Asexual propagation of bur oak (Quercus macrocarpa) Michaux
by Cheryl Louise Moore

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Sciences
Montana State University

Abstract:
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APPROVAL

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Cheryl Louise Moore

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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GLOSSARY

Accession – Seed sources, usually from different trees or provenances

Adventitious roots – Roots that form from an unusual place on a stem or leaf; other than a seedling root

Asexual propagation – A propagation method used to reproduce an exact genetic copy of the parent (stock) plant.

Banding – An adjunct treatment to etiolation, intended to maintain the etiolated condition of the base of the cutting after the etiolation material has been removed from the parent plant.

Basipetal movement – Movement of material toward the base of the plant, either from the shoot or root apex.

Clonal propagation – See asexual propagation

Dibble – A small hand tool used to make holes in planting media. Used in striking cuttings so applied rooting hormones are not removed during the process.

Endogenous – Synthesized within the plant

Epicormic shoots – Shoots emerging from a latent (dormant) bud

Etiolation – Growing plants or shoots in the absence of light

Exogenous – Introduced from outside the organism

Hardwood cutting – Propagules removed from the parent plant in winter when the buds are dormant.

Hedging – Severe pruning of stock plants to retain or promote the production of juvenile phase cutting material

Parent plant – See stock plant

Phenology – The study of the impact of seasonal variation in an organism.

Proximal – The portion of a plant organ closest to its attachment to the main part of the plant

GLOSSARY – CONTINUED

Quercus macrocarpa – Bur oak

Quercus spp. – Oak species

Seed orchard – Plantings of trees often comprised of phenotypically superior trees, intended to cross-pollinate to produce superior seeds

Softwood cutting – Vegetative propagule removed from the parent plant in early spring, when the tissue is actively growing and succulent.

Striking – Physically placing cuttings into propagation media.

Stock plants – Source plants for cutting material

Stooling – Use of a tree stump to provide cutting material through production of adventitious shoots.

Vegetative propagation – See asexual propagation

ABSTRACT

Seed orchards consisting of cloned superior parent trees are necessary to enable consistent propagation of trees with desirable traits. Cloning of superior trees for establishment of these seed orchards is typically accomplished by vegetative propagation. Because bur oak is a species well adapted for windbreaks in the northern plains, it would be desirable to establish a seed orchard from superior specimens, but bur oak cuttings are difficult to root. Superior 9-year-old trees from seed accessions were source plants for this study. In two greenhouse locations, hardwood cuttings were subjected to two media, two air temperatures, and nine concentrations of indole-3-butyric acid (IBA) in either a talc or a distilled water base, and various environmental treatments. Hardwood cuttings did not root. For the softwood study, parent plants were subjected to one or a combination of treatments, including full or localized light exclusion, banding, and/or hedging. Cuttings taken from these plants were treated with one of five IBA concentrations in a talc base. Cuttings were evaluated at 8 and 11 weeks for general health based on stem color, number of live leaves, petiole color, callus color and amount, and presence of roots. Highest rooting percentages in cuttings from plants that were hedged, etiolated and banded and treated with 2,500 ppm or 10,000 ppm IBA; hedging, etiolation and no-banding at 2,500 ppm IBA, and non-hedged, etiolated and not-banded at 10,000 ppm IBA. A combination of etiolation and hedging produced the healthiest cuttings. Rooting of cuttings correlated with cutting stem color, number of live leaves, petiole color, and survival rates. The amount and color of the callus correlated with the degree of rooting at one location and not at the other.

INTRODUCTION

Windbreaks and shelterbelts are particularly valuable in the Northern Plains, protecting homes, crops, and recreational areas from desiccating and chilling winds, and soil erosion. These belts of vegetation also provide wildlife habitat, buffers for riparian areas, carbon sequestration, nutrient cycling, and erosion control (Scianna, 1996). Using native species and adapted seed sources has been proven to increase the survival, establishment, performance and longevity of shelterbelt systems. Bur oak, *Quercus macrocarpa*, is a native species recommended for use in windbreaks by the Natural Resources Conservation Service. Twenty-four seed sources (accessions) of bur oak collected throughout the northern Great Plains are undergoing provenance testing and evaluation for growth rate, seedling survival and form at the Plant Materials Center (PMC) in Bridger, Montana. After nine years, the selection process is nearing completion, and a seed release is anticipated within 2 to 3 years. Lack of adequate asexual propagation techniques limits the establishment of clonal seed orchards and growers' ability to increase these selected parent plants for a growing commercial market.

The first step to commercially releasing these accessions is to establish seed orchards. These orchards need to be established rapidly and contain clones of superior parent material. This is accomplished in other species through vegetative propagation of mature phase tissue by cuttings. Because of a historically limited ornamental landscape market, few nursery growers or researchers have needed to propagate bur oak by cuttings. Additionally, former attempts to do so have met with limited success in *Quercus* spp.,

and hence very little information is available regarding asexual propagation of *Q. macrocarpa*. In some experiments, genetic variation in the ability of individuals within a given accession to root gives different results within the same experimental treatment.

The purpose of these experiments is to determine what requirements must be satisfied in order to produce rooted cuttings of this species. Standard variables include hardwood and softwood cuttings, differing levels of exogenously-applied rooting hormone, media composition, and cool vs. warm greenhouse air temperatures during rooting of hardwood cuttings. Techniques proven successful on other species will also be tested, including hedging of parent material, etiolation of parent trees and banding of resulting softwood, and blanching of light-grown softwood tissue through banding. Also, an attempt to force winter buds to elongate into material appropriate for softwood cuttings will be made.

LITERATURE REVIEW

Overview

Bur oak, *Quercus macrocarpa* Michaux, is a member of the Fagaceae, and is also known as mossycup oak. Based on leaf, pollen, fruit and flower characteristics, as well as the morphology of trichomes found on the foliage, molecular information and interspecific hybridization, it has been assigned Subgenus *Quercus*, Section *Quercus* (=Leucobalanus) or white oaks (Hardin et al., 2001). See Appendix A for infrageneric classification of important *Quercus* species. *Q. macrocarpa* is a true white oak native to a broad area of North America including the northern plains of the United States. It has been identified by the Natural Resources Conservation Service (NRCS) as a species suitable for inclusion in windbreak and shelterbelt systems (Scianna, 1996). In Montana, native stands are found only in Carter County, although the species has been widely planted throughout eastern Montana as a successful landscape specimen and street tree. Although globally (range-wide) ranked as "Demonstrably Secure," The Montana Natural Heritage Program lists bur oak as "Critically Imperiled" in Montana, "...because of extreme rarity or some factor making it especially vulnerable to extirpation" (NRIS, 2003). Bur oak is at the periphery of its native range in the northern plains, and does not attain the stature that the species typically reaches on more fertile sites in higher precipitation zones.

Asexual propagation is used to perpetuate the genetic and phenotypic characteristics of a desirable stock plant. Perpetuation of specific characteristics of *Q.*

macrocarpa has not been considered important, as this species grows too large to be considered for home landscape use, other than as a large shade tree, nor has it been considered a particularly ornamental oak. Generally, shade trees are propagated from seeds, as clonal propagation is not cost-effective (Davies et al., 1994). Because oaks propagate readily from seeds (Dirr and Heuser, 1987; Hartmann et al., 2002), there has been little reason to research the potential for asexual propagation of a previously non-ornamental species such as *Q. macrocarpa*. However, due to its suitability for inclusion in windbreaks and shelterbelts, propagation of selected parental material by cuttings has become a goal at the Plant Materials Center in Bridger, Montana (Scianna, 1996). The discovery of a reliable method for the perpetuation of specific genetic lines of bur oak could be valuable to both the nursery and forestry industries (Flemer III, 1962).

Seed-raised hybrids may be both useful and attractive, but their population has tremendous genetic variation, including differences in growth rate, height, color, insect and disease resistance, and winter hardiness (Hartmann et al., 2002; McGuigan et al., 1996; Morgan, 1985). Propagation through grafting, micropropagation or cuttings is necessary when the perpetuation of specific superior characteristics is desired. For example, *Q. macrocarpa* is known for slow growth. Stout (1944) documents family farms in Wisconsin with bur oaks on the property showing "scarcely any" growth during 85 years of property ownership. Discovery and clonal propagation of faster-growing trees would be beneficial to the nursery and forestry industries.

In sexually reproducing species, normal variation occurs through genetic recombination, mutations and genetic segregation (Hardin et al., 2001). The oak genus is

readily propagated from seeds, but the genetic recombination from cross pollination can, and does, form interspecific hybrids (Bassuk, 2001; Coombes, 2000; Dirr, 1998; Lamant and Sternberg, 2000; McGuigan et al., 1996; Sternberg, 1996). See Appendix A for further discussion. The resulting variability makes seedling phenotypes unpredictable (Deen, 1974). Cross-pollination of oaks appears to be favored over self-pollination (Scianna, 1996). This makes the classification of *Quercus* spp. complicated, confusing taxonomists and nurserymen alike, with the same name often applied or assigned to different species. For example, according to Lamant and Sternberg (2000), the binomial *Q. prinus* L. was formerly used to describe chestnut oak in the United States. They say the same tree is known as *Q. montana* Willd. in common usage, and more correctly by priority of publication, as *Q. michauxii* Nuttall. Since it was not clear which of these two species Linnaeus identified as *Q. prinus,* Lamant and Sternberg say that name has been discarded. However, according to the USDA PLANTS database (2002), chestnut oak is listed as both *Q. prinus* and *Q. montana*, while *Q. michauxii* is listed as swamp chestnut oak. Similar misnomers and confusion exist throughout the genus, and much more research is needed to determine species relationships (Hardin et al., 2001).

Commercial release of genetically superior trees requires the establishment of clonal seed orchards to provide a consistent supply of seeds of known parentage (Flemer III, 1962; Hartmann et al., 2002). Seed orchards are established from clonal parent material, or a collection of clones, usually obtained by grafting, budding or rooted cuttings, methods that have met with limited success in *Quercus* spp. (Drew III and Dirr, 1989).

Clonal propagation of the Fagaceae, including beech, chestnut and oak, is problematic. There has been some successful research on asexual propagation of oaks reported, but most of it has involved species other than *Q. macrocarpa.* Researchers agree that oak does not propagate easily using traditional clonal propagative methods, and successful methods are strongly species specific (Dirr and Heuser, 1987; Drew III and Dirr, 1989; Ferrini and Bassuk, 2002; Morgan, 1985; Skinner, 1952). Additionally, there appears to be no rooting relationship between members of the three groups comprising the subgenera of scale-cup oaks, or *Quercus* (=Leucobalanus): The true white oaks, the chestnut oaks and the live oaks (Drew and Dirr, 1989; Hardin et al., 2001; Skinner, 1952). Morgan et al., (1980) reports that within a group of *Q. virginiana* (live oak) of the same age grown in a single nursery from a single seed source, the parent trees would produce cuttings having rooting abilities that varied from 0 percent to 71 percent rooting.

The initiation of adventitious roots on woody cuttings usually occurs in undifferentiated, living parenchyma cells of the secondary phloem (Hartmann et al., 2002). However, depending upon the species, they may also originate from lenticels, callus, cambium, and vascular rays. Root initiation of easy-to-root species generally occurs just outside the central vascular system, while more difficult-to-root species often develop an interim non-directed cell formation, such as callus, prior to root initiation. Adventitious roots in some genera, such as *Prunus,* emerge in longitudinal rows along the stem of the cutting directly below buds.

Rooting of cuttings has been successful in a number of oak species, including *Q. macrocarpa* (Bassuk, 2000, 2001; Hawver and Bassuk, 2000). These studies utilized a modified stooling process in which adventitious roots were initiated on the stems of stock plants. Stock plants were usually 3- to 5-year old seedlings that had been pruned to the ground prior to spring bud break. A bottomless pot was placed around the remaining stock plant. When the stock plant produced new shoots, media was added to the pot, up to the shoot apex, and kept moist to encourage rooting. More media was added as the growing shoot elongated. The dormant rooted shoots were cut and potted at the end of the season.

Most nursery growers and researchers propagate oaks by seeds in containers within climate-controlled greenhouses (Dirr and Heuser, 1987; Hartmann et al., 2002). However, the *Q. macrocarpa* stock plants used in our study were field-grown, strongly taprooted, and could not be moved to greenhouses without damaging the root system and stressing the tree (Hendricks, 1996; Yanny, 1996). Therefore, modifications to existing propagation protocols were made to allow these trees to remain in place, in the extreme conditions of high winds and intense sun that characterize the climate at the Bridger, Montana location.

Research on oak cutting propagation has shown that there are many interacting factors that affect the rootability of cuttings. Ontogenetic age of the parent plant, species, provenance, time of year cuttings are taken, applied rooting hormone level and light exposure during growth of cuttings are major variables (Bassuk, 2000; Covan, 1986; Deen, 1974; Dehgan et al., 1977; Dirr and Heuser, 1987; Drew and Dirr, 1989; Ferrini

and Bassuk, 2002; Flemer III, 1962; Komissarov, 1938; McGuigan et al., 1996; Morgan, 1985; Thimann and Delisle, 1939; Zaczek et al., 1997).

Experimental rooting variables that were compared in this study included collection time of year, etiolation and banding of entire parent plants, etiolation and banding of individual branches, hedging of parent plants, etiolation and banding of hedged parent plants, applied rooting hormones of different concentrations, two rooting medias, and variable air temperatures during attempted rooting of hardwood cuttings. A secondary trial investigated forcing adventitious shoots to initiate and expand from hardwood branches, producing softwood cutting material from winter wood. This side study appears as Appendix B.

Plant Phenology – Time of Year for Collection

Wide variation in rooting success has been reported in cuttings taken from different species of stock plants grown under natural light conditions at various times of the year. This variation is attributed to temperature fluctuations, light intensity, photoperiod, and/or the interaction of photoperiod with light intensity (Scianna, 1998). With some species, the "window of rootability" may be as short as two weeks or less.

Fully dormant buds on cuttings taken in midwinter have either no effect or an inhibitory effect on rooting (Hartmann et al., 2002). The presence of active buds in softwood cuttings may promote rooting.

Some difficult-to-root species root more readily from hardwood cuttings, presumably due to a greater amount of stored carbohydrates (Maynard, 1994). Optimal

levels of carbohydrates for the development of adventitious roots have not been determined (Scianna, 1998).

A limitation of softwood cuttings is that active transpiration requires open stomata. If cuttings are water stressed, stomata close, limiting the intake of carbon dioxide required for photosynthesis (Larcher, 2003). Cuttings that become water stressed will remain in that condition until root formation allows water uptake from the media.

On the other hand, root initiation in difficult-to-root species is sometimes more successful with softwood cuttings. One theory is that expanding axillary buds produce large amounts of auxin, a plant hormone that stimulates adventitious root initiation. Endogenous auxin may be supplemented with exogenous auxin to increase rooting (Kramer and Kozlowski, 1979). Ideal amounts, types and concentrations of exogenous auxin vary by species and have not been established for *Q. macrocarpa.*

Propagation of softwood cuttings usually is more complex than hardwood cuttings and requires more sophisticated equipment (Hartmann et al., 2002). For this type of cutting, a high leaf to stem ratio favors rooting. Cuttings with thin stems and normal sized leaves and those with normal stems and larger than normal leaves tend to root more readily. Softwood cuttings are quite perishable, and need to be stored and handled with care. They are usually taken early in the day when tissues are turgid, immediately bagged, kept cool, and struck within 48 hours of harvest (Scianna et al., 1999).

Maynard and Bassuk (1987a) identified the "window of rootability," varying from days to weeks, for a large number of woody plant species, and reported that etiolation of the parent tissue lengthens this window. Periods of reduced rooting may surround those

of ideal rooting. Beakbane (1969) lists a number of genera, including *Quercus* spp., with sclerenchymatous rings that form with age within the stem cortex, and may inhibit rooting. Young material that has not yet developed these potential barriers to adventitious rooting is best for cuttings.

Table 1 summarizes research related to rooting percentages as a function of the time of year that the cuttings were collected. Bur oak has not typically been included in adventitious rooting studies for *Quercus* species, presumably due to the difficulty in rooting cuttings. This table demonstrates the extreme variability within the genera, and provided a starting point for our research. Other interacting factors impact the rooting of these species, and will appear in other tables within this document.

Table 1. Percent adventitious rooting of *Quercus* spp. based on collection time

Collection	Species	Percent Rooting - %	Ref.
February	*Quercus* spp.	82	7
June	*Q. ilex*	0-40	1
Late June	*Q. phellos* x *rubra*, *Q. palustris*	73 Terminal cutting 81 Subterminal cutting	3
Early July	*Q. robus fastigiata*	50	4
July	*Quercus* spp.	56 (8 yr old tree) 34 (20 yr old tree)	5
Summer	*Quercus* spp.	"good"	6
August	*Q. phillyreoides*	43	2

(1) Deen (1974); (2) Dehgan et al., (1977); (3) Dirr and Heuser (1987); (4) Flemer III (1962); (5) Komissarov (1938); (6) Morgan (1979); (7) Thimann and Delisle (1939).

Light Exclusion - Etiolation and Banding

Etiolation is recognized as a stock plant treatment that increases rooting potential in softwood cuttings of many difficult-to-root species, and may also extend their "window of rootability" (Blazich, 1988; Hartmann et al., 2002; Maynard and Bassuk,

1987a; Maynard and Bassuk, 1988; Moe and Andersen, 1988). Strictly defined, etiolation is the complete exclusion of light from the elongating tissues of the stock plant (Hartmann et al., 2002), although it also refers to plants grown in reduced light levels.

Etiolation causes anatomical and physiological changes in plant tissue that differ among species, and has been correlated with improved rooting since the 1920s. Typical responses to etiolation include lack of chlorophyll in tissues, increased internodal length, increased tissue succulence, smaller than normal leaves, and increased sensitivity to auxin. Etiolation also decreases the mechanical strength of stem tissues due to the increase in parenchymatous tissue, absence of sclerified tissues (such as continuous phloem fiber sheaths), and decrease in cell wall thickness. Lignin production is reduced and the metabolites normally used for their production may instead be used to enhance adventitious root formation (Englert et al., 1991; Griffin and Bassuk, 1996; Hartmann et al., 2002; Hawver and Bassuk, 2000; Bassuk and Maynard, 1987; Maynard and Bassuk, 1988).

The formation of adventitious roots is dependent upon cellular dedifferentiation to form new meristematic cells (Hartmann et al., 2002). Sclereids cannot dedifferentiate during the cutting/rooting process (Maynard and Bassuk, 1996). Because of this, the presence of a large number of sclereids may reduce rooting potential of the shoot by reducing the number of potential initiation sites. Beakbane (1969) used this argument to support his theory that propagation should be undertaken using very young, or juvenile, shoots that have not yet formed this sclerenchymatous tissue. Maynard and Bassuk, together and separately (Bassuk, 2000, 2001; Bassuk and Maynard 1987; Bassuk et al.,

1986; Maynard, 1994, 2002; Maynard and Bassuk, 1985, 1987a, 1987b, 1988, 1990, 1992, 1996), in their extensive research of this subject, recommend etiolation to avoid the formation of sclerenchymatous tissue, which, among other changes, prevents the formation of sclerified tissues.

