VEGETATIVE PROPAGATION AND TOPOPHYTIC RESPONSES
OF SELECTED BALDCYPRESS CLONES

A Thesis
by
ANDREW RICHARD KING

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2010

Major Subject: Horticulture
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Approved by:
Co-Chairs of Committee, Michael A. Arnold
Douglas F. Welsh
Committee Member, W. Todd Watson
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ABSTRACT

Vegetative Propagation and Topophytic Responses of Selected Baldcypress Clones. (August 2010)

Andrew Richard King, B.S., Stephen F. Austin State University

Co-Chairs of Advisory Committee: Dr. Michael A. Arnold
Dr. Douglas F. Welsh

Baldcypress, *Taxodium distichum* (L.) Rich., var. *distichum* is a highly adaptable tree of significant ecological importance in the Southeastern U.S. Including *Taxodium distichum* var. *imbricarium* (Pondcypress) and *Taxodium distichum* var. *mexicanum* (Montezuma cypress), the native range is extended into south Texas and Mexico. Previously, baldcypress selections were made for drought, foliar salt exposure and high pH soil tolerance. Experiments were conducted beginning in May, 2008 to determine commercial viability of vegetative propagation by shoot tip cuttings of these selections, to identify treatment combinations that led to optimal rooted cutting quantity and quality, and to determine whether baldcypress cuttings displayed a topophytic effect and if so, if there was a correlation between branch angle on the ortet and topophysis exhibited by ramets during nursery production. The propagation studies revealed that rooted cutting quantity and quality were optimal when softwood cuttings were taken from south Texas and/or Mexican provenances and treated with either 7,500 or 15,000 mg·L⁻¹ K-IBA (potassium salts of indole-3-butyric acid). A gradient was observed with cuttings from more southern and western provenances typically exhibiting greater rooted cutting quantity and quality than cuttings from more eastern and northern provenances. A
tradeoff between greater rooting percentages in a substrate with greater aeration (100% perlite) versus greater root quality (root numbers, length, or mass) in a substrate with a higher water-holding capacity (100% peat moss) was also displayed. Additional basal wounding proved to be detrimental to both rooted cutting quantity and quality. A significant ($P \leq 0.05$) topophytic effect was observed among genotypes for the divergence of central leaders from a vertical orientation during nursery production, but the branch angle of the cutting on the ortet was not a good predictor of this divergence on the ramet.
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CHAPTER I
INTRODUCTION

Baldcypress, *Taxodium distichum* (L.) Rich., var. *distichum* is a highly adaptable tree of significant ecological importance in the Southeastern U.S. (Arnold, 2008; Pezeshki and DeLaune, 1994). The species type's native range extends from southeast Texas to southern Illinois and Indiana in the west and from Florida to southern Delaware in the east (Wilhite and Toliver, 1990). With the addition of *Taxodium distichum* var. *imbricarium* (Pondcypress) and *Taxodium distichum* var. *mexicanum* (Montezuma cypress), the native range is extended into south Texas and Mexico (Denny and Arnold, 2007). The wide range of climates across this large geographic area has resulted in tolerances to an array of climatic conditions making it a wise choice of plant material in other regions as well. *Taxodium distichum* has been used effectively as an erosion-control species (Copes and Randall, 1993), grown for its ability to survive partial submersion (Arnold, 2008) and used in other locations where plant selection is limited. Research indicates that baldcypress is one of the most photosynthetically active species during periods of flooding (Nash and Graves, 1993), but it also has the ability to live in upland areas where water is not as plentiful (Arnold, 2008). The vast range of environments in which this species can thrive makes it a valuable landscape tree. While baldcypress exhibits durability and adaptability in landscapes, it does have limitations under some environmental conditions. It displays stress-induced foliar response

This thesis follows the style of the Journal of the American Society for Horticultural Science.
symptoms of chlorosis, marginal necrosis, and/or premature senescence when exposed to high pH soils, prolonged drought or salt-laden foliar spray from irrigation (Arnold, 2008). Though it will survive in a wide range of soil pH, high pH soils tend to cause manganese chlorosis in some provenances of baldcypress (Denny, 2007). Foliar chlorosis can be overcome temporarily however, through trunk injections of ferric ammonium citrate (Dirr, 1998). Prolonged drought causes browning of the leaves, beginning at the tip or margins and progressing through the entire leaf, which eventually fall off creating an ornamental liability. Though it can tolerate soil salts, salts from irrigation water can cause premature leaf drop (Arnold, 2008). In response to these stresses, initial screenings of baldcypress populations were made in an effort to identify more drought, salt and high pH soil tolerant selections (Denny, 2007). Denny (2007) found that of the 14 open-pollinated Taxodium families tested (ranging from southern Mexico to Alabama) there was a geographic gradient for tolerance to drought, foliar salts and soil alkalinity. Families from Mexico and south Texas were generally more tolerant of the aforementioned stresses while those from the more eastern provenances were least tolerant. Tolerance to soil salinity also was variable, but appeared to be more associated with variances among families and less strongly with differences in geographic origin (Denny, 2007). Field trials of clones selected from populations tolerant of one or more of these stresses are being conducted at twelve sites ranging from the Ohio west to Iowa, south to west Texas and east to central Florida (Arnold et al., 2010). Once these clones are field tested, it will be imperative that they fit into conventional nursery production systems, including being easily clonally propagated, for widespread acceptance by the nursery and landscape industry to occur.
A. Vegetative Propagation of Baldcypress

A considerable amount of research has been focused on studying the vegetative propagation of forest tree species, particularly conifers (Farrar and Grace, 1942a; Farrar and Grace, 1942b; Grace and Farrar, 1945). Of this research, limited studies focus on baldcypress. For commercial purposes, *Taxodium* is typically propagated asexually in two ways. It is grafted on seedling rootstocks or produced via adventitiously rooted shoot cuttings. Grafting baldcypress is a reliable method of propagation (Dirr, 1998), however it is more expensive than cuttings (Thomsen, 1978). Vegetative propagation by cuttings yields uniform plants and through selection can be used to expedite narrow sense heritability genetic improvement in the species (Pezeshki & DeLaune, 1994).

There are many methods for manipulating cuttings to encourage optimal adventitious root formation. Most of these methods for improving rooting of the ramets (individual plants derived from the original ortet) fall under three main categories: management of the ortet (original plant from which the clone is derived) or stockblock, treatment of cuttings during propagation, or management of the environment surrounding the cuttings during propagation (Hartmann et. al., 2002). The majority of research conducted on asexual propagation of baldcypress by cuttings focused on treatment of cuttings during propagation, specifically using different types and concentrations of auxin (Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005) or wounding the basal portion of the cutting (Zhou, 2005). Commonly, the management of the ortet is also tested by making the timing of cutting harvest the independent variable in an experiment (Zhou, 2005). In most instances the environment is a constant across all treatment combinations (Copes and Randall, 1993; Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005). Lu et
al. (2004) was the only study found that manipulated the surroundings of baldcypress cuttings by testing different rooting substrates.

