ABSTRACT

DREPS, HEATHER BLAKE. Production System Factors Affecting Rooting and Subsequent Performance of Northern Red Oak (*Quercus rubra* L.) Cuttings for Outplanting. (Under the direction of Daniel J. Robison.)

Northern red oak (NRO), *Quercus rubra* L., is a valuable tree species for timber, wildlife, restoration, and urban forestry. Seed production and growth responses from different maternal sources can be widely variable. Rooting choice stem cuttings of NRO could prove to be a time-saving nursery practice, yielding prime candidates for outplanting.

This study sought to develop effective protocols to root NRO stem cuttings at high frequencies and to examine the effects of several factors during rooting and subsequent containerized growth. Supplemental light and heat were administered to determine the effects of artificially extending the growing season of successfully rooted cuttings. Different container sizes were used to determine which size encouraged optimum containerized growth and survival. In addition, shoot production enhancement was studied through pruning of field grown stock seedlings so that stem cutting material could be readily available throughout the growing season; different seedling sources were used to examine provenance variability for shoot production. Also, early (ES) and late season (LS) cuttings were taken to determine the viability of rooting at different times during the growing season. After two seasons of containerized growth, final measurements of NRO rooted cuttings were recorded to determine efficacy of prior treatments.

Geographic source and prune height treatments were both found to have significant impact on the number of new shoots generated by NRO stock plants. We found large stem caliper may enhance donor plant ability to generate new shoots for rooting as compared to

small stem caliper. Geographic source and prune height treatment significantly affected rooting ability of ES cuttings, which rooted at 64% overall. Rooting percentages were lower for LS stem cuttings (53% overall), but showed the possibility of LS rooting for nursery operations; prune height alone was statistically significant for rooting ability of LS cuttings.

During the first growing season, both growing environment and container size were significant in encouraging shoot growth post-rooting. After the first growing season and subsequent overwintering, survival rates of rooted cuttings were 75% overall. In this study, the smallest container size used during the first growing season was the most beneficial to rooted cutting growth based on final attribute measurements of height, root collar diameter (RCD), root mass, shoot mass, and root-to-shoot ratio, all of which have been proposed to be good indicators of field performance. Extending the growing season immediately after rooting significantly affected RCD and root mass measurements as well.

Production System Factors Affecting Rooting and Subsequent Performance of Northern Red Oak (*Quercus rubra* L.) Cuttings for Outplanting

by

Heather Blake Dreps

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Approved by:

Daniel J. Robison
Chair of Advisory Committee

Barry Goldfarb

Douglas J. Frederick

Thomas R. Wentworth

DEDICATION

For Matt and Zack,
My Joy and Inspiration

BIOGRAPHY

Heather Blake Dreps was born on July 7, 1973 in Durham, North Carolina, to parents Ronnie and Kaye Blake. She is the oldest sibling of sister Stacie and brother Chris. Heather grew up in Durham where she graduated from the North Carolina School of Science and Mathematics in 1991. She then attended Meredith College in Raleigh, North Carolina as a N.C. Teaching Fellow. She graduated with a Bachelor of Science degree in Biology and a minor in Art in 1995. She began her teaching career at Athens Drive High School in Raleigh, where she taught Biology, Chemistry, and Physical Science; there, she also met her future husband, Matt, an English teacher. After four years of teaching, Heather had a unique opportunity to join an advertising agency where she learned graphic design from the ground up and was able to hone her skills as an illustrator. Although interesting and challenging, advertising did not hold the allure for Heather that the natural world did, so she left full time graphic design work in 2003 to begin a new journey: the pursuit of a Master of Science degree in Forestry at N.C. State University. After her first year of coursework, Heather's life changed again, but in the biggest way possible: she learned she was pregnant. Matthew Zachary Dreps was born on March 3, 2005, and has proven to be both the biggest inspiration and "speed bump" to her degree's completion! Heather hopes to one day incorporate her love for the environment, teaching, and design into a career that develops solutions for lessening human impact on the Earth and helps people realize their powerful individual influence.

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INTRODUCTION AND LITERATURE REVIEW

Species Overview

Quercus rubra L., northern red oak, is a widely distributed and abundant species found throughout the eastern half of North America. Northern red oak (NRO) is valuable commercially, second only to cherrybark oak, as a high quality lumber species (Preston and Braham, 2002). NRO has shown steadily increasing stumpage prices in the U.S. and Canada (Linehan *et al.*, 2003; Armstrong *et al.*, 1993). NRO is not only valued for its furniture-grade lumber and veneer, but is also a valuable urban forestry species. NRO shows promise in carbon sequestration, reclamation of compromised land and for wildlife habitat, soil stabilization, and water quality protection (Zaczek *et al.*, 1993; Huang *et al.*, 2004). NRO is naturally abundant and hybridizes easily with other red oaks occurring within the same regions (Isebrands *et al.*, 1994). It prefers mesic to dry-mesic conditions and is often a dominant species in its range (Johnson, 1994). NRO often performs best in shelterwood situations, as it prefers early development in light shade. Direct seeding of acorns is not recommended due to animal predation. Ideally, young trees, bareroot or containerized, should be planted and herbaceous competition controlled (Johnson, 1994). Rotations for unmanaged NRO saw timber can be 60 or more years.

Due to its versatile use and high value, NRO is a prime candidate for tree improvement as well as timber management. NRO is a long-lived species and is a moderate to fast grower in natural stands, usually reaching full maturity in 150-200 years; however, initial growth is slow, and newly planted NROs often experience dieback in field situations (Isebrands *et al.*, 1994; Johnson, 1994). Techniques that could improve propagation efficiency and genetic quality would be desirable (Kormanik *et al.*, 2004; Kreibel *et al.*, 1988).

Reproductive Biology

There is limited research on the complete reproductive biology of NRO since it has not been the subject of extensive breeding or plantation management research (Cecich, 1994). However, reproductive information is available for oaks in general and within its two major subgenera: white oaks (*Lepidobalanus*) and red oaks (*Erythrobalanus*). Lepidobalanus members achieve pollination and acorn development within one growing season. On the other hand, members of the erythrobalanus subgenus require two growing seasons for seed production; pollination occurs the first season, but acorn production does not occur until the following growing season. NRO is an erythrobalanus member and therefore, has an extended period of time over which problems can develop for its seed crop.

NROs are dioecious, with pistillate flowers usually developing at tops of trees. Staminate flowers responsible for pollen production are usually produced throughout the crown of the tree at the same time as their pistillate counterparts. The staminate flowers are ready to produce pollen usually one to two weeks after they appear, and they release their pollen over a 3-4 day period (Cecich, 1994). Problems can occur during pollen release and pollination of pistillate flowers. High relative humidity can cause high pistillate and staminate flower mortality, poor pollination, and subsequent poor seed crop (Cecich, 1994). Low temperatures can delay pistillate flower emergence as well lessen their receptivity to pollen (Cecich, 1994). Likewise, low temperatures, along with rainy weather that causes humidity to be higher, can delay or prevent staminate flower production and pollen dispersal.

Actual pollination success between male and female flowers contributes to yearly seed production fluctuations making sexual propagation problematic for NRO (Farmer, 1981).

Female flowers are an important factor in determining seed production success for trees. Pollen produced by staminate flowers may pollinate pistillate flowers on the same tree, or may be dispersed by wind to female flowers on surrounding trees. Pistillate flower numbers vary from tree to tree. Female flower production is genetically controlled, and therefore contributes to the lifetime seed production potential for each tree, which can vary throughout the NRO species (Cecich, 1994).

Although pistillate flower production and pollination have a large bearing on acorn production, other factors can contribute to the lack of success for seed production in NROs as well. High pistillate flower numbers do not ensure large acorn crops. Instead, other factors that inhibit or enhance flower development, pollination, fertilization, and embryogenesis may be just as important in determining seed production success (Cecich *et al.*, 1991; Gysel, 1956; Sharp and Chisman, 1961; Sork *et al.*, 1993).

Insects can damage seed crops and cause dramatic drops in production as well. Weevils (*Curculio* spp. and *Conotrachelus* spp.) have been shown to damage or destroy developing acorn crops (Cecich, 1994). Treehoppers (Membracidae) may be more damaging to NROs because they spend their entire lifecycles within the tree crowns. Treehoppers' late instars and young adults feed on meristematic tissue and can cause female flower abortion (Cecich, 1994; Cecich *et al.*, 1991). Therefore, these sucking pests can cause low seed production in NRO through reduction of pistillate flowers.