In woody species, root cells often originate immediately exterior to the vascular cambium, and are usually associated with interfascicular rays (Hartmann et al., 2002) (Appendix C). In some difficult-to-root species, including oak (Beakbane, 1969), a continuous ring of sclerified tissue is sometimes formed exterior to the point of origin of adventitious roots (Hartmann et al., 2002). In addition to this heavy sclerification, tissue may demonstrate extensive development of the fiber sheath, both of which have been correlated with poor rooting in cutting propagation. Some researchers propose that these may act either as a mechanical barrier to root emergence, or as a physiological barrier to root initiation in many difficult-to-root genera, including *Quercus* spp. (Beakbane, 1969; Deen, 1974). However, Maynard and Bassuk (1996) speculate that once roots have initiated, they are able to penetrate most sclerenchymatous tissues external to the root initiation site. Hartmann et al., (2002) describe the rooting pattern in several species where root primordia reach this ring and then grow downward to emerge from the cutting base. No studies of this type have included *Quercus* spp.

Research indicates that adventitious root initiation simply does not occur in some difficult-to-root species, so the sclereid-mechanical barrier hypothesis has been largely discounted (Hartmann et al., 2002; Maynard and Bassuk 1996). The hypothesis of

reduced initiation sites caused by dedifferentiation inhibition and the presence of sclerified tissue, a physiological process, remains valid.

Bassuk (2000) theorized that red light normally inhibits adventitious rooting through its action on phytochrome. In this study, cuttings from *Q. macrocarpa* grown in white light alone ("red light") had no rooting, while 35 percent of the etiolated cuttings rooted. Whereas red light stimulates "normal" growth responses through stimulation of the formation of the active form of phytochrome (P_{fr}), plants grown in the dark or in far-red light are stimulated to produce long internodes and chlorotic leaves.

The technique traditionally used to etiolate plant material involves covering all or a portion of the stock plant with light excluding material, such as black cloth or black polyethylene, fastened over a structure that encloses the portion of the plant to be etiolated. The etiolation response occurs in whatever part of the plant is covered (Maynard, 2002; Moe and Andersen, 1988). Exclusion of 100 percent of the light will result in etiolation of tissues, but similar results can occur at lower proportions of light exclusion (Hartmann et al., 2002). Researchers at the East Malling Research Station, Kent, England have conducted research on M.9 apple and other woody species demonstrating that as little as 80 percent light exclusion significantly increases rooting (Maynard and Bassuk, 1985). Softwood cuttings taken from etiolated growth typically have higher rooting percentages, even those of the 'Delicious' apple group, considered one of the most difficult-to-root cultivar groups (Anderson, 1981).

Etiolation is often followed by a banding procedure to maintain the etiolated condition of the base of the future cutting while allowing the distal portion of the cutting

to acclimate and grow normally. When etiolated shoots reach 5 to 10 cm in length, the etiolation material is removed for a short period of time to allow placement of a light-excluding band on the base of the shoot. The initial etiolation material is then replaced, but is gradually removed over a period of approximately one week, to allow the distal portion of the cutting to gradually adjust to higher light levels and environmental conditions while the band maintains etiolated tissue at the proximal end. Materials utilized for banding include black tape, paper, tubing, aluminum foil, and black paste. Material such as Velcro® may be used to simultaneously band and wound this area, and auxin in a talc base may be applied to the Velcro, further stimulating formation of adventitious roots (Hartmann et al., 2002).

Bassuk et al., (1986) reported a procedure that included etiolation, followed by banding of the new tissue using a one-inch wide strip of Velcro dipped in 0.8 percent talc-based indole-3-butyric acid (IBA) and pressed into the shoot base to wound the tissue. The strip remained in place for four weeks, at which time the shoot was removed just proximal to the band. All lower leaves were stripped from the cutting, and, in some cases, additional 0.8 percent IBA was applied prior to placement ("striking") in the propagation bench. The researchers reported that all deciduous cuttings, including those of four *Quercus* species, rooted in approximately 4 weeks, either as a result of etiolation and banding, or blanching (Bassuk et al., 1986; Maynard and Bassuk, 1988).

In two studies, successful etiolation trials were reported that included *Q. macrocarpa* and other oak species (Table 2). One-year-old (Griffin and Bassuk, 1996) and three- to five-year-old (Bassuk, 2000) greenhouse stock plants were cut back just

prior to bud break, leaving only one to two inches of stem above the root system. When adventitious buds formed on the cut stems, the plants were placed under black cloth tents in the greenhouse under 90 to 98 percent light exclusion, until the new growth reached 18 to 30 cm in length. Heat buildup inside the tents was problematic, but was dissipated by draping white plastic over the black cloth tents, placing fans inside the tents, opening the corners of the tents, or making cuts in the fabric near the top of the enclosure (Bassuk et al., 1986). The modified stooling procedure previously discussed (Bassuk, 2000, 2001), was used following etiolation.

Table 2. Percent adventitious rooting of *Quercus* spp. based on etiolation vs. light-grown tissue. IBA rate for all treatments was 8,000 ppm in a dimethyl sulfoxide (DMSO) carrier.

Species	Etiolated		Light grown	
	Study 1 Rooting %	Study 2 Rooting %	Study 1 Rooting %	Study 2 Rooting %
Q. acutissima	100.0	78.0	0.0	4.0
Q. bicolor	45.2	52.0	0.0	0.0
Q. macrocarpa	71.4	35.0	0.0	0.0
Q. palustris	100.0	25.0	0.0	5.0
Q. rubra	25.0	43.0	0.0	0.0

Study 1: Griffin and Bassuk (1996). Study 2: Bassuk (2000).

A further refinement of the etiolation and banding method (Ferrini and Bassuk, 2002) included *Q. macrocarpa, Q. robur* and *Q. bicolor*. The researchers compared results of 45 day-old seedlings to 3-year old plants. In all treatments, cuttings from older plants demonstrated reduced rooting when compared to the younger plants. Three-year old *Q. macrocarpa* plants displayed 47.6 percent rooting. The researchers also report that cuttings of both *Q. robur* and *Q. macrocarpa* responded strongly to etiolation and banding, and in *Q. macrocarpa*, the two treatments appeared to have a synergistic effect.

An important consideration with any etiolation technique is the removal of the material used to block light. At the time the cutting base is banded, the degree of shading of the rest of the potential cutting must be reduced slowly, after shoots reach about 5 to 10 cm in length (Bassuk and Maynard, 1987). This allows the shoot to gradually begin producing chlorophyll, and hence, photosynthates while avoiding desiccation. The basal band remains in place in order to maintain the etiolated condition of the underlying plant tissue until time of removal from the plant. Cuttings are removed from the parent plant by cutting proximal to the banding material. After transport to the propagation bench, the band is removed, a new cut made within the etiolated zone produced by the band, and the cuttings treated with additional rooting hormone and struck in media.

A procedure similar to banding is blanching. Blanching is the banding of light-grown shoots while they are still in the softwood stage (Bassuk and Maynard 1987; Hartmann et al., 2002) by covering the base of 5 to 7 cm long new shoots with hormone-coated Velcro bands (Maynard and Bassuk 1987a). Hartmann et al., (2002), however, report enhanced rooting of cuttings of *Quercus* spp. only when banding followed etiolation. In extensive studies, Bassuk found that the combination of etiolation and banding almost always improved the rooting percentage over either etiolation or banding alone.

Encouragement of Succulent Growth Having High Rooting Potential – Hedging

Juvenility is that ontogenetic phase of development characterized by rapid vegetative growth prior to reproductive maturity. It is a time when cell division is

concentrated in root tips, shoot tips and axillary growing points (Hartmann et al., 2002). It precedes the vegetative and reproductive adult phases and is retained throughout the life of the plant within the "cone of juvenility" – near the base of the plant (Morgan, 1985). Cuttings taken from juvenile plants or juvenile phase tissue often demonstrate improved or increased adventitious rooting compared to adult phase tissue, as the cuttings will be of the same ontogenetic phase as the stock plant at the point of removal (Hackett, 1988; Hartmann et al., 2002; Macdonald, 1986; Morgan, 1985). It is generally recognized that stock plant juvenility is the key to success in difficult-to-root species, such as oak (Hackett, 1988; Morgan et al., 1980; Scianna, 1998) with an inverse linear relationship between success of rooting and increased age of parent material. The higher rooting potential of juvenile cuttings may be maintained or retained by perpetuating this juvenile phase by techniques such as pruning or hedging (Hartmann et al., 2002; Skinner, 1952). The Plant Materials Center (PMC) oaks used in this study were 10 chronological years old the beginning of 2002 (Scianna, 2002). Late fall leaf retention in some species, such as oak and beech, can be an indication of juvenility. Based on this, these trees are slightly beyond the juvenile stage. Flowering and fruit set indicate a phase shift from juvenility to the ontogenetic adult phase. In trials of several *Quercus* species, it was found that rooting of cuttings strongly decreased with increasing age of the parent plant (Ferrini and Bassuk, 2002).

Hedging is severe pruning of the adult phase parent plant in order to promote a large flush of succulent and potentially juvenile shoots. *Quercus macrocarpa* is known for vigorous sprout growth following the cutting of pole-size or smaller trees (Johnson,

1990). If the lower part of the plant is "rejuvenated" by hedging and induced to sprout, the resulting sprouts may demonstrate improved or increased adventitious rooting. Hartmann et al., (2002) report successful vegetative propagation of some species by hedging seedlings and clonal populations established from juvenile material. They report that hedging is the most important propagation technique available for the production of large quantities of rejuvenated or juvenile cutting material.

When assessing a comparison of hedged vs. non-hedged clones, researchers have found that hedging arrested the decline in rooting percentage usually brought on by increasing ontological age of parent material. Hedged trees of seedling origin remained juvenile, and cuttings from them rooted more readily than cuttings from unhedged trees of the same age (Hackett, 1988). It is not clear why hedging increases rooting percentage. One theory is that maturation is arrested at the ontogenetic stage of the nodes from which hedge shoots were formed. Another is that hedged plants are physiologically invigorated (or rejuvenated) instead of reverting to true juvenility, as 1-year old shoots in stooling beds have been known to flower even though they have high rooting potential.

Etiolation, or light exclusion, was discussed in the previous chapter. Bassuk (2001) has successfully combined another method of severe pruning, stooling, with etiolation and shading treatments. Howard (1994) found that hedging created conditions in shoots that enhanced the response to localized blanching of softwood cuttings.

Stimulation of Adventitious Root Initiation – Auxin

Phytohormones are compounds synthesized by plants, that are 'made in one part of the plant while functioning at another' (Allaby et al., 1998). Phytohormones are used in various physiological processes. Auxins are a group of phytohormones that influence growth and developmental responses in plants. They regulate cell division, differentiation of vascular tissue, differentiation of roots in tissue culture applications, and initiation of roots on cuttings. Although auxins can inhibit rooting at some concentrations, they can stimulate the growth of lateral and adventitious roots at optimum concentrations (Palme et al., 1994).

Prior to the discovery of chemicals that could be applied to cuttings to enhance the adventitious rooting of difficult-to-root species, propagating oaks by cuttings was considered impossible (Skinner, 1952). While oaks are still considered difficult-to-root, plant propagators widely use exogenously applied auxin to promote rooting of cuttings from other species, even those among the most difficult-to-root.

It is well documented in the literature that different genera, species and even cultivars require specific types and concentrations of applied hormone to maximize rooting (Hartmann et al., 2002; Zaczek et al., 1997). Some easy-to-root species need no exogenous auxin, but moderately-difficult-to-root species benefit from application. Difficult-to-root species, such as oak, may not respond consistently to specific levels of exogenously applied auxin (Macdonald, 1986). Auxin's effects may also vary from year to year, a frustration to researchers and nursery growers unable to repeat earlier propagation successes.

Naturally occurring auxin is produced in the plant in the buds and near the shoot apex and moves basipetally. It degrades as it translocates through the plant, and extremely small amounts are capable of stimulating root initiation. Early attempts at applying auxin to stimulate rooting involved application to the apical end of the cutting, simulating the natural flow of the hormone in plants. It was soon discovered, however, that increased rooting resulted from basal applications. In leafy plum cuttings treated with a radioactive-tagged auxin, (indole-acetic acid IAA), researchers found that IAA was absorbed within 24 hours after application whether the application was basal or apical. They noted, however, that most of the IAA remained in the basal portion of the cutting when applied there. These studies also demonstrated that the same amount of auxin was absorbed regardless of the presence of leaves, discounting the theory of hormone translocation via transpirational pull (Hartmann et al., 2002).

IBA and naphthaleneacetic acid (NAA) are significant root-forming auxins, and are the most widely used hormones for stimulating adventitious rooting. The application of IBA is usually most effective. It is unknown why IBA is more effective in promoting rooting of many species than naturally-occurring IAA or other auxins, nor is the exact role of auxin in adventitious root initiation known (Blakesley, 1994; Blazich, 1988; Haissig and Davis, 1994).

Maynard and Bassuk's (1987a) research was previously discussed in the etiolation chapter. Their method combined 0.8 percent IBA application with etiolation and banding, and promoted callusing and root initiation much like air layering, but was reported to be faster and easier.

The question of whether or not callusing of cuttings precedes rooting in *Q. macrocarpa* is unresolved, but in one study of *Q. ilex* it was reported that adventitious rooting only originated from the stem above the callused cut.Re-treating the callused and unrooted cuttings with additional IBA after 40 days in the mist bench failed to induce rooting from the callus (Wang and Rouse, 1989).

As previously noted, individual genera and species require different auxin concentrations to facilitate root initiation. No research was found identifying the ideal hormone concentration for rooting *Q. macrocarpa.* Studies of other *Quercus* species were used as a basis for experimentation to determine the optimum hormone concentration for *Q. macrocarpa*, and are included in Table 3.

Table 3. Percent adventitious rooting of *Quercus* spp. based on hormone concentration and type

Hormone Concentration (ppm)	Species Tested	Percentage Rooting %	Reference
IBA 20,000	*Q. robus fastigiata*	50	4
10,000 K-IBA	*Quercus* spp.	"good"	6
10,000 K-IBA	*Q. virginiana*	Failed to root	3
10,000 K-IBA	*Q. phellos* x *rubra* *Q. palustris*	73 Terminal 81 Subterminal	3
10,000 IBA	*Q. alba*	16.7 (shaded bench)	8
10,000 IBA	*Q. palustris*	10 (shaded bench)	8
3,000 IBA	*Q. ilex*	0-40	1
2,000 IBA	*Q. phillyreoides*	43	2
"IBA" (conc. not given)	*Quercus* spp.	56 (8 yr old tree) 34 (20 yr old tree)	5
400 IAA	*Quercus* spp.	82	7

(1) Deen, 1974; (2) Dehgan et al., 1977; (3) Dirr and Heuser, 1987; (4) Flemer III, 1962; (5) Komissarov, 1938; (6) Morgan, 1979; (7) Thimann and Delisle, 1939; (8) Zaczek et al., 1997.

The Effect of Leaves on Adventitious Root Formation - Air Temperature

For adventitious rooting of cuttings from most temperate species, Hartmann et al., (2002) suggests optimal daytime air temperatures of 21° - 27°C (70° - 80°F), with nighttime temperatures of 15°C (60°F), with some species rooting better at overall lower air temperatures. However, these higher air temperatures promote bud elongation and increase evapo-transpirational losses from leaves that occur in advance of root initiation in hardwood cuttings. Dormant hardwood cuttings have high stored carbohydrates and low endogenous auxin levels. Lower air temperatures can delay bud break, preventing the diversion of stored carbohydrate reserves needed for rooting. Hardwood cuttings held under conventional daytime temperatures (21° - 27°C) will typically break bud within 4 to 8 weeks (Scianna, 2001). At that time, moisture stress will cause cutting deterioration if root formation is inadequate to meet transpirational demands (Howard, 1994). Moisture-stressed plants close stomata to reduce water loss (Larcher, 2003). However, closed stomata prevent carbon dioxide uptake by the leaf, limiting photosynthesis (Maynard, 1994). While root initiation is temperature-driven, subsequent root growth is dependent on carbohydrate availability (Hartmann et al., 2002). All of these processes rely upon finite carbohydrate resources until rooting occurs and photosynthesis can begin (Maynard, 1994).

Although photosynthates from leaves are not needed for root initiation, it is vital that emerged leaves be kept moist to avoid cutting desiccation due to lack of roots. Overhead misting is used to prevent desiccation, and is the cooling and irrigation system of choice for uniform water distribution in cutting propagation systems (Shaw, 1996).

OBJECTIVES AND HYPOTHESES

The purpose of this study is to develop a practical method to asexually propagate bur oak that would enable establishment of seed orchards by clonal reproduction of selected superior trees. In addition, the identified method should be practical and cost-efficient for the nursery industry. Specifically, to determine the significance of:

1. Appropriate time of year for cutting collection

   Hypothesis 1. Seasonal collection time influences root initiation

2. Optimum air temperature for propagating cuttings

   Hypothesis 2. Cool air temperatures increases root initiation of hardwood cuttings of *Q. macrocarpa*

3. Optimum exogenously-applied hormone concentration for root initiation

   Hypothesis 3. A specific applied hormone concentration increases root initiation

4. Etiolation and banding of parent plants

   Hypothesis 4. Etiolation and banding increases root initiation

5. Hedging for improved rooting of cuttings

   Hypothesis 5. Hedging increases root initiation

6. Media type

   Hypothesis 6. Cuttings root more readily in inorganic rather than organic media

7. Synergy

   Hypothesis 7. Some combination of treatments increases root initiation

MATERIALS AND METHODS

Conventional hardwood and softwood trials were conducted in Year 1 to establish a baseline for propagative success using traditional methods. Those treatments exhibiting promise were refined and repeated in Year 2. Additional treatments included variations of parent plant light exclusion, tested in conjunction with techniques proven to yield ontogenetically juvenile cuttings with higher rooting potential.

Cuttings were collected from selected bur oak trees at the Plant Materials Center (PMC) in Bridger, Montana. Propagation was undertaken at the Plant Growth Center (PGC) on the campus of Montana State University (MSU), Bozeman, Montana, with experimental replication at the PMC.

For the hardwood studies, 500 cuttings were taken February 1, 2002 and on January 10, 2003 from 50 trees representing five accessions. These cuttings were subjected to eight concentrations of applied IBA in a talc base, two media and two air temperatures at MSU and PMC.

For the softwood cutting study in 2002, 500 cuttings were taken from the same 50 trees. These cuttings were subjected to five hormone concentrations and two media. Branches of five trees chosen at random were subjected to localized etiolation and banding. Thirty cuttings were taken from two previously hedged trees and subjected to five hormone concentrations and two media.

For the softwood study in 2003, 160 cuttings were taken from 33 hedged and 33 non-hedged trees that had been selected at random, and subjected to full etiolation with and without banding. Two hundred fifty cuttings were taken from 25 non-hedged trees

that had been subjected to a localized treatment. All softwood cuttings were then subjected to a range of applied rooting hormones.

An attempt was made to collect only disease- and insect-free plant materials. Insecticidal soap was applied to source plants in the field prior to cutting removal to control aphids. Removed cuttings were sorted by tree, placed in Ziploc™ bags, treated with a fungicide mixture of Apron™ (metalaxyl) at the rate of 0.004 g/liter distilled water plus Benlate™ (benomyl) and Terraclor™ (PCNB or pentachloronitrobenzene) both at the rate of 0.1425 g/liter distilled water. The Ziplocs were placed in coolers with icepacks and transported to greenhouses.