Treating cuttings with differing auxin concentrations or types is one of the most common treatments in the research of baldcypress propagation by cuttings (Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005). Pezeshki and DeLaune (1994) found that when baldcypress cuttings were taken from one year-old trees and treated with a 1,000 mg·L\(^{-1}\) concentration of indole-3-butyric acid (IBA) (ROOTONE\(^{\text{®}}\), Ferti-lome Co., Bonham, TX) they rooted at a similar percentage than those that received no IBA, but showed significantly greater \((P \leq 0.05)\) shoot dry weights. St. Hilaire (2003) researched the rooting of *Taxodium distichum* (L.) Rich. var. *mexicanum* G. Gordon softwood cuttings. In this study an increase in rooting percentage was exhibited when cuttings were treated with increasing amounts of IBA. In one year of the study rooting percentages were 48 and 82 for 3000 and 8000 mg·L\(^{-1}\) IBA, respectively. Zhou (2005) found that rooting percentages increased with increasing levels of K-IBA (potassium salts of indole-3-butyric acid). Cuttings treated with 5,000 and 10,000 mg·L\(^{-1}\) K-IBA rooted at a significantly higher \((P \leq 0.05)\) rate than did cuttings treated with 0 and 2,500 mg·L\(^{-1}\) K-IBA.

All cuttings are by definition wounded; however, increasing the area of mechanical wounding has been proven to induce increased wound responses, including the division of the affected cells, which may lead to adventitious root formation in various species (MacKenzie et al., 1986). Zhou (2005) included a wounding treatment in a number of experiments conducted on a baldcypress clone called T302 (a reported baldcypress x Montezuma cypress hybrid). Cuttings were incision wounded by cutting 1cm along the
axis. One experiment conducted from 3 August 2006 to 9 November 2006 included a wounding treatment that did not significantly affect ($P \geq 0.05$) rooting percentage or root density ranking (RDR, a qualitative method of ranking root system quality). Conversely, another experiment commenced on 12 November 2006 and ended on 6 February 2007 and included a wounding treatment that did significantly affect ($P \leq 0.05$) both rooting percentage and RDR. Overall wounded treatments produced cuttings with a 1.6 and 1.8 times greater rooting percentage and RDR, respectively, than did non-wounded treatments.

Rooting substrates are often comprised of an organic component (i.e. peat moss) and a mineral component (i.e. perlite) which provides aeration. A rooting substrate serves a number of purposes including securing the cutting in an erect position, providing water for the cutting, supplying sufficient aeration for adventitious rooting and reducing the amount of irradiance that reaches the base of the cutting (Hartmann et al., 2002). The uptake of water in cuttings is proportional to the content of water, by volume, in the rooting substrate (Grange and Loach, 1983; Rein et al., 1991), while excess water prevents proper aeration (Erstad and Gislerød, 1994).

St. Hilaire (2003) published the first report of the propagation of Mexican baldcypress or Montezuma cypress [reported as *Taxodium mucronatum* Ten., but treated here as asserted by Denny and Arnold (2007) as a variety of baldcypress (*Taxodium distichum* var. *mexanum* Gordon)] by softwood cuttings. St. Hilaire took cuttings from the lower branches of an 11-year-old tree and from two two-year-old trees in separate locations in New Mexico. The cuttings were wounded by scraping off the epidermis and phloem on opposite sides of the basal end with a razor blade and treated with a talc
containing 3000 and 8000 µg · g⁻¹ IBA, respectively. Cuttings were destructively harvested after 13 weeks. The study was duplicated the following year. In both years the only cuttings that rooted were from the two-year-old tree located in Los Lunas, New Mexico (lat. 34° 48’ 18” N; long. 106° 43’ 42” W). In the first year the cuttings from that tree rooted at an average of 65%, while the following year the average was 10%. In this study the level of rooting hormone was determined not to be of statistical significance ($P \leq 0.05$). Zhou (2005) conducted propagation studies on a baldcypress called T302 (a baldcypress X Montezuma cypress cross). The studies measured the effect of wounding, rates of hormone and a plant growth stimulator called Regal Crown® (Regal Chemical Co., Alpharetta, Ga.) Cuttings were dispersed into four different experiments conducted at four times throughout the year. One experiment conducted in the summer of 2006 showed an increase in rooting percentage with an increase in hormone rate. Summer cuttings dipped in 5000 and 10000 mg·L⁻¹ K-IBA rooted at an average of approximately 58 and 68%, respectively. The control and 2500 mg·L⁻¹ K-IBA rooted at a much lower percentage. Zhou (2005) also reported a significant increase in rooted cuttings due to incision wounding. No effect on rooting was observed due to the Regal Crown® growth stimulator.

Copes and Randall (1993) searched for species to help prevent erosion on shores of reservoirs and chose baldcypress because of its ability to grow in partial submersion. Due to limited availability of baldcypress seed from trees in northern climates, asexual propagation from cuttings was investigated. The study showed that baldcypress cuttings rooted best in a substrate that averaged 50% water content by weight (the highest percentage tested in this study). It was suggested that the cuttings may have rooted at a
higher percentage if a higher average water content was tested. Pezeshki and DeLaune (1994) tested the influence of the age of the donor tree on adventitious rooting of cuttings and how rooting hormone affects cuttings. Two treatments were assessed, one with no hormone and one with the application of a commercial rooting powder, ROOTONE® (Ferti-lome Co., Bonham, TX). ROOTONE® is commonly used to stimulate adventitious rooting of shoot cuttings of many species and has an active ingredient of 1,000 mg · L⁻¹ indole-3-butyric acid, IBA. Cuttings from trees that were between 25 and 50 years old rooted at a rate of 12% regardless of the hormone treatment, while cuttings from one-year-old trees rooted at rates of 75% for cuttings treated with hormone and 88% for untreated cuttings. However, the hormone-treated cuttings taken from the one-year old plants that did root showed a significant (\( P \leq 0.05 \)) increase in shoot dry weight when compared to the rooted, untreated cuttings.

B. Topophysis / Cyclophysis

Topophysis has been defined in a number of different ways. One of the first attempts to reconcile these conflicting definitions was conducted by Olesen (1978) in which the term topophysis was proposed as “the phenomenon that scions, buddings and cuttings for some time after grafting, budding or rooting maintain the branch-like growth habit they had as shoots on the ortet”. This definition was challenged by Dodd and Power (1988) which defined topophysis alternatively as “states of differentiation of meristems on the ortet that vary according to the hierarchical order of the meristem within a branched system.” The meaning of the term cyclophysis is much less controversial. The term is defined as “the process of maturation of the apical meristems” (Olesen, 1978). Power et
al. (1988) discussed how the effects of topophysis and cyclophysis are often confounded in experiments which did not permit the two phenomena to be separated.

