi-hardwood cuttings, the entire stem of the shoot is undergoing varying stages of lignification (MacDonald, 1986). Hardwood cuttings are usually taken in the fall or winter when the previous season's growth is complete, terminal bud is present, stems are firm and woody, plants have usually been exposed to at least one frost, and leaves of deciduous plants have abscised or can be pulled off easily without tearing the bark (Barnes and Lewandowski, 1991).

Other morphological events such as flower induction can temporarily change the rootability of shoots. Rooting of untreated shoots of a Douglas fir (*Pseudotsuga menziessi* (Mirb.) Franco) clone coincided with bud break (Roberts, 1969).

A third method to determine the best cutting time for various species is based on mean ambient temperatures. The heat unit or degree-day system used today originated with Rene A.F. de Reaumur c. 1730 (Wang, 1960). Reaumur found the mean daily air temperature for 91 days during the months of April, May and June in his locality and determined the sum to be nearly a constant value for the development of any plant from year to year. He assumed his summation of temperatures, known as Reaumur's thermal constant of phenology, expressed the amount of heat required to reach a given stage of maturity.

Arnold (1960) devised a method to estimate accumulated heat units by using daily maximum and minimum temperatures and a specified threshold. The degree-day system has been used in horticulture to predict phenological events such as budbreak in pecans (Sparks, 1993) and blueberries (*Billardiera longiflora* Labill.) (Spiers, 1976), leaf emergence of sour cherry (*Prunus* L.) (Eisensmith et al., 1980), flowering in blueberry bushes (Mainland, 1986), and harvest time in stone and pome fruits (Fisher, 1962) and corn (*Zea mays* L.) (Gilmore and Rogers, 1958).

Chilling units, rather than heat units, were found effective to determine collection time for cuttings of *Juniperus* L. species (Major and Grossnickle, 1990).

B. Root Promoters

Auxin— Auxin is the plant hormone most often associated with adventitious rooting. Application of auxin in suitable concentrations hastens root development and increases the number of roots formed on most plants capable of rooting (Thimann and Delisle, 1939). Various phases in rooting may have different auxin requirements (De Klerk

et al., 1999). Auxin is required to induce roots but inhibits the outgrowth of root primordia, growth of roots and shoot growth of cuttings (De Klerk et al., 1990).

The auxin IBA is the most widely used root promoting chemical, although naphthaleneacetic acid (NAA) has worked better with some species (Dirr, 1986). Differences in effectiveness of various auxins may lie in the compound's affinity for an auxin receptor involved in rooting, or in the concentration of free auxin that reaches the target cells (De Klerk et al., 1999). The concentration that reaches the target cells may depend on uptake, transport and conversion of the auxin, as well as the amount synthesized by the plant itself (De Klerk et al., 1999). Indolebutyric acid treatments may control endogenous auxin levels in cuttings by regulating the IAA-oxidase system or indirectly through transport of auxin protectors (Mato and Vieitez, 1986).

Liquid formulations of IBA or NAA may be superior to talc formulations because the hormones are in solution and can be readily absorbed (Dirr, 1986). Alcohol used to dissolve hormones acts as a carrier and helps with penetration of the hormones into the cuttings (Dunn, 1995). Gray (1959) determined a 5 sec dip was as effective as a 160 sec dip in promoting rooting of cuttings, and suggested the quick dip method be used for hard-to-root species. Extremely concentrated quick dips of 20 to 40 $g \cdot L^{-1}$ IBA or NAA will often cause phytotoxicity at the base of a cutting, but rooting may occur in the untreated region just above the injured tissue (Chong, 1981; Dirr and Heuser, 1987).

Treating cuttings with 2% to 5% sucrose reduced cutting mortality (Thimann and Delisle, 1939), and Snow (1941) suggested that a sucrose treatment to sugar maple cuttings may reduce the toxicity of high auxin applications.

Ethylene apparently plays a critical role in auxin's stimulation of adventitious rooting. Clark et al. (1999) found that auxin treatments increased adventitious rooting of tomato (*Lycopersicon esculentum* Mill.) and petunia (*Petunia* × *hybrida* Hort. Vilm.-Andr.) but had little or no effect on rooting of ethylene-insensitive transgenic tomato and petunia plants.

Dipping stem cuttings in sulfuric acid prior to IBA treatments may promote rooting of plants native to alkaline soils, suggesting a short exposure to acid may break acid-labile linkages (calcium bridges) in cell walls of calciferous plants, loosen cell walls and increase permeability of applied auxin (Uhrstrom, 1974). Such an acid pretreatment reduced loss of foliage in woody species in which root initiation takes more than six weeks (Lee et al., 1976).

Haissig (1974) proposed that lack of adventitious root formation in response to auxin may be caused by lack of essential enzymes to synthesize root-inducing auxin-phenol conjugates, presence of enzyme inhibitors, lack of enzyme activators, lack of substrate phenolics, and physical separation of enzyme reactants.

Rooting Cofactors—The activity of rooting cofactors (root promoting substances that are synthesized in the leaves and buds, move down the stem and accumulate at the base of a cutting to stimulate root initiation) was summarized by Hess (1965). Cofactors that act synergistically with endogenous IAA to promote rooting include oxygenated terpenoids, isochlorogenic acid, three lipid-like compounds with functional alcohol and nitrile groups, IBA which may counteract the root inhibitory effects of gibberellic acid, and a phenolic compound (Hartmann et al., 1997). Phenolic compounds may act as antioxidants,

protecting IAA from oxidation and the plant tissue from oxidative stress caused by wounding (De Klerk et al., 1999).

If one or more of the cofactors is unavailable, auxin treatments will not be effective, regardless of the auxin concentration (Hartmann et al., 1997). Kling et al. (1988) made extracts from tissue samples of five *Acer* species and discovered that crude extracts containing a phenolic compound or a weak acid from young stems of easy-to-root *A. ginnala* Maxim., *A. rubrum* L. and *A. saccharinum* L. stimulated root initiation in mung bean (*Vigna radiata* L.) cuttings but extracts from difficult-to-root *A. saccharum* and *A. griseum* (Franchet) Pax had no effect on root initiation in mung bean.

C. Carbohydrates and Mineral Nutrition

A low C : N ratio is undesirable; instead cuttings should be selected from lateral shoots in which rapid growth has decreased and carbohydrates have accumulated, rather than selecting cuttings from succulent terminal shoots (Hartmann et al., 1997).

Smalley and Dirr (1987) speculated that the greater leaf area of larger cuttings with six or more leaves would provide more carbohydrates to support root development than the two leaves of single-node cuttings. Larger cuttings yielded more roots and longer roots than shorter cuttings for *A. rubrum* 'Red Sunset' (Smalley and Dirr, 1987) and *A. japonicum* 'Aconitifolium' (Dixon, 1980). Rooting percentages were higher with long cuttings than with shorter cuttings of sugar maple (Donnelly and Yawney, 1972).

Leaves on cuttings exert a strong stimulating influence on root initiation by serving as an auxin source and by translocating carbohydrates to the site of adventitious root

formation (Hartmann et al., 1997). Also, photosynthate production may be important for rooting (Davis, 1988).

Severing shoots from the parent plant affects photosynthesis and leaf water, carbohydrate and hormone status (Smalley et al., 1991). Smalley et al. (1991) determined that leaf water status of red maple cuttings deteriorated before rooting and improved after root emergence; leaf carbohydrate concentrations increased until rooting and decreased after rooting; and abscisic acid (ABA) levels increased when the cuttings were inserted into the rooting medium but decreased before rooting. The increase in ABA in water-stressed cuttings may have affected photosynthesis by triggering stomatal closure or affecting the mesophyll cell photosynthetic apparatus (Raschke and Hedrich, 1985).

Calcium was required in the late phases of adventitious rooting of poplar shoots grown in vitro (Bellamine et al., 1998) and a net influx of calcium in differentiating cells is presumed to accompany the auxin-induced differentiation response (Kaira and Bhatla, 1998).

D. Anatomical barriers

Several anatomical barriers have been suggested to explain the difficulty of rooting in some woody species.

Lignification— Lignin is a strong, waterproof polymer that can be deposited between cellulose microfibrils (Mauseth, 1988). This deposit strengthens the wall and makes it water-resistant. Deposition of lignin is called lignification, and because it is a characteristic of sclerenchyma cells, it is also called sclerification. The process seems to occur most often when the organ will persist for a long time. Sclerification may occur as rings of fibers

between the phloem and cortex, exterior to the origin of adventitious roots (Mauseth, 1988). Sclerenchyma cells typically occur in bundles, as in fibrous stems and bark (Mauseth, 1988).

Sclerification of phloem tissue has been proposed as the barrier in some species, possibly presenting a physiological barrier to the initiation of roots, or a mechanical barrier to their emergence (Beakbane, 1961). In many easy-to-root plants, adventitious roots arise in the secondary phloem, usually in association with a ray that has contact at its distal end with living cells; however, most of the rays of difficult-to-root plants abut on fibers, sclereids or other elements without living protoplasts (Beakbane, 1961). As Beakbane (1961) pointed out, sclerification is associated with senescence (or loss of cellular integrity) in tissues so there is at least a correlation between the degree of sclerification of the primary phloem of the stem and rooting ability.

Edwards and Thomas (1980) observed a relationship between sclerification and rooting ability of three Juniper species. The easy-to-root shore juniper (*Juniperus conferta* Parl.) had very little evidence of cells containing lignin; Chinese juniper (*Juniperus chinensis* L.) 'Pfitzeriana Aurea' which is a moderately-rooting species, contained a larger amount of sclerenchyma fibers; the difficult-to-root flaky juniper (*Juniperus squamata* Buch.-Ham.ex D. Don) 'Meyeri' had very heavy groupings of sclerenchyma. Sclerenchyma sheaths have been associated with the difficult-to-root species para rubber (*Hevea brasiliensis* (A. Juss.) Muell. Arg.), black wattle (*Acacia mearnsii* De Wildeman), cinnamon (*Cinnamomum zeylanicum* Bl.), heaths (*Erica* L. spp.), beech (*Fagus sylvatica* L.), Oregon grape (*Mahonia bealei* (Fort.) Carr.) and oaks (*Quercus* L. spp.), but were not believed to be the sole cause of the lack of rooting (Beakbane, 1961, 1969). Mature stems of English ivy (*Hedera helix* L.) were more lignified

than stems from juvenile plants, and the mature stems contained a discontinuous ring of sclerenchymous fibers encircling the phloem (Goodin, 1965). The increased fiber development was believed to contribute to the difficulty in rooting of mature plants, but was not considered the only source of the problem, since the fiber ring was not continuous.

Many woody genera such as *Cotoneaster*, *Ficus*, *Rubus* and *Salix* have initiation sites in the parenchyma cells of leaf traces and leaf gaps (Girouard, 1967b). In cuttings of *Griselinia lucida* Forst., a difficult-to-root species containing a continuous band of fibers, root initials formed in gaps in the fibers close to nodes where leaf traces entered the vascular system (White and Lovell, 1984). The root cap was present by the time the outer cortex was reached and vascular connections between the root and the stem were complete at or near the time of root emergence. Lovell and White (1986) noted that nodes are often sites where xylem differentiation is initiated and may be regions of high auxin concentration.

Not all researchers believe that lack of rooting is caused by anatomical factors, including Hartmann (1969) who said the differences in root initiation between easy- and difficult-to-root plants is likely due to biochemical factors. Studies by Sachs et al. (1964) failed to show any clear relationship between continuity of a sclerenchyma ring and rooting ability, and they believe that ease or difficulty of rooting is associated primarily with root initiation rather than subsequent development. 'Bartlett' pear (*Pyrus communis* L.), which is difficult-to-root, has an almost continuous sclerenchyma ring but can be rooted in fairly high percentages with the proper procedures (Hartmann et al., 1963).

Suberization— Suberin is a wax-like material that is deposited in cork cells of woody species to protect the cell walls from chemical and enzymatic attack (Mauseth, 1988).

Suberization of the cortex, and not sclerification, was correlated with poor rooting in cuttings from at least eight species of difficult-to-root Australian native plants (Williams et al., 1984).

E. Wounding

When a cutting is taken, living cells at the cut surfaces are injured and exposed, and the wound healing response begins (Cline and Neely, 1983). Wounding can be beneficial to cuttings where bands of sclerenchyma form a physical barrier to horizontal root emergence through the stem, possibly because the wound penetrates the sclerenchyma fibers and allows a gap for penetration of the adventitious roots and possibly for other reasons such as allowing free diffusion of root promoting substances and gases, and the ability of root initials to make vascular links (Edwards and Thomas, 1980).

Mexican orange (*Choisya ternata* HBK) and rosemary (*Rosmarinus officinalis* L.), containing no more than faint traces of discontinuous sclerenchyma tissue, showed no response to wounding; but holly (*Ilex aquifolium* L.), which had a strong response to wounding, had a continuous band of sclerenchyma cells (Edwards and Thomas, 1980). Wounding may result in heavier callus production and root development along the margins of the wound (Hartmann et al., 1997). Adventitious roots in the cuttings of holly often arose from the periphery of the wounded area (Edwards and Thomas, 1980).

Wounding of tissue destroys cell compartments which leads to synthesis or release of hormones and enzymes that degrade cell walls and membranes (De Klerk et al., 1999). The hormones and enzymes play a role in rooting, possibly by increasing auxin uptake, reducing conjugation or oxidation of applied auxin, or by enhancing the competence of the

tissue to respond to plant hormones (De Klerk et al., 1999). Wounding may also trigger activation of stress-induced compounds (De Klerk et al., 1999).

F. Physiological Age

The biological age of the tree from which cuttings are taken is the most important single factor affecting root initiation (Hartmann et al., 1997). Invigoration treatments such as severe pruning can change the status of food reserves, C : N ratio, auxin distribution, inhibitor/promoter balance, and levels of cytokinins, gibberellins and rooting cofactors in the remaining shoots (Fortainier and Jonkers, 1976).

Hedging—Hedging (or shearing) of mature trees to rejuvenate physiologically mature stock plants frequently improves rooting success (Hartmann et al., 1997).

Sugar maples form long vegetative shoots when cut back, and have been successfully propagated in Canada with hedging (Morsink, 1971). Morsink (1971) obtained roots on 75 to 89% of 35 to 55 cm-long cuttings collected from basal stump sprouts representing trees of many ages. The cuttings, with at least two pairs of leaves fully elongated and terminal growth still in progress, were severed by breaking the stems at the base of new shoots. The cuttings were all classified as juvenile and rooted without the use of auxin application. Roots appeared five weeks after cuttings were taken.

Cuttings from seedlings and 3- to 7-year-old hedged stock plants rooted better than cuttings from three-year-old Loblolly pines (Hamann, 1998). Cuttings from the three-year-old trees showed a general loss in rooting potential compared to cuttings from the seedlings and hedged plants, with less callus development and root initiation and a delayed wound

healing process. The emerging roots were thicker and more brittle than those of cuttings from hedges or seedlings. Within a 90-day period, around 80% of the cuttings from seedlings and hedged donor plants showed development of roots, while those from 3-year-old trees ranged from 0% to 20% rooting.

Mound Layering—Mound layering involves severe pruning of stock plants to induce adventitious shoots that are juvenile in appearance and vigorous in growth (Howard et al., 1988). When new growth begins in the spring, stock plants are severed near the ground level. New shoots emerging from buds in the severed stumps are allowed to develop. When the shoots have grown 7.6 to 12.7 cm in height, they are encouraged to root by mounding soil, sawdust or a soil-sawdust mixture around their bases; the mounding process is repeated as the shoots grow (Hartmann et al., 1997). The shoots are then removed by cutting below the new root system.

Mound layering is the main technique used in both Europe and North America to clonally propagate apple rootstocks such as Malling 9, Malling 26 and Malling Merton 106 (MacDonald, 1986). A mound layering bed can be used for 15 to 20 years if it is maintained in a vigorous condition (Hartmann et al., 1997). Sugar maples have a tendency to layer from a variety of sources, including branches and basal sprouts (Fayle, 1996).

Semi-hardwood shoots on mound layered pecan seedlings rooted when the shoots were girdled (Wood, 1989). Wood (1989) concluded that the girdling triggered, whereas IBA enhanced, rooting, when 46% of girdled shoots produced roots and 100% of girdled plus IBA treated shoots rooted. With Chinese pistache seedlings, 75% to 77% of shoots from mound layering beds rooted when wounded and treated with IBA (Dunn and Cole, 1995).

Successful rooting has also been obtained on cuttings taken from sprouts of severely pruned American elm (*Ulmus americana* L.) (Schreiber and Kawase, 1975), paperbark maple (*Acer griseum* (Franch.) Pax.) (Hoogendoorn, 1984), and Monterey pine (*Pinus radiata* D. Don) (Libby and Hood, 1976; Menzies, 1985).