All pots and propagation equipment within the greenhouses were cleaned and disinfected with a quaternary ammonium compound prior to use (Blackwell, 1996). All media was pasteurized. Mist systems were monitored regularly to minimize over-watering, leaking valves, and poor drainage. Applications of the fungicide BanRot™ 40 WP (etridiozole and thiophanate-methyl) at the rate of 1.75g/liter were applied weekly.

Mist chambers were constructed within the warm air greenhouse and cold room at the MSU PGC. Each consisted of a wood frame with retractable 6 mil construction grade poly film sides, with mist risers running the length of each chamber. A poly film cover was placed over the mist chamber in the PGC cold growth room to protect the light fixtures from mist system overspray. A light film of water was maintained on plant tissues by timing of mist application.

In the PMC warm air greenhouse, a similar mist chamber was constructed. The cuttings were hand watered in the cold air chamber.

Fiberglass screening was used to cover the warm air greenhouse mist chambers to reduce the solar load on delicate softwood cuttings by 50%. As heat mats were used for bottom heating of hardwood cutting media, fans were constantly run to minimize heat accumulation in MSU's cold growth room.

Ambient air temperature in the MSU and PMC warm greenhouses was 24°C (75°F) days and 18°C (65°F) nights. Light was provided in 16-hour photoperiods for this greenhouse with high-pressure sodium lamps supplementing daylight hours. Air temperature in the MSU cold growth room was 4°C (39.2°F) while the PMC cold growth room was 2.75°C (37°F). Light was provided at MSU in 12-hour photoperiods by fluorescents. No lights were used at the PMC.

For all trials, cuttings were bathed in water to remove field-applied fungicide and any insects and then trimmed to a final 10 to 15 cm in length. A 45° basal cut was made on all cuttings. Wounding of hardwood cuttings was accomplished with a 2 to 3 cm long, shallow vertical cut at the base of each cutting. This wounding of basal tissue prior to hormone application is thought to stimulate rooting by promoting cell division and increasing absorption of water or exogenous auxin, or by removing impervious tissue that may prevent adventitious root development and growth. Although typically used on evergreen plants, this wounding technique may also encourage rooting of deciduous cuttings (Larsen and Guse, 1997). If present, apical buds were removed from cuttings as endogenous auxin, formed in the apex, could interfere with exogenously-applied rooting hormone. With softwood cuttings, 2 to 3 leaves were left on each cutting, with the leaf blade area reduced by half to minimize transpirational losses. Cuttings were then dipped

in the fungicide Captan™ (N-((trichloromethyl)-thio)-4-cyclohexene-1,2-dicarboximide) at the rate of 42.53g/liter water. Bases were treated with a rooting hormone in talc at concentrations listed below, and placed into a predibbled hole in the media (Blazich, 1988).

Hardwood Cuttings Year 1, 2002

Hardwood cutting material was collected at the Plant Materials Center (PMC) in Bridger, Montana, in February, 2002 as previously outlined. Five hundred cuttings from 50 trees representing five accessions were collected and transported to Montana State University (MSU) for the time of year, rooting hormone concentration, and air temperature experiments. These experiments were duplicated at the PMC.

Prior to striking, bases of the cuttings were treated with specific concentrations of IBA in a talc base. Concentrations of 0 ppm, 2500 ppm, 5000 ppm, 7500 ppm and 10000 ppm IBA were used in an attempt to identify the optimum concentration that would promote maximum adventitious root initiation in this species.

Trays measuring 15 cm x 33 cm x 12 cm were planted with 25 cuttings each, each tray with five cuttings treated with each concentration of hormone. A total of one hundred cuttings were treated with each hormone concentration. The trays were placed in mist chambers in the warm air greenhouses and in the cool air growth rooms in a completely random design. Bottom heat in all locations was supplied and maintained at 25°C (77°F).

Softwood Cuttings Year 1, 2002

In June and July, 2002, 500 softwood cuttings were collected from the same 50 trees that were used for hardwood cutting collection earlier that year. These cuttings were pretreated as outlined above and transported to MSU, with experimental replication at the PMC. One hundred cuttings were treated with a basal dip in each of 0 ppm, 2500 ppm, 5000 ppm, 7500 ppm or 10000 ppm IBA in talc. Fifty cuttings treated with each hormone were struck in sand and 50 in 1:1 peat:perlite media. Cuttings were placed in trays of 25 each, and placed in a completely random design into a warm air greenhouse with daytime temperature of 24°C (75°F) and nighttime temperature of 18°C (65°F).

In the hedging experiment for 2002, two trees were severely pruned (hedged) in February, 2002. The leaders of both trees were removed, leaving approximately three feet of stump. All lateral branches were also removed at this time. Thirty softwood cuttings were taken from new growth from these trees in June, 2002, and transported in coolers to MSU. Each cutting was pretreated as previously outlined, and treated with one of five concentrations of applied IBA: 0 ppm, 2,500 ppm, 5,000 ppm, 7,500 ppm or 10,000 ppm. Six cuttings were treated with each IBA concentration. All hedged cuttings were struck in sand.

Additionally, five trees were chosen at random, and were subjected to localized etiolation and banding. For this experiment, a localized method for etiolating individual branches was developed for field-grown stock plants. In April, 2002, two selected parent plant branches on each selected tree were covered with white Cone-tainers™ filled with cored black closed-cell foam to exclude light. These cones were fastened to the tip of the

parent plant branch with wire, and secured with electrical tape. New shoots elongated into the dark environment of the cored cone. In early June, 2002, the black closed-cell foam was removed. Bases of new growth were banded five weeks after bud break of surrounding trees with Velcro® dipped in 7500 ppm IBA in talc to maintain the etiolated condition of the tissue at the base of the future cutting. The hook side of the Velcro was pressed into the basal end of the stem to lightly wound the tissue. Cone-tainers with seven tape-covered openings were replaced over the banded shoot. The tape was gradually removed over a period of one week, acclimating the etiolated shoots to field conditions. The cones were completely removed when shoots were 5 to 10 cm in length, while the Velcro remained in place.

Banded new growth remained in place on the parent plant for approximately 4 weeks, at which time the cuttings were removed from the stock plant with a cut made just proximal to the band and transported to MSU for striking.

Cuttings were subjected to the standard pretreatment outlined in the first section of this chapter. The Velcro band was removed, and a new 45° basal cut was made within the etiolated zone produced by the Velcro. Each cutting base was treated with IBA in talc at a rate of either 0 ppm, 2500 ppm, 5000 ppm, 7500 ppm, or 10000 ppm. These cuttings were all struck in sand. Cuttings for all treatments were held within the same greenhouses with conditions as previously noted.

Hardwood Cuttings Year 2 – 2003

In January 2003, hardwood cutting material was collected at the Plant Materials Center (PMC) from the same 50 trees utilized in Year 1. In addition to the standard pretreatment, these cuttings were surface-sterilized with quick dips in 95% ethyl alcohol, then 10% chlorine bleach at the propagation bench. Cutting disease problems occurring in Year 1 warranted additional disinfestations to reduce the presence of pathogens. In addition, the quantity of pathogens on cuttings in the peat:perlite media in Year 1 suggested that an inorganic media might prove superior. Therefore, fifty percent of the cuttings were struck in 1:1 peat:perlite and fifty percent in pasteurized sand. As no rooting occurred in the hardwood cuttings treated with lower concentrations of rooting hormone in Year 1, a series of higher hormone concentrations was tested in Year 2. Based on suggestions received at the annual International Horticultural Congress meetings in Toronto, 500 cuttings were treated with K-IBA (potassium salts of indole-3-butyric acid) in a distilled water base at greater concentrations than used in the previous year. One hundred cuttings were treated with each K-IBA concentration: 0 ppm, 10,000 ppm, 20,000 ppm, 30,000 ppm, and 40,000 ppm and then struck in sand. Twenty trays of twenty-five cuttings were placed in the cool air growth chamber with a maintained air temperature of 4°C (39.2°F). Bottom heat was maintained at 25°C (77°F).

Softwood Cuttings Year 2 – 2003

Softwood cuttings were collected from the PMC in June of 2003, and transferred to MSU for pretreatment or kept at the PMC for replication. Softwood cuttings were treated with the same rooting hormone series that was used in Year 1. Due to the increased presence of pathogens on softwood cuttings struck in the peat:perlite media as compared to those struck in sand in Year 1, all softwood cuttings were struck in pasteurized sand in Year 2. Greenhouse conditions were identical to Year 1.

Due to the complexity of experiments undertaken in Year 2, the treatments, number of trees involved and number of cuttings taken are summarized in Table 4.

Table 4. Summary of 2003 softwood treatments for MSU and the PMC

Treatment	Abbreviation	MSU		PMC	
		Number of Trees	Number of Cuttings	Number of Trees	Number of Cuttings
Non-Hedged + Non-Etiolated + Non-Banded	NH + NE + NB	25	125	17	85
Hedged + Non-Etiolated + Non-Banded	H + NE + NB	16	80	16	80
Non-Hedged + Fully Etiolated + Non-Banded	NH + E + NB	16	80	17	85
Hedged + Fully Etiolated + Non-Banded	H + E + NB	16	80	16	80
Non-Hedged + Fully Etiolated + Banded	NH + E + B	16	80	16	80
Hedged + Fully Etiolated + Banded	H + E + B	16	80	16	80
Non-Hedged + Local Etiolated + Banded	NH + LE + B	25	125	0	0

Hedging treatments were performed in January at the time of hardwood cutting collection. A total of thirty-three trees were severely pruned (hedged) to a height of approximately three feet. Leaders and large branches that were removed during hedging were used in the forcing experiment outlined in Appendix B.

The Year 1 localized etiolation trial was repeated at MSU in Year 2. The method for localized etiolation was the same, except the elongating shoot was provided more room for growth by stacking a bottomless Cone-tainer inside one with the bottom intact. Twenty-five trees had five branches each etiolated in this manner.

Banding of the localized etiolation group occurred five weeks after surrounding trees broke bud. Removal of the etiolation Cone-tainers was done as in the previous year, with acclimation occurring over a period of one week. Localized etiolated cuttings were removed from the parent plants four weeks after banding, transported to the PGC in coolers, and treated with the same concentrations of applied IBA in talc as Year 1. All cuttings were struck in pasteurized sand.

An etiolation by hedging by banding experiment was constructed for whole plants. Thirty-three hedged trees were entirely etiolated and thirty-three non-hedged (whole) trees were entirely etiolated. This method of etiolation was accomplished by first erecting an enclosing cage consisting of wooden snow fence in the case of hedged trees, and welded fencing wire for the whole trees. These frames were then covered with black woven polypropylene landscape cloth (5 oz. DeWitt™ Pro5 weed barrier) that was painted with whitewash to reduce the heat buildup inside the structure. These structures

resembled large cans in the field. The whitewashed landscape cloth excluded between 97.7 and 99.996 percent of the light for no more than 7 μmol m^{-2} s^{-1} inside the structures.

Ten shoots from each fully etiolated tree, sixteen hedged and sixteen non-hedged, were banded five weeks after etiolation material had been installed. Gradual removal of the landscape fabric, by rolling up fabric from the bottom of each structure, began the following day, and was completed a week later. Due to excessive elongation, cuttings were taken from etiolated trees, both hedged and non-hedged, three weeks after banding. These 160 cuttings were divided evenly between MSU and PMC for replication. Eighty H + NE + NB (hedged control – Table 4) cuttings were taken from sixteen trees for both MSU and PMC. One hundred twenty five NH + NE + NB (non-hedged control) cuttings were taken from twenty-five trees for MSU and 85 cuttings from seventeen trees were taken for PMC. The additional non-etiolated control cuttings for MSU were also to be used for the LE treatment, and were therefore taken from the same trees as those used in that experiment.

Measurements for Testing the Hypotheses

Parameters measured at Week 8 and 11 included: Greenhouse location, number of roots, number of visible root initials, and length of longest root (mm). Additionally, cutting health data was measured as stem color rating (stem color), number of live leaves, petiole color rating (petiole color), new bud elongation (bud break), callus amount rating (callus amount), and callus color rating (callus color) (Table 5). At Week 8, a survival

rating was given each cutting, indicating whether or not the cutting appeared able to survive.

Table 5. Cutting health evaluation criteria

Stem color Rating	# Live Leaves	Petiole Color Rating	Bud break	Callus Amount Rating	Callus Color Rating
5 – Green	#	3 – Red	Yes/No	5 – Maximum	4 – White
4 – Green-brown		2 – Green		4 -	3 – Light brown
3 – Brown with green nodes		1 – Browning		3 – Middle	2 – Brown
2 – Dark stem - green base		0 – Black		2 -	1 – Black
1 – Brown				1 – Discernable	0 – None
0 – Black				0 – None	

Data was analyzed using a factorial ANOVA and means were obtained for main effects and interactions significant at 0.05. These means were then evaluated using Tukey's Honestly Significantly Different (HSD) at the 0.05 level of significance.

RESULTS AND DISCUSSION

Year 1

Time of Year

Rooting of hardwood cuttings was compared to rooting of softwood cuttings in Year 1. No rooting of hardwood cuttings occurred in any treatment. A low percentage of softwood cuttings rooted. Results from other environmental variables are discussed below.

Year 1 – Hardwood Cuttings

Air Temperature

Fifty percent of the cuttings (250) were held in cool air conditions while the remaining cuttings were held in warm air. The warm air cuttings formed callus, broke bud three weeks after collection, the leaves desiccated and dropped, and the cuttings started dying at 5 weeks. Cuttings in the cool air did not break bud until 10 weeks. Callus tissue formed, but no rooting occurred. These cuttings also eventually died. From this, we determined that rooting of hardwood cuttings in Year 2 would be attempted only in the cool air treatment.

Media

The media used in Year 1 was a pasteurized 1:1 peat:perlite blend. High levels of fungus (Appendix D) suggested that we repeat the experiment using the same media and

compare it to an inorganic substrate, such as pasteurized sand, or determine better fungicide treatments.

Rooting Hormone Concentration

Bases of hardwood cuttings were treated at striking with indole-3-butyric acid (IBA) in a talc base at concentrations of 0 ppm, 2,500 ppm, 5,000 ppm, 7,500 ppm and 10,000 ppm. No cuttings rooted. Upon presentation of preliminary results at the International Horticultural Congress (IHC) annual meetings in Toronto in August, 2002, researchers present recommended using higher rates, up to 40,000 ppm for hardwood cuttings, in a liquid base, for the Year 2 trials.

Year 1 – Softwood Cuttings

Media

It was determined from the hardwood study results that peat:perlite, an organic media, may promote the presence of certain pathogens and fungi (Appendix D). Therefore, we struck fifty percent of the softwood cuttings (250) in the same organic media, and half in steamed sand, an inorganic media. Rooting only occurred in the inorganic media. Increased fungus gnat infestation and fungal presence was noted in the organic media (Appendix D). The survival rate of cuttings was lower in the organic media. Based on these results, only a sand media was used for softwood experiments in Year 2.

Hedging

Two trees were pruned to three-foot stumps in February to eliminate apical dominance and force adventitious bud break and elongation. Fifteen cuttings from each tree were subjected to the rooting hormone regimen outlined under Rooting Hormone Concentration. The highest percentage of rooting, 17 percent (1 of 6 cuttings), occurred with this treatment, combined with 7,500 ppm applied IBA, struck in sand. These results suggested that a more comprehensive study of hedging be initiated in Year 2.

Rooting Hormone Concentration

Softwood cutting bases were treated with the same concentrations of IBA used for the hardwood cuttings. Concentrations of 0 ppm, 2,500 ppm, 5,000 ppm, 7,500 ppm and 10,000 ppm IBA were applied in a talc base at striking. One cutting from a single non-hedged, non-etiolated tree in sand, rooted in the 5,000 ppm and 10,000 ppm treatments for rooting percentage of 2 percent for each treatment (1 of 50 each). One cutting in sand rooted from a hedged tree at 7,500 ppm for a rate of 17 percent rooting (1 of 6). The results of this experiment suggest applications of rooting hormone were probably necessary for adventitious rooting of softwood cuttings, probably within the concentration range 2,500 ppm to 10,000ppm. It was determined that this range would be repeated for softwood cutting trials in Year 2.

Callus formed on all cuttings, and more callus generally formed on cuttings treated with lower concentrations of applied IBA than higher. No correlation between callus formation and rooting could be established in Year 1.

Etiolation

No cuttings rooted with the localized etiolation treatment in Year 1. Since successful rooting has been documented with this technique for other difficult-to-root species, it was modified and included in Year 2 experiments. The initial design of our localized etiolation structure allowed new growth to double back onto itself when it reached the end of the cone, yielding distorted cuttings. A design change was instituted in Year 2 by nesting two cones together, a bottomless cone inside an intact cone, thereby lengthening the tube and creating adequate growing space.

An additional problem that developed in Year 1 may have resulted from the presence of too many apical buds. Bur oak characteristically forms a cluster of apical buds, and in Year 1, were left intact until the time of banding, when all but one of the elongated shoots were clipped. This may have resulted in inadequate space within the cone for normal shoot elongation. It was decided in Year 2 that the apical cluster of buds would be removed prior to installation of the etiolation cone, leaving the first lateral bud to act as a single terminal within the cone. It was also determined that complete etiolation of hedged and non-hedged (whole) trees should be attempted.

Summary

From Year 1 experiments it was determined that it is possible to root softwood cuttings, given the proper combination of treatments. From the results it appears that some concentration of exogenous rooting hormone is necessary for rooting, and that

hedging promotes adventitious root formation. Although logistical problems hindered success with one etiolation treatment in Year 1, successes reported in the literature warranted a repeat of this treatment in Year 2. Since all rooted cuttings were produced in sand, and more fungi were observed on the organic media, sand will be used exclusively in Year 2 for rooting softwood cuttings.

Whether hardwood cuttings can be rooted is less clear. Both organic and inorganic media, in conjunction with cool air temperatures, and higher concentrations of applied IBA would be used in Year 2.

Year 2

Time of Year

No hardwood cuttings rooted in any treatment in the second year of this study. Softwood cuttings rooted at higher numbers than in Year 1.

Year 2 – Hardwood Cuttings

Media

Fifty percent (250) of the hardwood cuttings in Year 2 were struck in 1:1 peat:perlite and fifty percent into steamed sand. These cuttings were evaluated at Week 5 for callus development on the base of the cutting and along the wounded stem. Cuttings in sand had more basal callus, whereas cuttings in peat:perlite had more callus along the stem wound.

Rooting Hormone Concentration

Hardwood cutting bases were treated with higher concentrations of K-IBA (potassium salts of indole-3-butyric acid) in distilled water than in Year 1. K-IBA rates of 0 ppm, 10,000 ppm, 20,000 ppm, 30,000 ppm and 40,000 ppm were used. This regime of high hormone concentrations failed to produce rooted hardwood cuttings.

Generally, hardwood cuttings callused by Week 5, at which point the callus began turning dark and deteriorated. At Week 5, 34 percent of hardwood cuttings in sand had budswell, whereas budswell on cuttings in peat:perlite measured 58 percent. Some budbreak occurred, but by Week 16, all cuttings were dead.