Much research has been conducted concerning topophysis and cyclophysis, but none could be found by name concerning the affects of either phenomena on baldcypress \[\textit{Taxodium distichum} \text{(L.) L.C. Rich.}\]. Upon further review however, a few studies conducted with baldcypress researched the effect of the age of the ortet on rooting quantity and quality (Pezeshki and DeLaune, 1994; St.Hilaire, 2003). This research does in essence test the effects of cyclophysis on baldcypress. Still, none was found that followed the cuttings through development to ascertain the effects of cyclophysis on growth habit. Black (1973) discovered that in Douglas-fir, \textit{Pseudotsuga menziesii} (Mirb.) Franco, cuttings branch position in the crown made a significant difference in rooting percentage of cuttings and that there was a varied response in timing for rooted cuttings to reorient from a plagiotropic (away from vertical) to an orthotropic (vertical) growth habit. Smith et al. (1956) emphasized topophysis in a vegetative propagation study of black cottonwood (\textit{Populus trichocarpa} Torr. and Gray) and Carolina poplar (\textit{Populus canadensis} var. \textit{eugenei} Simon-Louis). Cuttings were taken from three different positions on each donor plant. In each species there was a significant correlation between both cutting survival and growth and original orientation of the cuttings on the ortet. Average growth rates were calculated for both species. Cuttings from leaders averaged 30.5 cm of vertical growth, those taken from primary branches next to the leader averaged 20.3 cm of vertical growth and those taken from secondary branches grew 11.4 cm on average. Both of these studies were limited to measuring affects of topophysis on amount of growth, not orientation of the rooted cuttings (ramets).
Perhaps the most closely-related study, due to its qualitative nature, was by Power et al. (1988). Topophysis was studied in coast redwood \textit{[Sequoia sempervirens (D. Don) S.F.L. Endlicher]}, cuttings. Cuttings taken from terminal branches predominantly grew in an orthotropic manner, while primary and secondary branches grew progressively more plagiotropic in habit. It was also noted that more mature donor plants rendered a cutting that was more inclined to exhibit the negative effects of topophysis.

The purpose of experiments reported in this thesis were: 1) to examine the commercial viability of propagating the previously-selected clones of \textit{Taxodium} via cuttings based on rooted cutting quantity and quality 2) to determine which stages of development and hormone concentrations would yield optimum rooting percentages and highest quality adventitious root systems 3) to determine the combination of rooting substrate, wounding treatment and K-IBA concentration that yielded the optimal percentage of rooted cuttings and a root system of optimal quality 4) to determine whether or not topophysis significantly affects the orientation and/or growth of ramets in baldcypress and 5) to determine if branch angle divergence from the trunk of the ortet impacts the degree of topophysis observed on the ramets from rooted shoot tips.
CHAPTER II

INFLUENCE OF TIMING AND ROOTING HORMONE CONCENTRATIONS ON ROOTING QUANTITY AND QUALITY OF BALDCYPRESS

Baldcypress, *Taxodium distichum* (L.) Rich., is a highly adaptable tree of significant ecological importance in the Southeastern U.S. (Arnold, 2002; Pezeshki and DeLaune, 1994). The genus *Taxodium* Rich. includes three different varieties: baldcypress (*Taxodium distichum* (L.) Rich. var. *distichum*), pondcypress (*Taxodium distichum* var. *imbricarium* (Nutt.) Croom) and montezuma cypress (*Taxodium distichum* var. *mexicanum* Gordon) (Denny and Arnold, 2007). With the inclusion of these three varieties the native range of *Taxodium* extends from the east coast of the U.S. to southern Texas and into Mexico (Denny and Arnold, 2007). Though the species is highly adaptable, certain environments do affect its growth. *Taxodium* displays stress-induced responses when grown in high pH soils, exposed to prolonged drought or when salt-laden spray from irrigation contacts the foliage (Arnold, 2002, Denny, 2007, Dirr, 1998). Denny (2007) made selections for tolerance to these stresses and recommended that studies be conducted on the asexual propagation of these clonal selections by cuttings.

*Taxodium* is typically commercially propagated in one of three ways. It is grown from seed, grafted or produced via rooting shoot cuttings. Baldcypress seeds exhibit a dormancy that is easily overcome with stratification (Dirr, 1998); however, seedling material lacks uniformity (Pezeshki & DeLaune, 1994). Grafting baldcypress is a reliable method of propagation (Dirr, 1998); however, it is the most expensive of the three previously mentioned (Thomsen, 1978). Though a relatively small amount of research
has been conducted, vegetative propagation by cuttings yields uniform plants and through selection can be used to expedite narrow sense heritability genetic improvement in this species (Pezeshki & DeLaune, 1994). Relatively high percentages of successful rooting have been reported for *Taxodium* (Copes and Randall, 1993; Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005), supporting the practice of commercially propagating baldcypress by cuttings.

Copes and Randall (1993) reported 58% rooting of baldcypress when using 3 year old ramets that were initially taken from 20 year old ortets. The authors attributed this higher percentage to the 50% moisture content of the rooting substrate which was the wettest tested. Pezeshki and DeLaune (1994) found that the rooting percentage of hardwood baldcypress cuttings decreased significantly ($P \leq 0.05$) when cuttings were taken from branch tips of 25 – 50 year-old trees versus one year-old trees. Additionally, when the cuttings taken from the one year-old trees were treated with a 1,000 mg·L$^{-1}$ indole-3-butyric acid (IBA) (ROOTONE®, Ferti-lome Co., Bonham, TX) they rooted at a similar percentage as those that received no IBA, but IBA treated cuttings had significantly greater ($P \leq 0.05$) shoot dry weights. St. Hilaire (2003) researched the rooting of *Taxodium distichum* var. *mexicanum* softwood cuttings. In this study an increase in rooting percentage was exhibited when cuttings were treated with increasing amounts of IBA. In one year of the study rooting percentages were 48 and 82 for 3000 and 8000 mg·L$^{-1}$ IBA, respectively. Cuttings over all treatment combinations rooted at an average of 65% the first year and 10% the following year, indicating that the age of the ortet may be a limiting factor in successful propagation. This result agrees with the previously-discussed study by Pezeshki and DeLaune (1994), as well as work done by
Moore (1970). Zhou (2005) reported that rooting percentages increased on a baldcypress clone T302 (a baldcypress x Montezuma cypress cross) with increasing levels of K-IBA (potassium salts of indole-3-butyric acid). Cuttings treated with 5,000 and 10,000 mg·L⁻¹ K-IBA rooted at a significantly higher \( P \leq 0.05 \) rate than did cuttings treated with 0 and 2,500 mg·L⁻¹ K-IBA.

The objective of the current study was to examine the commercial viability of propagating the previously-selected clones of *Taxodium* via cuttings and to determine which stages of development and hormone concentrations would yield optimum rooting percentages and highest quality adventitious root systems.

