

Rooting Stem Cuttings of Several Species within the genus *Quercus* L.

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Introduction

Several species in the genus *Quercus* L. have been vegetatively propagated by means of rooted stem cuttings including *Q. alba* L. and *Q. palustris* Muenchh. (Zaczek et al. 1997), *Q. nigra* L. (Hare 1977), *Q. pagoda* Raf. (Farmer 1965), *Q. phillyreoides* A. Gray (McGuigan et al. 1996), *Q. robur* L. (Larsen 1946), *Q. rubra* L. (Zaczek et al. 2006, Dreps 2007), *Q. suber* L. (Kommisarov 1960) and *Q. turbinella* Greene (Davis 1970). Rooting trials for a number of additional oak species were conducted at North Carolina State University from 2004 to 2007.

These trials were the natural progression of a forest research project initiated at NC State in 2002 to develop protocol for large-scale production of rooted stem cuttings of *Quercus rubra* [northern red oak (NRO)] intended for deployment as reforestation planting stock.

Oak rooted stem cuttings and Forestry in eastern North America

Regenerating natural hardwood timber stands in upland regions of eastern North America, with NRO (and other native oak species) as a significant component, has proved challenging to foresters. Two major limitations to such regeneration efforts are the lack of advanced oak regeneration in pre-harvested or pre-disturbed stands and the slow growth and high mortality rates of existing advanced oak regeneration, after canopy removal, as a result of intense herbaceous and woody competition. In some cases, this regeneration dilemma has been addressed by employing artificial regeneration techniques, such as plantation systems and enrichment plantings.

Planting stock utilized in these systems is commonly obtained from bare-root nurseries growing seedlings from unimproved, bulk collected acorns. Efforts to genetically improve oak planting stock exist, but have been limited largely due to the costs associated with relatively long periods of juvenility, episodic acorn crops, abundant acorn predators, and long term progeny trials (Robison et al. 2004). However, recent advances in the vegetative propagation of oak by means of rooting stem cuttings may offer a viable alternative to conventional oak improvement practices, improving growth rates, consistency, and quality of artificial planting stock.

Additionally, the ability to vegetatively propagate oak species such as NRO (Gocke and Robison 2007), southern red oak (*Q. falcata* Michx.) and water oak (*Q. nigra*) (Duncan and Matthews 1969) from stump sprouts originating at the base of recently felled trees gives tree breeders a direct means of basing tree selection on phenotypic expression. Vegetative propagules could also save considerable time and money associated with NRO breeding strategies utilizing acorns.

As a result of its ecological and economic importance in eastern North America several techniques have been developed to vegetatively propagate NRO from

stem cuttings including rooting juvenile and mature semi-hardwood cuttings under conditions of high humidity (Teclaw and Isebrands 1987, Zaczek et al. 1993, Zaczek 1994) and rooting shoots originating from mature buds grafted onto juvenile root stocks (Zaczek and Steiner 1997, Zaczek et al. 2006). Of these techniques, rooting juvenile semi-hardwood cuttings has provided some of the most consistent results (Picture 1). For a detailed discussion on efforts to vegetatively propagate stem cuttings of NRO see Dreps 2007, Drew and Dirr 1989, Fishel et al. 2003, Gocke and Robison 2007, Teclaw and Isebrands 1987, and Zaczek et al. 2006.

Attempts to root juvenile and mature semi-hardwood cuttings of other oak species have also been successful under conditions of high humidity. On occasion some of these mature cuttings produce flowers and fruiting structures (Figure 3). Other mature traits exhibited by these mature rooted cuttings may include slower growth rates and greater branching. Field trials need to be established to determine the affect of the physiological age of oak stem cuttings on subsequent rooted cutting field growth.