Year 2 – Softwood Cuttings

Localized Etiolation

No locally etiolated cuttings rooted in Year 2, even with the design modification. We theorized that the single etiolated bud was stressed to the point that the bud immediately below the cone, expanding in sunlight, became dominant and elongated outside the cone, diverting carbohydrates to this actively growing shoot. The cuttings that survived the cone etiolation treatment in Year 2 were not distorted, but also did not root.

Rooting Hormone Concentration

IBA in talc was applied just prior to striking at concentrations of 0 ppm, 2,500 ppm, 5,000 ppm, 7,500 ppm and 10,000 ppm. All cuttings that rooted had been treated with IBA, but there was no consistent dose response.

Hedging and Etiolation

Rooting occurred in both the hedged and non-hedged groups. Etiolation was the common denominator in all rooted cuttings at MSU and all except one rooted cutting at the PMC. A statistical interaction occurred between hedging and etiolation at MSU and the PMC.

Rooting

Softwood cutting rooting was successful in the hedging, etiolation and banding study (Table 4). Data are presented as a percentage of total cuttings struck and as a percentage of surviving cuttings for MSU (Tables 6 and 7), and the PMC (Tables 8 and 9). Although total rooting percentage demonstrates that this technique would probably not be cost-efficient, rooting expressed as a percentage of surviving cuttings indicates that cutting health and longevity may be a primary limiting factor. Detailed listings of rooted cuttings sorted by greenhouse location, cutting number, tree number, parent treatment and applied IBA concentration are found in Appendix E.

Table 6. Percentage of total softwood cuttings rooted in Year 2 at MSU

	IBA Concentration (ppm)				
Parent Treatment*	0	2500	5000	7500	10000
NH+NE+NB	0	0	0	0	0
NH+E+NB	0	5.0	0	5.0	15.0
NH+E+B	0	0	0	8.33	4.17
NH+LE+B	0	0	0	0	0
H+NE+NB	0	0	0	0	0
H+E+NB	0	18.75	0	0	6.25
H+E+B	0	12.5	0	6.25	0

*See Table 4 for abbreviation explanation

Table 7. Percentage of softwood cuttings given a positive survival rating that rooted in Year 2 at MSU

	IBA Concentration (ppm)				
Parent Treatment*	0	2500	5000	7500	10000
NH+NE+NB	0	0	0	0	0
NH+E+NB	0	9.1	0	10.0	42.9
NH+E+B	0	0	0	15.4	9.1
NH+LE+B	0	0	0	0	0
H+NE+NB	0	0	0	0	0
H+E+NB	0	33.3	0	0	14.3
H+E+B	0	12.5	0	8.0	0

*See Table 4 for abbreviation explanation

Table 8. Percentage of total softwood cuttings rooted in Year 2 at the PMC

	IBA Concentration (ppm)				
Parent Treatment*	0	2500	5000	7500	10000
NH+NE+NB	0	0	6.67	0	0
NH+E+NB	0	0	0	0	0
NH+E+B	0	4.55	0	4.55	0
H+NE+NB	0	0	6.67	0	6.67
H+E+NB	0	0	0	0	0
H+E+B	6.25	0	12.5	6.25	18.75

*See Table 4 for abbreviation explanation

Table 9. Percentage of softwood cuttings given a positive survival rating that rooted in Year 2 at the PMC

	IBA Concentration (ppm)				
Parent Treatment*	0	2500	5000	7500	10000
NH+NE+NB	0	0	50.0	0	0
NH+E+NB	0	0	0	0	0
NH+E+B	0	33.3	0	25.0	0
H+NE+NB	0	0	25.0	0	33.3
H+E+NB	0	0	0	0	0
H+E+B	25.0	0	25.0	20.0	50.0

*See Table 4 for abbreviation explanation

Hypothesis 1 – Seasonal Collection Time Influences Root Initiation

Hardwood cuttings did not root in either Year 1 or Year 2. Approximately 2 percent of the softwood cuttings struck in sand and treated with 5,000 and 10,000 ppm IBA rooted in Year 1. In that year, 17 percent of softwood cuttings taken from hedged trees rooted. No other cuttings rooted in Year 1.

In Year 2, softwood cuttings rooted as shown in Tables 6, 7, 8, and 9 (previously presented) and further developed in Appendix E.

Since no hardwood cuttings rooted with any treatments or under any conditions, and softwood cuttings rooted at varying percentages, seasonal collection time influences root initiation.

Hypothesis 2–Cool Air Temperatures Increase Root Initiation of Hardwood Cuttings

Hardwood cuttings in the warm air greenhouses quickly broke bud and desiccated. It has become apparent in this study that maintaining the health of cuttings is critical to rooting. While no rooting occurred on any hardwood cutting in either air

temperature regime, those cuttings in the cool air greenhouse remained viable longer. Because all cuttings died before root initiation could occur, we cannot reject the null hypothesis and cannot clearly determine that cool air temperatures increase root initiation of hardwood cuttings.

Hypothesis 3 – A Specific Applied Hormone Concentration Increases Root Initiation

Researchers have identified ideal rooting hormone concentrations for many genera, species, and cultivars for maximization of root initiation. There does not appear to be an ideal hormone concentration for *Q. macrocarpa*. In this study, it was determined that while some concentration of IBA is required, rooting can occur at any concentration. This finding confirms Macdonald's statement (1986) for *Q. macrocarpa*, that difficult-to-root species may not respond to specific levels of exogenously applied auxin.

Because cuttings rooted at all IBA concentrations (Table 6, 8) it is difficult to pinpoint a specific "best" concentration for root initiation. Therefore, the null hypothesis that a specific applied hormone concentration will increase rooting cannot be rejected.

Hypothesis 4 – Etiolation and Banding Increases Root Initiation

No locally etiolated cuttings rooted in Year 1. As a result, the method for localized etiolation was modified in Year 2, elongating the tube to accommodate new growth as previously described. However, a greater percentage of surviving etiolated shoots were removed from the tree in Year 1 as compared to Year 2, due to lack of elongation of etiolated shoots in Year 2.

In addition to the localized etiolation treatment, entire trees both non-hedged and hedged were etiolated in Year 2. Some new growth elongated excessively in the dark frames and cutting material was already quite long when banded. Cuttings were much longer than the ideal length of 10 to 15 cm. Due to the position of the band and stem nodes, the lengths of some cuttings reached 35 cm. Although this excessive elongation of cutting material was non-conventional, some rooting occurred on cuttings as long as 28 cm and as short as 3.5 cm (Appendix E).

When the etiolation frames were opened for banding, the tops of most fully etiolated non-hedged trees were dead. This was presumably due to heat buildup. The higher branches and shoot tips of the fully etiolated hedged trees also appeared to have been heat-stressed. Many of the shoots that were used elongated as a result of latent bud elongation on the trunk of the tree as a result of apical dominance below the dead wood. It appears that these trees could be considered heat-treated or heat-hedged. However, hedging is a sudden act, with no possibility of translocation of nutrients or water to living parts of the plant. It is assumed that heat death of tissues would occur more gradually, thereby allowing translocation to perennating buds (Allaby, 1998).

Many species demonstrate the greatest heat resistance during winter dormancy (Larcher, 2003) suggesting that if these trees were fully dormant, they may not have responded to the heat treatment. Regeneration of plants after fires may depend upon new growth from perennating buds at the base of dead shoots. When aerial parts of a woody plant die back as a result of unfavorable conditions, food for new shoots are stored in buds on the stems of woody plants, or perennating buds (Allaby, 1998). *Q. ilex* and *Q.*

coccifera (as well as plants from other genera) are known to produce new shoots from stems after exposure to fire (Larcher, 2003), and it is possible that such an occurrence happened with these trees after being heated.

Of the cuttings that rooted, all at MSU had been etiolated and most at the PMC had been etiolated (Table 6, 8). Therefore, etiolation increases root initialization.

Hypothesis 5 – Hedging Increases Root Initiation

Older trees, regardless of species, do not readily propagate from cuttings. As *Q. macrocarpa* is difficult to root under favorable conditions, old *Q. macrocarpa* trees should be extremely difficult to root (Ferrini and Bassuk, 2002). In Year 1 of this study, the parent oak trees used were 9 years old, and cuttings were taken from them for two years. Many of these source plants had left the ontogenetic juvenile stage, and entered the reproductive phase of growth. Trees in the hedging study were severely pruned to force adventitious bud break and the resulting shoots to elongate. This new growth should originate within the "cone of juvenility," hence, cuttings taken from this new growth would exhibit the juvenile characteristic of improved adventitious rooting, regardless of chronological age of the tree. This theory was substantiated when, in Year 1, 17 percent of non-etiolated softwood cuttings from these hedged trees, treated with 7,500 ppm IBA, struck in sand, developed roots. One hedged source tree produced rooted cuttings in both study years, at MSU in Year 1 with IBA concentration of 7,500 ppm, and at the PMC in Year 2, at IBA concentrations of 5,000 ppm and 10,000 ppm. In

both years, cuttings from this tree were subjected to the same parent treatment, H + NE + NB.

In Year 2, the majority of rooted cuttings occurred in hedged trees at the PMC (Table 8). While equivalent numbers of hedged and non-hedged cuttings rooted at MSU (Table 6). Therefore, the hypothesis that hedging increases root initiation cannot be rejected.

Hypothesis 6 – Cuttings Will Root More Readily in Inorganic Rather Than Organic Media

Cuttings only rooted in sand, an inorganic media. Organic media had a higher rate of pathogen presence. Therefore, cuttings root more readily in inorganic rather than organic media.

Hypothesis 7 – Some Combination of Treatments Increases Root Initiation

While etiolation alone produced a significant amount of rooting, the addition of hedging to the parent plants increased the number of rooted cuttings (Table 6, 8; Appendix E). Therefore, the hypothesis that some combination of treatments increases root initiation cannot be rejected. More specifically, the combination of hedging + etiolation increases root initiation.

Clonal Variation

Although not a formal part of this study, it should be noted that a certain amount of clonal variation was observed, a phenomenon documented with other species of

Quercus (McGuigan et al., 1996). Multiple cuttings from nine different trees rooted, having received different treatments (Appendix E). Cuttings from four of these trees rooted at both the MSU greenhouse and at the PMC greenhouse with same parent treatments, but with different concentrations of applied IBA, giving further substantiation to the theory that difficult-to-root species do not respond uniformly to specific concentrations of IBA (Macdonald, 1986).

Correlation of Rooting and Cutting Health

It appears that maintaining the health of the cuttings is vital for rooting *Q. macrocarpa.* Rooting does not always occur quickly, as new roots were observed at Week 4 through 11 during this study. Maintaining healthy cuttings should therefore result in additional rooted cuttings. Cutting health observations made at Week 8 (Table 5) correlated to the hedging and etiolation treatments as observed for rooting, and will be discussed in the following MSU data section. Because of the different greenhouse types, and that two different observers made these measurements at MSU and the PMC, and due to the subjective nature of these measurements, discussion of cutting health is separated by location.

MSU Data - Softwood Cuttings Taken in 2003

At MSU, the hedging treatment was significant at the $P < .0001$ level in all evaluation categories (Table 10). Etiolation was significant in the presence of live leaves and petiole color. Banding was significant only for callus color. IBA treatment was

significant at varying levels in all categories, and was significant at P <.0001 for the presence of live leaves. The interaction of hedging and etiolation was significant for cutting survival, stem color and callus color.

Table 10. Summary of ANOVA main effects and interactions for the health of MSU cuttings.

	Model	Hedging vs. Non-Hedged	Etiolation vs. Non-Etiolated	Banding vs. Non-Banded *	IBA Levels	Hedging x Etiolation	Hedging x IBA	Etio-lation x IBA	Hedging x Etiolation x IBA
Cutting Survival	**<.0001**	**<.0001**	0.1040	0.0857	**0.0004**	**0.0107**	0.5915	0.0603	0.7281
Stem Color	**<.0001**	**<.0001**	0.2620	0.0660	**0.0003**	**0.0074**	0.6924	0.4010	0.6741
Live Leaves	**<.0001**	**<.0001**	**0.0002**	0.5064	**<.0001**	0.0690	0.3972	0.4709	0.7277
Petiole Color	**<.0001**	**<.0001**	**0.0007**	0.3906	**0.0009**	0.0599	0.4637	0.2538	0.8632
Amount of Callus	**<.0001**	**<.0001**	0.0557	0.1080	**0.0015**	0.2753	0.6511	0.4124	0.7179
Callus Color	**<.0001**	**<.0001**	0.1576	**0.0145**	**0.0012**	**0.0295**	0.9453	0.4927	0.6640

Significant main effects and interactions are shown in BOLD.
* Not all treatments included banding, therefore, only main effects were noted for that treatment.

An ANOVA analysis and subsequent mean separation test of rooting correlation to these six cutting health ratings revealed that cutting survival, stem color, live leaves, and petiole color were higher for rooted cuttings (Figure 1). Amount of callus and callus color did not correlate with rooting at MSU. Only ratings that correlated with rooting are discussed further.

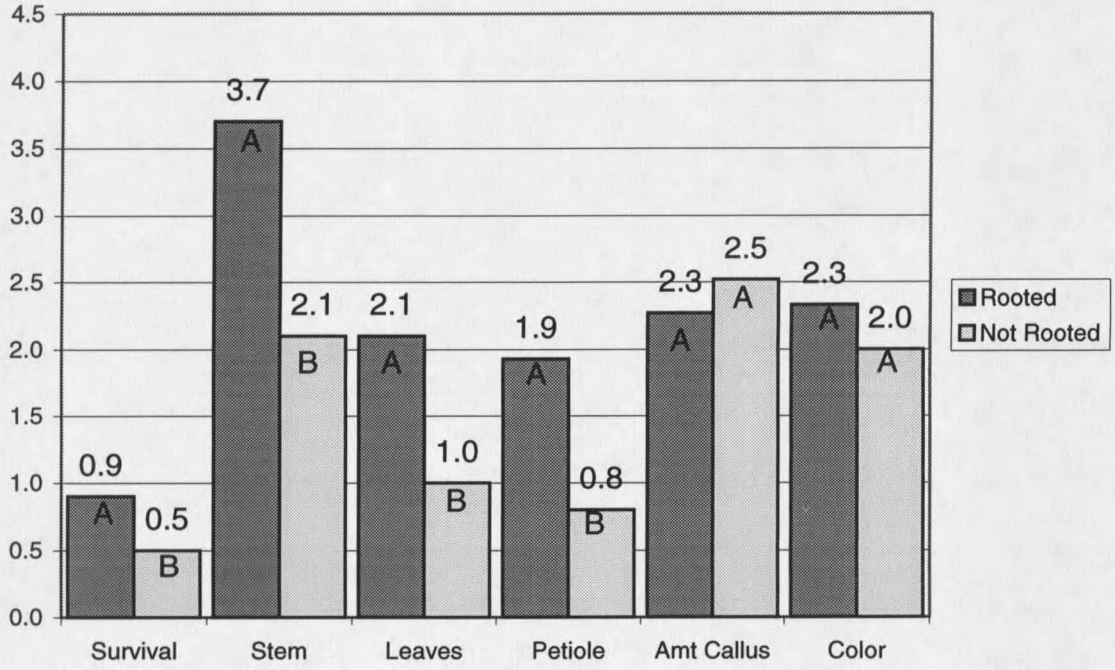


Figure 1. MSU Data: Correlation of rooted cuttings with cutting survival, stem color, live leaves, petiole color, amount of callus and callus color. Letters indicate significant differences at the 0.05 level of Tukey's HSD.

Etiolation x Hedging Interaction

Both cutting survival and stem color had significant etiolation by hedging interactions precluding any main effects (Table 10). Hedged plants had the highest survival rating regardless of etiolation treatment (Figure 2). But in the non-hedged treatments, etiolation enhanced survival.

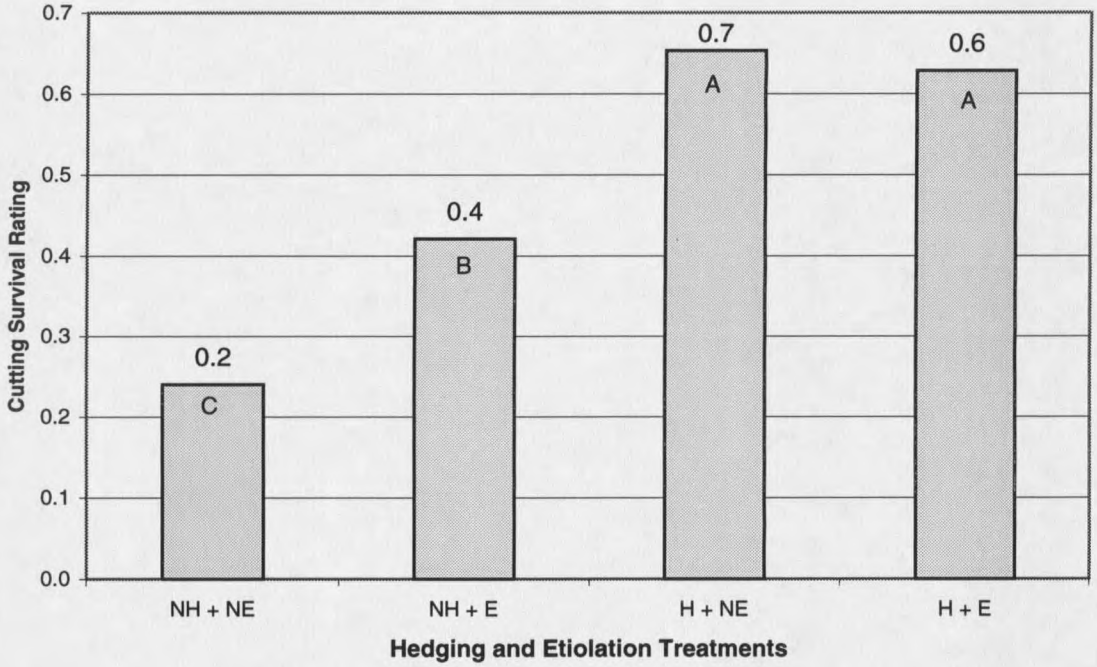


Figure 2. MSU Data: Effect of Hedging (H) vs. Not Hedging (NH) by Etiolation (E) vs. Not Etiolated (NE) interaction on survival of cuttings. ANOVA analysis with F = 6.55, P 0.0107, and R^2 = 0.16. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent LS Means.

The same trends were apparent for stem color (Figure 3). Hedging gave a higher rated or greener stem color. Etiolation only enhanced the stem color if the plants were not hedged. It is interesting to note that etiolated cuttings recovered to at least the stem color rating of non-etiolated cuttings by the eight-week observation.