**A. Materials and Methods**

Terminal shoot tip cuttings were taken from 4 year-old baldcypress selections grown in a research plot at Texas A&M University, College Station, Texas (lat. 30°37’645”N long. 96°22’319”W) or at the Texas AgriLife Research/Extension Center in Dallas, Texas (lat. 32°59’013”N long. 96°46’004”W). In an effort to test differing stages of stem maturity, cuttings were rooted at three times during the year. Softwood cuttings were taken from the College Station site on 16 May 2008 and from the Dallas site on 19 May 2008, semi-hardwood cuttings were taken from Dallas on 24 July 2008 and from College Station on 28 July 2008, and hardwood cuttings were taken from Dallas on 7 January 2009 and from College Station on 8 January 2009. All cuttings, except those taken from the College Station site on 16 May 2008, were kept overnight in a cooler at 8.7°C to ensure hydration. Cuttings were placed in plastic bags partially filled with water and stuck (planting the unrooted basal end in the substrate) the following day. Before
sticking, cuttings were trimmed to 10 cm to 15 cm in length and treated with one of three different concentration rates of potassium salts of indole-3-butyric acid (K-IBA) (Sigma-Aldrich Chemical, St. Louis, Mo.) The concentration rates of K-IBA were 0 (control), 7,500 and 15,000 mg·L⁻¹. The basal end of each cutting was submerged 5 cm deep into the solution for 5 sec. Cuttings were stuck 5 cm deep in a 1.5:1 peat:perlite (Sunshine® Peat Moss; Sunshine® Strong Lite coarse grade perlite, Sun Gro® Horticulture, Seba Beach, AB, Canada) substrate in black plastic flats, 10cm tall x 36 cm wide x 51 cm long (Dyna-flat™, Kadon Corp., Dayton, Ohio). The cuttings were placed in a greenhouse in a modified randomized completed block consisting of three blocks containing ten cuttings of each combination of clone and K-IBA treatment at each of the three stages of stem development. Reverse osmosis water was misted intermittently through high pressure mist nozzles for a period of 10 sec on a 16-min cycle during the daylight hours. Average substrate temperatures were measured with the Omega® HH309 Data Loggerthermometer (Omega Engineering, Inc., Stamford, CT.). Photosynthetically active radiation (PAR) was measured with a ceptometer (AccuPAR, Decagon Devices, Inc., Pullman, WA) randomly throughout the experiment or at concurrent times during the following year.

Cuttings were allowed to root for 8 weeks and then destructively harvested. Softwood, semi-hardwood and hardwood treatments were harvested 14 – 21 July, 22 September – 2 October, and 9 – 16 March, respectively. Information gathered from each cutting included determinations of the production of callus tissue and adventitious root formation and the number of roots produced per cutting. From each replicate treatment combination 5 rooted cuttings were randomly selected from each block on which root
length measurements and root and shoot dry masses were measured. In replicate treatment combinations that did not yield 5 rooted cuttings, cuttings that did root were selected along with randomly selected non-rooted cuttings until 5 samples were obtained for shoot dry mass measurements. Cuttings used for sampling that did not root were not included in mean estimations of root quality parameters, root number per cutting, root dry mass and total and average root length in order to ensure accurate estimates of adventitious root quality of those cuttings which rooted. Due to low rooting levels in hardwood cuttings, the cuttings which were not selected for destructive measurements at the predetermined harvest date were returned to the mist bed for an additional four weeks to determine if they might root if given additional time. Possible interactions between the clone propagated, K-IBA concentrations tested and stage of cutting maturation were analyzed using the General Linear Models Procedures in SAS in a stepwise fashion from beginning with the highest order interactions and progressing to the main effects if an effect was not significant in a higher order interaction. Means were compared using least squared means procedures (SAS 9.1 for Windows, Institute, Cary, N.C.).

B. Results and Discussion

Environmental conditions in the mist bench varied somewhat depending upon the time of year in which the stages of cutting development were rooted. The PAR measurements at canopy level were 403.1 µmol · m⁻² · s⁻¹, 461.1 µmol · m⁻² · s⁻¹ and 284.7 µmol · m⁻² · s⁻¹ for the softwood, semi-hardwood and hardwood treatments, respectively. Mean temperatures in the rooting substrates were 29.5 °C, 27.0 °C and 20.6°C for the softwood, semi-hardwood and hardwood treatments, respectively.
Significant two-way interactions (Table 1) were present for both rooting quantity measures, rooting percentage and callus percentage ($P \leq 0.0001$ and $P \leq 0.05$, respectively), while significant ($P \leq 0.01$) three-way interactions were shown for all rooting quality measures (root number, root length, and root dry mass). All parameters measured, except mean root length, were significantly affected by the main effects of K-IBA concentration, stage of cutting development and clone propagated (Table 1).

An analysis of variance of mean rooting and callus percentages indicated significant interactions among the stages of development and clones propagated ($P \leq 0.0001$) and among the stages of development and K-IBA concentrations ($P \leq 0.0001$, $P \leq 0.05$, for root percentage and callus percentage, respectively) (Table 1). For most clones (15 of 24 tested), the softwood stage of development produced a greater percentage of rooted cuttings (Figure 1). Cuttings tested in the semi-hardwood stage generally rooted at lower percentages than those in the softwood stage with the exception of clone EP5IC13, a provenance of *T. distichum* var. *imbricarium* (Table 2), which rooted at a significantly higher percentage in the semi-hardwood stage ($P \leq 0.01$). Callus percentages however displayed no significant difference between softwood and semi-hardwood in 20 of the 24 clones (Figure 2). No clone had rooting percentages above 2% in the hardwood stage when averaged across K-IBA concentrations after eight weeks in the mist bench. Callus percentages in the hardwood stage, though significantly lower than the softwood and semi-hardwood stages, were generally much greater than the hardwood rooting percentages realized in the current research. Since baldcypress roots emanate from callus tissue rather than pre-formed root initials (author’s observation) the greater number of callused cuttings than rooted cuttings suggests the potential for greater improvement in
Table 1. Levels of significance of analysis of variance for select rooting parameters and shoot dry mass.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Rooting %</th>
<th>Callus %</th>
<th>Root number</th>
<th>Total root length</th>
<th>Mean root length</th>
<th>Root dry mass</th>
<th>Shoot dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>**** z</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>ns</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>Clone</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>Stage x clone</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>K-IBA concentration</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>ns</td>
</tr>
<tr>
<td>Stage x K-IBA conc.</td>
<td>****</td>
<td>*</td>
<td>****</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Clone x K-IBA conc.</td>
<td>ns</td>
<td>ns</td>
<td>****</td>
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<td>**</td>
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<td>*</td>
</tr>
<tr>
<td>Stage x clone x K-IBA</td>
<td>ns</td>
<td>ns</td>
<td>****</td>
<td>**</td>
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<td>**</td>
</tr>
</tbody>
</table>

*significance at $P \leq 0.05, 0.01, 0.001, 0.0001 = *, **, ***, ****, respectively; ns = not significant at $P \leq 0.05$. *
Table 2. Localities of provenances of baldcypress used for propagation testing. Adapted from Denny (2007).