Oak rooted cuttings and Horticulture

Interest in the vegetative propagation of various oak species via rooted stem cuttings has also been encouraged by the horticultural industry. Here interest in rooted stem cuttings of oak has been stimulated by a desire to capture individual genotypes with unique traits (growth habit, leaf characteristics, drought tolerance, etc...) and market them as named oak cultivars. Until recently oak cultivars were mostly limited to grafted plants. Currently several oak cultivars on their own root systems have been introduced into the horticultural market. These recent introductions include several selection from Tree Introductions, Inc. [Highbeam™ overcup oak (*Q. lyrata* Walter), Hightower™ willow oak (*Q. phellos* L.), Highpoint™ nuttall oak (*Q. texana* Buckl.), Highrise™ live oak (*Q. virginiana* Mill.), Panache™ shumard oak (*Q. shumardii* Buckl.)], a single introduction from Southern Selections, LLC (Wynstar™ willow oak), and a selection from Shadowlawn Nursery [Cathedral Oak™ live oak]. The majority of these introduced cultivars were selected for their compact, narrow form and adaptability to urban environments as street and landscape trees.

Factors affecting adventitious root formation on oak stem cuttings

Several factors affect the ability of oak stem cuttings to form adventitious roots. These factors include maturation or aging patterns common in many woody plants, genetics and stock plant management. The rooting ability of NRO stem cuttings reflects ontogenetic aging patterns common in many woody plants (Robinson and Wareing 1969, Hartmann et al. 1997, Fishel et al. 2003), in which, rooting ability along with other morphological and physiological characteristics, follow distinct patterns closely related to the developmental age at which new shoots are produced (Hartmann et al. 1997). High rooting ability, considered mostly a juvenile trait, therefore, decreases as ontogenetic maturation and the adult phase of development capable of sexual reproduction, is gradually and eventually attained in shoots growing at increasing distances from the base of the tree (Hartmann et al. 1997, Fishel et al. 2003).

Maturation is generally accepted as the major obstacle to rooting stem cuttings from trees. Therefore, though large trees can produce a large number of stem

cuttings many fail to root (Naujoks et al. 1995). Whereas, stem cuttings collected from seedlings are few in number but root in high percentages. An example of an exception to this rule is the results of a study conducted by McGuigan et al. 1996 to evaluate the rooting ability of stem cuttings collected from two *Q. phillyreoides* trees, 40- and 8-years-old. Softwood cuttings were collected when the leaves were fully expanded on the initial flush of the growing season from the mid- to upper crown of the two trees. Optimal rooting occurred when individual stem cuttings were treated with 0.8% indole-3-butyric acid (IBA) in talc. In this treatment cuttings collected from the 40-year-old tree rooted in higher percentages than the 8-year-old tree (97% and 56%, respectively). Genetic differences between the two trees utilized in this study may have influenced the resulting disparity in rooting. Variation in rooting among individual genotypes of several woody plant species has been widely reported.

For many tree species maturation can be slowed by pruning near the base of the tree to encourage juvenile shoot production from sprouts (Hartmann et al. 1997, Zaczek and Steiner 1997). Juvenility has been maintained in this way for NRO by pruning seedlings into stock plant hedges (Drew and Dirr 1989). These stock plant hedges can be grown in containers or in the field and re-pruned multiple times to provide a continuous supply of stem cuttings for future rooting efforts.

Stem cutting collection time, rooting environment, and stem cutting preparation also impact the success of oak rooted cutting propagation. Teclaw and Isebrands (1987) reported optimal rooting for stem cuttings collected from early season, semi-hardwood NRO shoots collected during flush lag (Lag), the stage of development within a flush defined as the interval between the completion of one growth flush and the onset of the next flush of growth (Hanson et al. 1986).

Northern red oak, like other oak species, exhibits semi-determinate shoot growth characterized by recurrent, cyclic, or episodic flushes of growth during the growing season (Dickson 1994). Environmental conditions and the age of the tree impact the number of flushes produced within a given year (Dickson 1994). For example, young NRO seedlings are capable of producing a greater number of progressive flushes in one growing season than older, more mature NRO trees (Dickson 1994) (Figure 1).

NRO stem cuttings are most successfully rooted in conditions of high humidity, either under intermittent mist (Teclaw and Isebrands 1987 - stem cuttings from juvenile seedlings) or in a fog chamber (Zaczek et al. 2006 - stem cuttings from seedlings and mature trees). High levels of shade prior to rooting have further increased rooting percentages for NRO, especially among more mature cuttings, (Zaczek 1994) and *Q. phillyreoides* (McGuigan et al. 1996). New shoots collected from pruned containerized stock plants grown in partial shade (50%) rooted at higher percentages than those grown in full sun (84% and 68%, respectively) (unpublished data from NC State NRO rooting efforts).