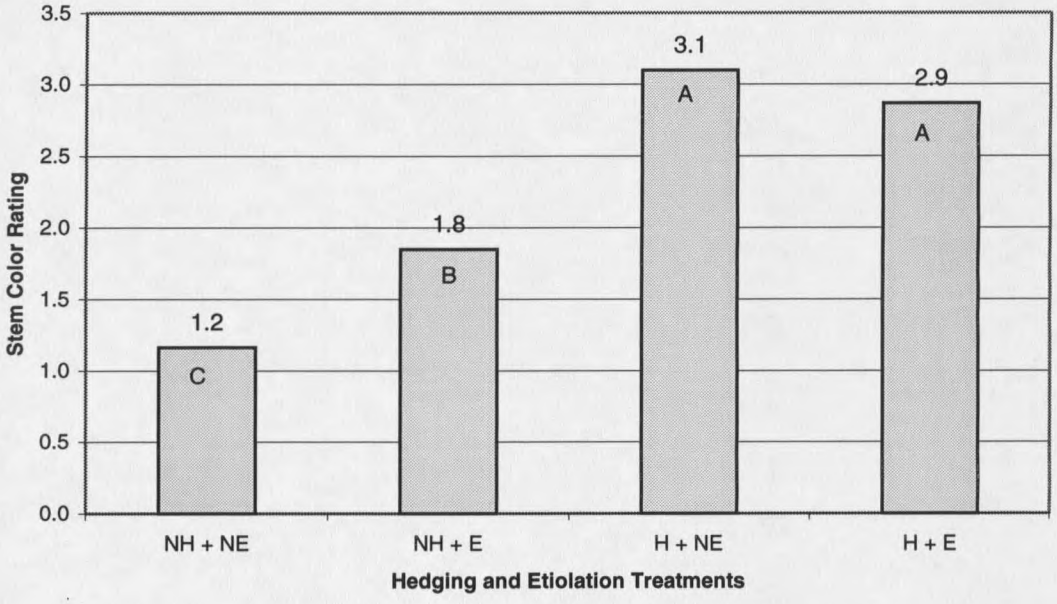


Figure 3. MSU Data: Effect of Hedging (H) vs. Not Hedging (NH) by Etiolation (E) vs. Not Etiolated (NE) interaction on stem color rating. ANOVA analysis with F = 7.21, P 0.0074, and R^2 = 0.17. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent LS Means.

Hedging and Etiolation Effects

Where hedging and etiolation did not interact, hedging and etiolation each had a positive effect on live leaf number and petiole color (Figures 4 and 5). This indicates that rooting, which is associated with a high live leaf number and petiole color (Figure 1), will be more likely to occur in hedged and/or etiolated cuttings.

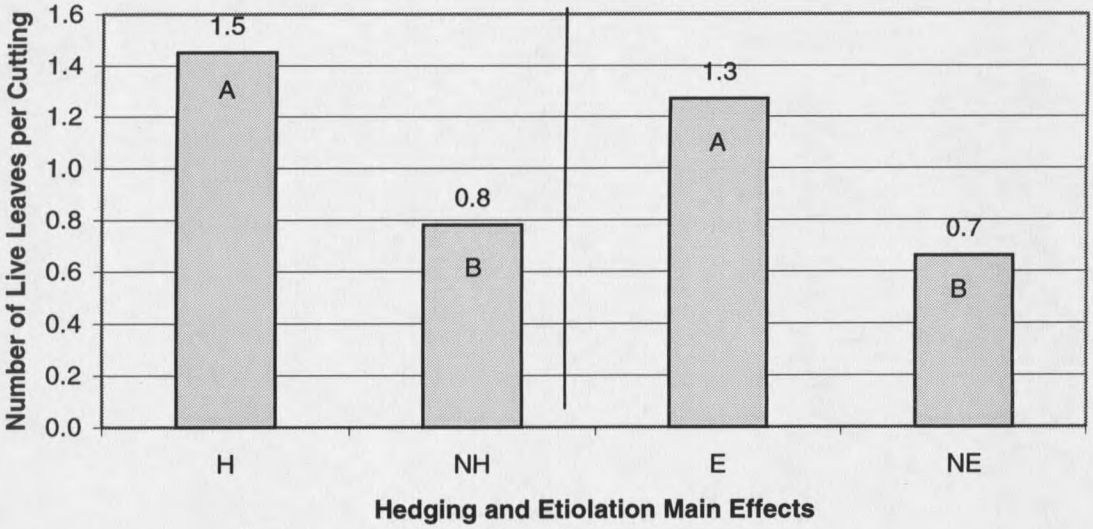


Figure 4. MSU Data: Effect of Hedging (H) vs. Not Hedging (NH) and Etiolation (E) vs. Not Etiolated (NE) on number of live leaves per cutting. ANOVA analysis with Hedging F = 41.47, P = <.0001; Etiolation F = 13.87, P = 0.0002 and R^2 = 0.16. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

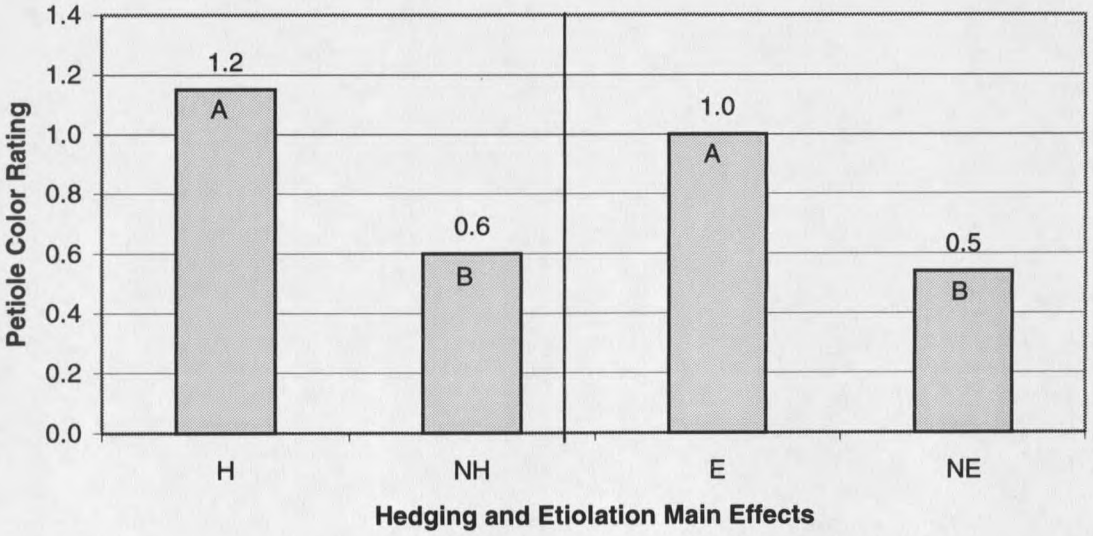


Figure 5. MSU Data: Effect of Hedging (H) vs. Not Hedging (NH) and Etiolation (E) vs. Not Etiolated (NE) on petiole color rating. ANOVA analysis with Hedging F = 48.89, P = <.0001; Etiolation F = 11.65, P = 0.0007, and R^2 = 0.16. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

Applied Rooting Hormone Main Effects

Effects of applied IBA were significant for survival, stem color, number of live leaves and petiole color (Table 10). Despite the observation that increasing concentrations of applied IBA appear to correlate with reduced cutting health, we saw consistently that levels of 7,500 ppm increase cutting health to levels not significantly different than 0 or 2,500 ppm IBA (Figures 6, 7, 8, and 9).

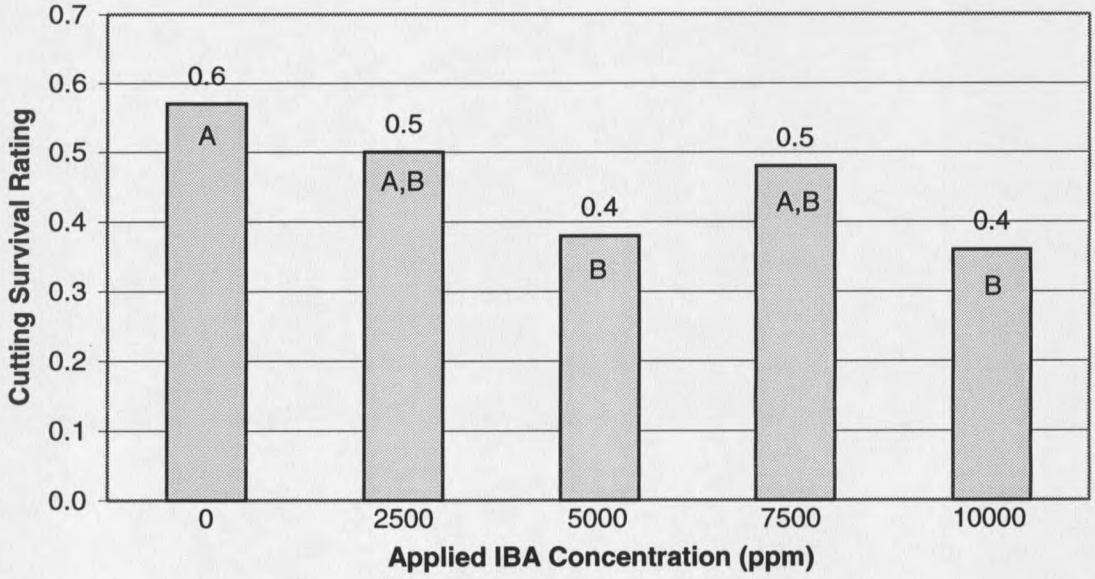


Figure 6. MSU data: Effect of applied IBA on cutting survival rating. ANOVA analysis with F = 6.55, P = 0.0107, R^2 = 0.16. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

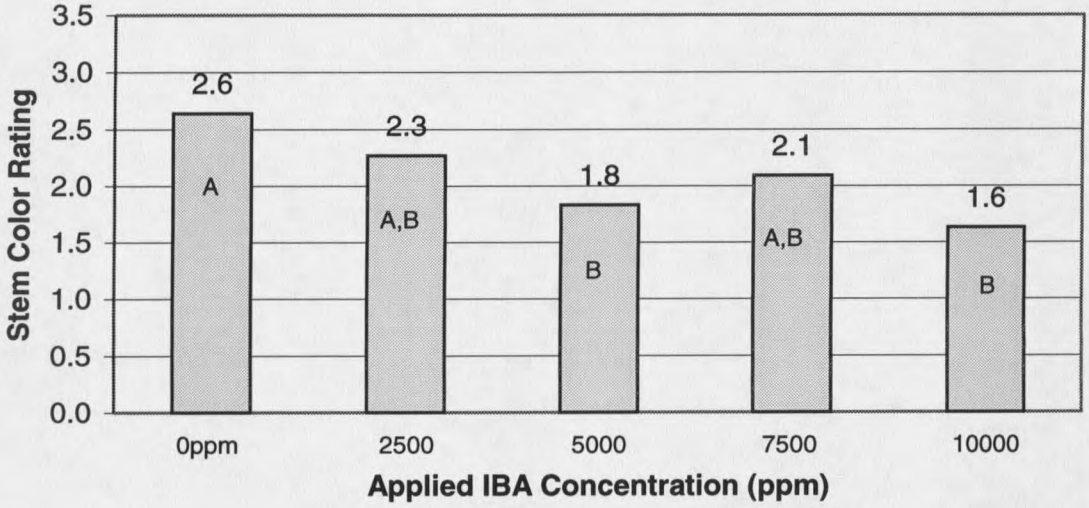


Figure 7. MSU Data: Effect of applied IBA on stem color rating. ANOVA analysis with F = 5.31, P = 0.0003, R^2 = 0.17. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

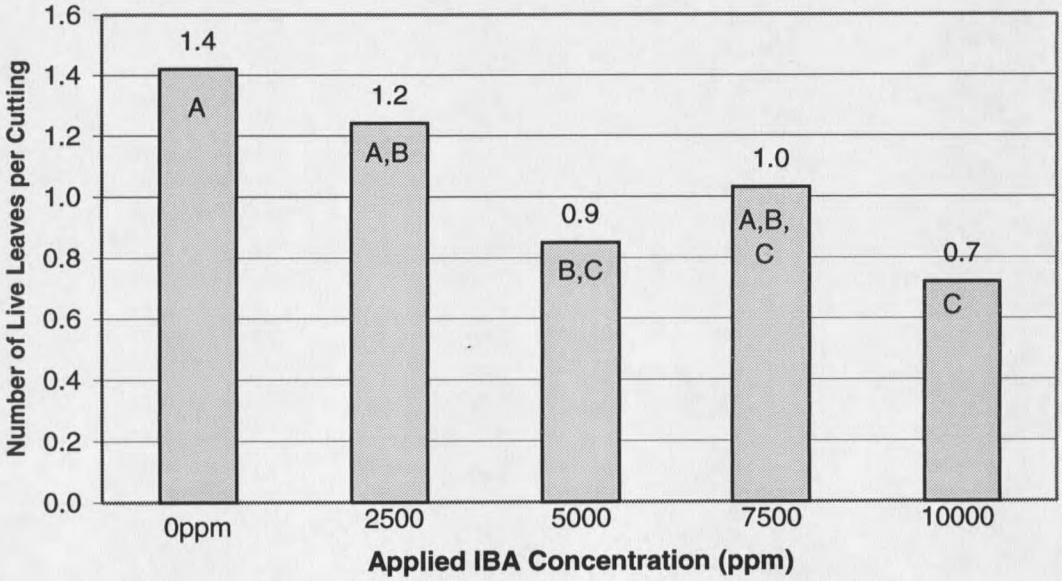


Figure 8. MSU Data: Effect of applied IBA on number of live leaves per cutting. ANOVA analysis with F = 6.66, P =<.0001, R^2 = 0.16. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

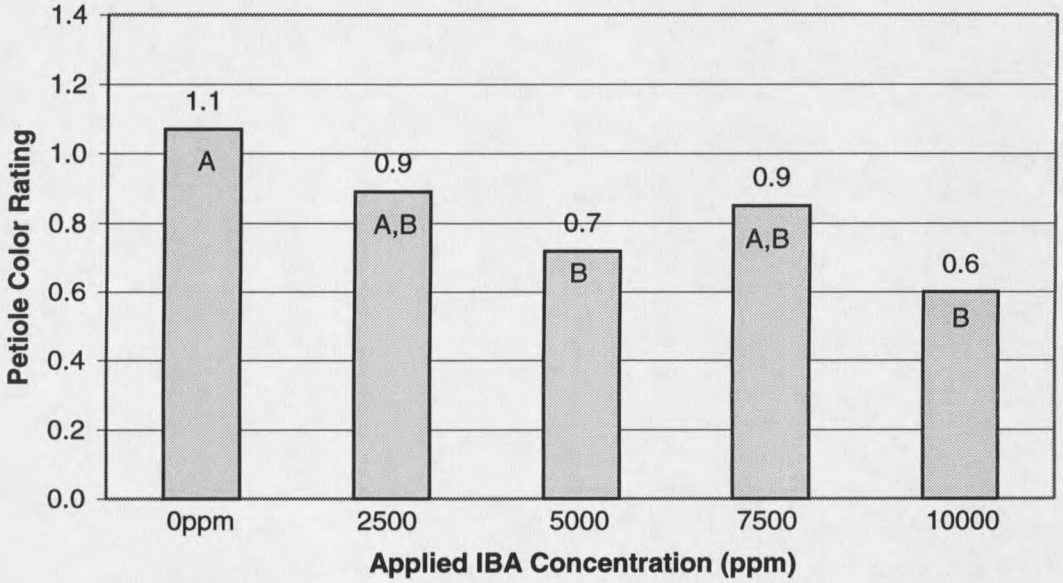


Figure 9. MSU Data: Effect of applied IBA on petiole color rating. ANOVA analysis with F = 4.75, P = 0.0009, R^2 = 0.15. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

PMC Data - Softwood Cuttings Taken in 2003

The ANOVA for the PMC shows higher levels of significance in responses for cutting survival, live leaves and petiole color than the models (Table 11). The stem color model significance was 0.0004, and both callus amount and callus color were at a P <.0001 level. The only interaction was for hedging x etiolation, in the stem color category.

Table 11. Summary of ANOVA main effects and interactions for the health of the PMC cuttings.

	Model	Hedging vs. Non-Hedged	Etiolation vs. Non-Etiolated	Banding vs. Non-Banded *	IBA Levels	Hedging x Etiolation	Hedging x IBA	Etiolation x IBA	Hedging x Etiolation x IBA
Cutting Survival	0.2355	**0.0006**	0.1193	**0.0482**	0.9189	0.8817	0.6250	0.9301	0.6533
Stem Color	**0.0004**	0.5142	**0.0001**	**<.0001**	0.8244	**0.0021**	0.4659	0.9684	0.4457
Live Leaves	0.4875	**0.0109**	0.2851	0.5186	0.6760	0.7325	0.5622	0.6274	0.8126
Petiole Color	0.5472	**0.0214**	**0.0317**	0.1052	0.8438	0.9524	0.6983	0.8842	0.7813
Amount of Callus	**<.0001**	**<.0001**	**<.0001**	**<.0001**	0.2592	0.3507	0.8160	0.2149	0.3869
Callus Color	**<.0001**	**<.0001**	**0.0001**	**<.0001**	0.5496	0.6969	0.9972	0.7340	0.3295

Significant main effects and interactions are shown in BOLD.
*Not all treatments included banding, therefore, only main effects were noted for that treatment.

At the PMC, the hedging treatment was significant for survival, live leaves, petiole color, and particularly callus condition, including both amount and color. Etiolation had a significant impact on stem color, petiole color, and callus condition. Banding was significant for survival, stem color, and callus condition.

An ANOVA analysis and subsequent mean separation test of rooting correlation to these six cutting ratings (Table 5) revealed that all health responses correlated with rooting (Figure 10). In all categories, health indicators were higher for cuttings that rooted than not rooted. Interestingly, for the PMC data, rooted cuttings had a higher amount of callus and better callus color than non-rooted cuttings. Because all health ratings correlated with rooting, all are discussed further.

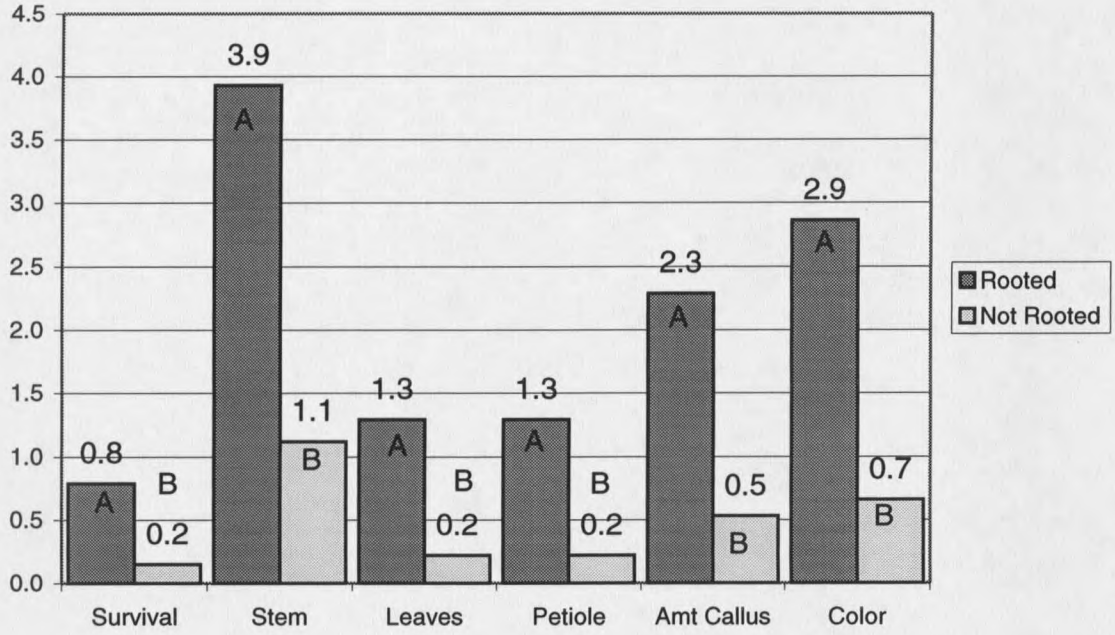


Figure 10. PMC Data: Correlation of rooted cuttings with cutting survival, stem color, live leaves, petiole color, amount of callus and callus color. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

Etiolation x Hedging Interaction

LS Means were compared for trends of those treatments demonstrating interaction: At the PMC, the only interaction noted was hedging x etiolation for stem color (Table 11). For the hedged x etiolated interaction for stem color, better stem color was observed in cuttings that were not etiolated, regardless of hedging treatment (Figure 11). Etiolation enhanced stem color of hedged cuttings and had a negative impact on non-hedged cuttings.