G. Rooting Environment

Media Fertilization—Fertilizer applied to media in which cuttings are placed before root initiation has little benefit and can reduce root and shoot growth (Wright et al., 1984). Nitrogen fertilization rates greater than 50 $mg \cdot L^{-1}$ decreased root dry weight in stem cuttings of sweetgum (*Liquidambar styraciflua*) (Rieckermann et al., 1999).

Media Temperature—For temperate climate species, the optimum air temperature for the rooting medium is 18°C to 25°C day and 15.6°C night (Hartmann et al., 1997). Dykeman (1976) found a high temperature of 30°C resulted in more rapid root initiation, shorter emergence time and more roots per cutting of *Forsythia* Vahl.; however, subsequent root development including elongation, diameter, root hair formation and secondary branching occurred more readily at 25°C. Burholt and Vant Hoff (1970) suggested the rate of cell division increases to a maximum between 30°C and 35°C.

Fungicides—Softwood cuttings under mist provide favorable conditions for the growth and spread of fungal diseases (Fiorino et al., 1969). Fungicide treatments have been shown to protect newly-formed roots from fungal attack, increase survival and increase overall quality of rooted cuttings (Hansen and Hartmann, 1968; Wells, 1963). Captan may protect newly-formed roots from fungal pathogens (Hartmann, 1969; Hartmann et al., 1997). Some systemic fungicides such as benomyl (methyl 1-(butylcarbomoyl) 2-

benzimadazolecarbamate) improved rooting of western sand cherry (*Prunus besseyi* Bail.) softwood cuttings (Fiorino et al., 1969). McGuire and Vallone (1971) found better rooting quality and in some cases higher rooting percentages were obtained in difficult-to-root *Ilex*, *Rhododendron*, *Sciadopytis*, *Magnolia* and *Vitis* clones with a combination of IBA and benomyl. McGuire and Vallone (1971) suggested that benomyl may act as a mobilizer to cause other endogenous materials to move to the site of root initiation and stimulate more rooting. Hoitink and Schmitthenner (1970) reported a shorter time required to obtain a good root system on *Rhododendron* cuttings when benomyl was combined with auxin.

IV. Anatomical Development of Adventitious Roots

Adventitious roots are roots that originate in locations other than the embryo or as branches of the primary root (Esau, 1965a). Adventitious roots may develop in response to damage when part of a plant is severed from the existing root system (Lovell and White, 1986). Adventitious root formation involves anatomical changes associated with wound responses as well as changes involved in root formation itself (Lovell and White, 1986).

Prerequisites for root initiation appear to be the availability of parenchyma cells that may be stimulated into cell division and a substrate such as phloem to support cell division and provide at least part of the stimulus for new root formation (Hess, 1969).

Anatomical changes that occur when roots form in wounded tissues have been divided into four stages (Davies et al., 1982; Girouard, 1967a): cellular dedifferentiation and cell division, formation of root initials, development of the initials into root primordia, and

growth and emergence of the new roots. On the genetic level, protein and RNA synthesis are critical early in the process of cell dedifferentiation (Oppenoorth, 1979).

Division of the first root initial cells is triggered by auxin, whether applied or produced by the plant (Haissig, 1972). The initials of an organized root tip first arise from cells containing a large, centrally located nucleus and a small vacuole (Lovell and White, 1986). At a later stage, the cells, often together with surrounding cells, form the root primordium: an organized mass of meristematic tissue in which some differentiation has occurred (Lovell and White, 1986). Later on, vascular connections form between the primordium and the existing vascular tissue of the plant and a functional root emerges (Esau, 1965a).

Starch accumulation may also signal the beginning of adventitious root formation. When IBA was applied to in vitro stem slices of *Malus* 'Jork 9,' starch grains accumulated during the first 24 h in cells of the cambial region and in cells in the vicinity of vascular tissue and in the primary rays (Jasik and De Klerk, 1997). The accumulation occurred only in the basal part of explants. After that, nuclei in the cells were activated, and cytoplasm density and cell organelle numbers increased. Cambium cells started to divide transversely and at 96 h, after several divisions, a continuous ring of cytoplasmic cells had appeared around the xylem near the basal cutting surface. Root meristemoids regenerated from the portions of the ring that were localized in the primary rays. Callus developed from other cells in the ring. The root meristemoids grew along the vascular bundles, emerged from the cutting surface, and were transformed into small, dome-like primordia.

Root formation was studied in callus isolated in tissue culture (Esau, 1965b). Xylem developed in the center, then became surrounded by cambium, and phloem elements

differentiated among cells produced to the outside of the cambium away from the xylem; eventually a root apex formed in the cambium and developed into a root (Esau, 1965b).

Adventitious roots can originate in almost any tissue, including the epidermis, stem cortex and pericycle, ray parenchyma, immature xylem and phloem cells, and the pith (Mauseth, 1988). Callus formation is often required for root initiation to occur (Lovell and White, 1986). In most species that are difficult to root, initiation of roots occurs within callus tissue (Davies et al., 1982; Hamann, 1998). Even though formation of callus and formation of roots are independent processes (Hartmann et al., 1997), origin of adventitious roots from callus tissue has been associated with difficult-to-root species such as Douglas fir (Bhella and Roberts, 1975) and creeping fig (*Ficus pumila* L.) (Davies et al., 1982).

Internal callus arose from continued division of the cambium in cuttings taken from *Griselinia littoralis* Raoul. (White and Lovell, 1984). In cuttings of the difficult-to-root loblolly pine (*Pinus taeda* L.), root initials formed in a zone near the wound cambium and wound phloem (Hamann, 1998). Often, root primordia are initiated at the intersection of a vascular ray with the phloem (Hess, 1969). Root primordia appeared to form in masses of cortical callus adjacent to the phloem parenchyma in the difficult-to-root golden mimosa (*Acacia baileyana* F. Muell.) with vascular connections established by vascular rays which forced apart a sheath of sclerenchyma fibers, often near a leaf trace (Schwarz et al., 1999). The proximity of root primordium formation to vascular tissue, particularly phloem, indicates an essential component of root initiation is translocated by the phloem (Hess, 1969).

Woody species including *Cotoneaster, Ficus, Rubus* and *Salix* have initiation sites in the parenchyma cells of leaf traces and leaf gaps (Girouard, 1967b).

The developmental sequence of root formation sometimes changes when plants mature, resulting in a loss of rooting capacity, as has been seen with ivy (Geneve et al., 1988) and Monterey pine (Smith and Thorpe, 1975), but in other species that lose rooting ability, the developmental sequence of root initiation is similar in cuttings from young or old sources, as was seen with loblolly pine (Hamann, 1998).

Although cuttings from individuals within a species may vary in rooting ability, the differences may not be caused by anatomical differences. Stem anatomy was similar in six genotypes of golden mimosa, regardless of their rooting ability, with a sclerenchyma fiber sheath between the cortex and phloem (Schwarz et al., 1999).

The objectives of this dissertation were to address the hypothesis that sugar maples found in Oklahoma possessing desirable ornamental characteristics can be successfully propagated by cuttings. I developed two research questions to help address the hypothesis. First, can the accumulation of heat degree-day units and the characterization of morphological markers be used to predict the ideal timing to take softwood stem cuttings from mature sugar maple trees, regardless of geographic location? Second, would mound layering hasten rooting of softwood cuttings? As a component of my research, I wished to identify superior sugar maples that respond well to cutting propagation so clones can be developed. I also desired to increase the scientific body of knowledge about sugar maple adventitious root formation by characterizing the anatomical changes that occur during the process of root formation in stem cuttings.

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CHAPTER 2

Effect of Timing and IBA on Rooting of Caddo Sugar Maple Stem Tip Cuttings

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Abstract. **In a study to determine the time period when the highest percentage of rooting occurs, cuttings were taken from selected sugar maple (*Acer saccharum* Marshall) trees in Stillwater, Okla., in 1999 based on calendar date and in 2000 based on stem morphology. Stem color was determined at each 2000 cutting date, based on Royal Horticultural Society color charts. Degree days from budbreak to each cutting date were calculated each year. Overall rooting percentage in 1999 ranged from a high of 35% in early May to a low of 10% in June. The rooting percentage in 2000 was 31% in mid-April and 27% in early May. Rooting in 1999 was greatest on cuttings in the green softwood stem stage and declined as the season progressed, coinciding with the progression of the stems into the red semi-hardwood stage and with no**

rooting after that stage. In 2000, cuttings in the early shoot expansion phase, after the leaves had fully emerged from the bud and had unfurled but before shoot elongation had ceased, rooted as well as cuttings taken two weeks later in the green softwood stem stage. The early shoot expansion stage occurred at 1,594 degree days and green softwood stem stage at 2,008 degree days. Timing did not affect the number of roots or length of roots among cuttings that rooted. Application of IBA did not significantly affect root number or length in 2000. In 1999 cuttings had more roots when treated with any of the IBA concentrations than those receiving no IBA, and they had longer roots when treated with 10 $g \cdot L^{-1}$ and shortest roots at 0 $g \cdot L^{-1}$ IBA.

Sugar maples are an attractive landscape ornamental, prized for their strong wood, pleasing form and vivid autumn color. However, sugar maples grown in the midwestern United States are subject to extreme heat and high winds, which may result in leaf tatter and scorch (Conley et al., 1995). Superior sugar maple specimens can be found in native stands in Oklahoma as well as other parts of the Midwest. Desirable specimens include *A. saccharum* 'Legacy' and 'Commemoration' (Pair, 1995) and 'Caddo,' an ecotype found in Red Rock Canyon, near Hinton in Caddo County, Okla. (Dent and Adams, 1983), as well as seedling-grown Caddo maples transplanted into urban settings. Many sugar maples growing in Oklahoma have desirable autumn color and are well-suited to the Midwest because of their resistance to drought, leaf tatter and scorch. Sugar maples tolerant of the Midwest's extreme heat and high winds would be a valuable addition to the nursery industry.

Sugar maples are generally propagated by seed or by bud grafts. Seed propagation is hampered because sugar maples tend only to produce a seed crop every two to seven years (Godman et al., 1990), and seed coats are often empty. Sexual propagation efforts are further complicated by the fact that individual trees show variable traits such as fall foliage color—a trait that is highly prized in ornamental plants. Vegetative propagation by grafting or cuttings allows the cloning of trees with superior horticultural traits, but often the desired traits are not evident until trees are mature. Nurserymen often avoid bud grafting because workers lack grafting skills and because of the need to produce numerous seedlings to use as root stock. Cutting propagation has not been feasible for mature sugar maples because it is difficult to successfully root cuttings from mature growth.

Because of the value of sugar maples in the landscape and their value as a source of maple syrup in the Northeast, several researchers have worked on methods to root stem cuttings but with mixed results (Enright, 1958; Greenwood et al., 1976; Snow, 1941; Yawney and Donnelly, 1981; Zaczek et al., 1997). Optimum timing to take cuttings has usually been reported as a calendar date (Enright, 1958; Greenwood et al., 1976; Zaczek et al., 1997) which is of limited use in other parts of the country where climate or other environmental factors may affect tree growth. Information also differs on the best auxin formulation (Khatamian and Pair, 1996; Snow, 1941; Yawney and Donnelly, 1981), propagation environment (Snow, 1941; Yawney and Donnelly, 1981) and amount of time for rooting to occur (Yawney and Donnelly, 1981; Zaczek et al., 1997).

The optimum time to take cuttings of sugar maples in the Burlington, Vt. area, according to Greenwood et al. (1976) is a two-week interval in June when the current year's growth reaches full size. Enright (1958) reported better rooting in June and July than April

and May for cuttings from trees in Maryland. Cuttings taken in early July in Pennsylvania of 'Legacy' sugar maples rooted very poorly (Zaczek et al., 1997).

Snow (1941) and Yawney and Donnelly (1981) recommended cutting dates based on stem morphology rather than calendar timing. Both reported better rooting of sugar maples when cuttings were in the green softwood stem stage. However, Snow's trials in Massachusetts were conducted with cuttings treated with weak concentrations of indole-3-butyric acid (IBA) for 24 hours and placed in outdoor propagating beds. Yawney and Donnelly (1981) reported no significant differences between the type of rooting hormone used but found that individual trees responded differently to hormone concentrations. Tree variability was also reported by Khatamian and Pair (1996), working with Caddo sugar maples in Kansas, when cuttings from one tree rooted best with an IBA treatment of 10 $g \cdot L^{-1}$ while cuttings from a second tree did not root regardless of hormone concentration.

Our objectives were to determine the effects of timing and IBA concentration on rooting of stem cuttings from selected sugar maples with superior drought and tatter resistance, using current common industry propagation methods. Calendar timing, morphological markers and the accumulation of heat degree-day units were used to predict the ideal timing to take stem cuttings from mature sugar maple trees.

Materials and Methods

Calendar Timing, 1999

Cuttings were taken from throughout the canopies of three adult trees, two believed to be about 30 years old, growing at the Oklahoma Botanical Garden and Arboretum

(OBGA) in Stillwater, Okla., and one of undetermined age growing on the Oklahoma State University campus in Stillwater, Okla. Cuttings of current season's growth were taken twice a month, from mid-April through early October. Cuttings were taken between 0900 HR and 1200 HR on each date.

Each stem tip cutting consisted of the entire current season's growth, from the terminal bud to just below the basal node. The cuttings ranged in length from about 7 cm to 20 cm (the tree growing on the university campus had shorter stem tips than the other two trees). Cuttings were immediately placed in 10°C water to prevent desiccation. They were taken to a greenhouse at OBGA and re-cut just below the lowest node. Seed clusters were removed from all cuttings. Leaves were removed from the bottom two nodes. All cuttings were wounded by scraping bark (the epidermis and phloem) from opposite sides of the lower 1-cm portion of the cutting.

Four concentrations of IBA were used. The basal 1 cm of cuttings was dipped for 10 sec in 0 $g \cdot L^{-1}$, 5 $g \cdot L^{-1}$, 10 $g \cdot L^{-1}$ or 15 $g \cdot L^{-1}$ IBA. The IBA was prepared by dissolving the appropriate amount of IBA in 50 ml of 70% isopropyl alcohol then using tap water to bring the solution to 100 ml. All cuttings were planted vertically to a depth of about 5 cm (or to their lowest set of nodes remaining on the cutting if the length of their stems between the first and second nodes was less than 5 cm) in plastic rooting flats 16 cm wide by 24 cm long by 8 cm deep, in pre-made holes in moist 1 peat : 4 perlite (by volume) medium, and kept in a polyethylene-covered greenhouse under natural photoperiod with a maximum photosynthetic photon flux (PPF) of 845 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Flats were placed on raised benches equipped with nozzles with an output of 32 L/hr placed 50 cm above the flats at 121-cm intervals. Shade cloth was placed over the greenhouse as necessary to prevent excessive heat

during the summer. Average high and low greenhouse temperatures were 29°C and 18°C, respectively. Cuttings received mist irrigation between 0800 HR and 1800 HR daily. Mist cycles were adjusted as necessary to allow foliage to dry between misting (avg. 2 sec duration every 8 min). Medium drenches of Gnatrol (*Bacillus thuringiensis* Serotype H-14, Abbott Laboratories, North Chicago, Ill.) for control of fungus gnat larvae were applied at a rate of 5 $ml \cdot L^{-1}$ on the following dates: 11 June, 18 June, 28 June, 20 Sept., 28 Sept., 7 Oct., 29 Oct. and 17 Nov. Cuttings were sprayed to runoff with Diazinon (0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl)-phosphorothioate, Novartis Crop Protection, Inc., Greensboro, N.C.) at a rate of 1.2 $g \cdot L^{-1}$ for aphid control on 9 Aug.

Twelve weeks after planting, cuttings were rated using the following scale: 0=dead, 1=no callus or roots, 2=callus present, 3=roots present. Rooting was defined as the presence of at least one root on a cutting. Rooted cuttings were evaluated for number and length of roots.

A split-plot with main units in a randomized complete block design was used with ten replications of five subsamples per treatment. Tree was the block, cutting date was the main plot treatment and IBA concentration was the subplot treatment. Statistical analysis was performed using analysis of variance (ANOVA) with mean separation among the cutting dates by the protected least significant difference (LSD) procedure. Trend analysis using IBA concentration as the independent variable was conducted on significant cutting date by IBA interactions and IBA main effects. Rating data were analyzed using a chi square analysis. Rooting occurred only on cuttings taken in late April and early May; therefore, cutting dates when no rooting occurred were omitted from the statistical analysis.

Timing Based on Morphology, 2000

Cuttings were taken from the two trees used in the 1999 study that were located at OBGA. The cuttings were collected on dates that corresponded with the onset of the following growth stages when a sufficient number of stems on the trees were in the appropriate stage: green budbreak (10 Mar. on one tree and 4 Apr. on the second tree), leaves of both trees fully emerged from the bud ("leaf emergence") (11 Apr.), leaves fully unfurled with shoots still expanding ("early shoot expansion") (21 Apr.), green softwood stem (5 May), red semi-hard stem (9 June) and brown hardwood stem (19 July).