Though exogenously applied rooting hormones are not required for adventitious root formation on juvenile NRO stem cuttings (Teclaw and Isebrands 1987) and juvenile stem cuttings of *Q. pagoda* (unpublished data from NC State oak rooting efforts), concentrations of IBA ranging between 0.5 % (5,000 ppm) and 1.5% (15,000 ppm) applied to the base of stem cuttings have led to high rooting percentages (Teclaw and Isebrands 1987, Zaczek et al. 2006). Increasing concentration of exogenously applied IBA has also been reported to result in a greater number of primary roots (Teclaw and Isebrands 1987).

Post-rooting care and subsequent growth

Once propagated, newly rooted oak stem cuttings should be acclimated to a less humid environment than the rooting chamber. This is completed in gradual steps over approximately two weeks. Following acclimation, if additional shoot growth is desired the cuttings are subjected to an extended photoperiod and warm temperatures to encourage a new flush of growth (forcing). Flushing after rooting improves overwintering survival for some oak species (McGuigan et al. 1996, Dirr and Heuser 2006). In one study conducted at NC State, NRO rooted cuttings that were forced demonstrated higher rates of overwintering survival than those not forced (91 % and 59 %, respectively), regardless of whether or not the cuttings flushed (Dreps 2007). The current author has observed that some oak species flush more readily following rooting than other oak species.

Rooting protocol for juvenile northern red oak stem cuttings

The following section is a description of the methods developed at NC State for rooting juvenile stem cuttings of NRO and follows one particular study. The collected stem cuttings originated as single flushes of growth from recently pruned containerized seedling stock plants.

Stock Plant Establishment

One-year-old, bare-root NRO seedlings (1-0) from the Clements State Tree Nursery (West Columbia, WV) were transplanted in April 2002 into 30 liter Treepot™ (Stuewe and Sons, Inc., Corvallis, OR) containers, filled with a medium mixture of 1 peat: 1 perlite: 1 field soil [Congaree series silt loam (USDA Soil Conservation Service 1970)]: 3 composted pine bark, by volume. These containerized seedling stock plants were grown outside at the North Carolina State University Horticulture Field Laboratory in Raleigh, NC and were spaced 0.3 m apart for one growing season without pruning.

In February 2003 these seedling stock plants (now entering their third growing season) were pruned to 2 cm above the base of 1) the first flush (B1); 2) the second flush (B2); or 3) the third flush (B3) of growth (Figure 1). The stock plants were organized into a split plot design with one half of the stock plants placed under partial shade (50%). Light level (sun vs. shade) was established as the whole plot factor and prune location, as the subplot factor. There were 15 stock plants per treatment (3 prune locations x 2 light levels) for a total of 90 stock plants.

The seedling stock plants were watered every one to two days for 5 minutes during the two growing seasons (2002 and 2003) with individual spray stakes (Antelco Shrubber™ 360°, Antelco Corporation, Longwood, FL). Irrigation frequency and duration during the growing season was controlled by a Hunter Smart Valve Controller (Hunter Industries, San Marcos, CA).

The stock plants were fertilized two months after the bare-root seedlings were transplanted into containers, with 50 grams of Coor's 14-14-14 slow release fertilizer plus minors (Coor Farm Supply, Smithfield, NC) applied as a top dressing. The shade cloth was supported by a wooden box frame and enclosed the entire area surrounding the shaded stock plants.

In May 2003 semi-hardwood stem cuttings were collected with by-pass pruners from the first flush of re-growth formed within 5 cm of each prune location on sun and shade grown seedling stock plants. Twenty-five stem cuttings were collected from each group of stock plants (3 prune locations x 2 light levels). There

were six treatments, five blocks, and five cuttings per treatment per block for a total of 150 experimental stem cuttings plus borders. For purposes of uniformity, only terminal stem pieces 16 cm or longer were collected for rooting. However, NRO stem cuttings ranging from 6 cm to 45 cm have been successfully rooted (unpublished data from NC State NRO rooting efforts). Following collection the stem cuttings were partially submerged in water and placed in shade until processing later the same day.