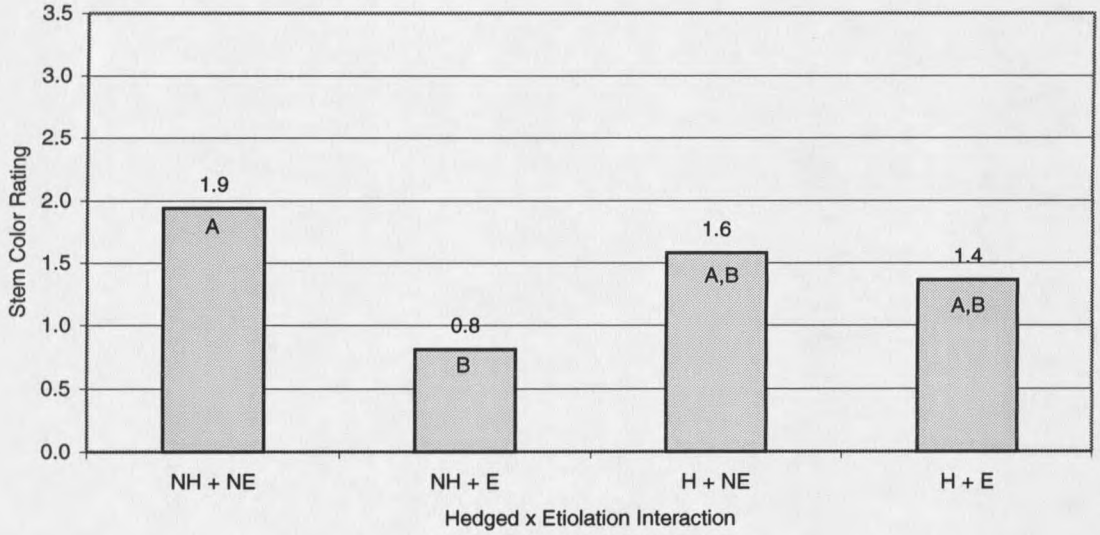


Figure 11. PMC Data: Hedging (H) and Not Hedging (NH) and Etiolation (E) and Not Etiolated (NE) interaction effects on stem color rating. ANOVA analysis with F = 9.55, P = 0.0021, $R^2 = 0.1$. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent LS Means.

Main Effects

Hedging had a positive effect on cutting survival (Figure 12), petiole color (Figure 13), number of live leaves per cutting (Figure 14), and callus amount and color (Figure 18). Banding had a positive effect on cutting survival (Figure 12), stem color (Figure 15), amount of callus and callus color (Figure 16). While the banding treatment P value was 0.0482 for survival, less than the 0.05 level of significance, Tukey's HSD did not find banding vs. not banding significant. It should be noted while evaluating these results that Tukey's test has a higher Type II error rate than other tests, failing to reject the null hypothesis as readily as other tests when it is false. Etiolation had a negative effect on callus amount, and callus color (Figure 17). While the etiolation treatment had

a P value of 0.0001 for survival in the ANOVA table (Table 11), Tukey's HSD did not find etiolated vs. non-etiolated to be significant. Non-etiolated cuttings had better petiole color (Figure 13), but not statistically better than etiolated cuttings. Applied IBA concentration was not significant (Table 11).

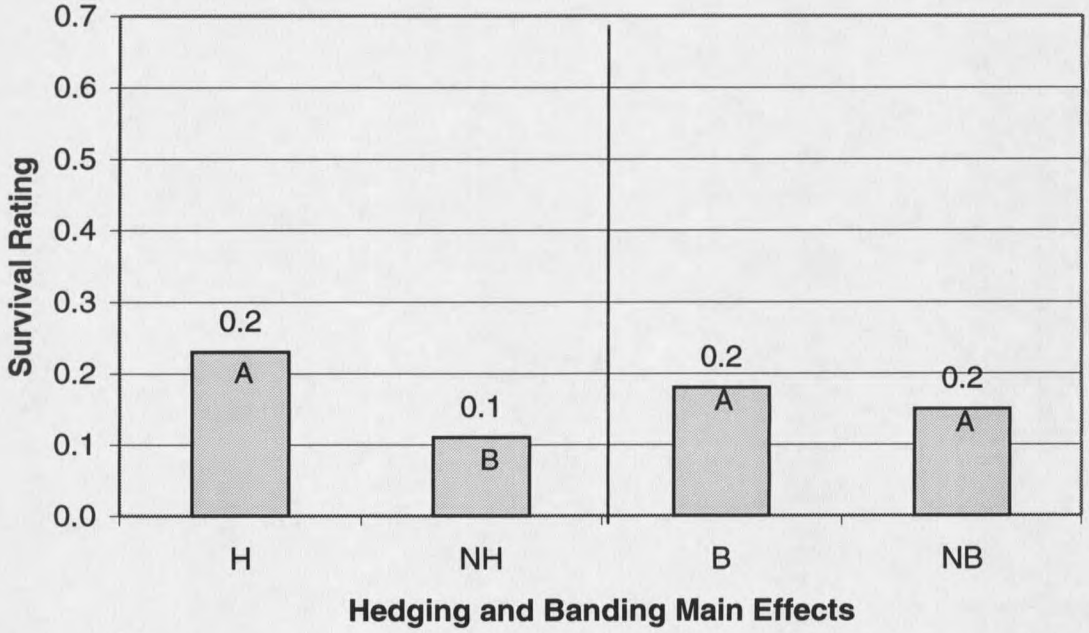


Figure 12. PMC Data: Effects of Hedging (H) vs. Not Hedged (NH) and of Banding vs. Not Banded (NB) on cutting survival rating. ANOVA analysis with Hedging F = 11.80, P = 0.0006; Banding F = 3.93, P = 0.0482, and R^2 = 0.05. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

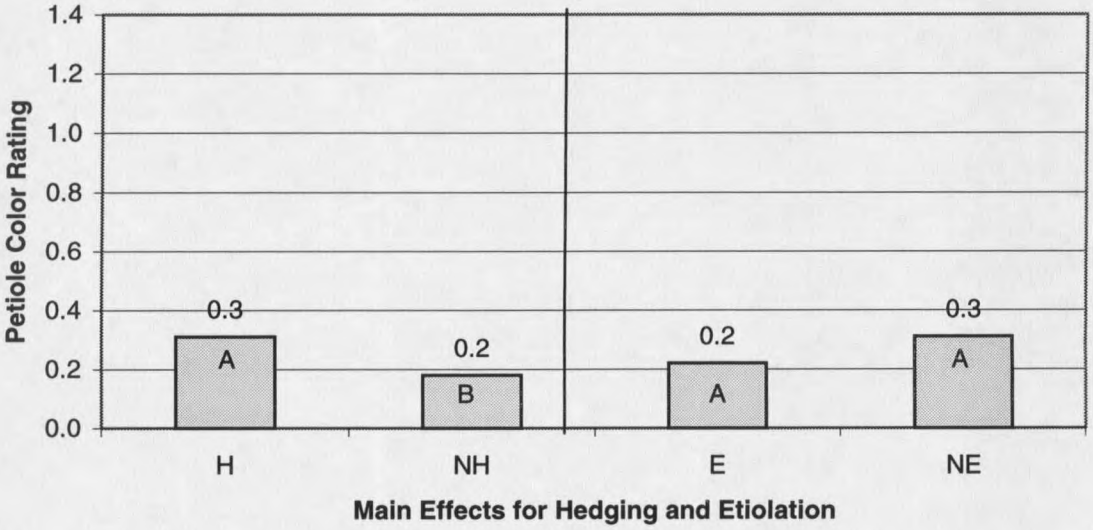


Figure 13. PMC Data: Effects of Hedging (H) vs. Not Hedged (NH) and of Etiolation (E) vs. Not Etiolated) on petiole color rating. ANOVA analysis with Hedging F = 5.33, P = 0.0214; Etiolation F = 4.64, P = 0.0317, and R^2 = 0.04. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

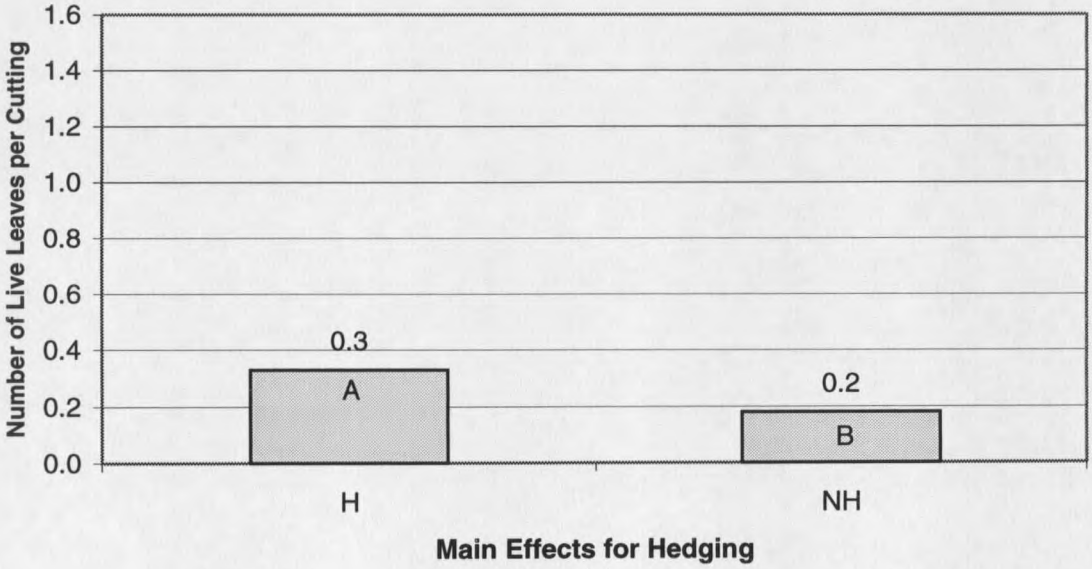


Figure 14. PMC Data: Main effects of Hedging (H) vs. Not Hedged (NH) on number of live leaves per cutting. ANOVA analysis with F = 6.54, P = 0.0109, and R^2 = 0.04. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

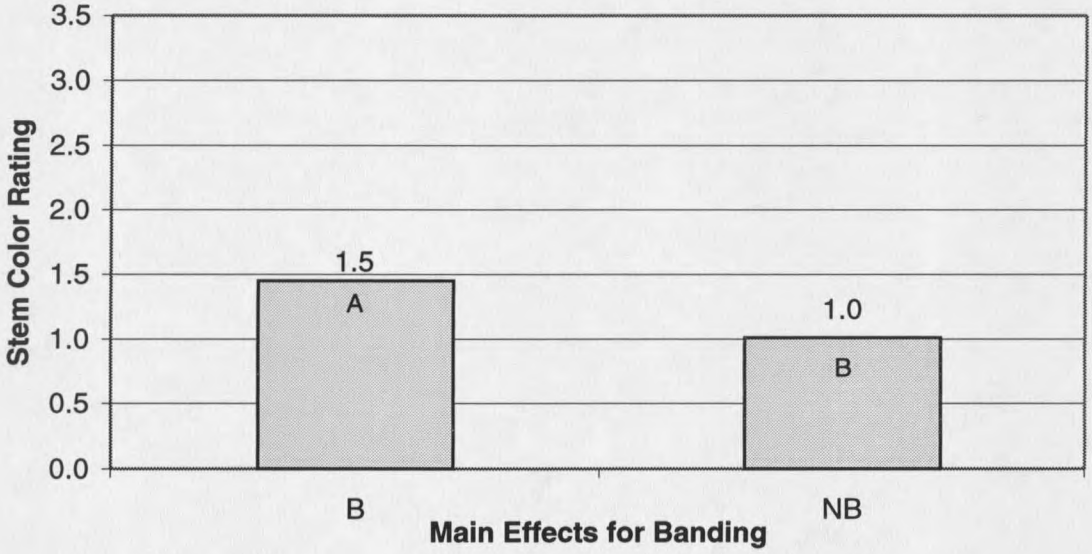


Figure 15. PMC Data: Main effects of Banding (B) vs. Not Banded (NB) on stem color rating. ANOVA analysis with F = 28.22, P = <.0001 and R^2 = 0.1. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

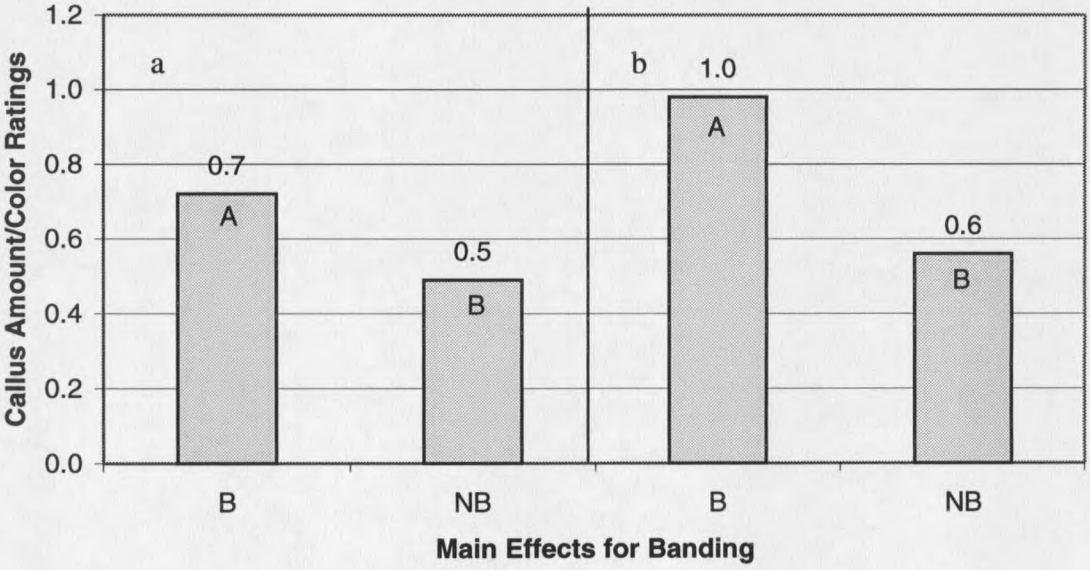


Figure 16. PMC Data: Effects of Banding (B) vs. Not Banded (NB) on a) callus amount and b) callus color. ANOVA analysis with Amount of callus/Banded F = 24.64, P = <.0001 and R^2 = 0.17; Callus color/Banded F = 35.0, P = <.0001 and R^2 = 0.15. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

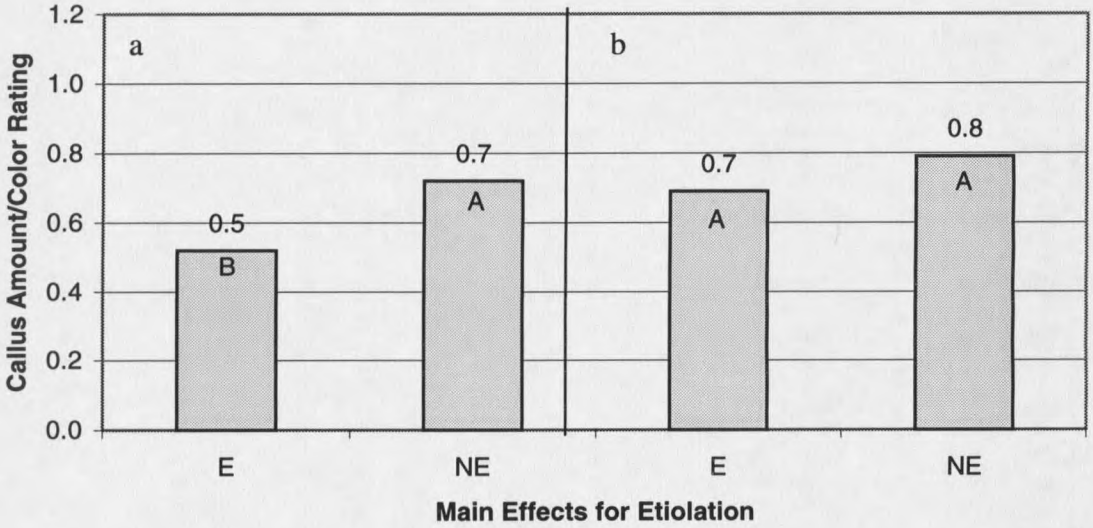


Figure 17. PMC Data: Effects of Etiolation (E) vs. Not Etiolated (NE) on a) callus amount and b) callus color. ANOVA analysis with Callus amount/Etiolation F = 19.48, P = <.0001, and R2 = 0.17; Callus color/Etiolation F = 14.89, P = 0.0001, R^2 = 0.15. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

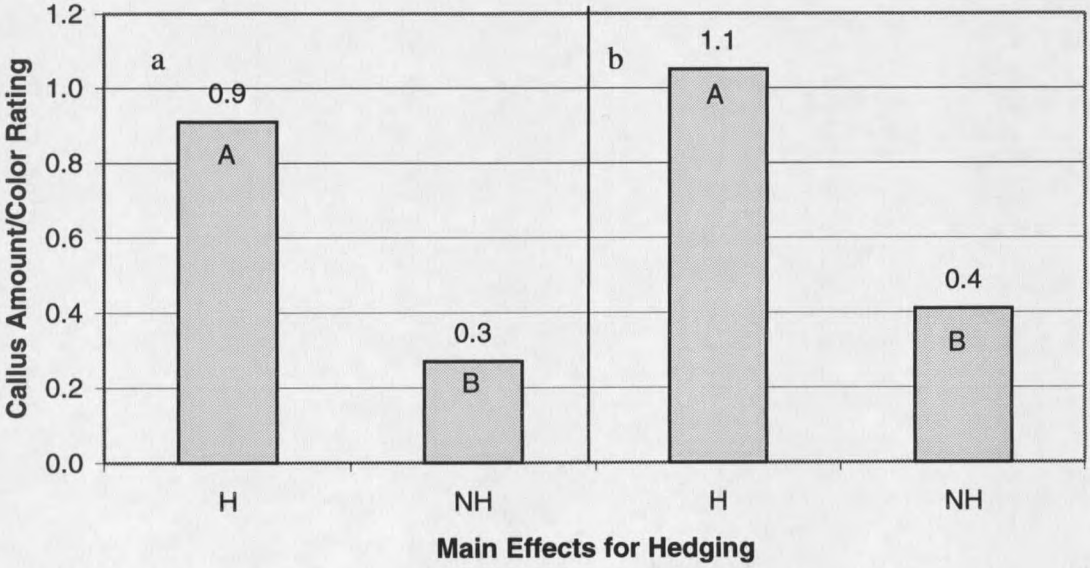


Figure 18. PMC Data: Effects of Hedging (H) vs. Not Hedged (NH) on a) callus amount and b) callus color. ANOVA analysis with Callus amount/Hedged F = 52.88, P = <.0001 and R^2 = 0.17; Callus color/Hedged F = 40.21, P = <.0001 and R^2 = 0.15. Letters indicate significant differences at the 0.05 level of Tukey's HSD. Bars represent means.