In the laboratory setting, studies have explored the effects of culture techniques on seedling emergence and development. From the very beginning of sexual propagation in controlled conditions, methods can cause different success rates. Simple collection procedures for acorns can affect the vitality of the seedlings to be cultured (Teclaw and Isebrands, 1986). Choosing seed sources that are registered for collection and have established superior characteristics is suggested to ensure premium seed for sowing; collecting from unrestricted sources is not recommended and does have bearing on seedling success (Buchschacher *et al.*, 1991). Sowing methods of acorns can also affect emergence and development of seedlings. Shallow seed sowing, amending planting media with peat, mulching, and planting at low densities are all recommended practices for encouraging vigorous NRO seedlings and decreasing seedling variability (Tomlinson *et al.*, 1997; Buchschacher *et al.*, 1991).

Episodic Growth Morphology

NRO displays shoot growth that is semi-determinate, meaning shoot growth occurs in episodic flushes during the growing season (Dickson, 1994). According to the *Quercus* morphological index (QMI), these multiple flushes of growth are characterized by four stages of development once the seed germinates and the epicotyl emerges from the soil: 1) bud swell, 2) linear stem elongation, 3) linear leaf expansion, and 4) lag phase (Hanson *et al.*, 1986; Dickson *et al.*, 2000). After a bud has swollen, internodes that comprise a flush will lengthen. Once stem elongation is completed, leaf expansion and growth will commence to form a flush of growth. When the growth flush ends, the plant will rest during lag phase until onset of the next flush of growth.

During each phase of episodic growth, photosynthesis levels differ and allocation of resources varies. During the first flush of growth for a seedling, much of the photosynthate remains in the flush. However, during the first lag phase and subsequent lag phases, photosynthate is directed to the roots for growth and storage (Dickson *et al.*, 2000). Subsequent flushes of growth are supported by their own photosynthate produced as well as that produced by the preceding flush. The more flushes achieved during a growing season, the more photosynthate is redirected away from flush growth to supporting root growth (Dickson *et al.*, 2000; Dickson *et al.*, 2000). As more leaf area is added through episodic growth, more photosynthate is available for root as well as shoot growth. For optimum photosynthate production and plant growth, Dickson and others (2000) propose seedlings with three flushes of growth per growing season are ideal; inhibiting further flushing (forcing lag phase) could enable the plant to allocate photosynthate to stem and root growth as well as storage for winter dormancy.

Asexual Propagation

Due to genetic variation among seedlings as well as the variety of seed production and growth responses exhibited by seeds from different maternal sources, research towards vegetative propagation of select NRO stock may yield some advantages. While seedlings can have different genetic and ontogenetic responses to varying environmental conditions, clones should theoretically have the same responses to environment when exposed collectively, making them more reliable for production purposes (Zaczek *et al.*, 1993). However, difficulty in vegetative propagation of oaks in general has not made them popular candidates for asexual reproduction techniques (Flemer, 1962; Hartmann *et al.*, 2002; Isebrands and Teclaw, 1990; Zaczek *et al.*, 1993; Zaczek *et al.*, 2006). Recent improvements in vegetative propagation techniques have made asexual reproduction possible for NRO as well as other oaks (Teclaw and Isebrands, 1987; Zaczek *et al*., 1993; Zaczek, 1994; Zaczek and Steiner, 1997; Zaczek *et al*. 2006).

Traditionally, NRO has been difficult to root from stem cuttings because identified desirable specimens are often too mature from which to obtain suitable rooting material (Zaczek *et al.*, 1993). However, consistently successful rooting at high rates with juvenile material from pruned stock has been achieved in recent years with developed protocols for NRO (Isebrands and Teclaw, 1990). Mature cuttings have been rooted, but with less consistent success. Differences in rooting response of cuttings from NRO seedlings have been attributed to source genotypic effects as well as ontogenetic age of cuttings (Zaczek *et al*., 1993; Drew and Dirr, 1989; Isebrands and Teclaw, 1990; Zaczek *et al.*, 1993). NRO has also shown variation in rooting ability depending on the timing of propagule harvest, which relates directly to the ontogenetic (including lag phase) age of cuttings (Zaczek *et al.*, 1993; Drew and Dirr, 1989). If stem cuttings for rooting could be collected throughout the growing season each year and at multiple times each season from donor plants, then larger numbers of cuttings would be available. In addition to examining rooting ability, Zaczek and others (1993) found that juvenile cuttings from seedlings produced more roots than mature cuttings, making ontogenetic age a contributing factor in root number potential as well. However, chronological age is not a dependable gauge of rooting ability, because mature cuttings have also been rooted at high percentages as well, which indicates the complexity of the mechanisms that control rooting ability (Zaczek *et al.*, 1993).

In general, the position from which cuttings are taken can have bearing on their rooting ability as well as their number of roots produced, subsequent shoot growth, and plagiotrophy (Reickermann *et al.*, 1999; Bentzer, 1988). In other tree species, distal (terminal) cuttings of *Populus* spp. have been shown to root less frequently than basal and mid-shoot (sub-terminal) cuttings (Schroeder and Walker, 1991). *Liquidambar styraciflua* has shown opposite results, with terminal cuttings rooting at higher percentages than sub-terminal cuttings and being deemed more plantable post-rooting (Reickermann *et al.*, 1999). However, Reickermann and others (1999) did find that sub-terminal *Liquidambar styraciflua* cuttings, once rooted, exhibited greater new shoot and root dry weights, greater number of shoots, higher percentage of shoots that flushed with new shoot growth, and greater shoot lengths than rooted terminal cuttings. *Populus* spp. and *Platanus occidentalis* have also exhibited higher shoot growth percentages from sub-terminal rooted cuttings rather than terminal cuttings (Schroeder and Walker, 1991; Nelson and Martindale, 1957). Reickermann and others (1999) proposed that sub-terminal cuttings are usually thicker in diameter, which may indicate more stored carbohydrates available for root and shoot development as opposed to terminal cuttings. Once rooted, these larger diameter cuttings could grow into larger transplants for outplanting; therefore, sub-terminal cuttings could prove to be potentially stronger competitors if they could be effectively rooted. Also, if sub-terminal cuttings could be encouraged to root at higher percentages, NRO and other species could be aided in increasing energy stores for overwintering through biomass accumulation during post-rooting shoot growth, and the total number of shoots available for rooting from donor plants would be greater as well.

Production System Factors: IBA

Successful rooting of NRO cuttings has been obtained by treating the basal cut end of cuttings with indolebutyric acid (1.5% IBA) when used in conjunction with irrigation/misting and proper timing of taking cuttings (Isebrands and Teclaw, 1990; Teclaw and Isebrands, 1987). NRO can be reliably rooted from a variety of sources of juvenile material with concentrations of IBA ranging from 0.5% to 1.5% (Teclaw and Isebrand, 1987; Zaczek *et al.,* 2006). Teclaw and Isebrands (1987) achieved highest rooting by blanching NRO stems at full leaf expansion, then following with 1.5% IBA treatment four days later. Ergo, timing of blanching as well as hormone concentration both contributed to the rooting ability of stem cuttings. Increasing concentration of applied IBA has also been shown to induce a greater number of first order lateral roots (Teclaw and Isebrands, 1987). In addition, Teclaw and others (1987) found that juvenile material provided the best rooting material, and high concentrations of IBA and blanching could not combat the detrimental effects of advanced ontogenetic age on rooting, a major limiting factor. Successful rooting of NRO stem cuttings with auxins other than IBA has not been reported.

Production System Factors: Misting/Shade Chamber

Misting chambers have been shown to contribute to successful rooting of NRO cuttings (Isebrands and Teclaw, 1990; Zaczek *et al.*, 1993). Cuttings must absorb water through extra-root means initially, and NRO is especially sensitive to humidity or dry conditions during the early critical stages of rooting. Humidity-irrigation conditions under which more robust rooters, such as poplar and sweetgum, can thrive can not be tolerated by NRO (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). Higher rooting success of cuttings has also been attributed to shading of cuttings during rooting with heavier shade inducing higher rooting than lower shade levels (Zaczek, 1994). However, ontogenetic age and genotypic effects of cuttings still affected rooting of cuttings even in heavily shaded trials.