Rooting Facility and Procedures

The cuttings were processed by removing the leaves and petioles from the bottom half of each 16 cm terminal cutting. The remaining leaves were trimmed 1/2 of their original size to facilitate sticking and reduce leaf transpiration rates. The base of each cutting was then recut (approximately 1 cm of stem was removed) and dipped 2 cm deep into a liquid solution of 1% IBA in an aqueous 50% EtOH (95%) solution for 5 seconds. After allowing the bases to dry for one minute, the cuttings were stuck 4 cm deep into 164 ml Ray Leach "RL" tubes (Ray Leach SC-10 Super Cell Cone-tainers™, Stuewe and Sons, Inc. Corvallis, OR) filled with a moist media mixture of 3 peat: 3 perlite: 1 vermiculite, by volume. The containers were placed into every other hole of RL-98 trays (Stuewe and Sons, Inc., Corvallis, OR) at a linear spacing of 8.6 cm and a diagonal spacing of 4.3 cm.

The trays were then placed in a rooting chamber constructed around a propagation table inside a polyethylene-covered, Quonset-styled greenhouse with shade cloth. The rooting chamber consisted of a rectangular PVC frame (2.5 m tall x 1 m wide x 5 m long) covered on all four sides with sheets of Frost Blanket™, a white UV stabilized non-woven fabric, (The Master Gardener Company, Spartanburg, SC) to contain humidity. A shade cloth (50%) was placed on top of the PVC frame to reduce light stress during rooting. Combined, the shaded greenhouse and shaded rooting chamber provided 15% ambient light (as compared to mid-day, outdoor ambient light) within the rooting chamber (measured with a Sunfleck Cep-tometer™, Decagon Inc., Pullman, WA).

Talstar™ Flowable insecticide (FMC Agricultural Products Group, Philadelphia, PA) at a rate of 3.25 mL/L and Gnatrol™ biological larvicide (Valent USA Corp., Walnut Creek, CA) at a rate of 6.25 mL/L were used on the cuttings during rooting to control for insects. Zero-tol™ broad spectrum algacide/fungicide (Biosafe Systems, Glastonbury, CT) at a rate of 3.25 mL/L was also applied to the cuttings and rooting chamber. The sheets of Frost Blanket™ covering the sides of the rooting chamber were replaced every 30 days to manage algae build up. Stem cutting leaf litter was removed regularly to maintain a clean rooting environment.

Overhead NaanDan™ "Water and Sprinkling" nozzles (Kibbutz Naan, Naan, Israel), with a flow rate of 41.6 liters/hour, provided mist initially every 12 minutes for 15 seconds in the rooting chamber. Irrigation frequency and duration during both years were controlled by a Davis Engineering Solar 6A Misting Controller (Davis Engineering, Winnetka, CA). The frequency of mist was tapered gradually after 50 days of rooting. Seventy-five days after sticking the rooted cuttings were acclimated to a non-misting environment and at 90 days were relocated to a propagation bench in the same greenhouse as the rooting chamber. The rooted cuttings were hand watered once daily for the remainder of the 2003 growing season.

Conditions in the greenhouse were conducive to new growth by extending the photoperiod to 18 hours per day with the aid of supplementary lighting (1.2 m,

40W GE Spectra Rays Full Spectrum Fluorescent Tubes, General Electric Company) hung 1 m above the cutting tops and a constant temperature of 26°C. This forcing step is not absolutely necessary but a period of extended growth can significantly improve overwintering survival for newly rooted NRO stem cuttings (Dreps 2007). After a three month period of extended growth, the day-length and temperature inside the greenhouse was slowly adjusted to those present outside. Once the cuttings were dormant (mid-January 2004) they were moved to an overwintering house covered with white polyethylene plastic until bud break the following growing season.