Stem colors on each date, determined by comparing representative stem samples to Royal Horticulture Society color charts in the early afternoon of each cutting date in a room with an east-facing window, were 165A Grayed-Orange for green budbreak and for the leaf emergence stage, 144A Yellow-Green at early shoot expansion and green softwood stem, 152A Yellow-Green at red semi-hard stem, and 200B Brown at the brown hardwood stem stage. Photographs of stem cuttings in the first four morphological stages are shown in Figs. 2.1-2.4.

Cuttings were treated as described above, except the IBA was dissolved in 100 ml of 70 percent isopropyl alcohol (no tap water used). Cuttings were planted into 10.2 cm by 10.2 cm by 10.2 cm square black plastic pots filled with Strong-Lite Hi-Porosity Mix (Strong-Lite Horticultural Products, Pine Bluff, Ark). Gnatrol (*Bacillus thuringiensis* Serotype H-14) drenches were applied to the media on 17 Apr. and 27 Apr. Diazinon media drenches at a rate of 0.9 $g \cdot L^{-1}$ for fungus gnat control were made on 5 May and 2 June. Average high and low air temperatures in the greenhouse were 32°C and 15°C, respectively. Cuttings were evaluated after eight weeks.

There were 20 replications of each treatment per tree, for a total of 40 replications of each treatment.

Degree Days

Degree days that accumulated prior to each cutting date were calculated using the following formula: Σ(average daily temperature – threshold temperature). Average daily temperatures recorded at the Oklahoma State University Agronomy Research Station in Stillwater were obtained from the Oklahoma Climatological Survey (Norman, Okla.). The Agronomy Station is located about 0.8 km east of OBGA. Average temperature was calculated by adding temperature observations taken every 5 min during a 24-HR period, and then dividing by the number of observations for the period.

A threshold temperature of 0°C was used. Degree days accumulated from 14 Jan. 1999 and 2 Feb. 2000, the earliest dates in which average daily temperatures remained above 0°C, through the day before each cutting date.

Results

No significant interactions occurred between date and IBA concentration (except for presence of callus in the 2000 study); therefore, main-effect means are presented and discussed.

1999.

The percentage of cuttings that died while on the mist bench increased with date (Table 2.1). Among the cuttings taken on 1 June, most died by the end of the 12-week

evaluation period. The percentage of cuttings that were alive but did not callus or root was highest on 18 May and lowest on 6 May. The IBA treatments did not significantly affect the percentage of dead cuttings or cuttings alive but without callus or roots. Cuttings taken on 6 May had a greater tendency to form callus without roots than cuttings taken on 18 May or 1 June. The IBA concentration affected callus production, although only between cuttings treated with the 0 $g \cdot L^{-1}$ and 15 $g \cdot L^{-1}$ rates. Rooting percentage was highest on 6 May. Rooting was lowest on 1 June, but not significantly different from the rooting percentage for cuttings taken on 18 May. Indole-3-butyric acid enhanced rooting on cuttings but as long as IBA was used the concentration did not affect rooting percentage. Timing did not significantly affect root number or root length among cuttings that rooted, but there were differences in root number and length as a result of IBA. Root number on rooted cuttings increased linearly as IBA concentration increased. A curvilinear relationship existed between root length and IBA concentration. Cuttings that rooted had longest roots when treated with 10 $g \cdot L^{-1}$ IBA and shortest roots at 0 $g \cdot L^{-1}$.

2000.

Cutting date did not significantly affect any of the rooting parameters (Table 2.2) except for the percentage of cuttings that callused (Fig. 2.5). Date interacted with IBA for callus such that percentages of cuttings with callus were similar regardless of IBA concentration on 21 Apr., but among cuttings taken on 5 May callus was more prevalent at lower IBA concentrations whereas rooting occurred at higher IBA concentrations (Fig. 2.5). As in 1999, exogenous IBA was required to produce roots on cuttings but the concentration did not affect rooting percentage (Table 2.2).

Discussion

Rooting occurred primarily in the spring when cuttings were in the early shoot expansion or green softwood stem stages. Similar results were observed for eastern sugar maples (Snow, 1941; Donnelly, 1977; Greenwood et al., 1976), although those trees did not reach the green softwood stem stages until June or July. Donnelly (1977) reported that a high percentage of cuttings from four trees rooted well when taken in mid-June at the green softwood stem stage of development even though cuttings from one tree rooted equally well when they were "quite immature" which may have been the early shoot expansion stage.

At least three theories have been proposed to explain why sugar maple cuttings appear to have only a narrow window of rootability near the start of the active growing season each spring. Greenwood et al. (1976) hypothesized that the cambium is the site of root initiation in sugar maples; therefore, the rooting period probably coincides with rejuvenation associated with annual cambial activity. A second possibility is that mature sugar maple shoots lack compounds that stimulate rooting or the compounds are not present in concentrations optimal for rooting. Kling et al. (1988) made extracts from tissue samples of five *Acer* species and discovered that crude extracts containing a phenolic compound or a weak acid from young stems of easy-to-root *A. ginnala* Maxim., *A. rubrum* L. and *A saccharinum* L. stimulated root initiation in mung bean (*Vigna radiata* L.) cuttings but extracts from difficult-to-root *A. saccharum* and *A. griseum* (Franchet) Pax had no effect on root initiation in mung bean. A third theory regarding sugar maples' brief rooting potential each year involves anatomical barriers such as a lignified sheath in maturing tissues (Beakbane, 1961; Edwards and Thomas, 1980) or suberization of the cortex (Williams et al.,

1984) which may act as physiological barriers to root initiation or mechanical barriers to root emergence. Barriers are not believed to be the sole cause of lack of rooting, however, since the rings often are not continuous barriers (Beakbane, 1961, 1969; Goodin, 1965), and occasionally they do not prevent rooting even when they are almost continuous (Hartmann et al., 1963).

Overall rooting percentages in 1999 and 2000 were similar even though the 2000 cuttings were on the mist bench for eight weeks compared to 12 weeks for the 1999 cuttings. Although no direct statistical comparisons were made between the 1999 and 2000 results, root length appeared to be greater among cuttings taken during early shoot expansion and green softwood stem stages in 1999 than in 2000 (Tables 2.1 and 2.2). This difference was likely the result of the additional four weeks the cuttings were given before rooting was evaluated in 1999 compared to 2000. Root number also tended to be greater in 1999 than in 2000.

Eight weeks probably was sufficient time for cuttings to form roots in 1999 as it was in 2000. The time required for root formation in sugar maple cuttings in other studies has taken 3 to 16 weeks. If cuttings were taken from trees known to have high rooting potential, roots could appear as quickly as three to four weeks after sticking, although it took a longer period to get any appreciable root length (Yawney, 1984). Roots appeared in five weeks on softwood stem cuttings taken from hedged sugar maples (Morsink, 1971). Six to seven weeks was required for rooting of sugar maple cuttings taken in Maryland (Enright, 1958), but 15 to 16 weeks was necessary for rooting of 'Legacy' sugar maple cuttings taken in July in Pennsylvania, and less than 10% of the cuttings rooted (Zaczek et al., 1997).

Roots always formed in the presence of callus in our study. Hartmann et al. (1997) indicate that wounding may result in heavier callus production and root development along the margins of the wound. Wounding may provide several benefits, including ethylene production in injured tissues that may promote adventitious root formation, creation of new sink areas by wounding resulting in the accumulation of auxins and carbohydrates, potentially more water absorption through the wound and more exogenous hormone uptake, and possible release of beneficial hormones and enzymes from destroyed cell compartments (DeKlerk et al., 1999). Callus formation is often required for root initiation (Lovell and White, 1986). In fact, in most difficult to root species, initiation of roots occurs within callus tissue (Davies et al., 1982) including internal callus (White and Lovell, 1984; Hamann, 1998; Schwarz et al., 1999). Callus is a mass of undifferentiated tissue from which new vascular connections and root primordia can arise. Esau (1965) reported that cultured callus tissue arising from undifferentiated parenchyma explants may spontaneously develop vascular tissue. She described the formation from cultured callus cells of xylem, cambium, phloem and eventually a root apex.

Root production was stimulated by IBA during both years of our study, regardless of IBA concentration used. Yawney and Donnelly (1982) had similar results in which hormone type and concentration did not affect the overall rooting response of cuttings from eastern sugar maples. In contrast, cuttings from a Caddo sugar maple at Wichita, Kan., rooted better when treated with IBA at 10 $g \cdot L^{-1}$ than at other (unspecified) hormone concentrations used, while cuttings from a second Caddo tree did not root regardless of IBA rate (Khatamian and Pair, 1996).

Treatment with IBA stimulates more uniform root production on cuttings (Hartmann et al., 1997). Among our cuttings that rooted in 1999, higher IBA rates resulted in more roots than did lower IBA rates, although only the 10 $g \cdot L^{-1}$ rate resulted in longer roots. All three IBA treatments resulted in longer roots than the control although only the 10 $g \cdot L^{-1}$ treatment was significantly different than the control (Table 2.1). It is possible the 10 $g \cdot L^{-1}$ rate was optimal for our sugar maple cuttings while the 15 $g \cdot L^{-1}$ rate slightly delayed emergence or slowed the growth of roots. This would be consistent with results of De Klerk et al. (1990) in which the outgrowth of root primordia was inhibited by auxin at a concentration optimal for root initiation.

There was a date by IBA interaction affecting callus formation in 2000 (Fig. 2.5). When callus formed on cuttings taken on 21 Apr., the cuttings tended to subsequently produce roots regardless of IBA concentration. However, cuttings taken on 5 May seemed to require the stimulus of higher IBA rates to produce roots after callusing. By 5 May, shoot growth was slowing. Perhaps endogenous auxin production was slowing, too, as the shoots matured, although researchers have been unable to detect any correlation between endogenous auxin content of cuttings and rooting ability of sugar maples (Greenwood et al., 1976).

The degree day system, based on the accumulation of heat units over a base or threshold temperature, has provided a useful tool for predicting the best cutting dates for Chinese pistache (*Pistacia chinensis*, Bunge.) (Dunn et al., 1996), as well as prediction of budbreak in pecan [*Carya illinoinensis* (Wangenh.) K. Koch] (Sparks, 1993) and blueberries (*Billardiera longiflora* Labill.) (Spiers, 1976), leaf emergence of sour cherry (*Prunus* L.) (Eisensmith et al., 1980) and flowering in blueberry bushes (Mainland, 1986).

Calendar dates and degree day accumulations for the various morphological stages other than the greenbud stage were surprisingly similar in 1999 and 2000 (Table 2.3), although a direct comparison may not be appropriate since the dates for 1999 were not necessarily when the morphological stages first began. Instead, in 1999, the cutting dates were based on calendar timing—taking cuttings on the first and third Tuesdays of each month. The greenbud stage occurred earlier in 1999 than in 2000, particularly on one tree that apparently needed a substantially higher number of degree days for buds to break. We believe this may have been the result of the tree's heavy seed crop in 1999 which may have depleted its carbohydrates and other reserves needed to begin the subsequent growing season. The second tree from which cuttings were taken in 2000 had a very small seed crop in 1999. Neither tree had seeds in 2000. Sugar maples are an irregular bearing species. Good or better crops usually occur every 1 to 4 years on trees in north central Wisconsin, every 2 to 5 years in other portions of the United States, and every 3 to 7 years in Canada (Godman et al., 1990).

From this study we recommend taking cuttings at the early shoot expansion or green softwood stages which occur around mid-April through early May in Oklahoma and upon the accumulation of around 1600 to 2200 degree days. Our cuttings rooted best when IBA was applied at a rate of 5 to 15 $g \cdot L^{-1}$.

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Table 2.1. Effect of date (pooled IBA levels) and auxin (pooled dates) on *Acer saccharum* softwood stem cuttings taken in 1999. n=50 for cutting date. n=150 for IBA concentration.

	Percentage of All Cuttings				Cuttings that Rooted	
Tmt	Dead[z]	Alive[y]	Callus[x]	Rooted[w]	Root Number	Root Length (cm)
Cutting Date						
6 May	18c[v]	3c	45b	35a	2.9	7.2
18 May	33b	27a	22a	20b	5.9	8.3
1 June	62a	14b	16a	10b	2.9	8.0
P =	0.0001	0.0001	0.0027	0.0022	NS	NS
IBA Rate ($g \cdot L^{-1}$)						
0	38	13	44b	7b	1.2	5.9
5	33	11	27ab	31a	3.4	7.8
10	34	16	27ab	23a	3.8	8.5
15	46	18	13a	25a	5.0	7.0
P =	NS	NS	0.0216	0.0459	0.0279	0.0501
Linear	--	--	--	--	0.0015[u]	NS
Quadratic	--	--	--	--	NS	0.0332
Cubic	--	--	--	--	NS	NS

[z] Dead represents cuttings that died while on the mist bench.
[y] Alive represents cuttings that were green but did not produce callus or roots.
[x] Callus represents cuttings that were alive and produced callus but no roots.
[w] Rooted represents cuttings that had at least one root at the end of the 12-week period.
[v] Means within treatment and column followed by the same letter are not significantly different. ($LSD_{0.05}$).
[u] Not significant (NS) or P-value.

Table 2.2. Effect of date (pooled IBA levels) and auxin (pooled dates) on *Acer saccharum* softwood stem cuttings taken in 2000. n=40 for cutting date. n=80 for IBA concentration.

	Percentage of All Cuttings			Cuttings that Rooted	
Tmt	Dead[z]	Alive[y]	Rooted[x]	Root Number	Root Length (cm)
Cutting Date					
21 April	22	30	31	3.4	4.4
5 May	11	20	27	2.8	3.8
P =	NS	NS	NS	NS	NS
IBA Rate ($g \cdot L^{-1}$)					
0	13	30	14b	2.5	3.1
5	10	22	39a	2.9	4.9
10	18	21	35a	3.2	4.1
15	25	28	28a	3.6	3.7
P =	NS	NS	0.0182	NS	NS
Linear	--	--	--	NS[w]	NS
Quadratic	--	--	--	NS	NS
Cubic	--	--	--	NS	NS

[z] Dead represents cuttings that died while on the mist bench.
[y] Alive represents cuttings that were green but did not produce callus or roots.
[x] Rooted represents cuttings that had at least one root at the end of the 8-week period.
[w] Not significant (NS).

Table 2.3. Degree day units for each cutting date in 1999 and 2000.

	1999		2000	
Stem Morphology	**Calendar Date Cuttings Taken**	**Degree Days[z]**	**Calendar Date Cuttings Taken**	**Degree Days[z]**
Green Bud	3 March	864	10 March (Tree 1) 4 April (Tree 2)	638 1,130
Leaf Emergence	5 April	1,341	11 April	1,319
Early Shoot Expansion	19 April	1,712	21 April	1,594
Green Softwood	5 May	2,209	5 May	2,008
Red Semi-Hardwood	31 May	3,145	9 June	3,413
Brown Hardwood	20 July	5,334	19 July	5,272

[z]Degree days were calculated using the following formula: (average daily temperature – threshold temperature).

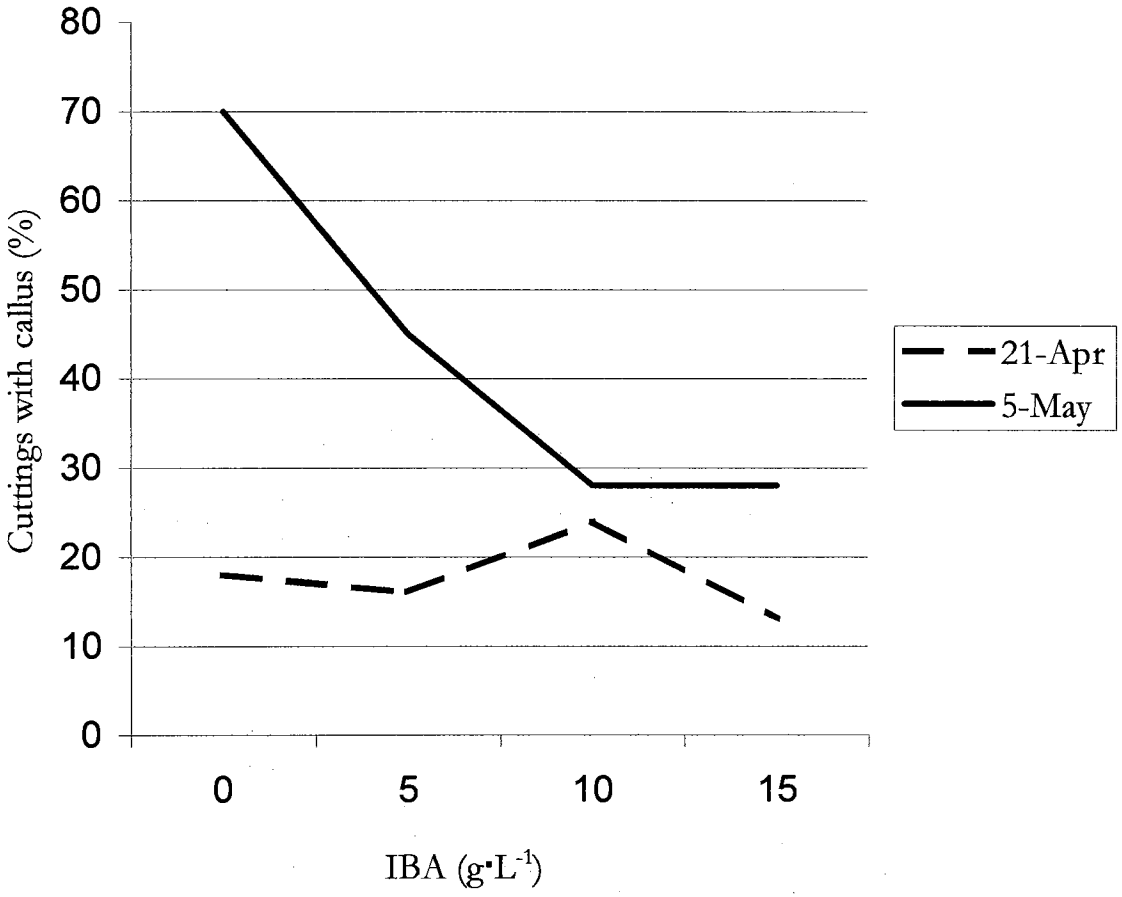
Figure 2.1. Green Bud Stage.