Production System Factors: Container Size

In the quest to enhance plant development from rooted cuttings, studies have also examined the effect of container size on development. Hanson and others (1987) found that for NROs, which can develop a large taproot, containers with a high surface area to volume ratio produced the greatest seedling growth. Their studies showed that a container with dimensions of 15.4 cm x 36 cm and a volume of 6333 cm^3 showed optimum conditions for encouraging NROs seedlings to grow largest in the shortest amount of time. They proposed that longer containers provided too much room for the NRO taproot to develop which, in turn, hindered top growth. Likewise, they showed that wide containers supported seedling growth when container depth was held constant, further supporting their claim for high surface area to depth ratios for optimum growth; increased shoot growth in more shallow containers could be a result of restricted taproot development.

Other studies have shown that container-grown NRO seedlings consistently outperform their bareroot and direct-seeded counterparts once transplanted to the field (Zaczek *et al.*, 1993; Zaczek *et al.*, 1996; Johnson *et al.*, 1984; Kerr, 1994). Zaczek and others (1993, 1996) also found keeping seedlings in containers longer (2 years versus 1 year) to be advantageous to survival and vigor once transplanted to the field; the larger older seedlings handled deer browsing and cicada damage more easily than their smaller, younger counterparts. They also found that growing seedlings in the same containers and not transplanting within the nursery setting produced the highest quality seedlings at outplanting time. Transplanting within the nursery resulted in poorer field performance. Stronger, high quality transplants may offset the initial cost of raising seedlings in containers versus high-density nursery bed operations. Fewer seedlings may be needed at transplant time because of fewer culls and higher survival and growth rates of containerized seedlings than with bareroot seedlings (Zaczek *et al.*, 1996).

Production System Factors: Root Manipulation

Manipulating root growth has also been shown to be an effective method of encouraging stronger, more vigorous plants for outplanting in field conditions. Planting seedlings in small volume containers discourages large tap root formation and encourages first order lateral roots (FOLR), which in turn, increases survival and diminishes transplant shock at outplanting time (Struve *et al.*, 2000). Undercutting seedlings has been shown to increase FOLR number and development (Buchschacher *et al.*, 1991). Struve (1996) also found that copper-treating inner surfaces of containers before planting discouraged tap root growth and encouraged FOLR development. In addition, amending planting media with peat has also shown to increase lateral root development as well, presumably by improving soil texture and lowering soil pH (Buchschacher *et al.*, 1991). The undercutting method, or even root trimming at transplant time, could potentially encourage improved lateral root development, making first season grown NRO seedlings better equipped for outplanting the following spring. But Struve (1996) argued that encouraging fibrous root development often eliminates the need for root pruning so that root tips remain intact, which improves performance at transplant time.

Production System Factors: Supplemental Light and Heat

The growth of seedlings and rooted cuttings of many woody species depends on daylength; long and short days encourage and stop shoot growth, respectively (Rieckermann *et al.*, 1999; Borthwick, 1957). Consequently, adding supplemental light or providing an interruption of the dark period can extend the photoperiod for these species. Extending the photoperiod for *Acer rubrum* L. "Red Sunset" by using a four hour night interruption has been shown to significantly increase shoot growth after rooting of cuttings has taken place (Still and Lane, 1984). Several other studies have also shown increased shoot growth for multiple species with use of extended photoperiods (Still and Lane, 1984; Cathey *et al.*, 1975; Hanover *et al.*, 1978). Calme and others (1994) observed that NROs exhibited high cellular activity past the end of the normal growing season while other species had become dormant; this suggests NRO is quiescent rather than dormant at growing season's end and could be encouraged to put forth new shoot growth under favorable growing conditions, such as extended photoperiod or warmer greenhouse temperatures. Increased shoot growth indicates possible increased biomass accumulation for cuttings, which could potentially increase overwintering survival (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). NROs have also had increased flush numbers during the growing season as a response to full sunlight versus shaded growing conditions. Warmer spring temperatures also encouraged increased flush numbers during the growing season as compared to seedlings exposed to cooler spring temperatures (Farmer, 1975). Both temperature and sunlight intensity can be controlled in a greenhouse setting for rooted cuttings.

However, there are concerns associated with extending the growing season artificially for NROs. Since the species responds positively to favorable growing conditions once the natural growing season is complete, NRO may have difficulty meeting its chilling requirements for bud dormancy release if its growing season is artificially lengthened (Calme *et al.*, 1994). Optimal rest (so that days to bud break are maximal) is necessary after the growing season to ensure high overwintering survival for oaks and subsequent bud break the following spring. Also, if the growing season is artificially lengthened through greenhouse conditions, plants may not naturally acclimate to changing seasonal conditions (*e.g.* freezing temperatures), which can threaten their overwintering survival (Calme *et al.*, 1994). An overwintering structure could prove necessary to protect roots with frost sensitivity that have not hardened in response to naturally cooling temperatures once the artificially-lengthened growing season (in the greenhouse) has ended and plants have been moved to outdoor conditions.

Transplant Grading for Outplanting

When containerized plants are ready for transplant to the field, many factors contribute to the potential for survival and performance once outplanted. A grading system must be developed to identify good candidates for transplanting. Opinions vary as to which morphological features indicate high potential for field growth and survival. High FOLR number and consequently, high root volume, are good indicators for grading NRO bareroot seedlings at transplant time (Shultz and Thompson, 1996; Jacobs *et al.*, 2005; Davis and Jacobs, 2005). However, counting FOLR for containerized seedlings as a grading procedure could be destructive and impractical. Instead, root collar diameter (RCD) has been shown to be a good predictor of survival and performance (Thompson and Schultz, 1995; Dey and Parker, 1997; Clark *et al.*, 2000). RCD also shows a strong correlation with root system size and volume without being destructive in measurement at outplanting time. Height has also been shown to correlate with field performance; taller plants can compete more easily with weeds and establish themselves more quickly (Jacobs *et al.*, 2005). Root and shoot growth and their relationship to one another are strong indicators of performance as well; consequently, root and shoot dry mass and their relative ratios to one another are also good indicators of performance (Davis and Jacobs, 2005; Johnson, 1994).

STUDY OBJECTIVES

NRO, along with other oaks, has proven to be a difficult plant to propagate and efficiently grow reliably in nursery and silvicultural situations. Seed-based propagation without genetic improvement can lead to trees with morphological and physiological challenges that detract from planting success and future stand value. Therefore, a commercially efficient protocol for producing superior clones from elite selections could be valuable in producing faster maturing and robust planting stock.

To develop an asexual cutting-based propagation protocol for superior stock plants of NRO and to enhance their first and second years' growth and development, the following objectives in four experiments were pursued--

- Shoot production following donor plant pruning at different heights examined the ability of seedling stock plants to generate flushes of regrowth for rooted cutting use.
- Early and late season cuttings were tested for their rooting ability.
- Rooted cuttings were exposed to supplemental heat and light within a greenhouse to determine the effects of a simulated growing season extension and different container sizes on shoot growth, overwintering survival, and final plant size.
- After two growing seasons, morphological measurements of rooted cuttings were evaluated to determine the relationship between plant characteristics and field growth potential.

MATERIALS AND METHODS

Experiment 1: Shoot Production Following Pruning of Field Grown Stock Plants

Preparation of stock plants to encourage juvenile shoot growth

Three-year-old NRO field grown seedling stock plants were evaluated during Experiment 1. The stock plants originated as 1-0 bareroot seedlings from three geographic locations: Georgia (GA), Tennessee (TN), and West Virginia (WV). The GA and TN seedlings were obtained in early 2002 from the Georgia Forest Commission's Flint River Nursery in Byromville, GA. The WV seedlings were obtained from the Clements State Tree Nursery in West Columbia, WV. The GA and WV source seedlings originated from open-pollinated acorns collected within each state. The TN source seedlings were germinated and grown at the Flint River Nursery but originated from acorns produced at the Watauga Northern Red Oak Seed Orchard under the care of Dr. Scott Schlaurbaum and the University of Tennessee's Tree Improvement Program.

One hundred eighty seedlings from each geographic source, GA, WV, and TN, were shovel-planted in Raleigh, NC at the N.C. State University-Reedy Creek Field Laboratory on February 3, 2002, February 20, 2002, and March 29, 2002 respectively. The planting site was mechanically ripped at four foot intervals and hardwood mulch was incorporated during a disking treatment into the top 6 inches of soil (Cecil sandy loam soil series; Cawthorn, 1970) prior to planting. When planted, the seedlings were organized into a randomized complete block design with four blocks of 45 stock plants. Within each block, three 15 tree row plots were planted for each geographic source (GA, WV, and TN).