In early May 2004 the surviving rooted cuttings were transplanted into 6.23 L Treepot²™ containers (Stuewe and Sons, Corvallis, Oregon). Composted pine bark mulch was used during transplanting. The same day each transplanted rooted cutting was top dressed with 1/8 cup of Nutricote Total (13-13-13) slow release fertilizer (Chisso-Asahi Fertilizer Co., Tokyo, Japan).

The newly transplanted rooted cuttings were then placed in a Quonset-style shade house (50%) and irrigated by hand every two to three days until shoot height and stem diameter were measured at the end of the growing season in November 2004 (Picture 3). The average rooted cutting stem height at the end of approximately 18 months of growth was 75 cm tall and 11.2mm in diameter.

Analysis of variance (ANOVA) for rooting percentage was conducted using the mixed model procedure in SAS (SAS Institute, Cary, NC) and included both fixed (treatment) and random (block) factors and their interactions. When ANOVA results indicated treatment differences of $p \leq 0.05$, a pairwise comparison was utilized to separate the means at $p \leq 0.05$. Rooting results for this NRO study are summarized in Tables 1.

Rooting trials for additional oak species 2004-2007

Rooting trials for a number of additional oak species were conducted at NC State University from 2004 to 2007. These trials were the natural progression of the NRO rooted cutting efforts at NC State conducted from 2002 to 2007. The same basic protocol described in the above section on NRO rooted cutting propagation was used for these trials. For some of the oak species evaluated during this period, stem cuttings were collected from pruned seedling stock plants.

Others were collected from the most juvenile material obtainable on non-pruned seedling specimens. Because the most physiologically juvenile portion of a tree is centered around the base of the trunk, shoots originating within the general vicinity of this area also provided potential juvenile rooting material including coppice sprouts, stump sprouts and epicormic sprouts. When this material was not available stem cuttings were collected from the lower third of the canopy of a tree.

All NRO stem cuttings collected were from first flush material in a semi-hardwood stage of development or LAG. In some cases it was difficult to obtain a sizeable number of stem cuttings for certain species. However successful rooting of even a single stem cutting demonstrates the potential for rooted cutting propagation of these species. The results of this work are presented in Table 2.

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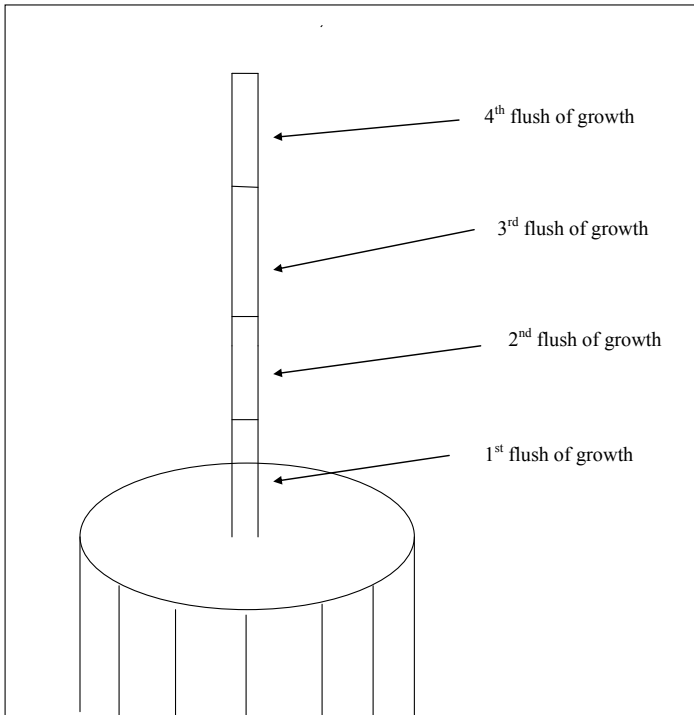


Figure 1. A representation of an oak seedling stock plant, prior to pruning, with four flushes of growth.