Figure 2.2 Leaf Emergence Stage.

Figure 2.3 Early Shoot Expansion.

Figure 2.4. Green Softwood Stage.

Figure 2.5. Date by IBA interaction for proportion of cuttings with callus in 2000. Date*IBA P = 0.0155.


80
70
60
50
40
30
20
10
0
Cuttings with callus (%)
0
5
10
15
IBA (g·L-1)
21-Apr
5-May

CHAPTER 3

Sugar Maple Trees Differ in Rooting Potential

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Additional Index Words: *Acer saccharum*, Caddo sugar maple, vegetative propagation, difficult-to-root ornamentals, IBA, NAA, clones

Abstract. **Green softwood stem cuttings were taken from nine adult sugar maples (*Acer saccharum* Marshall) in Stillwater, Okla., chosen for superior horticultural traits including lack of leaf tatter and scorch during drought conditions. The cuttings were treated with 2.5 g·L^{-1} IBA, 5 g·L^{-1} IBA, 2.5 g·L^{-1} NAA, 5 g·L^{-1} NAA, 2.5 g ·L^{-1} IBA + 2.5 g·L^{-1} NAA, 5 g·L^{-1} IBA + 5 g·L^{-1} NAA, 0 g·L^{-1} IBA + 0 g·L^{-1} NAA (alcohol control). Cuttings were evaluated after about seven weeks for number and length of roots. Rooting response varied by tree and there were significant differences among auxin treatments. Trees with greater rooting percentages tended to have the highest number of roots per rooted cutting and the longest roots. Seventy-five percent of cuttings from Tree 1 rooted when treated with 2.5 g·L^{-1} IBA + 2.5 g·L^{-1} NAA. Eighty percent of cuttings from Tree 9 rooted when treated with 5 g·L^{-1} IBA. Tree 7 cuttings rooted well regardless of hormone treatment, with 48% rooting overall and 54% rooting when auxin was greater than 0 g·L^{-1}. Cuttings from**

Tree 8 did not root. Chemical names used: indolebutyric acid (IBA), naphthalene acetic acid (NAA).

Cloning, as described by Kester (1983), is the vegetative regeneration of a single genotype. Cloning allows a rapid increase in plant material having desirable chemical and horticultural characteristics (Raviv and Putievsky, 1987) and could lead to the development of new cultivars. New cultivars of sugar maples tolerant of the extreme heat and drought in the south and west are needed, but development of such cultivars has been hampered because of limited success with vegetative propagation of individual trees possessing the desirable characteristics.

Sugar maples are commonly propagated by seed, which results in genetically variable offspring, or by grafting, which is a slow and laborious process. Cutting propagation could avoid these problems, but sugar maples are among woody ornamental species that are difficult to propagate by cuttings. While many techniques are available for successfully rooting cuttings, sugar maples vary widely in rooting ability and in response to rooting hormone treatments (Donnelly, 1971, 1974; Koelling, 1968; Khatamian and Pair, 1996). Snow (1941) found that sugar maple cuttings required different lengths of time to form roots and concluded the differences could be the result of differences in physiological maturity at the time of collection or because of variation among parent trees.

Since variability in genotype is expected to cause differences in rootability of cuttings taken from individual sugar maples, the purpose of this study was to identify sources (specific superior individuals) for clone material that can be rooted in the nursery industry to allow the development and introduction of new cultivars. Anticipated benefits included

the capability to introduce sugar maple cultivars with desirable landscape characteristics, and to provide the information that will result in higher rooting percentages to help growers realize more efficient use of bench space, labor, supplies and plant materials.

Materials and Methods

Cuttings were taken randomly from the entire canopy of nine adult sugar maple trees between 0800 HR and 1400 HR on 9 May 2000, the time of year considered optimum for rooting (Chapter 2). All trees were located at the Oklahoma Botanical Garden and Arboretum (OBGA) or at the main campus of Oklahoma State University, Stillwater, Okla. (Appendix A).

The estimated age of trees 5, 6 and 7 was 10 to 15 years old, while all other trees were estimated to be 20 to 30 years old. All of the trees were characteristic of Caddo sugar maples except tree 3. Characteristics of Caddo sugar maples include leaves that are smaller, thicker and with a darker green color than eastern sugar maples (Steve Bieberich, personal communication). Tree 3 was of unknown origin but had large, thinner leaves that were not as deeply lobed as leaves from the other trees, and displayed bright yellow foliage in the fall, unlike the more orange-colored foliage of the other trees.

The cuttings consisted of the entire current season's growth. Stems were green with white lenticels, with lengths varying among trees and even within samples from the same tree. Length of the cuttings ranged from about 13 cm to 46 cm. The number of nodes on the cuttings from most trees ranged from three to five. The cuttings were removed from branch tips throughout the canopy and immediately placed in buckets containing tap water. The cuttings were taken to a polyethylene-covered greenhouse and recut just below the lowest node. The bottom leaves were stripped from the cuttings and bark was removed

from each side of the base, perpendicular from the nodes where the leaves had been removed.

Cuttings were given the following treatments immediately after wounding: 2.5 $g \cdot L^{-1}$ IBA, 5 $g \cdot L^{-1}$ IBA, 2.5 $g \cdot L^{-1}$ NAA, 5 $g \cdot L^{-1}$ NAA, 2.5 $g \cdot L^{-1}$ IBA + 2.5 $g \cdot L^{-1}$ NAA, 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA, 0 $g \cdot L^{-1}$ IBA and 0 $g \cdot L^{-1}$ NAA (alcohol control). The IBA and NAA formulations were prepared on 8 May 2000, by dissolving the appropriate amount of the rooting hormones in 70% isopropyl alcohol. The cuttings were dipped about 1 cm deep into the rooting hormone formulations for 10 sec, then inserted into 10 cm by 10 cm by 10 cm square black pots filled with pre-moistened Strong-Lite High-Porosity Mix (Strong-Lite Horticultural Products, Pine Bluff, Ark.) and placed on mist benches. The cuttings were planted into the medium to the depth of the second lowest nodes that had leaves still attached or to about 5 cm deep if the internode between the first and second sets of nodes was longer than 5 cm. Most cuttings were placed less than 5 cm deep. The cuttings were kept under natural photoperiod with a maximum photosynthetic photon flux (PPF) of 845 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Average maximum and minimum air temperatures in the greenhouse were 31° C and 17°C, respectively. Mist cycles were adjusted slightly depending on ambient temperature but ran about 2 sec every 2 min.

The cuttings were arranged on benches in a randomized complete block design with 20 replications per treatment per tree. Cuttings were evaluated for rooting on 29 June, about seven weeks after they were taken. Number and length of roots per cutting was also determined.

Data for percentage of cuttings rooted within each tree and auxin treatment were transformed using an arcsin transformation and analyzed using analysis of variance (GLM). A protected least significant difference (LSD) test was used to indicate differences in

rooting percentage among trees and auxin treatments. Data for number and length of roots per cutting were analyzed using analysis of variance (GLM) procedures, and an LSD was used to compare auxin treatments to controls within each tree (SAS Institute, Cary, N.C.).

Results

Cuttings from tree 8 did not root, so tree 8 has been excluded from all statistical analyses.

The trees exhibited different rooting responses (Fig. 3.1). A greater percentage of cuttings from trees 1, 2, 7 and 9 rooted than from trees 3, 4, 5 or 6.

A smaller percentage of cuttings rooted when no auxin was applied than when auxin was applied as 2.5 $g \cdot L^{-1}$ IBA, 5 $g \cdot L^{-1}$ IBA, 2.5 $g \cdot L^{-1}$ NAA or 2.5 $g \cdot L^{-1}$ IBA + 2.5 $g \cdot L^{-1}$ NAA (Fig. 3.2). The percentage of cuttings rooted with the 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA treatment did not differ from that of cuttings receiving no auxin. The percentage of cuttings rooted with 5 $g \cdot L^{-1}$ NAA did not differ from percentage of cuttings rooted in any other auxin treatment except the control.

An auxin treatment by tree interaction affected the average root number per cutting among cuttings that rooted (Fig. 3.3). Cuttings from tree 1 receiving 2.5 $g \cdot L^{-1}$ IBA, 5 $g \cdot L^{-1}$ IBA, 2.5 $g \cdot L^{-1}$ NAA, or no auxin had similar numbers of roots per cutting, but cuttings treated with 5 $g \cdot L^{-1}$ NAA or either of the IBA + NAA combinations produced more roots than cuttings in the control group. None of the cuttings in the control treatments on trees 2-5 rooted. Cuttings from tree 2 that received IBA, NAA or both averaged from 2 to 6 roots per cutting. Cuttings from trees 3 and 4 that received 2.5 $g \cdot L^{-1}$ NAA had more roots (8.0 for tree 3 and 7.0 for tree 4) than cuttings in other hormone treatments that rooted. No

rooting occurred in tree 3 with 5 $g \cdot L^{-1}$ NAA or 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA; in tree 4 with 5 $g \cdot L^{-1}$ IBA or 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA; or in tree 5 with 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA. Auxin formulation did not affect number of roots among rooted cuttings from trees 6, 7 or 9.

Roots on rooted cuttings from tree 6 were longer than those of cuttings from trees 3, 4, 7 and 9 (Fig. 3.4). Root length of cuttings from trees 1, 2 and 5 was similar to root length on the tree 6 cuttings but roots on tree 5 cuttings were longer than cuttings from trees 3 and 9. Tree 3 cuttings had the shortest roots.

Auxin treatments impacted average root length per rooted cutting (Fig. 3.5). Root length was greatest when cuttings were treated with 5 $g \cdot L^{-1}$ IBA, 2.5 $g \cdot L^{-1}$ NAA, 2.5 $g \cdot L^{-1}$ IBA + 2.5 $g \cdot L^{-1}$ NAA, or 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA. The shortest roots occurred on cuttings treated with 2.5 $g \cdot L^{-1}$ IBA, 5 $g \cdot L^{-1}$ NAA or the control, although root length with the 2.5 $g \cdot L^{-1}$ IBA treatment was not significantly different from any other auxin treatment except 5 $g \cdot L^{-1}$ IBA.

Discussion

Sugar maples are generally propagated by seed in North America (Van Gelderen et al., 1994) and cuttings taken from plants in a species that is usually seed-propagated can differ widely in rooting ability (Hartmann et al., 1997). As expected, the trees in our study exhibited different rooting responses (Fig. 3.1) even though all cuttings were in the green softwood stage which is the optimal morphological stage for rooting (Chapter 2). Variability in rooting among individual trees was also seen with eastern sugar maples (Koelling, 1968; Donnelly, 1971; Donnelly and Yawney, 1972), Caddo maples (Khatamian and Pair, 1996), red maples (*Acer rubrum* L.) (Snow, 1939), Norway spruce (*Picea abies* (L.) Karst.) and white

pine (*Pinus strobus* L.) (Deuber, 1940) and sweetgum (*Liquidambar styraciflua* L.) (Rieckermann et al., 1999). Snow (1941) speculated that differences in rooting of sugar maple cuttings could be the result of differences in parent stock. Yawney (1984), summarizing ten years of research on sugar maples at northeastern U.S. forestry research stations, said rooting potential of trees can vary from 0% to 100% but trees tend to be consistent from year to year in their rooting ability.

The differences in rooting response are probably not related to endogenous auxin content in the good versus poor rooting trees, but cuttings from good rooters have been shown to accumulate at least twice as much auxin at their bases as cuttings from poor rooters (Greenwood et al., 1976). The cuttings from good rooters may be more effective at synthesizing or activating rooting cofactors (Kling et al., 1988; Hess, 1962; Ashiru and Carlson, 1968) or essential enzymes (Haissig, 1974).

Genetic differences may also play a role in rooting ability of individual trees (Greenwood et al., 1976; Haissig, 1986). Protein and RNA synthesis are critical early in the process of cell dedifferentiation and formation of adventitious roots (Oppenoorth, 1979). It was suggested by Gabriel (1972) that genetic differences might explain why some sugar maples produced sap with up to 100% higher sucrose content than neighboring trees.

While the trees in our study responded differently in percentage of rooted cuttings, the type and concentration of auxin used made little difference as long as rooting hormone was used (Fig. 3.2). Yawney (1984) reported similar findings when evaluating powder and liquid formulations of auxins on eastern sugar maples, with maximum rooting achieved when auxin treatments were around 10 $g \cdot L^{-1}$. In contrast, Enright (1958) reported large differences in rooting of May cuttings (12% vs. 66%) between IBA treatments of 10 $g \cdot L^{-1}$ and 20 $g \cdot L^{-1}$, respectively, but lesser differences in rooting of June cuttings (78% rooting

with the 10 $g \cdot L^{-1}$ and 90% rooting with the 20 $g \cdot L^{-1}$). In our study, only the 5 $g \cdot L^{-1}$ IBA + 5 $g \cdot L^{-1}$ NAA treatment resulted in rooting as low as that of the controls (Fig. 3.2), possibly because high concentrations of auxin injure or kill tissues at the base of cuttings (Hess, 1969).

We saw interactions between auxin treatment and trees for the number of roots per cutting (Fig. 3.3) but not for the length of roots per cutting. Both the frequency and rate of root development varied considerably among genotypes of eastern sugar maples (Yawney and Donnelly, 1981).

Vegetative cloning is an essential method to develop new cultivars (Kester, 1983), and we have established that cuttings from Oklahoma's heat- and drought-tolerant sugar maples, like those of eastern sugar maples, vary in the ability to produce adventitious roots. It seems logical to identify trees that are good rooters to allow the potential for the nursery industry to use those trees as sources for clone material. We found four sugar maples with outstanding horticultural characteristics that show evidence of good rooting ability (50% or better rooting of cuttings) given specific auxin treatments. We would recommend any of the four trees (trees 1, 2, 7 and 9) as potential parents to a cultivar because of their desirable ornamental characteristics, stress resistance and rootability.

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Fig. 3.1. Percentage of cuttings from eight sugar maple trees rooted. Data are pooled over IBA rates. n = 140.