The seedling stock plants evaluated in Experiment 1 were irrigated periodically throughout their establishment with overhead irrigation nozzles. On June 15, 2002, the stock plants were each fertilized with 50 mL of Coor's 14-14-14 Slow Release with Minors fertilizer (Coor Farm Supply, Smithfield, NC). On April 1, 2003, the stock plants were each fertilized with 5 g of Hi-Yield™ Ammonium Sulfate (Voluntary Purchasing Groups, Inc., Bonham, TX). On April 14, 2004, the stock plants were each fertilized with 59 mL Espoma Tree Tone (9-5-4) Plus All Essential Nutrients (The Espoma Company, Millville, NJ).

On March 4, 2004, sixty of the 180 stock plants representing each geographic source were pruned to a height of 5cm, 15cm, or 25 cm from the soil line, by hand with by-pass pruners. Within each of the four blocks, the nine "geographic source by prune height" treatments were represented by a 15-tree row plot of similarly pruned stock plants.

May 17-19, 2004, new shoot number was recorded at the completion of the first flush of re-growth during the lag phase of episodic shoot growth. Shoots were counted if they were formed within 5 cm of the prune cut. Stock plant basal caliper ($\pm$0.1 mm) was also recorded. There were 540 stock plants measured for shoot number in 2004.

Experiment 2: Rooting Trials for NRO Stem Cuttings

In Experiment 2, stem cuttings were collected at two times, during early season (ES) and late season (LS), and were evaluated for rooting ability. The stem-cuttings rooted in Experiment 2 were collected from the pruned (March 4, 2004) NRO seedling stock plants evaluated in Experiment 1.

Preparation of ES cuttings for rooting

On May 23, 2004, terminal stem cuttings (>10cm) were collected from the pruned NRO stock plants to evaluate the rooting ability of ES NRO stem-cuttings. The ES stem cuttings were collected in the late afternoon to early evening and were submerged in water-filled coolers until the time of sticking. At the time of collection, the ES stem-cuttings were in a semi-hardwood stage of development, characterized by green or green and brown coloring and the presence of visually apparent developing lignification (completion of first flush or growth/lag phase).

All cuttings were prepared for "sticking" in containers in a quonset-style greenhouse at the Horticultural Field Lab (HFL) at N.C. State University, Raleigh, NC. The exterior of the greenhouse was shaded for the duration of the study with shade cloth producing 46% ambient light within the greenhouse.

At the time of sticking, ES stem cuttings were prepared by removing any leaves and petioles from the lower half of each cutting by hand with by-pass pruners. The remaining upper leaves were trimmed to one half of their original size. The basal end of each stem cutting was then re-cut and dipped 2 cm deep for 5 seconds in a 1% indole-3-butyric acid (IBA) in 50% EtOH solution. The final length of the ES stem cuttings ranged between 9 and 15cm. After allowing the basal end of the cutting to dry for one minute, the ES stem cuttings were then stuck 5 cm deep in 64.5 cm^3 Ray Leach™ Supercell Cone-tainers™ (Stuewe and Sons, Corvallis, Oregon) filled with a media mixture of 5 peat: 4 perlite: 1 vermiculite mix by volume. ES stem cuttings in Ray Leach tubes were placed in Cone-tainer™ trays (RL-98) (Stuewe and Sons, Corvallis, Oregon) and were arranged in a shaded rooting chamber constructed inside the greenhouse. Within the trays, the ES stem cuttings were organized into a randomized complete block design with the three geographic sources (GA, TN, and WV) and three prune height treatments (5cm, 15cm, and 25cm) represented in equal numbers in all six blocks. Within a block, there were 5 stem cuttings per geographic source by prune height treatment (organized in 5 cutting row plots), for a total of 270 cuttings plus borders.

The misting/rooting chamber consisted of a box-shaped PVC frame (2.5m tall x 1m wide x 5m long) which was completely covered on sides and top by sheets of Frost Blanket™, a white UV stabilized non-woven fabric (The Master Gardener Company, Spartanburg, SC 29301). Shade cloth (50%) was also draped over the top surface of the PVC frame to reduce light within the chamber during rooting. Within the shaded rooting chamber, ambient light levels were measured at 15% (relative to light outside) with a Sunfleck Ceptometer™ (Decagon, Pullman, WA). Overhead NaanDan™ "Water and Sprinkling" nozzles (Kibbutz Naan, Naan, Israel 76829), with a flow rate of 41.64 liters/hour, provided mist every 12 minutes for 15 seconds for the first fifty days. After this initial establishment period, misting was decreased gradually while cuttings remained in the rooting chamber. Irrigation frequency and duration were controlled by a Davis Engineering Solar 6A Misting Controller (Davis Engineering, Winnetka, CA).

For insect control, Talstar™ Flowable insecticide (FMC Agricultural Products Group, Philadelphia, PA) and Gnatrol™ biological larvicide (Valent USA Corp., P.O. Box 8025, Walnut Creek, CA 94596-8025) were used on the cuttings while they were enclosed in the rooting chamber. Zero-tol™ broad spectrum algaecide/fungicide (Biosafe Systems, Glastonbury, CT, 06033) was also applied to the cuttings and rooting chamber during the duration of rooting. Frost Blanket™ sheets were also replaced every thirty days to deter algae accumulation within the chamber. Leaf litter was also removed on a regular basis to maintain the rooting environment and discourage disease and pests.

On July 21, 2004, after 60 days of rooting, the stem cuttings were evaluated for rooting success (survival). A stem cutting was considered rooted if at least one root could be seen emerging from the drainage hole of the Ray Leach Supercell Cone-tainer™. Or, if no root was visible, the Cone-tainer™ was inverted, the potting media was partially pulled out, examined for roots, and gently re-inserted into the Cone-tainer™.

Preparation of RC stock plants to encourage shoot growth for LS cuttings

When the ES stem cuttings were collected on May 23, 2004, all stock plants were also repruned by hand to 2cm above the initial prune heights of 5cm, 15cm, and 25cm. The stock plants were repruned as a matter of routine care and to encourage new juvenile shoot production for potential rooting purposes.

Preparation of stem cuttings for LS rooting

On June 27, 2004 after the completion of one flush of regrowth (shoots in lag phase), the stock plants were repruned again to 2cm above the previous prune location. This third pruning was conducted to encourage new juvenile shoots to grow for evaluation in the LS rooting trial. Stem cuttings intended for the LS rooting trial were collected from shoots at the completion of one flush of regrowth on July 26, 2004. The collection, sticking, and rooting protocol described for the ES rooting trial was followed during the LS rooting trial as well. The same irrigation schedule as well as fertilizer/pesticide regimens were used for the LS cuttings as for the ES cuttings.

LS stem cuttings were organized into a randomized complete block design with five blocks and two geographic sources represented: Georgia or Mixed Origin (TN and WV combined). The Mixed Origin source was necessitated by the production of a lower number of stem cuttings during the LS collection for WV and TN stock plants. The two sources were combined (thoroughly mixed) to create the mixed origin geographic source. Within each of the five blocks, there were 10 stem-cuttings (organized in 10 stem-cutting row plots) representing each of the six geographic source (GA and mixed origin) by prune height treatments evaluated, for a total of 300 stem cuttings.

After 60 days on September 23, 2004, the LS stem cuttings were evaluated for rooting success in the same manner used for the ES rooting trial.