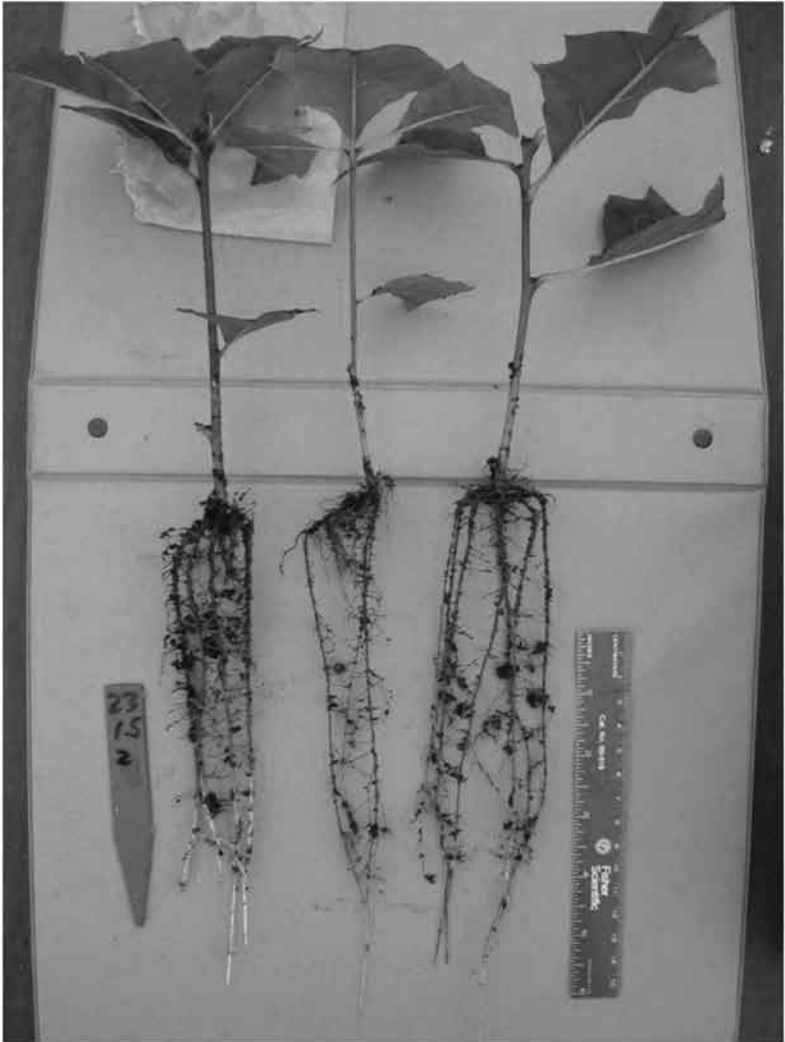


Figure 2. *Quercus rubra* L. (northern red oak; rooted juvenile stera cuttings after 12 weeks under intermittent mist in a rooting chamber.



Figure 3. A rooted stem cutting of *Quercus wutaishanica* Mayr. (Liaodong oak) in flower.



Figure 4. *Quercus rubra* L. (northern red oak) rooted juvenile stem cuttings after 12 weeks under intermittent mist in a rooting chamber.

Table 1. The effect of prune locations (B1, B2, and B3) and light level [sun versus shade (50%) grown stock plants] on rooting ability of northern red oak stem cuttings

Prune Treatment	Rooting Percentage		
	Sun	Shade	Total
B1	64	84	74 a
B2	56	72	64 b
B3	36	56	46 c
Total	52 b*	70.7 a	N/A
Anova Results (5 cuttings/5 blocks/6 treatments)	<p style="text-align: center;"><u>Sources of Variation</u></p> <ul style="list-style-type: none"> • Prune location (p-value) = 0.0366 • Light level (p-value) = 0.0354 • Prune location*light level (p-value) = 0.9744 <p style="text-align: center;">(Proc Mixed - SAS)</p>		

* Prune location and light level rooting means with the same letter did not differ significantly (ANOVA Mixed model, pairwise comparisons, $p < 0.05$).

Table 2. Rooting results for stem cuttings of twenty-nine oak species (*Quercus* L.)