<table>
<thead>
<tr>
<th>Open-pollinated family</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Locale</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP8D</td>
<td>31°33’36’N</td>
<td>91°26’24’W</td>
<td>Mississippi River, LA</td>
</tr>
<tr>
<td>EP7D</td>
<td>30°23’24’N</td>
<td>88°55’48’W</td>
<td>Biloxi, MS</td>
</tr>
<tr>
<td>EP5I</td>
<td>30°27’0’N</td>
<td>88°6’36’W</td>
<td>Fowl River, AL</td>
</tr>
<tr>
<td>EP3D</td>
<td>29°5’24’N</td>
<td>91°12’6’W</td>
<td>Bayou Teche, LA</td>
</tr>
<tr>
<td>EP2D</td>
<td>29°48’0’N</td>
<td>91°47’24’W</td>
<td>Iberia Parish, LA</td>
</tr>
<tr>
<td>EP1D</td>
<td>32°20’24’N</td>
<td>94°42’0’W</td>
<td>Lake Cherokee, TX</td>
</tr>
<tr>
<td>TX3D</td>
<td>29°47’24’N</td>
<td>99°35’24’W</td>
<td>Sabinal River, TX</td>
</tr>
<tr>
<td>TX8D</td>
<td>29°52’48’N</td>
<td>97°55’48’W</td>
<td>San Marcos River, TX</td>
</tr>
<tr>
<td>MX1M</td>
<td>25°52’48’N</td>
<td>97°27’0’W</td>
<td>Southmost, TX</td>
</tr>
<tr>
<td>MX2M</td>
<td>25°18’36’N</td>
<td>104°38’24’W</td>
<td>Rio Nazas, MX</td>
</tr>
<tr>
<td>MX4M</td>
<td>27°51’0’N</td>
<td>101°7’48’W</td>
<td>Rio Sabinas, MX</td>
</tr>
<tr>
<td>MX5M</td>
<td>26°4’12’N</td>
<td>97°54’36’W</td>
<td>Progreso, TX</td>
</tr>
<tr>
<td>MX6M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*location of the provenance is unknown*
Figure 1. Percentage of rooted cuttings for each of 24 clones in the three developmental stages tested (Softwood, Semi-hardwood and Hardwood). Each graph contains clones from similar provenances: (A) clones from eastern provenances (EP), (B) three clones from eastern provenances (EP) and three clones from central Texas provenances (TX), (C) clones from south Texas and Mexican provenances (MX), (D) clones from south Texas and Mexican provenances, n = 9.
Figure 2. Percentage of callused cuttings for each of 24 clones in the three developmental stages tested (Softwood, Semi-hardwood and Hardwood). Each graph contains clones from similar provenances: (A) clones from eastern provenances (EP), (B) three clones from eastern provenances (EP) and three clones from central Texas provenances (TX), (C) clones from south Texas and Mexican provenances (MX), (D) clones from south Texas and Mexican provenances, n = 9.
rooting percentages of hardwood cuttings may be possible in the future as optimal rooting conditions are further defined. Those cuttings returned to the mist bench for an additional four weeks did not root in increased numbers to any substantial extent, suggesting that slower root development under the slightly cooler temperatures and lower irradiances present during the winter rooting of hardwood cuttings was not likely the cause of the poor rooting responses of the hardwood cuttings.

The interaction in rooting and callus percentage among stages of development and K-IBA concentrations (Figures 3A and 4A), however appears to be an artifact due to the severe lack of rooting and the decreased incidence of callus tissue formed in the hardwood stage of development. A supplemental analysis of rooting and callus percentage data omitting the hardwood stage showed no significant ($P \geq 0.05$) interaction among stages of development and K-IBA concentrations. The main effect of K-IBA concentration on rooting and callus percentage showed no significant difference ($P \geq 0.05$) between the 7,500 and 15,000 mg·L$^{-1}$ concentrations, while the 0 mg·L$^{-1}$ control treatment produced significantly fewer callused and rooted cuttings than the other two treatments (Figures 3B and 4B). These results follow those of previous research on seedling (St. Hilaire, 2003) or clonal cuttings (Zhou, 2005) of *Taxodium*. With the 15,000 mg·L$^{-1}$ concentration, many cuttings exhibited tissue toxicity in the basal portions of the cuttings which in most cases did not affect the ability of the cutting to root above the affected tissue, but rather the overall quality of the cutting was affected. Typically the toxicity was manifested by the basal 5 cm of the cutting (the portion that had been dipped into the K-IBA solution) becoming necrotic followed by root formation at the lower-most portion of the remaining live tissue (Figure 5).
Figure 3. Rooted cutting percentage for the interaction among stage of development and K-IBA concentration and main effect of K-IBA concentration. (A) Percentage of cuttings rooted for each of three stages of development (Softwood, Semi-hardwood and Hardwood) and three concentrations of K-IBA (0, 7,500 and 15,000 mg·L⁻¹ K-IBA), n = 72. (B) Percentage of cuttings rooted across three concentrations of K-IBA (0, 7,500 and 15,000 mg·L⁻¹ K-IBA), n = 216.
Figure 4. Callused cutting percentage for the interaction among stage of development and K-IBA concentration and main effect of K-IBA concentration. (A) Percentage of cuttings callused for each of three stages of development (Softwood, Semi-hardwood and Hardwood) and three concentrations of K-IBA (0, 7,500 and 15,000 mg·L⁻¹ K-IBA), n= 72. (B) Percentage of cuttings callused across three concentrations of K-IBA (0, 7,500 and 15,000 mg·L⁻¹ K-IBA), n = 216.
Figure 5. Necrotic tissues at the basal portion of a cutting treated with 15,000 mg·L⁻¹ K-IBA was likely due to toxicity caused by the high rate of rooting hormone.
Significant three-way interactions ($P \leq 0.01$) were found among stages of development, K-IBA concentrations and the clones propagated for all root quality parameters, as well as shoot dry mass (Table 1). The three-way interaction for number of roots per rooted cutting proved to be highly significant ($P \leq 0.0001$). The greatest mean number of roots per rooted cutting were generally found on softwood cuttings from the south Texas and Mexico region which were treated with either 7,500 or 15,000 mg·L$^{-1}$ K-IBA (Figure 6). Cuttings treated with 15,000 mg·L$^{-1}$ K-IBA had significantly greater root numbers per cutting which agrees with St. Hilaire (2003), but contradicts that found in Pezeshki and DeLaune (1994) where root number per cutting was not significantly ($P \leq 0.05$) affected by treatment with IBA. The root density ranking (RDR) discussed in Zhou (2007) presumably included a qualitative measurement of root number per cutting however no direct quantitative measurements were given. Zhou’s RDR followed the results found in the current research in that significantly greater root quality measures were found on cuttings treated with relatively high rates of hormone (10,000 mg·L$^{-1}$ K-IBA) than with the control (0 mg·L$^{-1}$ K-IBA). Very few roots per cutting were found in hardwood cuttings across all families and K-IBA concentrations (Figure 6). Generally in the softwood and semi-hardwood stages, cuttings treated with 0 mg·L$^{-1}$ K-IBA grew fewer roots per cutting than did the two greater concentrations across all families (Figure 6). A geographic breakdown of the 24 families tested shows three relatively distinct groups. The majority of the clones from south Texas and Mexico (clonal designations beginning with MX) were in the group that yielded the greatest number of roots per rooted cutting while the bulk of the clones from eastern U.S. provenances (clonal designations beginning with EP) were found in the group that
Figure 6. Mean number of roots per cutting for the 24 clones tested. A-F are the mean number of roots per cutting for six clones from the eastern provenances (EP) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 6. Continued. G-L are the mean number of roots per cutting for three clones from the eastern provenances (EP) and for the three clones from central Texas provenances (TX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 6. Continued. M-R are the mean number of roots per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 6. Continued. S-X are the mean number of roots per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
performed most poorly. An intermediate group contained two south Texas/Mexico clones as well as a clone from the eastern provenances. Clones from central Texas provenances (clonal designations beginning with TX) were found in both the greatest and least roots per cutting groups. This distribution by provenance conforms to results found by Denny (2007) for regional variation in several stress tolerances associated with *Taxodium* and indicates that perhaps baldcypress cuttings taken from trees with more southerly and westerly provenances show more vigor in propagation than those taken from more eastern and northern provenances.