Experiment 3: Growth and Survival of Rooted Stem Cuttings Post-Rooting

Transplanting rooted cuttings to different container sizes and manipulating the growing environment

Experiment 3 evaluated the effects of container size and extended day length/warm temperatures on subsequent shoot growth and overwintering survival for newly rooted NRO stem cuttings. Rooted cuttings used in this experiment included the successfully rooted ES West Virginia stem cuttings from Experiment 2 combined with successfully rooted stem cuttings from a companion NRO rooted cutting study conducted at NC State University, during the same growing season, by the Hardwood Research Cooperative (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). The stem cuttings from this companion study originated from seedling stock plants representing the same WV geographic source as in Experiment 2. However, the stock plants for the companion study were grown in 30.28 L TPOT8™ Containers (Stuewe and Sons, Corvallis, Oregon) at the HFL site, about 3 miles from the field grown stock plants (used in Experiments 1 and 2) at the Reedy Creek site. These stock plants were grown in a medium of 1 peat: 1 perlite: 1 field soil [Congaree series silt loam (USDA Soil Conservation Service, 1970)]: 3 composted pine bark, by volume (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). The seedlings were watered every two days for 5 minutes during the three growing seasons with individual spray stakes (Antelco Shrubbler ™ 360^0, Antelco Corporation, Longwood, FL) and approximately once per month by hand during the dormant seasons (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). The WV seedlings were fertilized twice during the preceding study (pers. comm., Matthew Gocke, Dept. of Forestry, N.C. State University, 1 March 2007). Stem cutting (ES) collection time for both Experiment 2 and the companion study occurred within a week of one another. Sticking and rooting protocols were identical for both studies. The stem cuttings from both studies were rooted adjacent to one another in the same rooting chamber during the same period of time in the same greenhouse.

The combined rooted stem cuttings were then randomly divided into three groups of 50 plants. These three groups were then each assigned to one of three treatments to evaluate the post-rooting effects of container size: 1) transplant to “large” container, 6.23 L Treepot2™ (Stuewe and Sons, Corvallis, Oregon), 2) transplant to “medium” container, 2.83 L Tall One™ Container (Stuewe and Sons, Corvallis, Oregon), or 3) remain in “small” container, 64.5 cm^3 Ray Leach™ Supercell Cone-tainers™ (Stuewe and Sons, Corvallis, Oregon) in which stem cuttings were originally rooted. If the assigned treatment required transplanting to the medium or large containers, rooted stem cuttings were each gently pulled with the soil intact from the container it was rooted in, the bottom 2.5 cm of the root system was pruned off, and each was transplanted into its assigned container containing only composted pine bark as soil media.

The rooted stem cuttings in three different container sizes were then each divided into two groups to evaluate the effect of growing environment. One half, or 150 rooted stem cuttings, were placed in an "nonforced" growing environment of outdoor conditions under partial shade (50%) at the HFL on August 18, 2004. The other half of the rooted cuttings remained in the climate-controlled greenhouse under "forced" conditions with extended photoperiods and warm temperatures intended to mimic a longer growing season. In the greenhouse, cuttings in the different containers were placed on a table without the misting chamber around them. The rooted cuttings placed in the two growing environments were organized into two identical randomized complete block designs. In each block, the three container sizes treatments were represented by 10-tree row plots. Small and medium size containers were elevated to the same height as the large size containers using stacked trays at appropriate heights so tops of all containers were at the same height in both growing environments. With five blocks per growing environment and two growing environments, there were a total of 300 rooted cuttings evaluated for Experiment 3, plus border cuttings.

The rooted cuttings were watered by hand every two days, ensuring that the soil did not dry out between waterings. All rooted cuttings in Experiment 3 were fertilized with nitrogen (100 ppm) on September 3, 2004, October 1, 2004, and October 20, 2004.

Greenhouse conditions were maintained from September 24, 2004 to October 30, 2004 at 15.6° C for the heater setting, 26.7° C for the fan setting, and 32.2° C for the wet wall setting. While these settings were strictly adhered to, the actual environmental conditions were not monitored during this experiment. Within the greenhouse, full spectrum fluorescent bulbs were lit from 7pm-1am (5 hour extended photoperiod) from August 18, 2004 until September 17, 2004. On this date, the fluorescent bulbs were powered from 5pm to 1am (7 hour extended photoperiod) until experiment completion 74 days since August 18, on October 30, 2004. During this same period, from August 18 to October 30, 2004, the environmental conditions outside of the greenhouse were: mean daily temperature of 20.3° C (http://www.nc-climate.ncsu.edu/cronos/index.php?station=REED&temporal=monthly, accessed 1 March 2007) and daylight decreasing from 13.5 hours to 11 hours over the period (http://aa.usno.navy.mil/data/docs/RS_OneDay.html, accessed 1 March 2007). During this period, cuttings inside and outside the greenhouse were regularly hand irrigated so that the soil media never dried.

On October 30, 2004, the extended photoperiod regimen began to taper off and the presence of new shoot growth was recorded for all rooted stem cuttings. Those rooted cuttings exhibiting new shoot growth were marked with a red twist-tie around the stem base for future reference. The "forced" environment rooted cuttings remained in the greenhouse until the end of January. During this time, the extended day length and warm temperatures of the "forced" environment were gradually reduced to mimic outdoor environmental conditions and in turn, transition the "forced" rooted cuttings toward a state of full winter dormancy. On October 30, 2004, the heater setting was lowered to 10°C and the fan setting to 21.1°C. Subsequently, the heater setting was lowered to 7.2°C and the fan setting to 18.3°C on November 8, 2004. Then on November 17, 2004, the heater setting was lowered to 4.4°C and the fan setting to 12.8°C.

On December 2, 2004, the rooted stem cuttings from the “unforced” environment under shade outside the greenhouse were transferred to a quonset-style unheated shade house covered with white opaque polyethylene plastic for overwintering. The “forced” environment cuttings were transferred to the same overwintering house on January 25, 2005. The experimental design used during the earlier “forced” vs. “nonforced” portion of Experiment 3 was maintained within the overwintering house.

Once in the overwintering house, rooted cuttings were irrigated twice a month for the remainder of winter. The white plastic was removed from the overwintering structure in late April 2005.

Overwintering survival for both sets of rooted cuttings was measured the following spring in early May 2005. Rooted cuttings were considered successfully overwintered when they broke bud and produced new shoot growth.

Experiment 4: Second Season Growth

On May 5, 2005, 35 surviving rooted cuttings from each container size and growing environment (forced and nonforced) in Experiment 3 were randomly selected and randomly assigned to new treatments to evaluate their subsequent growth during the 2005 season—their first year post-rooting. All of these rooted cuttings either remained in or were transplanted to the “large” size containers used in Experiment 3, with composted pine bark mulch as the soil medium. The same day, each rooted cutting was top-dressed with 30 mL of Nutricote Total (13-13-13) slow release fertilizer (Chisso-Asahi Fertilizer Co., Tokyo, Japan).

These rooted cuttings were then placed in a quonset-style shade house (50% shade) and organized into a randomized complete block design with five blocks and one 7-tree row plot per three container size treatments tested in Experiment 3, for a total of 105 rooted cuttings plus borders. Containers were kept far enough apart to prevent mutual shading by the developing crowns.

On November 15, 2005, at the end of the second growing season, the rooted cuttings in this experiment were measured for root collar diameter (± 0.1 mm) and shoot height (± 0.1 cm) before overwintering commenced. The cuttings were then overwintered in the same manner as the previous winter beginning December 2, 2004. Survival was recorded for these rooted stem cuttings on February 4, 2006, which were then immediately destructively harvested. At this time, roots were rinsed free of potting media and rooted cuttings were severed at the root collar into “roots” and “shoots”. The roots and shoots were bagged individually and were oven-dried at 70°C for 65 hours. Subsequently, root and shoot dry mass (± 0.1 g) were measured. Root-shoot ratios were then calculated by dividing root dry weight by shoot dry weight.

Overall Timeline

This study was conducted over a two year period, with the following breakdown of major events:

2/3/02-3/29/02: NRO stock plants were planted at the Reedy Creek site, Raleigh, NC.

3/4/04: The stock plants were pruned to one of three heights (Exp 1).

5/17/04-5/19/04: New shoots were counted and stem caliper was measured (Exp 1).

5/23/04: Early season cuttings were collected and rooted (Exp 2).

6/27/04:	NRO stock plants were repruned to one of three heights (Exp 2).
7/26/04:	Late season cuttings were collected and rooted (Exp 2).
8/18/04:	Successfully rooted cuttings were repotted to one of three container sizes and the growing season was artificially extended for half of the rooted cuttings (Exp 3).
10/30/04:	New shoot growth was recorded for the rooted cuttings (Exp 3).
5/1/05:	Overwintering success was recorded for the rooted cuttings before the second growing season commenced (Exp 3).
5/05/05:	Rooted cuttings were transplanted to or remained in the large container size (Exp 4).
11/15/05 & 2/04/07:	After a second growing season, final measurements were recorded for the rooted cuttings (Exp 4).