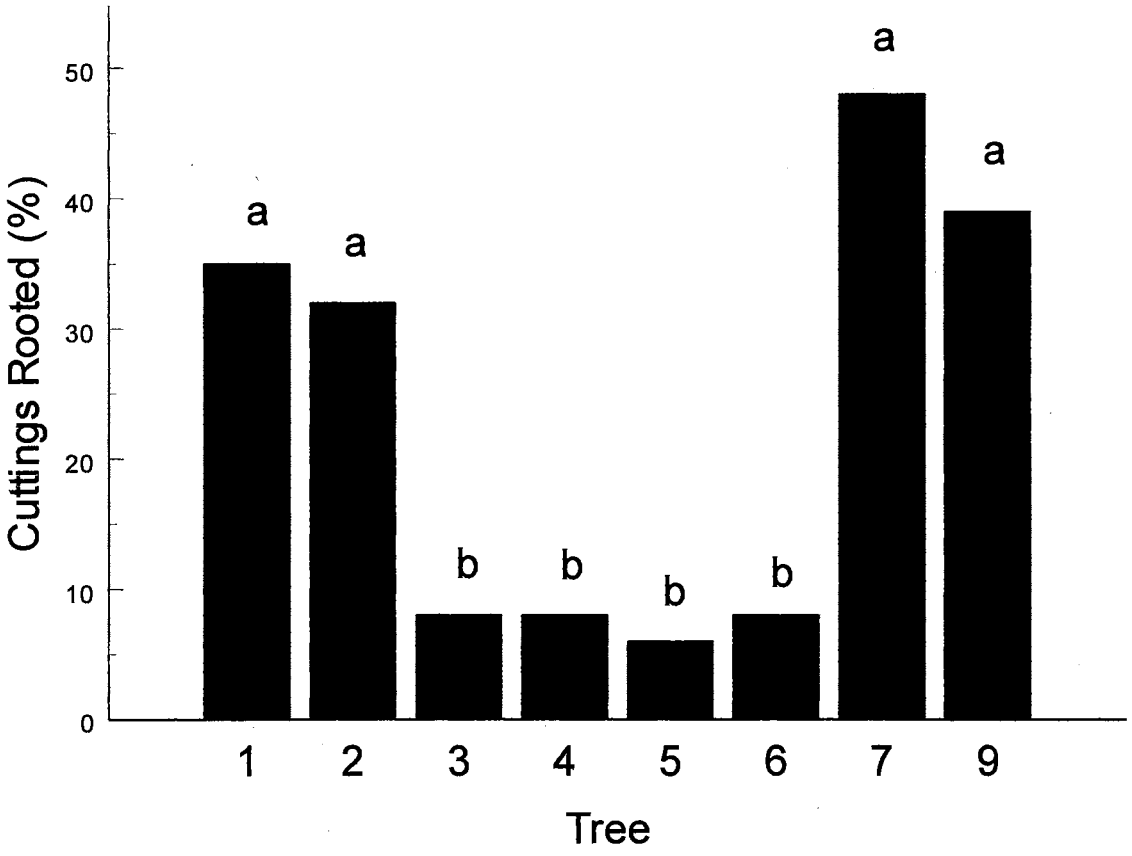

Cuttings Rooted (%)
50
40
30
20
10
0
a
a
b
b
b
b
a
a
1
2
3
4
5
6
7
9
Tree

Fig. 3.2. Percentage of sugar maple cuttings that rooted with various auxin treatments. Data are pooled over trees. n = 160.

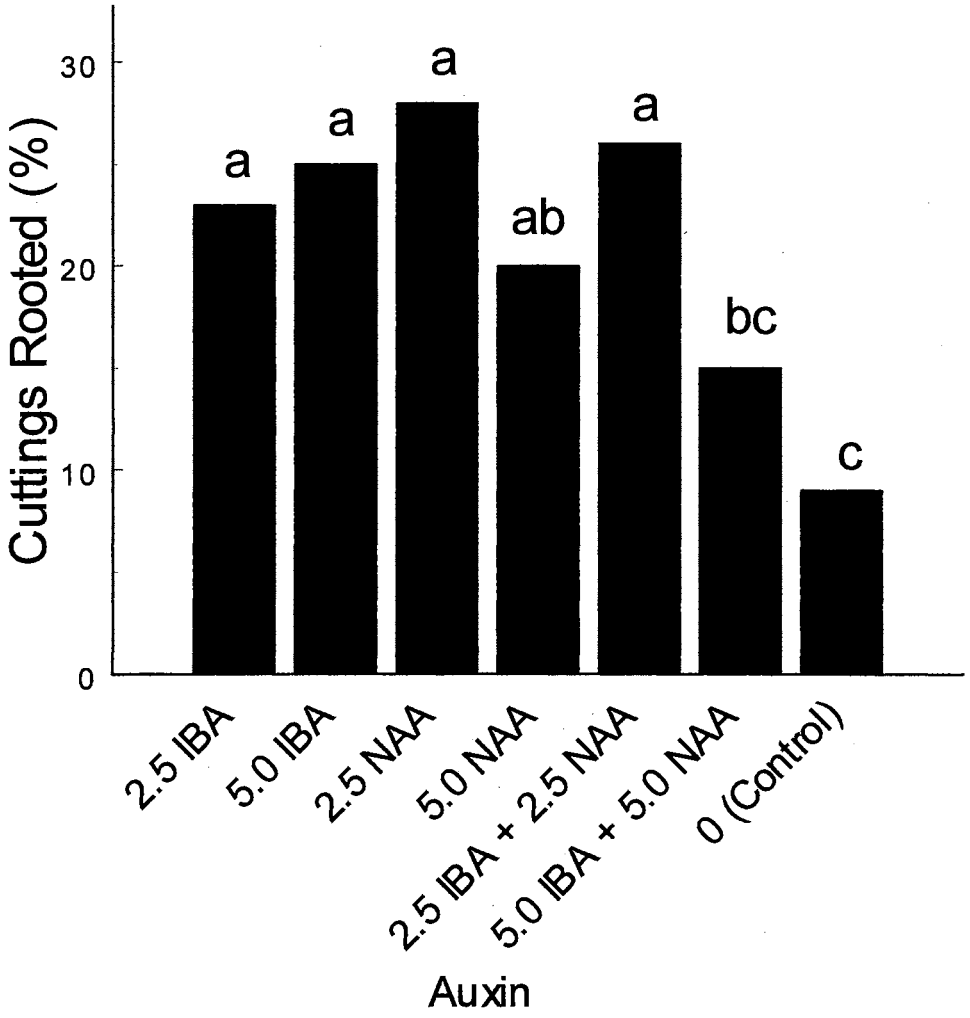

Cuttings Rooted (%)
30
20
10
0
a
a
a
ab
a
bc
c
2.5 IBA
5.0 IBA
2.5 NAA
5.0 NAA
2.5 IBA + 2.5 NAA
5.0 IBA + 5.0 NAA
0 (Control)
Auxin

Fig. 3.3. Average root number for rooted cuttings taken from eight sugar maple trees and receiving various auxin treatments. For trees 1, 6, 7 and 9, hormone treatments that differed from controls are marked with *** (P = 0.001). For trees 2, 3, 4 and 5, cuttings in the control treatment did not root.

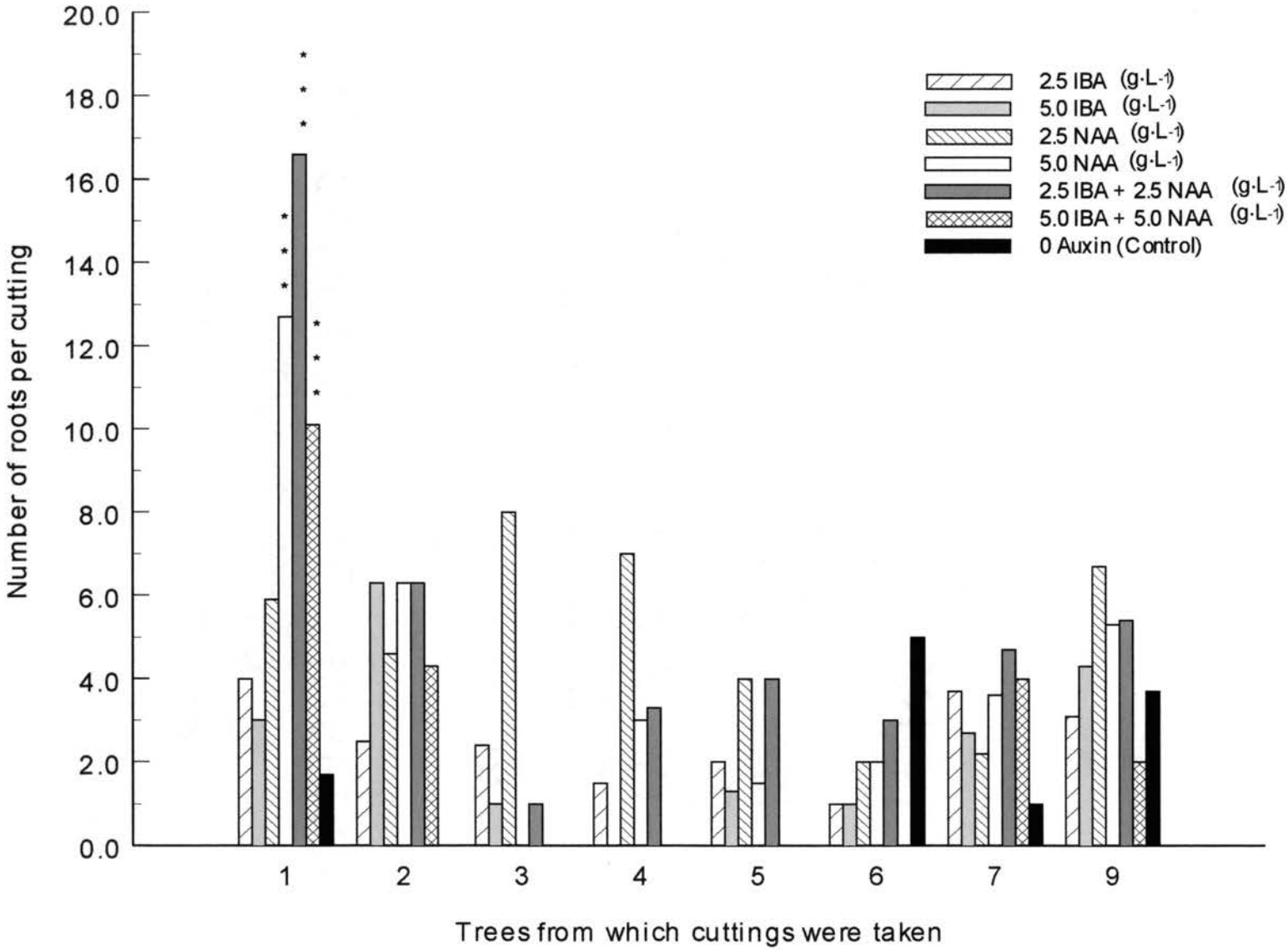

20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Number of roots per cutting
1
2
3
4
5
6
7
9
Trees from which cuttings were taken
2.5 IBA (g·L-1)
5.0 IBA (g·L-1)
2.5 NAA (g·L-1)
5.0 NAA (g·L-1)
2.5 IBA + 2.5 NAA (g·L-1)
5.0 IBA + 5.0 NAA (g·L-1)
0 Auxin (Control)

Fig. 3.4. Average root length of rooted cuttings taken from eight sugar maple trees (pooled over hormone treatments). tree P = 0.0008.

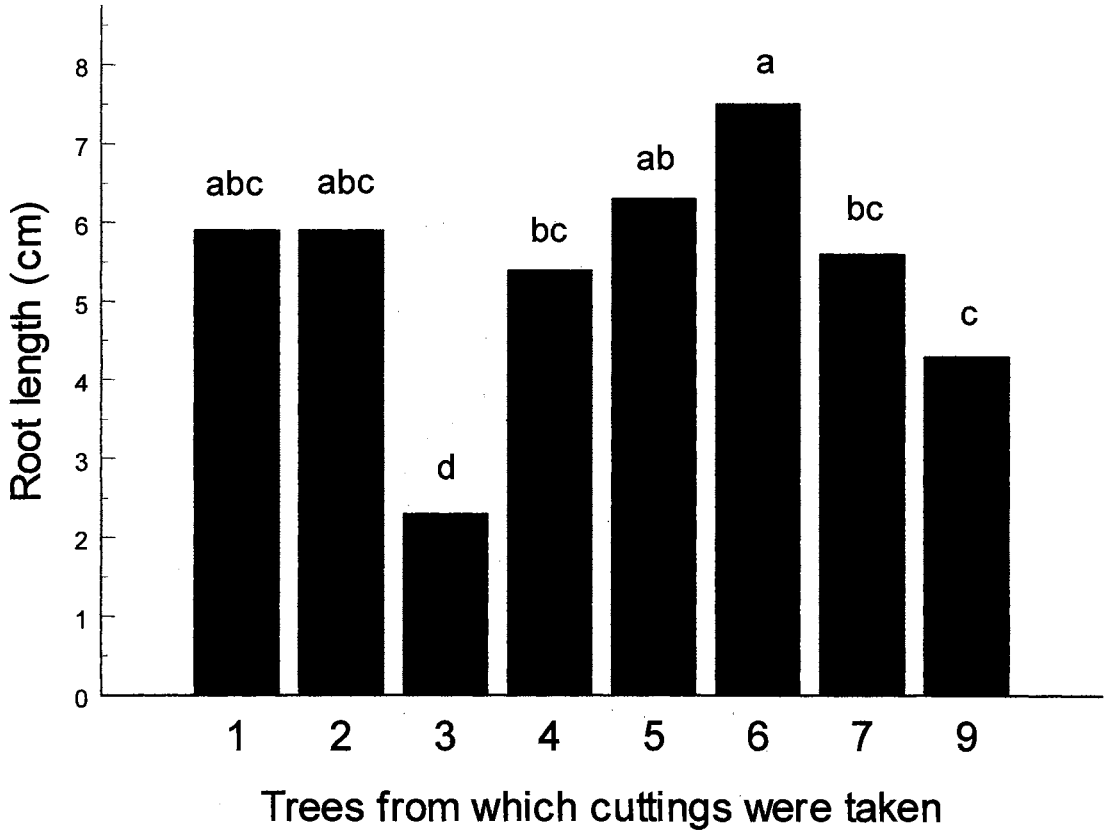
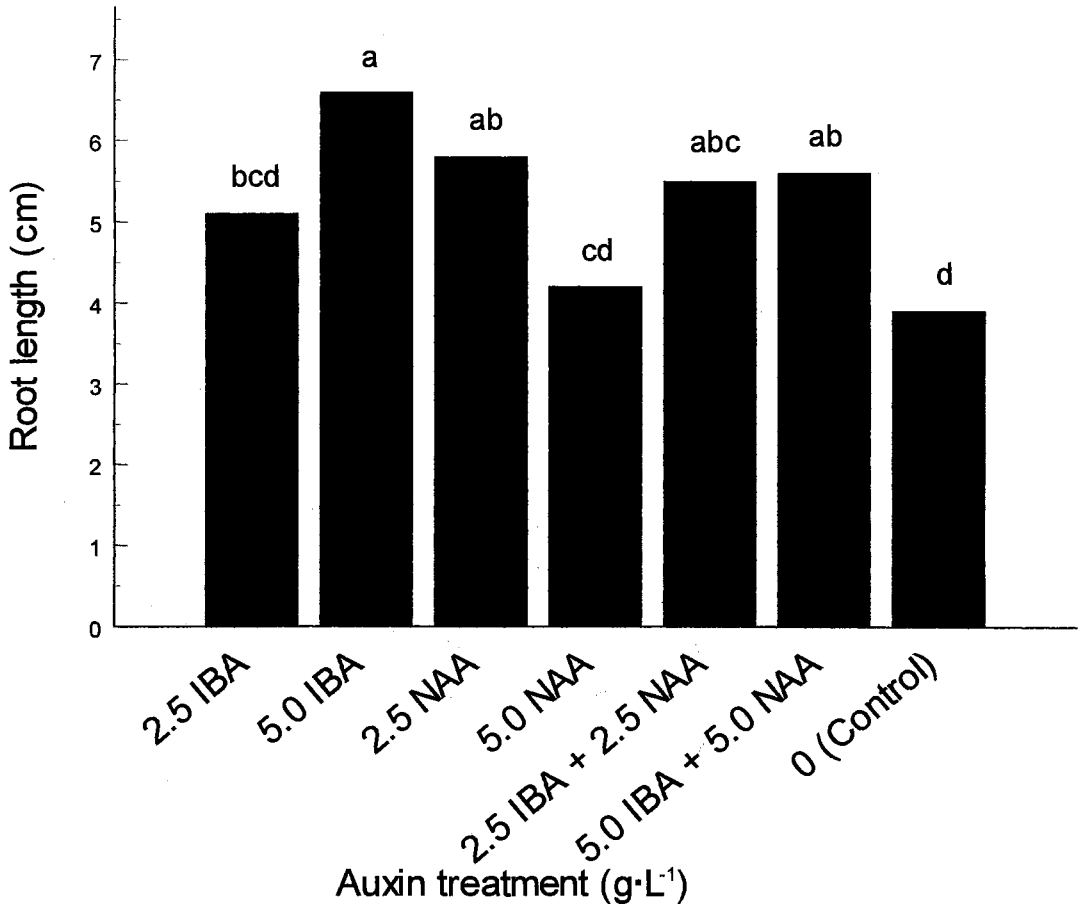

Root length (cm)
0
1
2
3
4
5
6
7
8
abc
abc
d
bc
ab
a
bc
c
1
2
3
4
5
6
7
9
Trees from which cuttings were taken

Fig. 3.5. Average root length per rooted cutting with various auxin treatments (pooled over trees). Rooting hormone treatment P = 0.0014.