Total and mean root length were also significantly affected \( (P \leq 0.01) \) by three-way interactions among stages of development, K-IBA concentrations and the clones propagated (Table 1). For both root length parameters measured, responses followed geographical gradient patterns reported by Denny (2007) for drought, soil alkalinity, and foliar salinity exposure responses. Total root length was generally greater in the south Texas and Mexican provenances, particularly in the softwood stage of development when treated with either 7,500 or 15,000 mg·L\(^{-1}\) K-IBA (Figure 7). Cuttings from eastern provenances had smaller total root lengths than the south Texas/Mexican and central Texas provenances, and statistically significant differences in total root length were rare across stages of development and K-IBA concentrations. Central Texas propagules tended to follow the same trend as the eastern provenances with the exception of TX8DC38 which had the greatest total root length among clones observed by greater than 50% (Figure 7L). The closely related parameter of mean root length differed from total root length in that statistical differences amongst stages of development were more common in cuttings from eastern provenances (Figure 8). Cuttings taken in the semi-
Figure 7. Total root length per cutting (cm) for the 24 clones tested. A-F are the total root length per cutting (cm) for six clones from the eastern provenances (EP) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 7. Continued. G-L are the total root length per cutting (cm) for three clones from the eastern provenances (EP) and for the three clones from central Texas provenances (TX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 7. Continued. M-R are the total root length per cutting (cm) for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 7. Continued. S-X are the total root length per cutting (cm) for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 8. Mean root length per cutting (cm) for the 24 clones tested. A-F are the mean root length per cutting (cm) for six clones from the eastern provenances (EP) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 8. Continued. G-L are the mean root length per cutting (cm) for three clones from the eastern provenances (EP) and for the three clones from central Texas provenances (TX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 8. Continued. M-R are the mean root length per cutting (cm) for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 8. Continued. S-X are the mean root length per cutting (cm) for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
hardwood stage of development generally had greater mean root lengths than did softwood and hardwood cuttings. Softwood cuttings from the south Texas and Mexican provenances had generally greater mean root lengths than did semi-hardwood cuttings however the differences were less marked. This decrease in mean root length in cuttings taken in the softwood stage of development was caused by the greater root numbers seen in softwood cuttings, as opposed to a reduction in total root length. Mean root lengths were generally greater in cuttings treated with 15,000 mg·L⁻¹ K-IBA (Figure 8) which concurs with results found in one year of the study reported by St. Hilaire (2003) but contradicts the results found in Pezeshki and DeLaune (1994).

Significant three-way interactions (\(P \leq 0.01\)) among stages of development, K-IBA concentrations and clones propagated were also observed for root and shoot dry mass (Table 1). Both dry mass parameters also displayed the aforementioned geographical gradient responses described in Denny (2007). Root dry masses were greater in the south Texas and Mexican provenances than in the eastern provenances (Figure 9). South Texas and Mexican cuttings taken in the softwood stage generally had greater root dry masses, especially in the 7,500 and 15,000 mg·L⁻¹ K-IBA concentrations (Figure 9). Semi-hardwood cuttings from these provenances were intermediate between softwood and hardwood cuttings. Similar to the root length parameters, the clone TX8DC38 was the lone clone from central Texas that had greater dry masses. TX8DC38 cuttings taken in the softwood stage and treated with 7,500 and 15,000 mg·L⁻¹ K-IBA concentrations had the greatest root dry mass compared to all clones (Figure 9L). The root dry mass of all cuttings taken in the hardwood stage was not significantly different from 0 g (Figure 9). Conversely, shoot dry mass was generally greater in the cuttings from the eastern
Figure 9. Root dry mass (g) per cutting for the 24 clones tested. A-F show the root dry mass (g) per cutting for six clones from the eastern provenances (EP) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 9. Continued. G-L show the root dry mass (g) per cutting for three clones from the eastern provenances (EP) and for the three clones from central Texas provenances (TX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 9. Continued. M-R show the root dry mass (g) per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 9. Continued. S-X show the root dry mass (g) per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 10. Shoot dry mass (g) per cutting for the 24 clones tested. A-F show the shoot dry mass (g) per cutting for six clones from the eastern provenances (EP) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 10. Continued. G-L show the shoot dry mass (g) per cutting for three clones from the eastern provenances (EP) and for the three clones from central Texas provenances (TX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 10. Continued. M-R show the shoot dry mass (g) per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
Figure 10. Continued. S-X show the shoot dry mass (g) per cutting for six clones from south Texas and Mexican provenances (MX) for each of the three stages of development and K-IBA concentrations tested, n = 3.
provenances. This is may be due to the ability of the trees from eastern provenances to continue more meristematic growth through cooler fall temperatures than their south Texas and Mexican counterparts. Shoot dry masses from the eastern provenances were also variable in reaction to K-IBA concentrations and cutting stages of development. The south Texas and Mexican provenances were similarly variable but with a slight tendency for greater shoot masses in the semi-hardwood stage (Figure 10). Again, the hardwood stage produced the lowest masses of shoot growth. This was likely due to the fact that all hardwood cuttings were void of foliage.

*Taxodium* cuttings in this study generally rooted at a greater percentage and were higher in quality when collected as softwood cuttings from provenances in south Texas and Mexico and treated with either 7,500 or 15,000 mg·L⁻¹ K-IBA. Rooting quantity and quality from semi-hardwood cuttings were generally found to be great enough to warrants commercial propagation. Cuttings from the hardwood stage in this study rooted so poorly that the practice of taking hardwood cuttings of baldcypress is unadvisable at this time. Though the clones from south Texas and Mexican provenances rooted at greater quantities and quality on the whole, the clone that performed best overall was TX8DC38, a central Texas clone.
CHAPTER III

INFLUENCE OF SELECT FACTORS ON ROOTING QUANTITY AND QUALITY OF BALDCYPRESS

Baldcypress, *Taxodium distichum* (L.) Rich., is a highly adaptable tree of significant ecological importance in the Southeastern U.S. (Arnold, 2008; Pezeshki and DeLaune, 1994). Baldcypress is typically propagated commercially in one of three ways. It is grown from seed, grafted or produced via cuttings (Dirr, 1998; Thomsen, 1978). Baldcypress seeds exhibit a dormancy that is easily overcome with stratification (Dirr, 1998); however, seedling material lacks uniformity (Pezeshki & DeLaune, 1994). Grafting baldcypress is a reliable method of propagation (Dirr, 1998); however, it is the most expensive of the three previously mentioned methods (Thomsen, 1978). Though a relatively small amount of research has been conducted, vegetative propagation by cuttings yields uniform plants and through selection, can be used to expedite narrow sense heritability genetic improvement in this species (Pezeshki & DeLaune, 1994). Relatively high percentages of successful rooting have been reported for *Taxodium* (Copes and Randall, 1993, King (previous chapter of this thesis), Pezeshki and DeLaune, 1994, St. Hilaire, 2003, Zhou, 2005), supporting the practice of commercially propagating baldcypress by cuttings, but rooting percentages and rooted cutting quality vary among genotypes.