Statistical Analysis

Analyses of variance (ANOVA) were conducted to examine treatment effects within each experiment of this study. Due to unbalanced data in experiments 1 and 4, the “Proc Mixed” model was used. For experiment 1, stock plant stem basal caliper was used as a covariate in the analysis. The “Proc Corr” model was used to examine correlations between stock plant basal stem caliper and new shoot numbers produced overall (regardless of treatments) and among individual geographic source by prune height treatments . The “Proc GLM” model was used for experiments 2 and 3 to analyze treatment effects. Sources of variation were considered significant at $p \leq 0.05$. Means were separated by the LS Means procedure in SAS. The homogeneity of variance was assessed for each data set through examination of diagnostic residual plots, and no data transformations were required. Pairwise comparisons were also performed for treatments which exhibited significance. All analyses were accomplished using SAS software (SAS, Inc., Cary, NC).

RESULTS

Experiment 1: Shoot Production Following Pruning of Field Grown Stock Plants

Original geographic source and prune height treatments were both found to have significant impact on the number of new shoots generated by NRO stock plants (Table 1.1). Overall, GA stock plants produced the highest mean shoot counts (9.6 shoots) regardless of prune height treatment. TN and WV generated significantly lower mean shoot counts of 8.0 and 7.5 respectively. Median values per geographic source may more accurately reflect the shoot numbers generated due to extreme values for some stock plants. However, there were several GA stock plants which produced few or no shoots regardless of prune height treatment. Also, there were extremely high shoot counts generated by many GA stock plants. GA stock plants' widely variable shoot counts created a wide distribution of shoot number values, resulting in a standard deviation of 6.67 (mean=9.6). Conversely, TN and WV's stock plants did not produce as many extreme shoot values as GA and, therefore, had smaller standard deviations: 3.6 and 3.0 respectively (means 8.0 and 7.5 respectively). For all geographic sources, prune height treatments were found to induce greater shoot generation when pruning heights increased.

Stem caliper was measured and included as a covariate during analysis. When stem caliper was compared among stock plants from different geographic sources, it was found to be significantly correlated with number of new shoots generated for stock plants regardless of pruning height treatment. As stem caliper increased, higher shoot numbers were achieved by stock plants, indicating potential capacity for increased new shoot generation associated with larger stemmed stock plants. The larger caliper size of GA stock plants may account for higher shoot counts than TN and WV's smaller caliper stock plants (Table 1.1).

For GA stock plants pruned at 5cm, the median shoot count was 12.3 versus the calculated LS mean of 8.2. Individual low shoot producing stock plants led to the lower mean value. For 15 cm and 25 cm pruning treatments, the converse was true for GA stock plants, where the median values were lower than the means. Stem caliper by shoot number analysis revealed that larger diameter stock plants yielded greater numbers of new shoots overall and for each geographic source regardless of prune height (Table 1.2). Within each geographic source, stock plants pruned to 15 cm had better correlation between stem caliper and new shoot number than the 5 cm and 25 cm pruned stock plants (Table 1.2).

Experiment 2: Rooting Trials for NRO Stem Cuttings

Early season stem cuttings rooted at 64% overall. Geographic source as well as prune height treatment significantly affected rooting ability of ES cuttings (Table 2.1). Georgia stock plants had the highest rooting percentage. The lowest prune height of 5 cm resulted in the highest rooting percentage consistently during the ES trial. Within geographic source treatments, prune height was not significant for rooting ability of GA and TN cuttings. For WV, however, cuttings rooted at significantly lower percentages when stock plant prune heights were at the 15 cm and 25 cm level.

Rooting percentages were lower for late season stem cuttings (53% overall) than for early season cuttings. For late season cuttings, geographic source did not statistically affect rooting ability between the GA and TN/WV sources (Table 2.2). Prune height did impact rooting ability for the 15 cm prune height, where rooting was significantly decreased compared to the 5 cm and 25 cm prune heights (Table 2.2).

Experiment 3: Growth and Survival of Rooted Stem Cuttings Post-Rooting

During the first growing season after rooting, 27% of stem cuttings exhibited new shoot growth. Both growing environment and container size proved to be significant in encouraging shoot growth post-rooting (Table 3.1). There was also a significant interaction between growing environment and container size in generating new shoot growth.

Thirty-nine percent of rooted cuttings displayed new shoot growth within the "forced" environment while only 15% produced new shoot growth in outdoor "not forced" conditions. Rooted cuttings in small container sizes displayed shoot growth less frequently (12%) than those in medium and large containers (33% and 36% respectively) for both environments combined. Rooted cuttings in medium and large containers under "forced" conditions exhibited the shoot growth most frequently of all container/environment combinations (50% and 52% respectively) (Table 3.1).

After the first growing season and subsequent overwintering, survival rates were 75% overall. Growing environment did not impact overwintering survival, but survival was lower in large containers overall (Table 3.1).

Experiment 4: Second Season Growth

After the 2nd growing season and subsequent overwintering, final height of rooted cuttings stems was significantly influenced by the 1st growing season's container size (Table 4.1). Smaller initial containers produced taller plants by the completion of the study, versus the medium and large size containers. Growing environment did not impact final heights (Table 4.1).

Final RCD was significantly affected by both growing environment and container size (Table 4.1). The forced growing environment produced larger RCD measurements overall. Within both the "forced" and "not forced" growing treatments, the small size container proved to significantly increase RCD for rooted stem cuttings.

Final root mass was significantly influenced by both growing environment and container size (Table 4.1). The forced growing conditions produced significantly higher root mass. Regardless of growing conditions, the small container size exhibited significantly higher root mass overall.

Final shoot mass was significantly affected by container sizes, with smaller containers yielding significantly higher shoot mass versus the medium and large containers (Table 4.1). Growing environment did not influence final shoot mass for any container size.

The resulting root-shoot ratios (R/S) calculated from final root and shoot masses were found to be significantly impacted by container size, but not growing environment (Table 4.1). The small and medium container sizes yielded significantly smaller R/S ratios than the large size containers.

Mortality was not significantly affected by the 1st growing season's container size or growing environment during the 2nd growing season (Table 4.1).

DISCUSSION

Provenance was found to have significant impact on shoot generation (Table 1.1), but this could also be due to the vigor and health of the original stock plants at initial planting time. Caliper measurements showed GA stock plants were larger stemmed than TN and WV overall. Irrigation, fertilization, and general care were different at the three separate parent nurseries for the stock plants, which could have produced stock plants of different quality. Also, because of Georgia's relative proximity to North Carolina and adaptation to the southern growing season, GA stock plants may have been better-suited to Raleigh, N.C.'s growing season and field conditions than WV and TN. Or, WV and TN stock plants may not have been as vigorous at planting time. Since environment was the same for all three sources, the data suggests that genetic characteristics of GA stock plants are responsible.

However, GA stock plants' response to pruning was more variable than that of the WV and TN sources (Table 1.1) and resulted in more extreme values for shoots generated. Future operational stock plants sources would benefit from genetic tree improvement studies to reduce their variability in response to pruning to produce superior stock plants geared towards rooting operations (Jacobs and Davis, 2005).

GA cuttings did not root as well during late season sticking as did WV and TN cuttings (Table 2.2). The same kinds of factors previously described as influencing provenance performance could account for this weaker performance during the late season.

Previously published studies have not evaluated source variation in NRO cutting characteristics (shoot production, rootability, vigor), which could be a critical factor in selecting genotypes for operational purposes (Cunningham, 1989).

Prune height had a significant impact on shoot growth and rooting during Experiments 1 and 2 (Tables 1.1, 2.1, and 2.2). In Experiment 1, high pruning at 25 cm was the most productive for both shoot generation and rooting during the early season trial (Tables 1.1 and 2.1). This was determined by multiplying the average number of new shoots produced following early season pruning by the percentage of stem cuttings rooted for each prune height. The late season rooted cutting experiment had overall lower rooting than the early season trial (Table 2.2). The case for this difference between early and late season cutting performance may be related to relative age/maturity, plant history, and/or changes in the physiological readiness of pruned NRO for shoot production and rooting due to environmental conditions such as heat, relative humidity, daylength, etc. In other rooted cutting systems, similar differences in seasonal performance have been reported (Zaczek *et al.,* 1993; Drew and Dirr, 1989; Blakesley *et al.*, 1991). However, the current study has demonstrated that, without modifying stock plant environment, a second set of excised cuttings can be successfully rooted and contribute to the productivity of an operational system. Furthermore, stock plant condition and/or rooting conditions could perhaps be better optimized for late season performance.