Conclusions

There were marked differences in results from the experiments at Montana State University (MSU) and the Plant Materials Center (PMC). Differences could, in some cases, be attributed to subjectivity, as different people were evaluating cuttings at each location, even though the differences were minimized through defining of the rating systems. However, limited rooting at MSU and the PMC may have resulted from an inability to keep cuttings viable long enough for adventitious root formation. While the MSU Plant Growth Center facilities were constructed as state-of-the art in the 1980s, with computer regulated environmental control systems, and the buffering of two adjacent greenhouses within the greenhouse range, the PMC greenhouse is a stand-alone glass house, fully exposed to the elements on three sides and the roof. Control of the greenhouse environment is not straightforward. Additionally, environmental conditions are less favorable for vegetative propagation at Bridger, Montana than at Bozeman, with a high number of solar days, low relative humidity, infrequent cloud cover, intense sun, and high winds.

Softwood cuttings at the MSU greenhouse remained healthier for a longer period of time than at the PMC greenhouse, with cutting health declining rapidly at the PMC in Year 1, with improvement in Year 2. Since rooting and cutting health correlate (Figures 1 and 10), less rooting would be expected with declining cutting health. At both facilities, rooted cuttings were much healthier than non-rooted cuttings, at a given point in time, presumably because they were healthy enough to initiate root. This is supported by the subsequent rooting of cuttings late in the study that were given a high health rating

at Week 8 (Appendix E). MSU's non-rooted cuttings were healthier at Week 8 than those at the PMC, again presumably a function of MSU's environmental conditions.

One area of interest was callusing. At MSU, the amount of callus and callus color ratings were statistically insignificant for both rooted and non-rooted cuttings. In contrast, both callus amount and color correlated with rooting at the PMC. Non-rooted cuttings at the PMC had statistically less callus and worse callus color at evaluation than rooted cuttings. It is possible that non-rooted cuttings at the PMC did not have the necessary resources for callus production and maintenance, or rooting, once cutting health began declining. Whether callus production is necessary for rooting, or if callusing competes with rooting for available carbohydrates is unclear. Since roots did not emerge directly from callus tissue, it suggests that callus formation is not a precursor to root initiation in this species. Adventitious rooting experiments designed to compare treatments that interfere with callus production with controls may clarify the role of callus with root initiation.

Another factor is the role of auxin in maintaining cutting health. Early studies with applied auxin determined that, regardless of apical or basal application, most auxin was translocated to the basal end of the cutting (Hartmann et al., 2002). Auxin applied basally, however, remains at the basal end, and is more effective in promoting adventitious rooting when applied there. Studies of auxin rate and dose response, including this one, require the removal of the apical bud, a source of endogenous auxin that could interfere with experimental results. At MSU, one of the first signs of cutting decline was the browning of the petiole and subsequent leaf abscission. Leaves would

often drop that appeared green and turgid, with stems that also appeared green and healthy. Leaves did not appear desiccated in the same manner that cuttings placed in a container of water look after a week. Auxin is involved in leaf abscission, with low rates late in the growing season resulting in leaf drop in deciduous plants. Since auxin is formed in the apex and moves basipetally, this source may supplement the production of auxin by young leaves and promote leaf retention. We have determined in this study that some concentration of applied auxin is required for adventitious rooting of this species, but an ideal concentration does not appear necessary. It would be interesting to leave cutting apices intact, and to apply a range of IBA concentrations to determine if endogenous auxin contributes to the maintenance of cutting health in this species.

The combination of the hedging and etiolation treatments indicates promise of healthier cutting production. At MSU, hedged plants had the highest survival rating regardless of etiolation treatment, while in the non-hedged treatments, etiolation enhanced survival. The same trend was seen for stem color. Hedging improved stem color, whereas etiolation improved stem color of non-hedged plants. At the PMC, cuttings that were not etiolated had the best stem color. Etiolation improved stem color only in hedged cuttings.

Based on the success of hedging and etiolation in this study, future attempts at rooting this species should include stoolbed propagation. In this method, parent plants are pruned almost to the ground, and the surrounding soil is mounded over the stump as new shoots emerge. This method simultaneously hedges the tree, and etiolates the cutting base, allowing the emerging shoot to adapt gradually to environmental conditions

and reducing over-elongation of the shoot. Since localized etiolation attempts failed both years, it would appear that either the design of the etiolation cones was faulty and needs further refinement, or else it is, indeed, necessary to root rejuvenated shoots from adventitious buds rather than new growth of the current year.

Further histological research is necessary to determine the cause of poor adventitious rooting of this species. Preliminary anatomical research (Appendix C) indicates there is reason to pursue the mechanical barrier theory presented in this work. If rooting can only occur at the xylem ridges, rooting potential is drastically reduced from species with a vascular cylinder without these ridges. Further complicating the barrier issue, phloem fibers and other sclerified tissues are producing a barrier distal to the xylem ridges. If the alignment of xylem ridges with lenticels is required for root protrusion, or if the cuttings are producing callus instead of roots, there is indeed a formidable combination of barriers to the formation of adventitious roots to be overcome by this species.

Based on our results, researchers should begin follow-up studies by beginning their studies with the combination of etiolation and banding, while applying IBA at a rate of 7,500 ppm IBA to the cuttings. These treatments were successful at both MSU and the PMC (Tables 6, 8). Some method of rejuvenation of parent trees should also be included, as hedging improved formation of adventitious roots.

APPENDICES

APPENDIX A

CLASSIFICATION OF *QUERCUS MACROCARPA*

CLASSIFICATION OF *QUERCUS MACROCARPA*

System of classification (Hardin et al., 2001)

Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Hamamelidae
Order	Fagales
Family	Fagaceae
Genus*	*Quercus*
Species*	*Quercus macrocarpa* (bur oak)

*There are two subgenera and three sections within the genus *Quercus*:

I. Subgenus *Cyclobalanopsis*. The cycle-cup oaks. Asia.

II. Subgenus *Quercus*. The scale-cup oaks. Northern Hemisphere.

a. Section *Quercus* (=*Leucobalanus*) – White oaks.

i. True white oaks: *Q. alba, macrocarpa, lyrata, stellata, margaretta, douglasii, garryana, lobata, gambelii*

ii. Chestnut oaks: *Q. michauxii, prinus, bicolor, muehlenbergii*

iii. Live oaks: *Q. virginiana, geminata, arizonica, oblongifolia.*

b. Section *Lobatae* (=*Erythrobalanus*) – Red and black oaks

i. True red and black oaks: *Q. rubra, velutina, shumardii, falcate, pagoda, coccinea, palustris, ellipsoidalis, texana, marilandica, laevis, kelloggii*

ii. Willow, laurel, and water oaks: *Q. phellos, nigra, hemisphaerica, laurifolia, imbricaria, incana*

iii. Western live oaks: *Q. wislizeni, agrifolia, emoryi*

c. Section *Protobalanus* – The intermediate oaks. *Q. chrysolepis, palmeri, cedrosensis, tomentella, vaccinifolia*

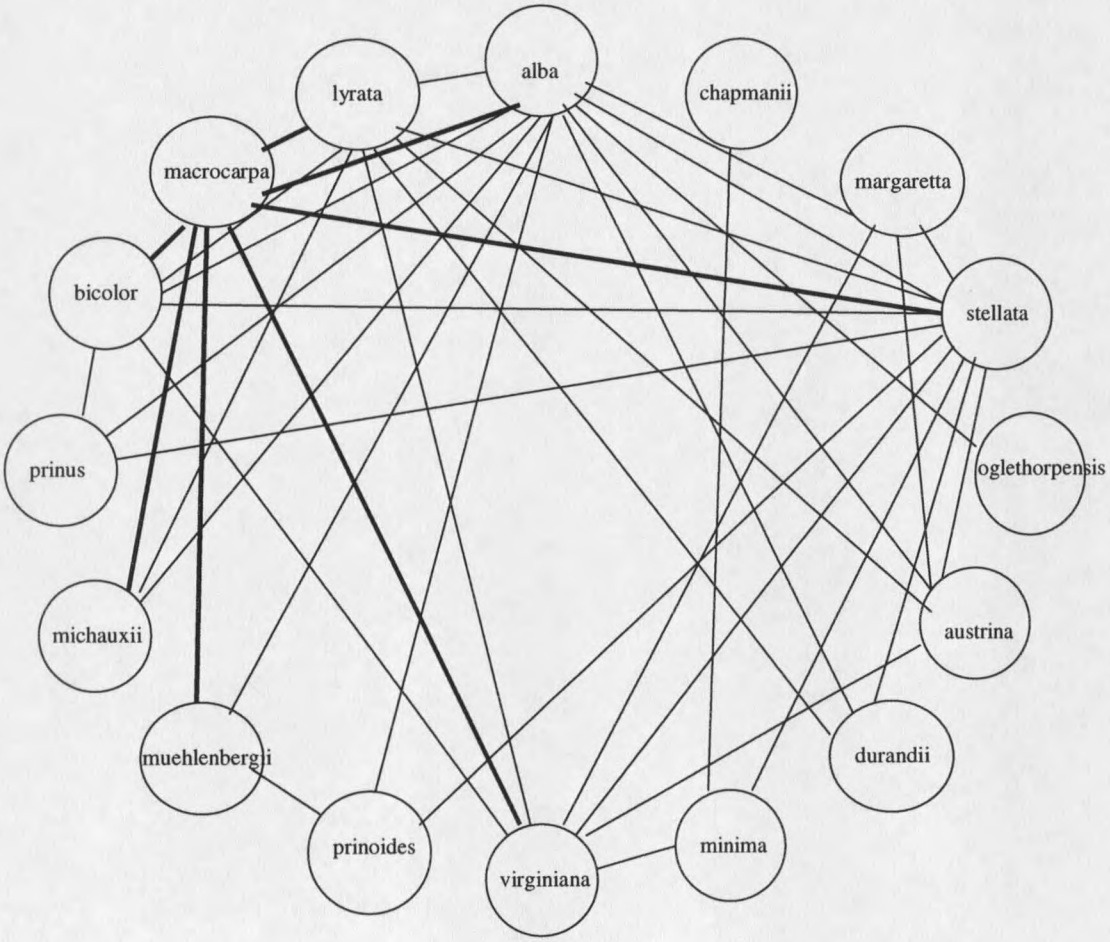
The White Oak Syngameon

A species is literally a group of organisms that closely resemble one another. In taxonomy, a species is one or more groups that can interbreed, but cannot exchange genes with other groups, or "an interbreeding group of biological organisms that is isolated reproductively from all other organisms" (Allaby, 1998). However, a few species can interbreed, often producing infertile offspring, while a small number produce fertile offspring. In the event of geographical barriers, reproductive isolation may lead to genetic drift, resulting in subspecies or semi-species (Grant, 1981). These subspecies could interbreed if they were reintroduced to one another.

A syngameon is a large pairing community with many species (or sub-species) having the ability to hybridize with those others geographically close enough for frequent or occasional pollen exchange. The syngameon group behaves as if it were a single biological species, made up of subspecies. The individual components, however, are treated as individual species in formal systematics.

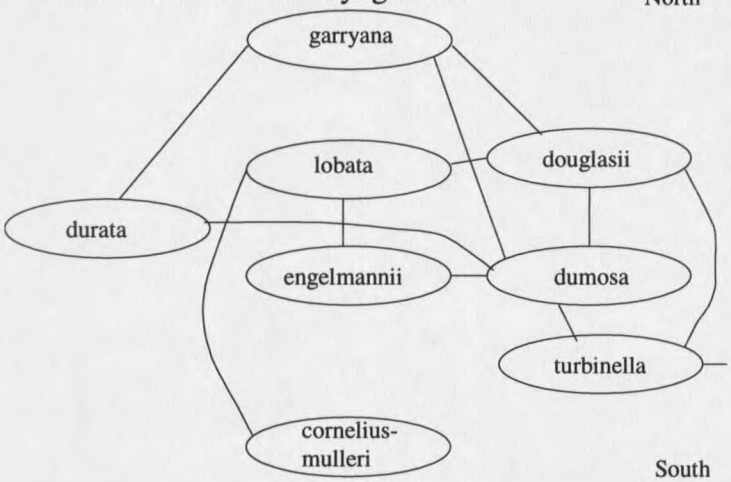
The white oak syngameon is taxonomically and geographically 'large' with hybridization occurring at the edge of each species' natural range (Grant, 1981). Undoubtedly, such hybridization will eventually result in a single taxon (Burger, 1975).

Below is the white oak syngameon of eastern North America, including *Q. macrocarpa* (Grant, 1981; Hardin et al, 2001). Those species that will cross with *Q. macrocarpa* are shown with bold connecting lines:



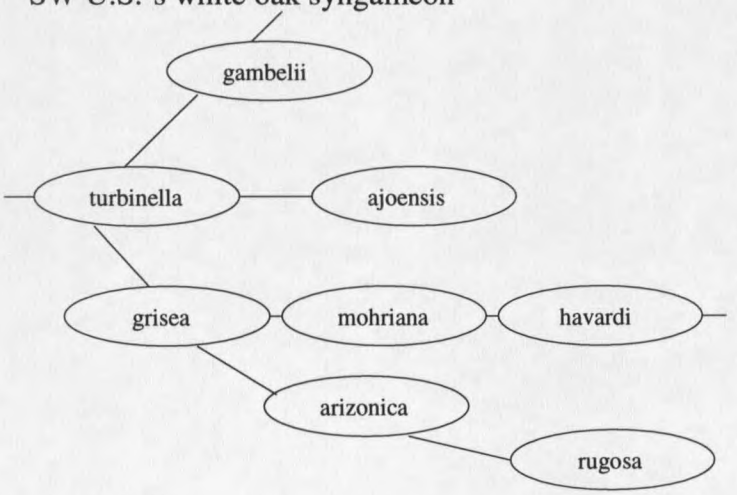
Interestingly, there are other white oak syngameons, such as the one found in California (Grant, 1981). These appear in approximate geographical orientation:

California's white oak syngameon



And the white oak syngameon found in the southwest United States (Grant, 1981):

SW U.S.'s white oak syngameon



APPENDIX B

GROWING SOFTWOOD SHOOTS FROM WOODY STEM SEGMENTS

GROWING SOFTWOOD SHOOTS FROM WOODY STEM SEGMENTS

Introduction

Rooting protocols vary according to species, and many difficult-to-root species will not root from hardwood cuttings. It was not known whether successful rooting of *Q. macrocarpa* would take place with hardwood or softwood cuttings. As softwood cuttings are only available for a short time each year, a novel method was developed by Dr. John E. Preece and colleagues to force elongation of shoots from latent buds, resulting in softwood cutting material from the hard wood to extend the softwood collection season (Preece et al., 2002). In this study, lower limbs of trees or large stems of shrubs were pruned from the parent tree in winter or early spring, any time after the chilling requirement of the species had been satisfied. Branches of ½" minimum diameter were cut into 16 to 18 inch lengths and placed horizontally in perlite-filled flats in a greenhouse mist chamber, with mist set to run for six seconds every six minutes. Air temperature was 24° C (75°F) and a 16-hour photoperiod was used. Budswell in most species was usually noted in two to four weeks, with shoots elongating in five to seven weeks. When the shoots were two inches or longer, they were cut from the horizontal limb and treated as softwood cuttings collected in the field.

Using this method, Preece has forced softwood shoots from a number of difficult to root species, including: *Acer ginnala* (amur maple), *A. palmatum* (Japanese maple), *A. saccharinum* (sugar maple), *Fraxinus Americana* (white ash), *Castanea dentata* (American chestnut), *Juglans nigra* (black walnut), *Quercus bicolor* (swamp white oak),

and *Q. rubra* (northern red oak). Preece reports the number and vigor of the resulting shoots varies greatly by species. He does not give any rooting percentages for the cuttings taken from the two *Quercus* species in his study.

Materials and Methods

In 2003, the second year of this study, 33 9-year old bur oak trees were severely pruned in early January for the hedging experiment. Any removed branches that were larger than ½" in diameter were transported to MSU to duplicate Preece's forcing experiment using *Q. macrocarpa*. Branches were trimmed to 18" in length, sprayed with 95% ethanol, and given a 10% chlorine bleach dip. Eighteen flats with 6 branches each were prepared from these 108 branches. A total of 6 flats (36 branches) were placed on each media: 1:1 (v/v) peat:perlite, 100% perlite, or 100% steamed sand. These flats were then placed in the warm air (24°C/75°F) greenhouse mist chamber, with mister set to go off for 6 seconds every 6 minutes. Light was provided in a 16-hour photoperiod. Cuttings that were produced with this method were treated with a talc-based IBA at 7500 ppm prior to striking in sand. Two small flats of willow branches were treated in the same manner and placed in the mist chamber. A BanRot 40% WP drench (1.75g/liter water) was applied weekly to reduce fungal growth.

Results and Discussion

The goal of this experiment was to force adventitious buds to elongate, forming shoots that could be removed from the branches and used as softwood cuttings. The

branches were not expected to root. Oak branches had no budswell at week #2, whereas willow branches had bud elongation in week #2 and roots formed on the branches at week #3.

At week #4, fungal growth was observed, which continued to flourish throughout the duration of this experiment, in spite of the weekly BanRot drench.

At week #7 the oak branches still did not have budswell, but the willows had produced many shoots and roots. Dr. Preece was contacted at this time via e-mail. He felt that if the oak had not as yet had any budswell, it would not in the future. At his suggestion, more branches were cut from the parent material at this time and placed in the mist chamber to investigate the possibility that the chilling requirement of the original oak branches had not been satisfied.

At week #9 budswell was noted on four of the original oak branches, at the same time budswell was noted in the cool air mist chamber on the cuttings in the simultaneous year 2 hardwood cutting study.

By week #16, the branches had produced many short leafy shoots, but only 6 shoots sufficiently elongated to remove as softwood cuttings. These shoots were removed from the branches, treated with 7500 ppm IBA, and struck in sand, but did not root.

APPENDIX C

ANATOMY OF CUTTING BASES

ANATOMY OF CUTTING BASES

Introduction

During these experiments, an attempt was made to observe anatomical changes occurring during the attempted rooting of cuttings and subsequent formation of callus tissue. We wanted to determine if root initials were actually forming, and if so, if they formed within stem or callus tissue.