Root length (cm)
0
1
2
3
4
5
6
7
bcd
a
ab
cd
abc
ab
d
2.5 IBA
5.0 IBA
2.5 NAA
5.0 NAA
2.5 IBA + 2.5 NAA
5.0 IBA + 5.0 NAA
0 (Control)
Auxin treatment (g·L-1)

CHAPTER 4

Mound Layering as a Propagation Method for Caddo Sugar Maples

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Additional Index Words: *Acer saccharum*, vegetative propagation, difficult-to-root ornamentals, IBA, etiolation

Abstract. **One-year-old Caddo sugar maple seedlings were transplanted to a field at the Oklahoma State University Nursery Research Station, Stillwater, Okla., and one year later subjected to a 2 by 2 factorial arrangement of wounding vs. no wounding and 5 $g \cdot L^{-1}$ IBA vs. no IBA. Soil was mounded around the treated shoots. Shoots were later evaluated for rooting. Wounding + IBA produced more roots than the other treatments, although rooting was poor regardless of treatment.**

Superior specimens of sugar maple (*Acer saccharum* Marshall) with desirable autumn color and resistance to drought, leaf tatter and scorch can be found in native stands in

Oklahoma as well as other parts of the midwestern United States. Desirable specimens include Caddo sugar maple, an ecotype found in Red Rock Canyon, near Hinton in Caddo County, Okla., as well as seedling-grown Caddo sugar maples transplanted into urban settings. In addition to resistance to leaf tatter and scorch (Conley et al., 1995; Pair, 1994), Caddo sugar maples are tolerant of high pH soils (Simpson and Hipp, 1993). Sugar maples tolerant of climates with extreme heat and high winds would be a valuable addition to the nursery industry.

Sugar maples are generally propagated by seed or by bud grafts. Seed propagation is hampered because sugar maples tend only to produce a seed crop every two to seven years (Godman et al., 1990), and seed coats are often empty (Dirr, 1998). Vegetative propagation allows the cloning of trees with desirable characteristics, but often the desired traits are not evident until trees are mature. Nurserymen often avoid bud grafting because workers lack grafting skills and because of the need to produce numerous seedlings to use as root stock. Cutting propagation has not been feasible for mature sugar maples because it is difficult to successfully root cuttings from mature growth.

Mound layering involves severe pruning of stock plants to induce adventitious shoots that are juvenile in appearance and vigorous in growth (Howard et al., 1988). The reversion from adult to juvenile form, with an accompanying shift from a difficult-to-root to an easy-to-root phase, can occur through formation of adventitious growing points in which new shoots are more juvenile than the original (parent) plant (Kester, 1983). Mound layering also involves the exclusion of light (etiolation) from the area to be rooted (Ryan, 1969). Etiolation results in physiological and anatomical changes in stem tissue, including alterations in meristematic activity, hormone action and the ability of tissues to support the growth and development of root primordia (Maynard and Bassuk, 1988). The combination

of severe pruning plus darkness accounts for the success of mound layering in enhancing rooting of cuttings (Howard et al., 1988).

In mound layering, stock plants are severed near the ground level when new growth begins in the spring. New shoots emerging from buds in the severed stumps are allowed to develop. When the shoots have grown 7.6 cm to 12.7 cm in height, they are encouraged to root by mounding soil, sawdust or a soil-sawdust mixture around their bases; the mounding process is repeated as the shoots grow (Hartmann et al., 1997). The shoots are then removed by cutting below the new root system.

Mound layering is the main technique used to propagate apple (*Malus* Mill.) rootstocks (MacDonald, 1986) clonally. A mound layering bed can be used for 15 to 20 years if it is maintained in a vigorous condition (Hartmann et al., 1997). Sugar maples have a tendency to layer from a variety of sources, including branches and basal sprouts (Fayle, 1996).

Mound layering has proven successful in producing rooted cuttings in difficult-to-root plant species including Chinese pistache (*Pistacia chinensis* Bunge.) (Dunn and Cole, 1995), pecan (*Carya illinoinensis* (Wangenh.) K. Koch.) (Wood, 1989); American elm (*Ulmus americana* L.) (Schreiber and Kawase, 1975), paperbark maple (*Acer griseum* (Franch.) Pax.) (Hoogendoorn, 1984), Monterey pine (*Pinus radiata* D. Don) (Libby and Hood, 1976; Menzies, 1985) and 'Cleopatra' mandarin (Duarte and Medina, 1971).

The objective of this study was to determine if mound layering is a feasible method for vegetatively propagating Caddo sugar maple seedlings.

Materials and Methods

Experiment 1

Seeds from seedling-grown Caddo sugar maples, collected in Oklahoma City, Okla. in fall 1998 by Steve Bieberich, a nurseryman and expert on Oklahoma native plant species, were stratified about three months by placing the seeds in a tray of slightly moist peat moss inside a sealed polyethylene bag and placed in a 4.4°C cooler. The seeds were planted in a polyethylene-covered greenhouse in January 1999, under natural photoperiod with a maximum photosynthetic photon flux (PPF) of 845 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Maximum/minimum air temperatures in the greenhouse were 29°C/18°C. The seedlings were placed in a shade house with a maximum PPF of 1150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for one week to acclimate to outdoor conditions, then transplanted on 13 Apr. 1999 to an outdoor mound layering bed at the OSU Nursery Research Station, Stillwater, Okla. The seedlings were spaced about one meter apart in one long row. Immediately after planting, an over-the-top application of Simazine (2-chloro-4, 6-bis(ethylamino)-S-triazine) at a rate of 6.4 $L \cdot ha^{-1}$ was made to control grassy weeds. The seedlings were fertilized based on soil test recommendations with ammonium nitrate.

The seedlings were cut to about 5 cm above the soil line on 17 Apr. 2000, as leaves were emerging. Root-inducing treatments were applied on 12 May, when most shoots were 12 cm in height.

Treatments consisted of a 2 × 2 factorial arrangement of wounding vs. no wounding and 5 $g \cdot L^{-1}$ IBA (dissolved in 70% isopropyl alcohol) application vs. no IBA. Wounding consisted of slicing into the phloem and cambial tissue about 1 mm deep and 8 mm long on

top of the horizontal section of the shoot base. The auxin treatment was IBA lightly rubbed into the wounded or non-wounded horizontal section of the shoot with a cotton swab.

When treatments were applied, soil was mounded around the shoots to a depth of about 9 cm. Additional soil was mounded around the shoots as they grew. The trees received drip irrigation once or twice weekly to keep the soil moist.

The stool shoots were evaluated on 9 Sept. 2000 for number and length of primary roots.

The experiment was arranged in a randomized complete block design. Blocking was generally done by mother tree if there were at least four shoots produced per tree. When trees had fewer than four shoots, the replication was completed on the subsequent block. Some trees produced three or fewer shoots; others produced five to eight shoots. There were 17 mother trees. Treatments were compared using a chi square analysis.

Experiment 2

One-year-old container-grown sugar maple seedlings, donated by Greenleaf Nursery, Park Hill, Okla., and originating from F.W. Schumacher, Sandwich, Mass., were transplanted into mound layering beds at the OSU Nursery Research Station in Stillwater in January 2000. The seedlings received the same treatments as seedlings in Experiment 1. The treatments were replicated six times. Evaluation for number and length of roots was 9 Sept.

Results

None of the shoots in Experiment 2 rooted, so results are presented only for Experiment 1.

Rooting percentage was significantly greater (P = 0.0238) among shoots treated with the wound + IBA treatment than root number for the control treatment. There were no significant differences in rooting among the other three treatments (data not presented).

Four of the 18 shoots receiving the wound treatment rooted, with an average of 2.8 roots per rooted shoot and an average root length of 8.2 cm. Six of the wounded shoots did not root. Eight of the wounded shoots died. Among the 17 shoots treated with IBA, five rooted with an average of 3.6 roots per rooted shoot and average length of 10 cm. Five of the IBA-treated shoots did not root; seven of the shoots died. Among the 17 shoots receiving the wound + IBA treatment, nine rooted with an average of three roots per shoot and average root length of 9.3 cm, while four shoots did not root and four shoots died. Among the 18 control shoots, three rooted with an average of two roots per shoot and average root length of 8.2 cm, while five shoots did not root and ten shoots died.

Discussion

Rooting of mound layered sugar maples was poor, and among the shoots that did root, the root system was probably not extensive enough to support the new plant. It is possible lighting was a factor in the poor rooting. Because of heavy rains in late spring, we were sometimes unable to enter the field to mound additional soil around plants as shoots grew. More shoots are produced when the crown of a plant is left exposed to light until the shoots have made some growth, but rooting is best if the plant is lightly covered with soil before budbreak and more soil is added at intervals as the shoots grow, so the basal portion of shoots where roots will develop is never exposed to light (Ryan, 1969). However, the bases of our shoots were not exposed to light other than the period when treatments were applied.

It is more likely that the age of our seedlings contributed to poor rooting. Howard (1977) determined that apple stoolbeds were more vigorous when the bed was established with larger plants than with smaller plants. Howard (1977) also found that the overall percentage of heavily rooted shoots of the difficult-to-root M.9a clone increased as the stoolbeds grew older. Vasek and Howard (1984) reported improved rooting of difficult-to-root apple rootstocks with biennial harvesting or when only large shoots were harvested the first year, allowing the smaller shoots to grow for a second season. This effect of larger plants used in establishing the stoolbed could also explain the difference in rooting seen in our study between the Caddo seedlings that had been planted for one year before mounding treatments were applied (Expt. 1) and the sugar maple seedlings planted shortly before the treatments were applied (Expt. 2). When pecan was successfully propagated by mound layering, four-year-old seedlings were used to establish stoolbeds (Wood, 1989). Three-year-old plants were used to establish beds for the propagation of mandarin (Duarte and Medina, 1971). Quamme and Brownlee (1990) waited three years after planting to begin assessing mound layering ability of 27 apple clonal rootstocks. On the other hand, Chinese pistache was successfully propagated by mound layering when beds were established with 10-month-old seedlings (Dunn and Cole, 1995).

It is possible the Caddo sugar maple shoots required a longer period to root than allowed in our experiment. The M.9a stool shoots develop roots relatively late in the season, possibly using resources available during autumn after the cessation of shoot growth (Howard, 1977).

Caddo sugar maple seedlings may not be suitable candidates for mound layering propagation, although we would like to see the study repeated on trees that have been planted in the field for several years before mounding treatments are applied.

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CHAPTER 5

The Anatomy of Root Initiation and Emergence in Caddo Sugar Maple Softwood Stem Cuttings

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Additional Index Words: *Acer saccharum,* adventitious roots

Abstract. **Adventitious root formation in softwood stem cuttings of Caddo sugar maples treated with IBA was studied using light microscopy. Root primordia were found near a leaf trace, both in the wound-generated callus tissue exterior to the stem's sclerenchymous sheath and interior to the sheath near the cambium.**

Mechanisms of adventitious root formation in sugar maples (*Acer saccharum* Marshall) are poorly understood. Woody plants produce adventitious roots in several areas including the cambium, wound callus and bud phloem (Raviv and Putievsky, 1987). In most species that are difficult to root, initiation of roots occurs within callus tissue (Davies et al., 1982; Hamann, 1998). According to Greenwood et al. (1976), unpublished Northeast Forest Experiment Station work by J.P. Rier revealed that the cambium is the site of root regeneration in sugar maples. Since rooting of stem cuttings of sugar maples occurs only during a two week period in June in Vermont, the period when rooting occurs may coincide with a rejuvenation associated with annual cambium activity (Greenwood et al., 1976).

In some species, rooting ability declines when the plants mature (Geneve et al., 1988). Some researchers believe anatomical barriers may contribute to the loss of rooting ability, including lignification (sclerification) of phloem tissue (Beakbane, 1961; Edwards and Thomas, 1980) or suberization of cork cells (Williams et al., 1984). Beakbane (1961) pointed out that lignification is associated with senescence in tissues, so there is at least a correlation between lignification of primary phloem of the stem and rooting ability. However, she said lignified sheaths are probably not the sole cause of the lack of rooting since root initials do not form within the sheath.

This study was designed to explore possible causes of rooting difficulties in sugar maples and to understand better the anatomical processes involved in adventitious root formation in sugar maple stem cuttings.

Materials and Methods

Green softwood stem cuttings were collected from a mature sugar maple growing at the Oklahoma Botanical Garden and Arboretum (OBGA) in Stillwater, Okla. Ten replicate cuttings were taken every three days and grown under conditions that had previously resulted in optimum rooting (Chapter 2). Cuttings were taken between 8 May and 2 June, 2000. On 2 June, the first set of cuttings taken had produced visible roots. On 8 June the basal 2 cm of all the cuttings was harvested and prepared for microscopy.

To check whether anatomical changes occurred in intact plants as well as in cuttings, basal stem segments were also collected periodically from the tree. These samples were taken on 8, 17 and 24 May and 2 June, and the tissue prepared for microscopy on the same dates.

To prepare the plant tissue for light microscopy, emerged roots were severed from the stems that had rooted, and stem segments were quartered and cut into pieces about 0.3 cm in length. The material was fixed in 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), dehydrated in an increasing series of ethanol concentrations (20 min immersion in 30%, 50%, 70%, 90% and 95% ethanol, and 100% three times for 20 min each), infiltrated with ethanol and LR White (London Resin Co., Berkshire, England), and embedded in LR White (procedures described in Electron Microscopy Laboratory Manual, 2000, College of Veterinary Medicine, Oklahoma State University, Stillwater, Okla.). The embedded tissue was serially sectioned with an ultramicrotome using a diamond knife. Sections were about 10 μm thick. The serial sections were transferred individually from the knife onto glass slides and stained with azure II methylene blue.

Results

Figure 5.1 shows the cross-sectional anatomy of a cutting base at the time it was severed from the sugar maple shoot on 8 May 2000. The vascular system had already formed a closed ring, with short rays usually a few cells wide. There was a nearly continuous ring of sclerenchyma fibers between the cortex and the phloem. Figure 5.2 shows cross-sections of cuttings taken from the tree on 17 May, 24 May and 2 June. Stem anatomy appeared similar at each date except the xylem and phloem increased in width over time.

Figure 5.3 shows root primordia formation in a cutting 29 days after treatment with 5 $g \cdot L^{-1}$ indolebutyric acid (IBA). One of the primordia appeared to form near a leaf trace, in cortical callus adjacent to the phloem parenchyma. The primordium was separated from the phloem parenchyma by the sclerenchyma sheath. A second primordium (Fig. 5.3G and Fig.

5.4) appeared to develop in phloem tissue near the original cambium, and was also near the leaf trace. In contrast to Figs. 5.3 and 5.4, Fig. 5.5 shows the cross section of a stem cutting that did not form adventitious roots.

Discussion

Lignification (a sheath of sclerenchyma fibers) has been proposed as a mechanical barrier to adventitious root formation in difficult-to-root species (Beakbane, 1961; Edwards and Thomas, 1980) but the sclerenchyma sheath in Caddo sugar maple stem cuttings did not prevent root formation. Root primordium formation occurred both to the interior and exterior of the sheath in one stem (Fig. 5.3) in our study. In a stem that did not root (Fig. 5.5) there were obvious gaps in the sclerenchyma sheath where roots could have emerged had they formed inside the sheath. Similarly, Goodin (1965), who studied the anatomy in stems of mature ivy, found that the ivy failed to root even though gaps in a sclerified sheath surrounding the phloem would have allowed roots to emerge. Based on our observations, we agree with Hartmann (1969) and Sachs et al. (1964) that biochemical rather than anatomical factors cause the differences in root initiation between easy- and difficult-to-root plant species.

Cambium has been reported as the site of root regeneration in sugar maples (Greenwood et al., 1976). Although we observed adventitious root formation in the cambial region (Fig. 5.4) we also discovered root initiation in wound callus tissue in the cortex near a nodal leaf trace (Fig. 5.3). Girouard (1967), White and Lovell (1984) and Schwarz et al. (1999) also reported root initiation sites in the parenchyma cells of leaf traces and leaf gaps. As Schwarz et al. (1999) pointed out, however, presence of a leaf trace did not guarantee root formation in cuttings but may have facilitated root initiation.

Caddo sugar maples are an ornamental species with desirable landscape characteristics including resistance to leaf scorch and leaf tatter (Conley et al., 1995). We have shown that stem cuttings of Caddo sugar maples will produce adventitious roots during the softwood stem stage. Cutting propagation may allow the production of clones of superior Caddo specimens.