Despite overall differences in early and late season performance (Tables 2.1 and 2.2), the trend for higher rooting was the same in late season for low (5 cm) and high (25 cm) pruned stock plants versus the mid-range prune height (15 cm) (Table 2.2). This response may be of note when devising pruning regimens for rooting operations during the late season.

Experiment 1 showed that provenance and pruning height can both have effects on number of shoots produced. However, stem caliper may be a better indicator of proclivity to produce new shoots (Table 1.2), though it is not the only factor controlling new shoot growth. Also, cuttings from plants with larger stem caliper may root at higher percentages, as they did from GA source plants, but this relationship was not consistent for all sources and remains uncertain. Large stem caliper alone can not be an indicator for rooting ability, because, for example, GA cuttings did not root as well as TN and WV cuttings during late season trials. Factors other than pruning height and provenance are at work as well.

Neither extended growing season nor container size had an immediate effect on overwintering survival immediately following the season of rooting (Table 3.2). However, repotting to different container sizes immediately after rooting and exposure to extended growing season conditions did have significant impact on the rooted cuttings in the form of new shoot growth (Table 3.1). The benefits of first season additional shoot growth are questionable when correlated with the final attribute measurements taken at the end of growing season 2. However, extending the growing season after rooting did have a positive impact on RCD and root mass at the end of the second growing season (Table 4.1), which have been proposed to be good indicators of future field performance (Reickermann *et al.*, 1999).

It should be noted that in Experiment 4, there were substantive mortality differences between container sizes even if not recognized statistically (Table 4.1). Rooted cuttings in medium size containers during the first growing season experienced 47.4% mortality as compared to 34.5% and 24.3% for small and large size containers, respectively, by the end of growing season 2 (Table 4.1). At the commencement of growing season 2, all rooted cuttings in small or medium containers were repotted to large containers while those in large containers remained. One uncontrolled factor that can not be accounted for with respect to the higher mortality rate for the medium container grown rooted cuttings is that they were transplanted twice (once each during growing seasons 1 and 2) while cuttings in the small and large containers grown during the later part of the first season were each transplanted only once during the entire experiment. Transplanting once (or not at all) would save time and labor, and perhaps enhance survival rates if these observations are found to be consistently true in other trials. Based on this study and others (Zaczek *et al.*, 1993; Zaczek *et al*., 1996), leaving rooted cuttings in small containers during the rooting process, first growing season, and overwintering period is advantageous for survival at outplanting. In the current study, the bottom 2 cm of roots of each rooted cutting were trimmed during transplant activities, and this, too, may have affected subsequent growth and survival rates.

Grading rooted cuttings before transplanting or outplanting is laborious, but several plant attributes, including height, root collar diameter, and root-to-shoot ratio have been shown to be good predictors of field performance (Thompson and Schultz, 1995; Dey and Parker, 1997; Clark *et al*., 2000; Jacobs *et al*., 2005; Davis and Jacobs, 2005; Johnson, 1994). Rooted cuttings grown in small containers throughout the first season produced the best specimens for outplanting based on these attributes (Table 4.1). Medium and large container grown rooted cuttings exhibited significantly lower heights and smaller root collar diameters, and their root-to-shoot ratios were higher than in small container grown rooted cuttings (Table 4.1). Rooted cuttings initially grown in small containers performed better overall, but the reasons are unclear. However, it could be related to taproot inhibition and well balanced root and shoot growth. In most cases, lower root:shoot ratios indicate a less balanced growth rate between root and shoot tissues, which could lead to poor field performance. Therefore, the plant attributes related to medium and large container size in the current study are not correlated with the attributes of those containerized seedlings expected to perform best in the field based on previous studies and protocols (Davis and Jacobs, 2005; Johnson, 1994).

Extending the growing season artificially during growing season 1 did have a significant impact on root collar diameter (Table 4.1). This could be attributed to a leaf duration effect allowing for more time for photosynthesis leading to more diameter growth. However, during the period of the extended growing season, container size also had a strong effect on RCD (Table 4.1). One factor which was not investigated in the current study, which could be important, is the size of the cutting itself at the time of sticking, regardless of the stem caliper or prune height of the donor plant.

Root mass at the end of the second overwintering period was highest for those plants that had remained in the small containers for the longest time (Table 4.1). Higher root mass suggests a more well-developed root system and potentially more first order lateral roots (FOLR), which are good indicators of field performance (Schultz and Thompson, 1996; Jacobs *et al*., 2005; Davis and Jacobs, 2005). The extended growing season also had a significant impact on root mass accumulation (Table 4.1). Specific examination of rooted cutting root system attributes, such as FOLR numbers and root system morphology, would be helpful in further identifying useful characteristics from which to predict outplanting success.

For practical application in nursery rooted cutting operations, provenance and prune height should be carefully considered when shoot production for rooting purposes is desired. Large caliper stems may enhance donor plant ability to generate new shoots for rooting as compared to small caliper stems. Donor plant prune height may also have a significant effect on excised shoots' rooting ability. These data suggest lower pruning (5 cm) may encourage more rooting than mid-range or high pruning (15 cm 25 cm) during the early season. As this study suggests, late season rooting is also possible, allowing for higher productivity in rooted cutting operations. In this study, all final attribute measurements were significantly impacted by container size. Leaving rooted cuttings in small containers throughout the entire first growing season may ensure more successful candidates for outplanting later when compared to those transplanted to medium or large containers during the first growing season.

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Table 1.1
Mean count ± SE (median) of new shoots produced from northern red oak stock plants of three geographic origins after pruning at 5 cm, 15 cm, or 25 cm above ground line on March 4, 2004 at N.C. State University-Reedy Creek Field Laboratory, Raleigh, NC. New shoot counts were recorded May 17-19, 2004. Stem basal caliper was used as a covariate in the analysis. Interactive effects were not significant and are therefore, not reported. Means within a column for "Prune Height" or within the row for "All Prune Heights Combined" followed by different letters differed significantly by ANOVA* protected LS means separation ($p \leq .05$).

Prune Height Treatment	***GA***	***TN***	***WV***	***All Geographic Sources Combined***
5cm	8.2 ± .963 a (12.3)	6.0 ± .694 c (6.0)	4.7 ± .627 e (5.0)	6.2 ± .631 x
15cm	11.8 ± .970 b (10.0)	8.5 ± .704 d (7.0)	6.2 ± .624 f (5.5)	8.9 ± .632 y
25cm	13.6 ± .973 b (7.0)	8.8 ± .679 d (8.0)	7.4 ± .621 g (7.0)	9.9 ± .628 z
All Prune Heights Combined (Median Value)	9.6 ± .648 h (10)	8.0 ± .638 i (7.0)	7.5 ± .643 i (6.0)	

Note*:
Prune Height for GA ANOVA: $F=13.75$, $p<.0001$
Prune Height for TN ANOVA: $F=12.10$, $p<.0001$
Prune Height for WV ANOVA: $F=16.04$, $p<.0001$
Prune Height for All Geographic Sources ANOVA: $F=35.48$, $p<.0001$
Geographic Source for All Prune Heights ANOVA: $F=7.80$, $p=.0005$

Table 1.2
Correlation coefficients accounting for the association between stock plant stem caliper and number of new shoots produced from northern red oak stock plants of three geographic origins after pruning at 5 cm, 15 cm, or 25 cm above ground line on March 4, 2004 at N.C. State University-Reedy Creek Field Laboratory, Raleigh, NC. New shoot counts and stem caliper were recorded May 17-19, 2004. Stem basal caliper was used as a covariate in the analysis, while geographic source and prune heights were used as the main experimental treatments.

Geographic Source/ Prune Height Treatment	*Correlation Coefficient Between Stock Plant Stem Caliper & Number of New Shoots*	*Associated P-value*
GA/5 cm	.39573	p=.0019
GA/15 cm	.58767	p<.0001
GA/25 cm	.32520	p=.0127
TN/5 cm	.18844	p=.1900
TN/15 cm	.40452	p=.0048
TN/25 cm	.25932	p=.0559
WV/5 cm	.40582	p=.0017
WV/15 cm	.41528	p=.0012
WV/25 cm	.26947	p=.0390
All Geographic Sources and All Prune Heights	.32371 *	p<.0001

Note:
* indicates a single partial correlation coefficient calculated to describe the association between number of new shoots and stock plant stem caliper after accounting for the effects of experimental factors geographic source and prune height.