Much has been written about preparation of wood samples for anatomical observation, but little has been written regarding preparation of young stem tissue, which is comprised of both small quantities of wood and much softer undifferentiated tissues. Preparation of stem samples for microscope study was problematic, and a completely satisfactory procedure was not found.

Adventitious roots are those originating in a manner other than from the root pole of the embryo or branches formed from the normal sequence of rooting (Esau, 1953; Kramer and Kozlowski, 1979). These roots may form from a stem, leaf, underground stems and also on old roots. They may form on nodes, internodes or on seedling hypocotyls. They may form on young tissue, or on older tissues, provided they have not lost their meristematic potentiality. They may develop from dormant root primordia that have previously been formed and are stimulated to grow, or may be formed *de novo* from new 'induced' primordia (Kramer and Kozlowski, 1979).

Adventitious roots are also those formed on cuttings and may develop either from callus tissue, or directly from the stem. They are usually formed near the site of active

differentiation. In older tissue, the origin of adventitious roots is near the vascular cambium, usually from a vascular ray. These roots may form from divisions within the cambium, or within the pericycle, a part of the primary phloem, or the interfascicular region between bundles of primary phloem.

Emergence of the root through distal tissues can be problematic, and may be blocked either physically or physiologically. Oak cuttings are considered to be difficult to root. In some difficult-to-root species, including oak (Metcalfe and Chalk, 1950), a continuous ring of sclerified tissue and extensive phloem fiber sheath may be formed exterior to the origin of adventitious root formation (Hartmann et al., 2002). These structures may either form a mechanical barrier or may interfere with the dedifferentiation process required for cuttings to form adventitious roots (Beakbane, 1969; Deen, 1974).

It was desirable to cross-section oak cuttings to make a histological study of the normal oak stem, callus development, and possible physical barriers to rooting that may occur or develop during attempted rooting.

Materials and Methods

Basal sections of cuttings were collected at the conclusion of the 2002 softwood cutting experiment. Samples were collected from cuttings treated with each of the 5 levels of exogenously-applied rooting hormone IBA (indole-3-butyric acid): 0 ppm, 2500 ppm, 5000 ppm, 7500 ppm, and 10000 ppm. Basal sections of the three cuttings that

rooted were also collected, along with attached roots. These samples were fixed and maintained in FAA (formalin-acetic acid-alcohol) (Sass, 1958).

Tissue was paraffin-embedded and sectioned at a thickness between 10-14μm with a rotary microtome. Following deparaffinization, the tissue was stained with safranin and fast green (Johansen, 1940; Ruzin, 1999).

The above procedure was marginally satisfactory, as the cuttings had formed a small amount of wood. The differences of density within the cutting made the infiltration of paraffin and sectioning difficult. Sass (1958) reports that perfect slides from young woody stems of tougher woods like oak are not made with any sort of consistency. In spite of these problems, usable slides were made.

Observations

A star-shaped vascular cylinder with five xylem ridges, or pentarch xylem (Esau, 1977) was consistently observed when the stems were cross-sectioned. The xylem ridges lined up with buds along the cutting, and are assumed to be the site of fascicular cambium. The inside angle of these ridges were aligned with the corky ridges of periderm that typically formed on older stems.

Cells that could be construed as mechanical barriers to root emergence were observed in the cross-sections. Phloem fibers were observed in groups and bands surrounding the vascular cylinder. Sclerified tissue was observed in the cortex around the periphery of the cuttings. Both types of tissues have been implicated in the mechanical barrier theory.

Again in cross-section, what appeared to be root initials at the ridges of the vascular cylinder appeared to swell towards lenticels.

Multi-faceted druse crystals occurred throughout the parenchyma. These crystals are typically made of calcium oxylate, and could, perhaps, interfere with dedifferentiation of cells during root initiation. The literature mentions the presence of rhomboidal crystals in the axial parenchyma and rays of *Quercus* but makes no mention of druses in any members of the family Fagaceae (Carlquist, 2001).

Unidentified gray matter occupied many of the xylem vessels. Perhaps these were tyloses, fungal growth, or both. Tyloses are formed by wounded tissue as a barrier to invasion by microorganisms. These tyloses block the xylem and cause dehydration of tissues. Fungal growth within the xylem could also block uptake of water.

The callus tissue observed had little organization. Occasionally, xylem was observed within the callus with some appearing in a whirlpool-like configuration, or tracheary nests (Hartmann et al., 2002). This confirmed Esau's statement (1965) that vascular tissue appearing in callus would usually appear as nodules or short strands, and is not organized into a system.

Theory I

Given the pentarch vascular cylinder and the alignment of buds and corky ridges, and given the apparent root initials forming at the base of lenticels, perhaps root initials only have a chance to penetrate the formidable combined mechanical barriers of fibers and schlerenchyma if the xylem ridge aligns with a lenticel. This theory has support in

that the roots that did occur from these cuttings emerged from the sides of cuttings with what appears to be a collar of white material at the root base, and aligned with the xylem ridge as observed in further studies in 2003. Kramer and Kozlowski (1979) report that some species form hypertrophied lenticels and adventitious roots as a response to flooding (Kozlowski, 1984) the protrusion of these roots from hypertrophied lenticels in *Fraxinus pennsylvanica* appears identical to those observed emerging from *Quercus macrocarpa.*

Sections were not completed of the rooted cuttings in 2002 or 2003 as the techniques used were not perfected, and quality of resulting slides unreliable.

Theory II

Quercus wood is ring-porus, having wide conducting vessels. These water-conducting vessels are known to be lost from the conducting system during winter; new ones must be formed every spring prior to leaves unfolding (Baas, 1982). A single ring is formed each year to conduct water, therefore there is an inherent risk involved: Should those conducting rings become blocked or diseased, no conduction will occur, perhaps one reason why hardwood cuttings don't "go." Vessels in oak are about four times wider and 30 times longer than *Acer* (maple), a diffuse-porus genus with more than one ring of water conducting vessels. Maple needs about 7000 times as many vessels as oak. In oak, if a single vessel is lost by some damage, that damage is 7000 times as serious than in maple. Oak is known to form tyloses (Kramer and Kozlowski, 1979), and as previously mentioned, these are often formed by wounded tissue as a barrier to invasion by

microorganisms. If our cuttings had xylem blocked by either fungal growth or tyloses, water uptake would be blocked and the cutting tissues would dehydrate.

APPENDIX D

MIST CHAMBER FUNGI

MIST CHAMBER FUNGI

A number of fungi were identified in the mist chamber surviving epiphytically on the oak cuttings. These fungi appeared to be more prevalent when an organic-based media was used, such as the 1:1 v/v peat:perlite combination. Fungi were cultured on PDA (potato dextrose agar) for further identification. Not all fungi were deemed significant, and therefore were not identified. Organism identification was confirmed by various Plant Pathology professors.

A fungicidal drench of BanRot 40 WP (1.75g/liter) was applied weekly to reduce the fungal presence.

Paecilomyces 3/1/02

This deuteromycete appeared as a fuzzy white coating on the bases of hardwood cuttings. It was quite prevalent in the cold air greenhouse in 2002, and was present in small quantities in 2003. This is not a pathogenic fungus but a saprophyte living on dead plant material. *Paecilomyces* is used as a biological control agent against whiteflies, and is one of the causal organisms of soft roots of wood that is held in a continuously high moisture content (Agrios, 1997). This identification was confirmed by Dr. Barry Jacobsen, Professor of Plant Pathology, Montana State University, Bozeman.

Ceratocystis pluriannulata 5/15/02

Dubbed the 'single-whisker phenomenom,' the beaked perethecia of this pyrenomycete resembles a tiny bowling ball embedded in the bark of hardwood cuttings, with a single annula from which spores emerged. As another *Ceratocystis* spp., *C. fagacearum* produces the vascular disease oak wilt, a devastating disease of oak trees, it

was important to rule out this species. Dr. Don Mathre, retired Professor of Plant Pathology, Montana State University, and expert in *Ceratocystis* identification, made the identification. This organism was seen only in 2002.

Trichoderma 4/4/02

Trichoderma was observed in 2002 on hardwood cutting bases, but only very occasionally. Trichoderma is a pyrenomycete, and grows as a green cushion on cutting bases, and is often used as a biological control of plant pathogenic fungi. Identification was confirmed by Dr. Bill Grey, Assistant Research Professor of Plant Pathology.

Epicoccum purparescens 2/14/03

Epicoccum is an ubiquetious fungus, therefore it was not surprising to find it on our horizontal forcing experiment. It is also used as a biological control for plant pathogenic fungi. Identification was confirmed by Dr. Bill Grey.

Fusarium spp. 2/14/03

Several *Fusarium* species were observed in masses on the horizontal log experiment. *Fusarium* spp. were also isolated directly from forced cutting tissue. It is postulated that the application of BanRot may be killing fungi that are naturally antagonistic to *Fusarium*. Plant material with black lesions were brought back from the Plant Materials Center and plated directly. It is possible that this *Fusarium* is growing within the oak trees in the field.

APPENDIX E

ROOTED CUTTING TREATMENT COMPARISONS

SOFTWOOD 2003 ROOTED - BOTH LOCATIONS - SORTED BY CUTTING NUMBER

Rooted Cutting Number	Tree Location	Tree ID	Hedging Treatment	Etiolation Treatment	Banding Treatment	I.B.A. Conc	Notes
			H=Hedged	E=Etiolated	B=Banded	ppm	
			NH=Not	NE = Not	NB=Not		
MSU			Hedged	Etiolated	Banded		
A	B5R1 EB	TASII-10	H	E	NB	2,500	Roots at Week 11
B	B3R1 EB	TASII-29	H	E	NB	2,500	
C	B3R1 EB	TASII-29	H	E	NB	10,000	
D	B543 EB	TASII-08	H	E	NB	2,500	
F	B7R2 P9	061-42	NH	E	NB	10,000	Roots in star arrangement
G	B3R2 P13	054-02	NH	E	NB	2,500	
H	B6R3 P11	060-12	NH	E	NB	10,000	ROOT WAS BROKEN OFF
I	B6R3 P4	042-15	NH	E	NB	10,000	
J	NB P3	237-05	H	E	B	7,500	Tall Cutting
K	B3R2 EB	TASII-26	H	E	B	2,500	Very tall cutting (28cm)
M	B3R2 P13	054-02	NH	E	B	7,500	Very short cutting (3.5cm)
N	B7R1 P10	064-21	NH	E	B	10,000	Very short cutting (3.5cm)
P	B6R3 P4	042-15	NH	E	B	7,500	Very tall cutting (21.5cm)
R	B6R3 P11	060-12	NH	E	NB	7,500	Roots at Week 11
S	B7R3 EB	250-17	H	E	B	2,500	Roots at Week 11
PMC							
1	SB P7	128-17	H	NE	NB	5,000	cutting dying
2	SB P7	128-17	H	NE	NB	10,000	This tree produced rooted cuttings in 2002
3	NB P3	237-05	H	E	B	10,000	
4	B4R1 EB	TASII-15	H	E	B	10,000	
5	B3R1 EB	TASII-29	H	E	B	5,000	2 new leaves, no old ones
6	B2R3 EB	TASII-33	H	E	B	0	
7	B2R3 EB	TASII-33	H	E	B	5,000	
8	B2R3 EB	TASII-33	H	E	B	7,500	
9	B2R3 EB	TASII-33	H	E	B	10,000	
10	B2R2 P7	060-09	NH	E	B	7,500	
11	NB P4	142-19	H	E	NB	10,000	
12	B5R1 EB	TASII-10	H	E	NB	5,000	
13	B7R2P9	061-42	NH	E	B	2,500	

SOFTWOOD 2003 ROOTED - BOTH LOCATIONS - SORTED BY PARENT TREATMENT

Rooted Cutting Number	Tree Location	Tree ID	Hedging Treatment	Etiolation Treatment	Banding Treatment	I.B.A. Conc	Notes
			H=Hedged	E=Etiolated	B=Banded	ppm	
			NH=Not Hedged	NE = Not Etiolated	NB=Not Banded		
MSU							
J	NB P3	237-05	H	E	B	7,500	Tall cutting
K	B3R2 EB	TASII-26	H	E	B	2,500	Very tall cutting (28cm)
S	B7R3 EB	250-17	H	E	B	2,500	Roots at Week 11
A	B5R1 EB	TASII-10	H	E	NB	2,500	Roots at Week 11
B	B3R1 EB	TASII-29	H	E	NB	2,500	
C	B3R1 EB	TASII-29	H	E	NB	10,000	
D	B543 EB	TASII-08	H	E	NB	2,500	
M	B3R2 P13	054-02	NH	E	B	7,500	Very short cutting (3.5cm)
N	B7R1 P10	064-21	NH	E	B	10,000	Very short cutting (3.5cm)
P	B6R3 P4	042-15	NH	E	B	7,500	Very tall cutting (21.5cm)
F	B7R2 P9	061-42	NH	E	NB	10,000	Roots in star arrangement
G	B3R2 P13	054-02	NH	E	NB	2,500	
H	B6R3 P11	060-12	NH	E	NB	10,000	ROOT WAS BROKEN OFF
I	B6R3 P4	042-15	NH	E	NB	10,000	
R	B6R3 P11	060-12	NH	E	NB	7,500	Roots at Week 11
PMC							
3	NB P3	237-05	H	E	B	10,000	
4	B4R1 EB	TASII-15	H	E	B	10,000	
5	B3R1 EB	TASII-29	H	E	B	5,000	2 new leaves, no old ones
6	B2R3 EB	TASII-33	H	E	B	0	
7	B2R3 EB	TASII-33	H	E	B	5,000	
8	B2R3 EB	TASII-33	H	E	B	7,500	
9	B2R3 EB	TASII-33	H	E	B	10,000	
11	NB P4	142-19	H	E	NB	10,000	
12	B5R1 EB	TASII-10	H	E	NB	5,000	
10	B2R2 P7	060-09	NH	E	B	7,500	
13	B7R2P9	061-42	NH	E	B	2,500	
1	SB P7	128-17	H	NE	NB	5,000	cutting dying
2	SB P7	128-17	H	NE	NB	10,000	This tree produced rooted cuttings in 2002

SOFTWOOD 2003 ROOTED - BOTH LOCATIONS - SORTED BY IBA CONCENTRATION

Rooted Cutting Number	Tree Location	Tree ID	Hedging Treatment	Etiolation Treatment	Banding Treatment	I.B.A. Conc	Notes
			H=Hedged	E=Etiolated	B=Banded	ppm	
			NH=Not Hedged	NE = Not Etiolated	NB=Not Banded		
MSU							
K	B3R2 EB	TASII-26	H	E	B	2,500	Very tall cutting (28cm)
S	B7R3 EB	250-17	H	E	B	2,500	Roots at Week 11
A	B5R1 EB	TASII-10	H	E	NB	2,500	Roots at Week 11
B	B3R1 EB	TASII-29	H	E	NB	2,500	
D	B543 EB	TASII-08	H	E	NB	2,500	
G	B3R2 P13	054-02	NH	E	NB	2,500	
J	NB P3	237-05	H	E	B	7,500	Tall cutting
M	B3R2 P13	054-02	NH	E	B	7,500	Very short cutting (3.5cm)
P	B6R3 P4	042-15	NH	E	B	7,500	Very tall cutting (21.5cm)
R	B6R3 P11	060-12	NH	E	NB	7,500	Roots at Week 11
C	B3R1 EB	TASII-29	H	E	NB	10,000	
N	B7R1 P10	064-21	NH	E	B	10,000	Very short cutting (3.5cm)
F	B7R2 P9	061-42	NH	E	NB	10,000	Roots in star arrangement
H	B6R3 P11	060-12	NH	E	NB	10,000	ROOT WAS BROKEN OFF
I	B6R3 P4	042-15	NH	E	NB	10,000	
PMC							
6	B2R3 EB	TASII-33	H	E	B	0	
13	B7R2P9	061-42	NH	E	B	2,500	
5	B3R1 EB	TASII-29	H	E	B	5,000	2 new leaves, no old ones
7	B2R3 EB	TASII-33	H	E	B	5,000	
12	B5R1 EB	TASII-10	H	E	NB	5,000	
1	SB P7	128-17	H	NE	NB	5,000	cutting dying
8	B2R3 EB	TASII-33	H	E	B	7,500	
10	B2R2 P7	060-09	NH	E	B	7,500	
3	NB P3	237-05	H	E	B	10,000	
4	B4R1 EB	TASII-15	H	E	B	10,000	
9	B2R3 EB	TASII-33	H	E	B	10,000	
11	NB P4	142-19	H	E	NB	10,000	
2	SB P7	128-17	H	NE	NB	10,000	This tree produced rooted cuttings in 2002

SOFTWOOD 2003 ROOTED - BOTH LOCATIONS - SORTED BY TREE

Rooted Cutting Number	Tree Location	Tree ID	Hedging Treatment	Etiolation Treatment	Banding Treatment	I.B.A. Conc	Notes
			H=Hedged NH=Not Hedged	E=Etiolated NE = Not Etiolated	B=Banded NB=Not Banded	ppm.	
	MSU CUTTINGS ARE LETTERED WHILE PMC CUTTINGS ARE NUMBERED						
P	B6R3 P4	042-15	NH	E	B	7,500	Very tall cutting (21.5cm)
I	B6R3 P4	042-15	NH	E	NB	10,000	
G	B3R2 P13	054-02	NH	E	NB	2,500	
M	B3R2 P13	054-02	NH	E	B	7,500	Very short cutting (3.5cm)
10	B2R2 P7	060-09	NH	E	B	7,500	
R	B6R3 P11	060-12	NH	E	NB	7,500	Roots at Week 11
H	B6R3 P11	060-12	NH	E	NB	10,000	ROOT WAS BROKEN OFF
13	B7R2P9	061-42	NH	E	B	2,500	
F	B7R2 P9	061-42	NH	E	NB	10,000	Roots in star arrangement
N	B7R1 P10	064-21	NH	E	B	10,000	Very short cutting (3.5cm)
1	SB P7	128-17	H	NE	NB	5,000	cutting dying
2	SB P7	128-17	H	NE	NB	10,000	This tree produced rooted cuttings in 2002
11	NB P4	142-19	H	E	NB	10,000	
J	NB P3	237-05	H	E	B	7,500	Tall cutting
3	NB P3	237-05	H	E	B	10,000	
S	B7R3 EB	250-17	H	E	B	2,500	Roots at Week 11
D	B543 EB	TASII-08	H	E	NB	2,500	
A	B5R1 EB	TASII-10	H	E	NB	2,500	Roots at Week 11
12	B5R1 EB	TASII-10	H	E	NB	5,000	
4	B4R1 EB	TASII-15	H	E	B	10,000	
K	B3R2 EB	TASII-26	H	E	B	2,500	Very tall cutting (28cm)
B	B3R1 EB	TASII-29	H	E	NB	2,500	
5	B3R1 EB	TASII-29	H	E	B	5,000	2 new leaves, no old ones
C	B3R1 EB	TASII-29	H	E	NB	10,000	
6	B2R3 EB	TASII-33	H	E	B	0	
7	B2R3 EB	TASII-33	H	E	B	5,000	
8	B2R3 EB	TASII-33	H	E	B	7,500	
9	B2R3 EB	TASII-33	H	E	B	10,000	

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