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Figure 5.1. Cross section of Caddo sugar maple stem. Tissue was processed for light microscopy immediately after removal from the tree on 8 May 2000. **x** 48

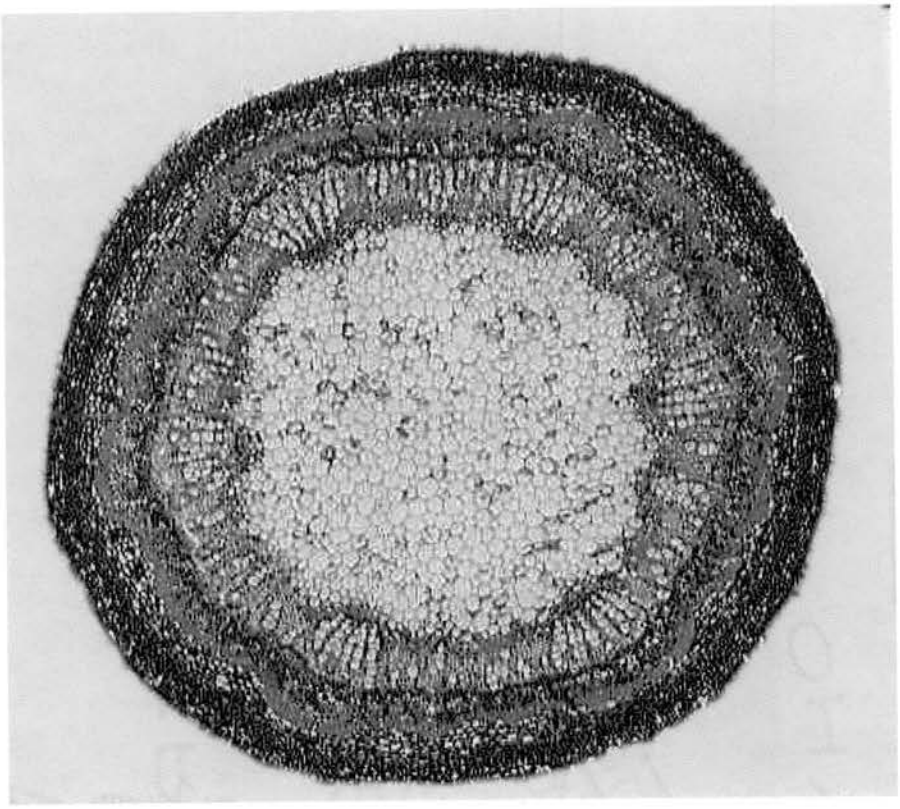
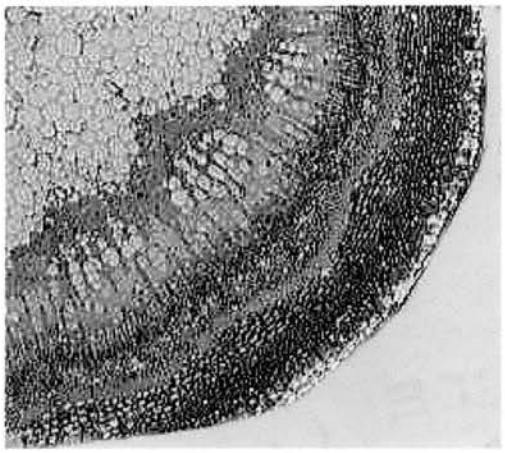
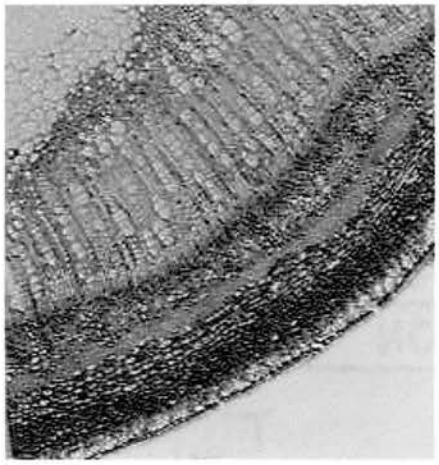


Figure 5.2. Cross sections of Caddo sugar maple stem. Tissue was processed for light microscopy immediately after removal from the tree on 17 May 2000 (top), 24 May (middle) and 2 June (bottom). x 43





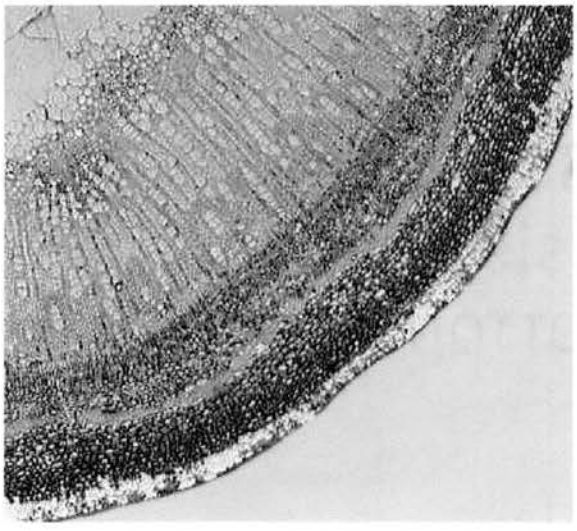


Fig. 5.3 Root primordia seen in cross section of Caddo sugar maple stem 29 days after treating with IBA. Micrograph shows leaf gap (A), root primordium forming from wound callus tissue (B), vascular ray penetrating sclerenchyma sheath (C), cambium (D), phloem (E), xylem (F) and adventitious root arising from original cambial activity (G) (see Fig. 5.4 for close-up of G). **x** 60.

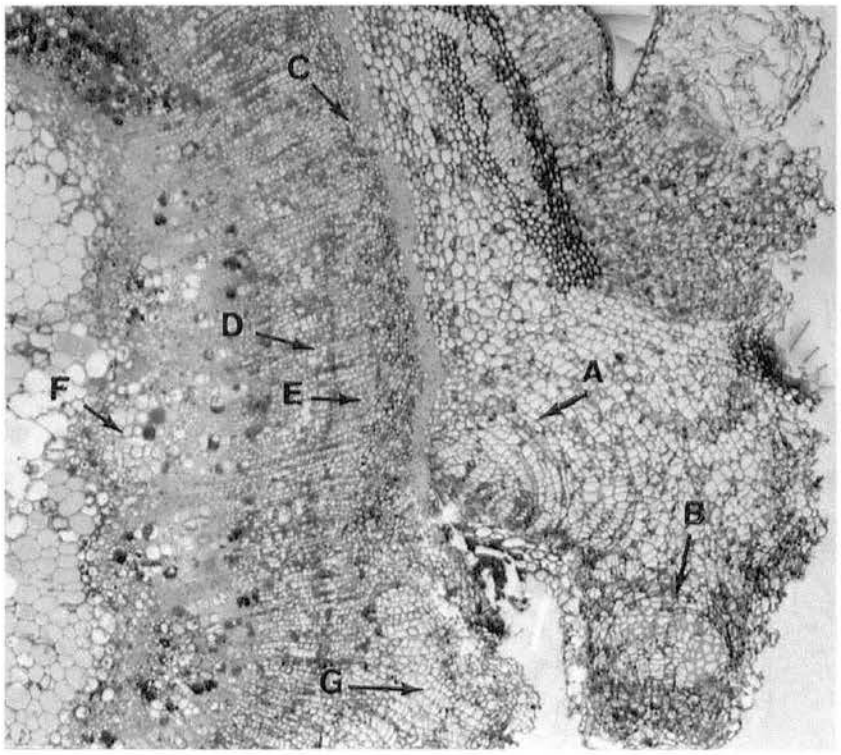

C
D
F
E
A
B
G

Fig. 5.4. Adventitious root arising from original cambial area in rooted stem cutting of a Caddo sugar maple. Cambium can be seen at base of arrow. X 60.

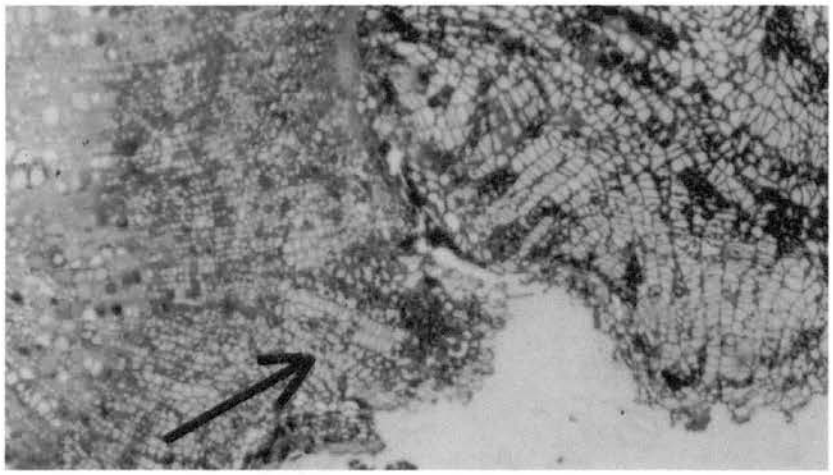
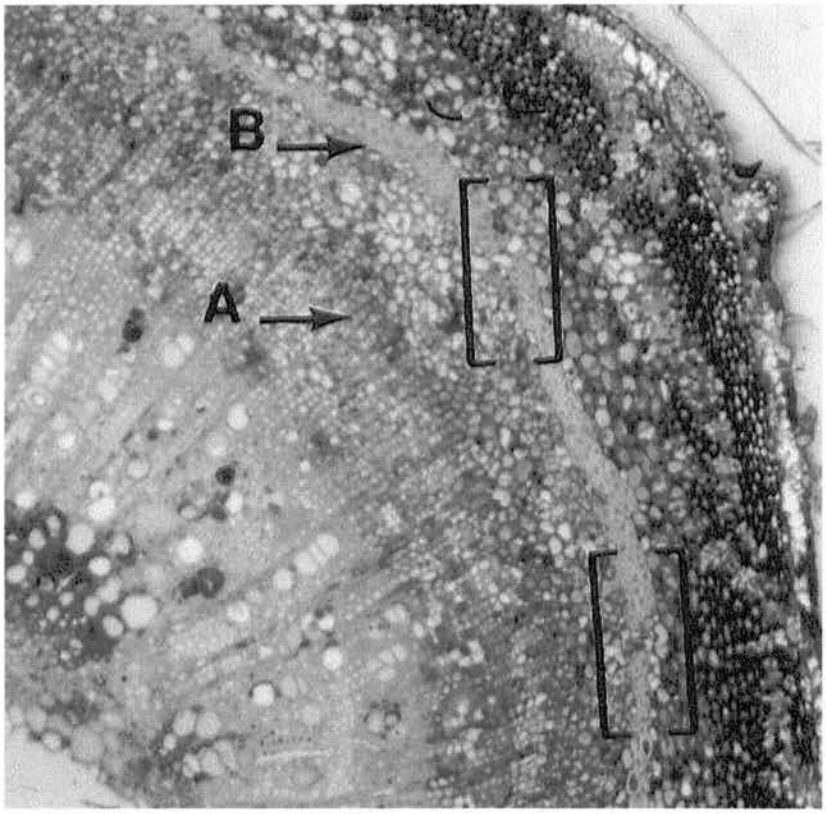


Fig. 5.5. Stem cutting that did not form adventitious roots. The cutting was taken on 17 May, 2000, placed on a greenhouse mist bench and processed for light microscopy on 8 June. The arrow marked A indicates the location of the cambium. The arrow marked B points to the sclerenchyma sheath. Gaps in the sheath are shown in brackets. x 100.


B
A

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Appendix A

Description of the location of sugar maple trees (Chapter 3).

Tree 1—just north of the OSU Nursery Research Station building known as Charlie's Barn.

Tree 2—across low-water bridge near pecan trees on the south side of the creek at OBGA.

Tree 3—north side of creek, east of low-water bridge.

Tree 4—east edge of OBGA, just west of creek, almost due east of Charlie's Barn.

Tree 5—Centennial Grove on OSU campus, western-most sugar maple on south bank.

Tree 6—Centennial Grove, center sugar maple on south bank.

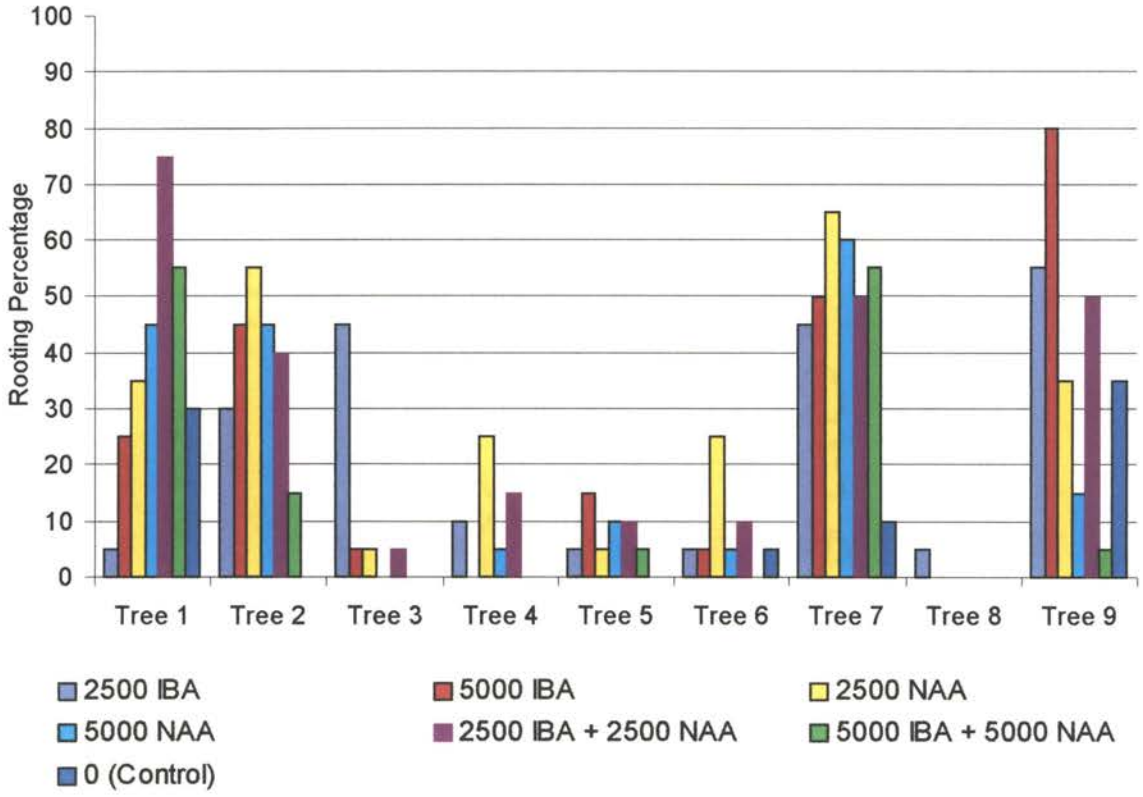
Tree 7—Centennial Grove, eastern-most sugar maple on south bank.

Tree 8—north side of OSU Human Environmental Science (HES) building, sugar maple on east.

Tree 9—north side of HES building, sugar maple on west.

Appendix B

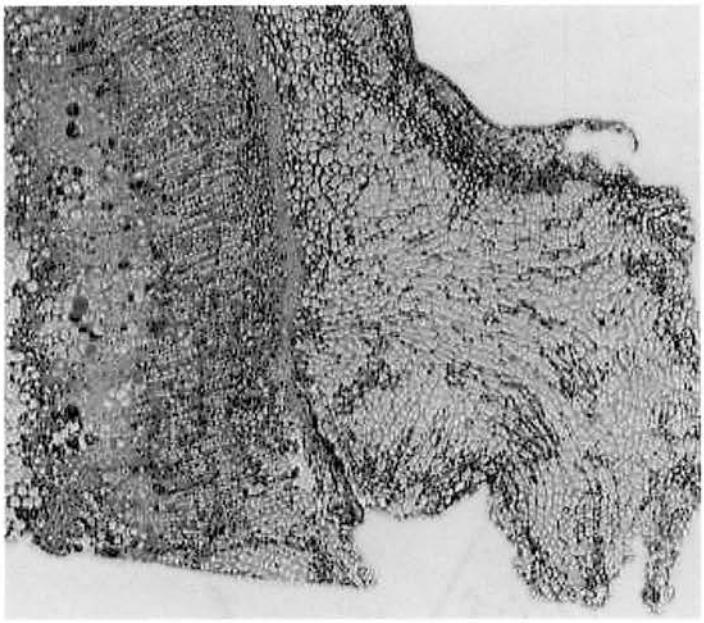
Rooting response for each tree and auxin combination (Chapter 3).