Table 2.1
Rooting percentage ± SE of early season (May 23, 2004) stem cuttings taken from northern red oak stock plants at N.C. State University-Reedy Creek Field Laboratory, Raleigh, NC. Stock plants were from three geographic origins and had been pruned to heights of 5 cm, 15 cm, or 25 cm on March 4, 2004. Interactive effects were not significant and are therefore, not reported. Means within a column for "Prune Height" or within the row for "All Prune Heights Combined" followed by different letters differed significantly by ANOVA* protected LS means separation ($p \leq .05$).

Prune Height Treatment	***GA***	***TN***	***WV***	***All Geographic Sources Combined***
5cm	83 ± .081	73 ± .087	73 ± .089 c	77 ± .049 x
15cm	70 ± .081	67 ± .087	40 ± .089 d	59 ± .049 y
25cm	67 ± .081	60 ± .087	47 ± .089 d	58 ± .049 y
All Prune Heights Combined	73 ± .049 g	67 ± .049 gh	53 ± .049 h	

Note*:

Prune Height for GA ANOVA:	F=1.18, p=0.3107
Prune Height for TN ANOVA:	F=0.59, p=0.5577
Prune Height for WV ANOVA:	F=3.95, p=0.0227
Prune Height for All Geographic Sources ANOVA:	F=4.62, p=0.0106
Geographic Source for All Prune Heights ANOVA:	F=4.27, p=0.0150

Table 2.2
Rooting percentage ± SE of late season (July 26, 2004) stem cuttings taken from northern red oak stock plants at N.C. State University-Reedy Creek Field Laboratory, Raleigh, NC. Stock plants were from two geographic origins and had been pruned to heights of 5 cm, 15 cm, or 25 cm on June 27, 2004. Individual geographic sources and interactive effects were not significant and are therefore, not reported. Means within a column for "Prune Height" or within the row for "All Prune Heights Combined" followed by different letters differed significantly by ANOVA* protected LS means separation ($p \leq .05$).

Prune Height Treatment	***GA***	***TN/WV mix***	***All Geographic Sources Combined***
5cm	58 ± .071	60 ± .070 a	59 ± .050 x
15cm	46 ± .071	38 ± .070 b	42 ± .050 y
25cm	58 ± .071	60 ± .070 a	59 ± .050 x
All Prune Heights Combined	54 ± .040	53 ± .040	

Note*:

Prune Height for GA ANOVA:	F=0.96, p=0.3856
Prune Height for TN/WV Mix ANOVA:	F=3.31, p=0.0391
Prune Height for All Geographic Sources ANOVA:	F=3.92, p=0.0209
Geographic Source for All Prune Heights ANOVA:	F=0.05, p=0.8160

Table 3.1
Percentage of northern red oak rooted cuttings exhibiting new shoot growth $\pm$ SE on October 30, 2004. Cuttings were exposed to one of two growing environments at the end of 2004's growing season: "forced" greenhouse conditions or "not forced" outside conditions. Rooted cuttings were also in one of three container sizes: small, medium, or large. Means for container sizes, collectively for "forced" and "not forced" environments, followed by different letters were significantly different by ANOVA* protected means ($p \leq .05$). Means for all container sizes as well as for all growing environments followed by different letters were significantly different by ANOVA* protected means ($p \leq .05$).

Container Size Treatment	***"Forced" Environment***		***"Not Forced" Environment***		***All Growing Environments Combined***	
Small	16 $\pm$ .058	a	8 $\pm$ .058	a	12 $\pm$ .041	x
Medium	50 $\pm$ .058	b	16 $\pm$ .058	a	33 $\pm$ .041	y
Large	52 $\pm$ .058	b	20 $\pm$ .058	a	36 $\pm$ .041	y
All Container Sizes Combined	39 $\pm$ .034	g	15 $\pm$ .034	h		

Note*:
Container Size for All Growing Environments ANOVA: $F=10.03$, $p<.0001$
Growing Environment for All Container Sizes ANOVA: $F=26.78$, $p<.0001$
All Growing Environments*Container Size ANOVA: $F=3.07$, $p=.0479$

Table 3.2
Percentage of northern red oak rooted cuttings surviving overwintering conditions ± SE on May 2005. Cuttings were exposed to one of two growing environments at the end of 2004's growing season: "forced" greenhouse conditions or "not forced" outside conditions. Rooted cuttings were also in one of three container sizes: small, medium, or large. Individual treatment effects and interactions for overwintering were not significant and therefore, not reported.

Growing Environment	***All Container Sizes Combined***	***Container Size***	***All Growing Environments Combined***
"Forced"	80 ± .035	Small	80 ± .043
"Not Forced"	71 ± .035	Medium	79 ± .043
		Large	67 ± .043

Note:
Container Size for All Growing Environments ANOVA: F=2.87, p=.0585
Growing Environment for All Container Sizes ANOVA: F=3.58, p=.0595

Table 4.1
Final attribute measurement means for northern red oak rooted cuttings by treatment. Measurements were taken in the early spring of 2006 prior to bud break. Means within a column by attribute for all "Growing Environments" columns or within the row for "All Container Sizes Combined" for each attribute followed by different letters differed significantly by ANOVA* protected LS means separation ($p \leq .05$). Means for container sizes, collectively for "forced" and "not forced" environments, followed by different letters were significantly different by ANOVA* protected means ($p \leq .05$). Where individual treatment effects and interactions were not significant, values were not reported.

Final Attribute Measured & Container Size	"Forced" Growing Environment	"Not Forced" Growing Environment	All Growing Environments Combined	Statistical Notes*:
Height (cm)				
Small	-	-	66.5 ± 3.90 x	Ht for All Envir: F=10.16 p=.0002
Medium	-	-	51.6 ± 4.47 y	Ht for All Cont: F=2.58 p=.1134
Large	-	-	42.4 ± 3.78 y	
All Containers Combined	57.4 ± 2.83	49.6 ± 3.93	-	
RCD (mm)				
Small	11.82 ± .66 a	10.09 ± .77 a	11.0 ± .46 x	RCD for All Envir: F=5.86 p=0.0186
Medium	9.36 ± .69 b	7.40 ± .99 b	8.60 ± .52 y	RCD for All Cont: F=17.35 p<.0001
Large	7.62 ± .59 c	7.33 ± .92 b	7.31 ± .44 y	
All Containers Combined	9.64 ± .33 g	8.27 ± .46 h	-	Force x RCD: F=18.41 p<.0001 NoForce x RCD: F=3.55 p=.0530
Root Mass (g)				
Small	40.3 ± 4.34 a	31.8 ± 5.18 a	36.1 ± 3.16 x	RtMass for All Env: F=4.86 p=.0314
Medium	31.1 ± 4.54 ab	18.2 ± 6.69 a	25.6 ± 3.62 y	RtMass for All Cont: F=6.06 p=.0040
Large	24.1 ± 3.66 b	19.6 ± 6.19 a	21.2 ± 3.06 y	
All Containers Combined	32.0 ± 2.29 g	23.3 ± 3.18 h	-	Force x RtMass: F=4.98 p=.0120 NoForce x RtMass: F=1.74 p=.2068
Shoot Mass (g)				
Small	-	-	27.9 ± 2.45 x	ShtMass for All Envir: F=3.41 p=.0698
Medium	-	-	15.8 ± 2.81 y	ShtMass for All Cont: F=14.92 p<.0001
Large	-	-	9.6 ± 2.37 y	
All Containers Combined	20.6 ± 1.78	15.0 ± 2.47	-	
Root:Shoot Ratio				
Small	-	-	1.41 ± .1491 x	R/S for All Envir: F=1.71 p=.1961
Medium	-	-	1.54 ± .1638 x	R/S for All Cont: F=14.05 p<.0001
Large	-	-	2.23 ± .1459 y	
All Containers Combined	1.83 ± .1251	1.63 ± .1502	-	
Mortality (%)				
Small	-	-	34.5 ± .0802	Mort for All Envir: F=.37 p=.5462
Medium	-	-	47.4 ± .0815	Mort for All Cont: F=2.02 p=.1389
Large	-	-	24.3 ± .0835	
All Containers Combined	32.5 ± .0597	38.4 ± .0757	-	