There are many methods for manipulating cuttings to encourage optimal adventitious root formation. Most of these methods fall under three main categories: management of the ortet, treatment of cuttings during propagation, or management of the environment
surrounding the cuttings during propagation (Hartmann et al., 2002). The majority of the research conducted on asexual propagation of baldcypress by cuttings focuses on the treatment of cuttings during propagation, specifically using different types and concentrations of auxin (King, previous chapter of this thesis; Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005) or wounding the basal portion of the cutting (Zhou, 2005). Commonly, the management of the ortet (plant from which the clone is derived) is also tested by making the timing of cutting harvest the independent variable in an experiment (King, previous chapter; Zhou, 2005). It is not common, however, to manipulate the environment surrounding the cuttings during propagation. In most instances the environment is a constant across all treatment combinations (Copes and Randall, 1993; King, previous chapter; Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005). Lu et al. (2004) was the only study found that manipulated the surroundings of baldcypress cuttings by planting them in three different rooting substrates.

Rooting substrates are typically comprised of an organic component (i.e. peat moss) and a mineral component (i.e. perlite) which provides aeration. A rooting substrate serves a number of purposes including securing the cutting in place, providing water for the cutting, supplying sufficient aeration for adventitious rooting and reducing the amount of irradiance that reaches the base of the cutting (Hartmann et al., 2002). The uptake of water in cuttings is proportional to the content of water, by volume, in the rooting substrate (Grange and Loach, 1983; Rein et al., 1991) while water in excess prevents proper aeration (Erstad and Gislerod, 1994). Copes and Randall (1993) found that baldcypress rooted at greater percentages when stuck in the wettest portion of a mist
bench tested (50% water content, by weight) as opposed to two more aerated portions (40% and 29% water content by weight, respectively). Little is known about the specific substrate requirements for rooting baldcypress cuttings.

All cuttings are by definition wounded, however increasing the area of mechanical wounding has been proven to enhance wound responses, including the division of the affected cells, which may lead to adventitious root formation (MacKenzie et al., 1986). Zhou (2005) included a wounding treatment in a number of experiments conducted on a baldcypress clone called T302 (a baldcypress x Montezuma cypress cross). Cuttings were incision wounded by cutting 1cm along the vertical axis. One experiment conducted by Zhou (2005) from 3 August 2006 to 9 November 2006 included a wounding treatment that did not significantly affect ($P \geq 0.05$) rooting percentage or root density ranking (RDR) (a qualitative method of ranking root system quality). Conversely, another experiment which commenced on 12 November 2006 and ended on 6 February 2007 included a wounding treatment that did significantly affect ($P \leq 0.05$) both rooting percentage and RDR. Overall wounded treatments produced cuttings with a 1.6 and 1.8 times greater rooting percentage and RDR, respectively, than did non-wounded treatments.

Treating cuttings with differing auxin concentrations or types is one of the most common treatments in the research on baldcypress propagation by cuttings (King, previous chapter; Pezeshki and DeLaune, 1994; St. Hilaire, 2003; Zhou, 2005). Pezeshki and DeLaune (1994) found that when baldcypress cuttings were taken from one-year-old trees and treated with a 1,000 mg·L$^{-1}$ concentration of indole-3-butyric acid (IBA) (ROOTONE®, Ferti-lome Co., Bonham, TX) they rooted at a similar percentage than
those that received no IBA but showed significantly greater ($P \leq 0.05$) shoot dry weights. St. Hilaire (2003) researched the rooting of *Taxodium distichum* (L.) Rich. var. *mexicanum* G. Gordon softwood cuttings. In this study an increase in rooting percentage was exhibited when cuttings were treated with increasing amounts of (IBA). In one year of the study rooting percentages were 48 and 82 for 3000 and 8000 mg·L$^{-1}$ IBA, respectively. Zhou (2005) found that rooting percentages increased with increasing levels of K-IBA (potassium salts of indole-3-butyric acid). Cuttings treated with 5,000 and 10,000 mg·L$^{-1}$ K-IBA rooted at a significantly higher ($P \leq 0.05$) rate than did cuttings treated with 0 and 2,500 mg·L$^{-1}$ K-IBA.

The objective of the current research was to determine the combination of rooting substrate, wounding treatment and K-IBA concentration that yielded the optimal percentage of rooted cuttings and a root system of optimal quality.

**A. Materials and Methods**

Softwood baldcypress cuttings were taken on 3 June 2009 from four-year-old trees grown in a research plot in College Station, Texas (lat. 30°37’645”N long. 96°22’319”W). Cuttings were kept overnight in a cooler at 8.7° C in plastic bags partially filled with water to ensure hydration and planted the following day, 4 June 2009. Before planting, cuttings were randomly assigned a factorial combination of a rooting hormone concentration, a wounding treatment, and a rooting substrate. Four concentrations of K-IBA (Sigma-Aldrich Chemical, St. Louis, Mo.) were tested: no hormone (control); low (5,000 mg·L$^{-1}$ K-IBA); medium (10,000 mg·L$^{-1}$ K-IBA); or high (15,000 mg·L$^{-1}$ K-IBA). Hormone treatments were administered by submerging the basal
end of the cutting 5 cm into the randomly assigned solution for 5 seconds. Half of the cuttings taken were wounded by making a 1 cm incision along the axis into the basal end of the cutting; the other half were not wounded (control). Three substrates were tested, 100% perlite (Sunshine® Strong Lite coarse grade perlite, Sun Gro® Horticulture, Seba Beach, AB, Canada), 100% peat moss (Sunshine® Peat Moss, Sun Gro® Horticulture, Seba Beach, AB, Canada) and a mix 1:1 perlite:peat moss. Bulk density, macro-pore space, micro-pore space (water-holding capacity), pH, electrical conductivity (EC) and particle size distribution measurements were taken for each substrate. Sieve sizes used for particle size distribution were 3.35 mm, 1.68 mm, 0.422 mm, 0.251 mm, 0.125 mm and 0.066 mm. Cuttings were planted 5 cm deep into the randomly assigned rooting substrate in black plastic flats, 10 cm x 36 cm x 51 cm (Dyna-flat™, Kadon Corp., Dayton, Ohio). Each factorial combination of auxin, substrate and wounding treatment was tested on 30 cuttings, ten in each of three blocks, which yielded a total of 720 total cuttings. Cuttings were planted in a modified randomized completed block design and flats were placed on benches in a polyethylene greenhouse at Texas A&M University Horticultural Gardens (College Station, Texas) in a randomized block pattern. Reverse osmosis water was misted intermittently through high pressure mist nozzles for a period of 10 sec. on a 16-min. cycle during daylight hours. Nozzles were located 46 cm above the rooting substrate. Substrate temperatures were measured with the Omega® HH309 Data Logger thermometer (Omega Engineering, Inc., Stamford, CT.). The photosynthetically active radiation encountered by the cuttings was measured with a ceptometer (Accupar, Decagon Devices, Inc., Pullman, WA) at canopy level randomly throughout the experiment. Cuttings were allowed to root for 8 weeks and were
destructively harvested on 5-10 August 2009. Information gathered from all cuttings included determinations of the production of callus tissue and root formation and the number of roots produced per cutting. From each replicate treatment combination, 5 rooted cuttings were randomly selected from which root length measurements and root and shoot dry mass were measured. In replicate treatment combinations that did not yield 5 rooted cuttings, cuttings that did root were selected along with randomly selected un-rooted cuttings until 5 samples were obtained. Possible interactions among K-IBA concentrations, wounding treatments and rooting substrates were analyzed using the general linear models procedure in SAS and means were compared using least squared means procedures (SAS 9.1 for Windows, Institute, Cary, N.C.). An arcsine transformation was applied to the rooting and callus percentage data to ensure a more normal distribution. The highest order interactions which were significant ($P \leq 0.05$) for a given parameter are presented. When higher order interactions were non-significant, data were pooled and significant lower order interactions or main effects are presented. When the interval concentrations K-IBA concentrations were significant, polynomial regression equations for those effects were fitted to the data and are presented when significant ($P \leq 0.05$).