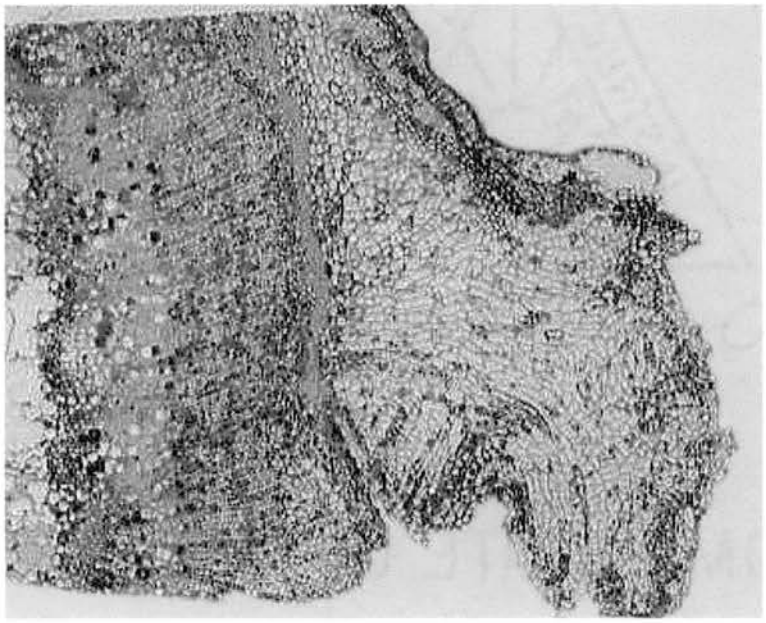


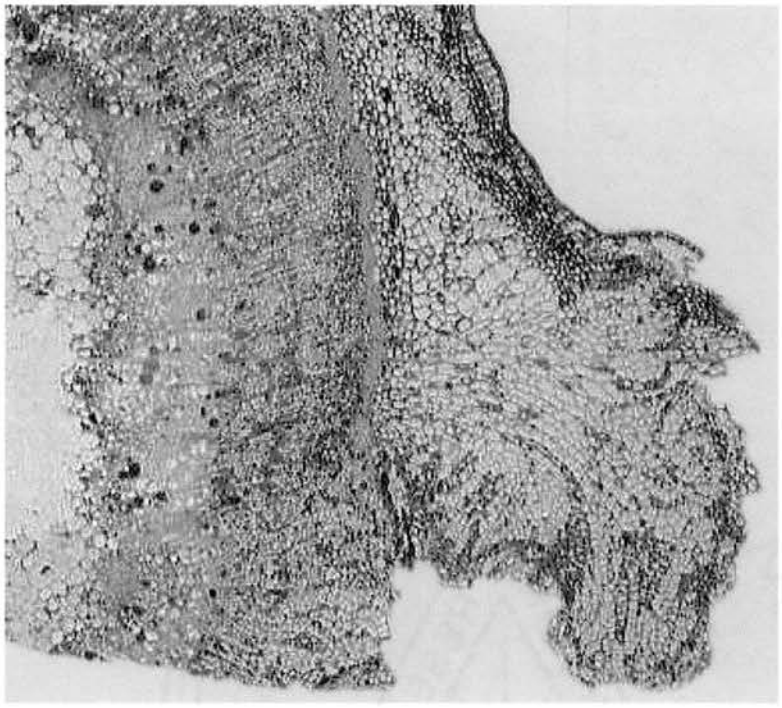
Appendix C

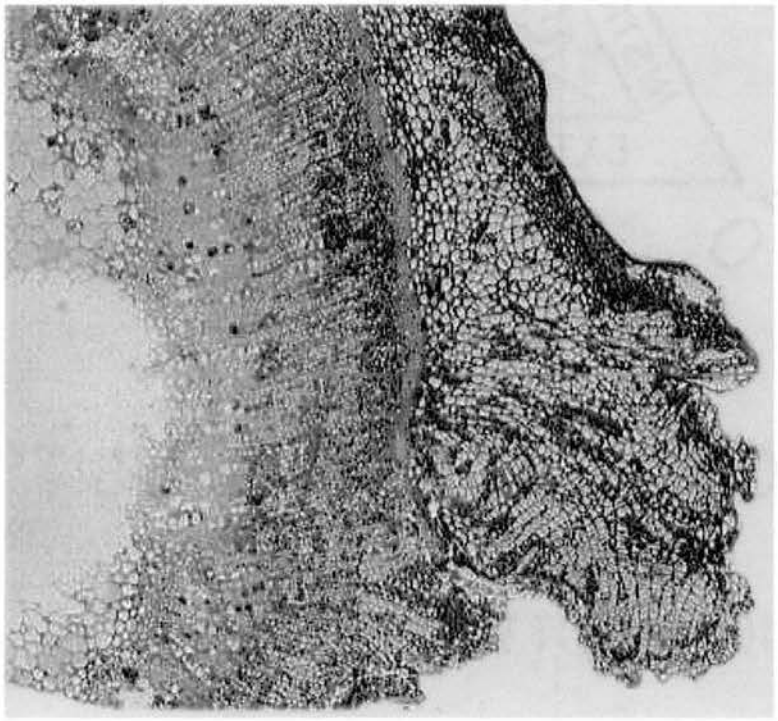
Micrographs of adventitious roots (Chapter 5).

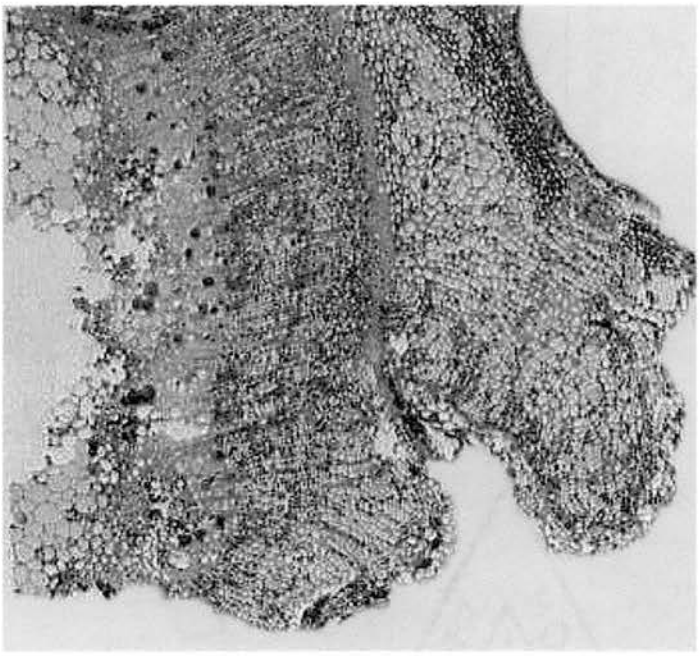
Series of thick sections (about 10 μm thick) from a single Caddo sugar maple stem cutting showing possible adventitious root formation and emergence. The cutting was taken on 11 May 2000, placed on a greenhouse mist bench, and processed for light microscopy on 8 June, when root emergence was visible. The distance between each tissue section shown is about 250 μm.

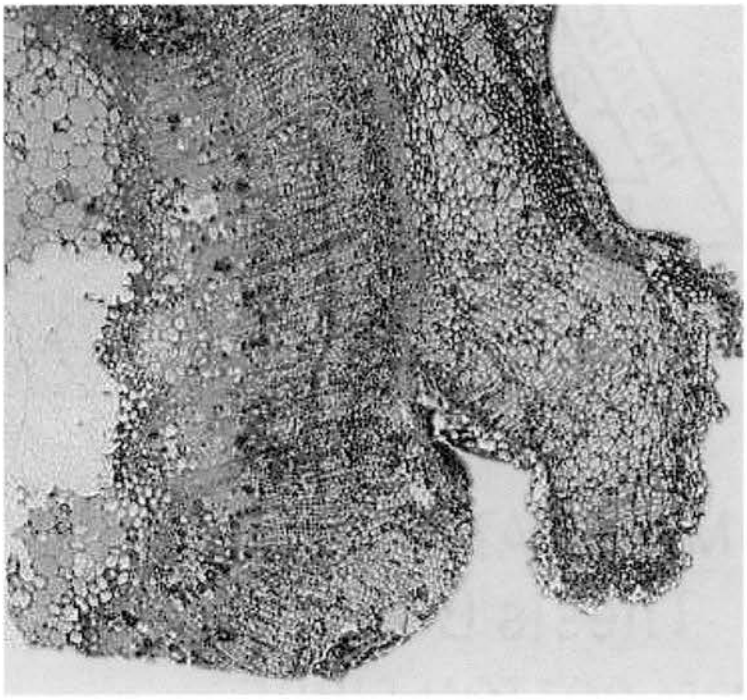


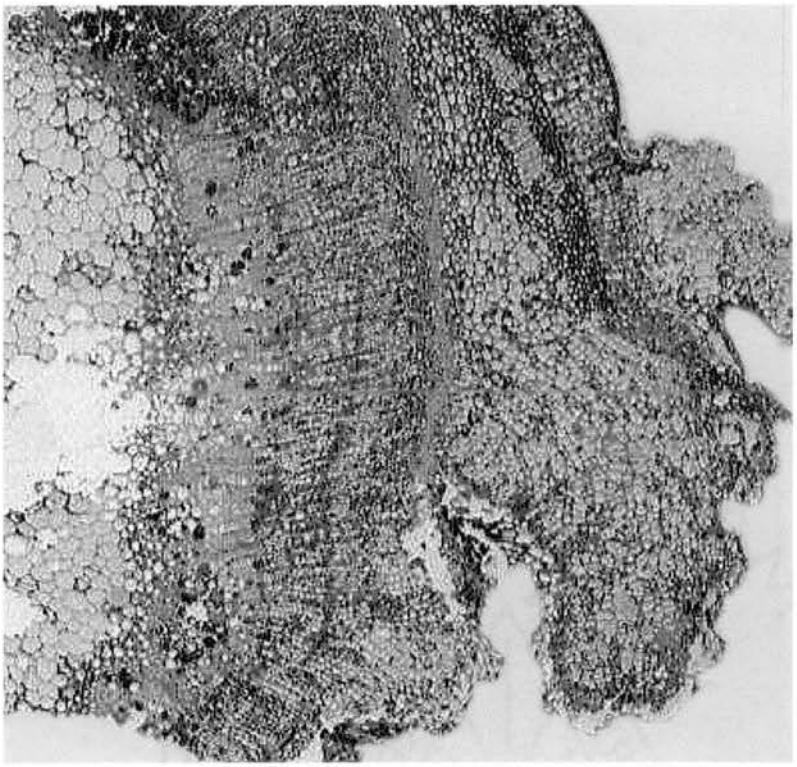


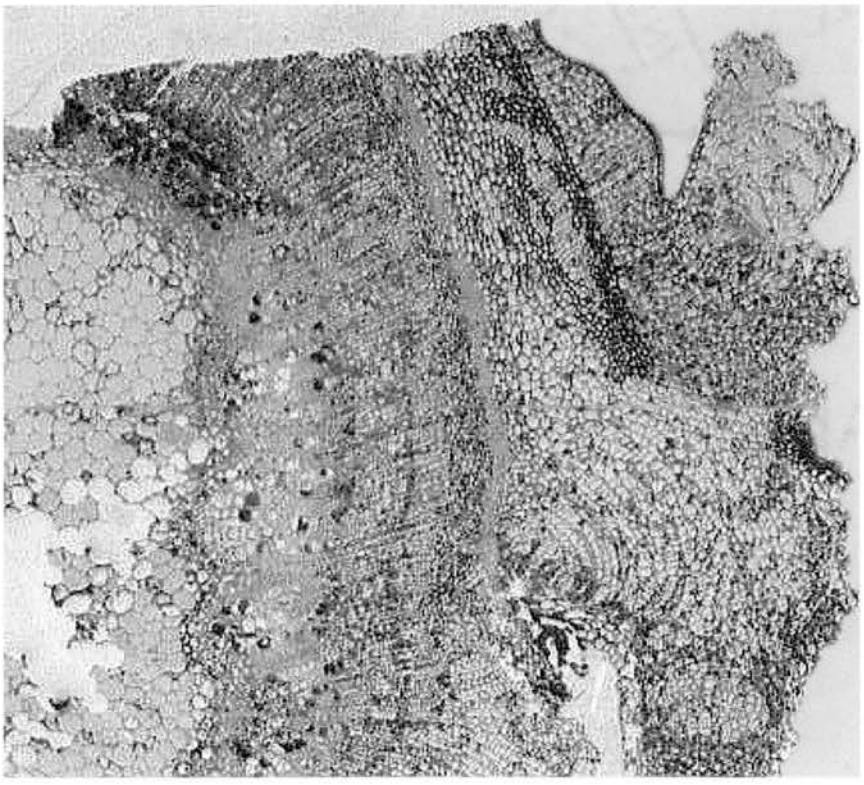


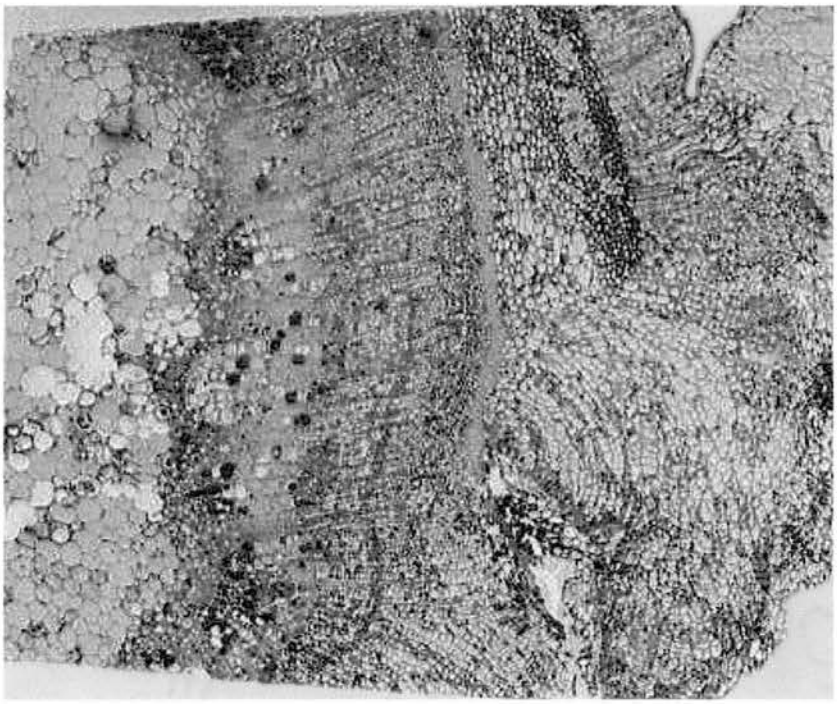


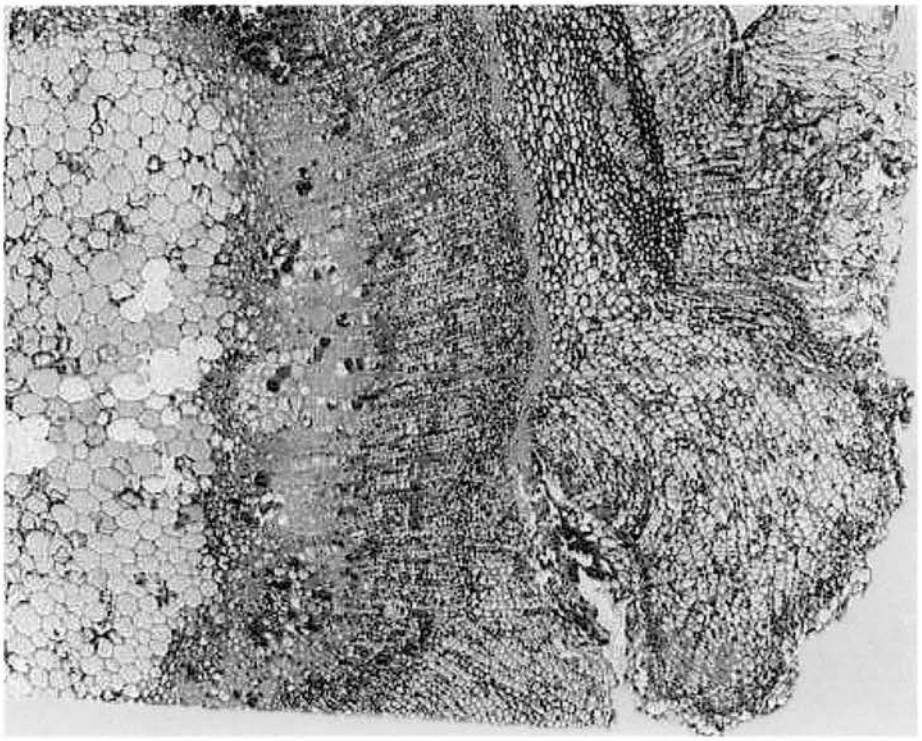












VITA

Clydette M. Alsup

Candidate for the Degree of

Doctor of Philosophy

Thesis: VEGETATIVE PROPAGATION AND ANATOMY OF ROOT INITIATION IN *ACER SACCHARUM* 'CADDO' STEM CUTTINGS

Major Field: Plant Science

Biographical:

Personal Data: Born in Pawnee, Okla. on 12 April 1960.

Education: Received Bachelor of Science degree in Horticulture from Oklahoma State University, Stillwater, in 1996; and Master of Science degree in Horticulture, with an emphasis in vegetable production, from Oklahoma State University in 1998. Completed the requirements for the Doctor of Philosophy degree in Plant Science at Oklahoma State University in May 2001.

Experience: Employed by Oklahoma State University Department of Horticulture and Landscape Architecture as a graduate research assistant and teaching assistant from 1996 to present. While attending college, have also worked at the OSU Teaching Greenhouses, Research Greenhouses, Turfgrass Research Center and Oklahoma Botanical Garden and Arboretum, and was a co-author of Oklahoma Cooperative Extension fact sheets while serving as a consumer horticulturist assistant.