Oak Species	Common Name	Stem Cutting Origin*	Rooting Hormone (IBA)**	Rooted / Collected = Rooting %
<i>Quercus acuta</i> Thunb.	Japanese evergreen oak	NP – J	2%	3/3 = 100%
<i>Q. alba</i> L.	eastern white oak	P – J	Control 0.5% 1.0%	6/20 = 30% 11/20 = 55% 10/20 = 50%
<i>Q. canbyi</i> Trel.	Canby's oak	NP – J	1.0%	1/2 = 50%
<i>Q. castaneifolia</i> C.A. Mey.	chestnut-leaved oak	NP – J	1.5%	3/4 = 75%
<i>Q. chapmanii</i> Sarg.	Chapman oak	NP – J	1.5%	8/15 = 53%
<i>Q. chenii</i> Nakai	a relative of sawtooth oak	P – J	1.0%	15/19 = 79%
<i>Q. crassifolia</i> H. & B.	Mexican leather leaf oak	M – C	2.0%	1/3 = 33%
<i>Q. durandii</i> Buckl.	Bigelow oak	NP – J	2.0%	6/8 = 75%
<i>Q. falcata</i> Michx.	southern red oak	NP – J	1.5%	11/12 = 92%
		M – E	1.5%	2/2 = 100%
<i>Q. fruticosa</i> Brot.	gall oak	NP – J	1.0%	2/8 = 25%
			1.5%	7/8 = 87.5%
<i>Q. georgiana</i> Curtis	Georgia oak	P – J	1.5% 2.0%	14/14 = 100% 11/13 = 85%
<i>Q. germana</i> Schiltl. & Cham.	Mexican royal oak	NP – J	1.0%	2/4 = 50%
<i>Q. glauca</i> Thunb.	Japanese blue oak	NP – J	1.5%	14/15 = 93%
<i>Q. graciliformis</i> C.H. Mull.	Chisos oak	M – C	1.5%	3/6 = 50%
<i>Q. lusitanica</i> Lam.	Portuguese oak	NP – J	2.0%	2/4 = 50%
<i>Q. macranthera</i> Fisch. & B. Mey.	Caucasian oak	NP – J	2.0%	1/2 = 50%
<i>Q. michauxii</i> Nutt.	swamp chestnut oak	P – J	1.5%	5/10 = 50%
<i>Q. macrocarpa</i> Michx.	bur oak	P – J	1.5%	27/39 = 69%
<i>Q. montana</i> Willd.	chestnut oak	P – J	1.0%	30/30 = 100%
			1.0%	9/9 = 100%
<i>Q. nigra</i> L.	water oak	P – J	1.5%	9/9 = 100%
<i>Q. oglethorpensis</i> Duncan	Oglethorpe oak	NP – J	1.5%	7/16 = 44%
			0%	84/84 = 100%
<i>Q. pagoda</i> Raf.	cherrybark oak	P – J	0.5%	82/84 = 98%
			1.0%	77/84 = 92%
			1.5%	9/10 = 90%
<i>Q. palustris</i> Munchh.	pin oak	P – J	2.0%	10/10 = 100%
<i>Q. phillyreoides</i> A. Gray	ubame oak	M – C	1.5%	13/15 = 87%
<i>Q. robur</i> 'Cupressoides' L.	English oak	M – C	2.0%	1/6 = 17%
<i>Q. rugosa</i> Nee.	netleaf oak	NP – J	1.0%	6/6 = 100%
<i>Q. sinuata</i> Walter	bastard oak	NP – J	2.0%	1/4 = 25%
<i>Q. texana</i> Buckl.	Nuttall's oak	NP – J	2.0%	5/5 = 100%
<i>Q. virginiana</i> Mill.	southern live oak	M – C	1.5%	2/2 = 100%

*N P-J = stem cuttings were collected from the lower third of the canopy or epicormic sprouts of a non-pruned juvenile tree. A juvenile tree is one that has not yet acquired the ability to produce flowering and fruiting structures (seedling to young tree).

P-J = stem cuttings were collected from pruned juvenile trees

M = stem cuttings were collected from epicormic sprouts (E) near the base of the tree or the lower third of the canopy (C) of a mature tree

**The various rooting hormone (IBA) concentrations were applied to the base of the cutting in a 45% EtOH (95%) solution.