**B. Results and Discussion**

Environmental conditions in the mist bench included a mean PAR measurement at canopy level of 461.1 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). The mean temperature in the rooting substrate was 27.9° C.
All substrate characteristics were significantly different ($P \leq 0.05$) among the three substrates tested with the exception of the particle size distribution in the smallest sieve used (0.066 mm) (Tables 3 and 4). The 100% peat moss and 100% perlite substrates were significantly ($P \leq 0.05$) different for most characteristics measured with the 1:1 mixed substrate typically being intermediate. The mixed substrate was not significantly different from the peat moss in bulk density, pH and EC, while the mixed substrate was not significantly different from the perlite in macro-pore space.

### Table 3. Characteristics of three rooting substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Bulk density (mg/cm³)</th>
<th>Macro-pore space (%)</th>
<th>Micro-pore space (%)</th>
<th>pH</th>
<th>Electrical conductivity (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Peat moss</td>
<td>104.0 a</td>
<td>0.168 b</td>
<td>0.567 a</td>
<td>4.87 b</td>
<td>168.3 a</td>
</tr>
<tr>
<td>1:1 Mix</td>
<td>95.5 a</td>
<td>0.388 a</td>
<td>0.475 b</td>
<td>4.90 b</td>
<td>152.3 a</td>
</tr>
<tr>
<td>100% Perlite</td>
<td>70.4 b</td>
<td>0.471 a</td>
<td>0.290 c</td>
<td>7.60 a</td>
<td>78.7 b</td>
</tr>
</tbody>
</table>

z – Means within columns, followed by the same letter are not different using least squares means comparisons at $P \leq 0.05$. Values represent means of 3 observations.

### Table 4. Particle size distribution of three rooting substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sieve 1 (3.35 mm) (g)</th>
<th>Sieve 2 (1.68 mm) (g)</th>
<th>Sieve 3 (0.422 mm) (g)</th>
<th>Sieve 4 (0.251 mm) (g)</th>
<th>Sieve 5 (0.125 mm) (g)</th>
<th>Sieve 6 (.066 mm) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Peat moss</td>
<td>41.77 a</td>
<td>11.27 c</td>
<td>38.13 a</td>
<td>7.93 ab</td>
<td>0.82 b</td>
<td>0.04 a</td>
</tr>
<tr>
<td>1:1 Mix</td>
<td>24.87 b</td>
<td>23.73 b</td>
<td>38.20 a</td>
<td>10.13 a</td>
<td>2.83 a</td>
<td>0.16 a</td>
</tr>
<tr>
<td>100% Perlite</td>
<td>26.43 b</td>
<td>41.00 a</td>
<td>25.00 b</td>
<td>5.83 b</td>
<td>1.71 ab</td>
<td>0.06 a</td>
</tr>
</tbody>
</table>

z – Means within columns, followed by the same letter are not different using least squares means comparisons at $P \leq 0.05$. Values represent means of 3 observations.
suggesting that both the peat moss and the perlite lent distinct characteristics to the 1:1 mix. To further reinforce this point, there were significant ($P \leq 0.05$) differences in micro-pore space (water-holding capacity) for all three substrates, with the mixed substrate exhibiting an intermediate water-holding capacity.

In this study all root and shoot parameters measured were significantly affected by at least one of the treatments tested with the exception of rooting percentage (Table 5). Two-way interactions were found for root dry mass and average root length and three-way interactions occurred in the shoot dry mass and root number parameters. Main effects were found for all other parameters measured.

Rooting percentage was not significantly ($P \leq 0.05$) affected by rooting substrates used, wounding treatments or K-IBA concentrations. The lack of significance in the

Table 5.
Levels of significance of analysis of variance for select rooting parameters and shoot dry mass.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Root Percent</th>
<th>Callus R:S ratio</th>
<th>Total length</th>
<th>Mean length</th>
<th>Number</th>
<th>Dry mass</th>
<th>Shoot Dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>ns</td>
<td>****</td>
<td>****</td>
<td>**</td>
<td>****</td>
<td>**</td>
<td>****</td>
</tr>
<tr>
<td>Wound</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Substrate x wound</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>K-IBA concentration</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td>Substrate x K-IBA conc.</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Wound x K-IBA conc.</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Substrate x wound x K-IBA</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

$^z$ – significant at $P \leq 0.05$, 0.01, 0.001, 0.0001 = *, **, ***, ****, respectively; ns = not significant at $P \leq 0.05$. 

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wounding and K-IBA treatments directly contradict the results of other experiments (St. Hilaire, 2003, Zhou, 2005) while Copes and Randall (1993) found similar results using much lower IBA treatments. Rooting substrates, though non-significant at $P \leq 0.05$, were significant treatment at $P \leq 0.1$ for rooting percentages. Callus percentage however, was significantly affected ($P \leq 0.0001$) by rooting substrates used (Table 5) and therefore gives cause to suggest that with time the rooting substrates might have significantly affected rooting percentage to a greater degree. Perlite, the substrate with the lowest water-holding capacity tested (Table 3), yielded the greatest percentage of callused cuttings at 85%, while peat moss, the substrate with the greatest water-holding capacity tested, yielded the fewest callus percentage at 53% (Figure 11). The mix of substrates was intermediate (62%). No other treatment tested significantly affected the percentage of callused cuttings (Table 5).

The root:shoot ratio was also significantly affected ($P \leq 0.0001$) only by the rooting substrates used (Table 5, Figure 12). The results encountered for root:shoot ratio were opposite of those observed for callus percentage in that the root:shoot ratio increased as the water holding capacity of the substrate increased, whereas the callus percentage decreased. Thus, when adventitious rooting occurred, a greater water holding capacity favored subsequent root growth, but a more porous substrate appeared to favor callus development. Peat moss yielded cuttings with a root:shoot ratio of 0.059, while cuttings planted in perlite had a ratio of only 0.013 with the 1:1 peat moss:perlite mix yielding an intermediate ratio (0.021) (Figure 12). No other treatment significantly affected root:shoot ratio (Table 5).
Figure 11. Percentage of callused cuttings for each of the three rooting substrates tested (100% peat moss, 100% perlite and a 1:1 mix of peat moss and perlite), n = 24.
Figure 12. Root:shoot ratio (g/g) of cuttings in three rooting substrates tested (100% peat moss, 100% perlite and a 1:1 mix of peat moss and perlite), n = 24.
For the total root length parameter, only main effects of rooting substrates, wounding treatments and K-IBA concentrations were significant \( (P \leq 0.01) \) (Table 5). Rooting substrates significantly affected the total root length per cutting (Figure 13) and the results were similar to those found for root:shoot ratio (Figure 12). Again, the substrate with the greater water holding capacity, peat moss, yielded cuttings with the greatest total root length (14.9 cm) while cuttings rooted in the peat moss: perlite mix and perlite alone grew root systems of 12.7 cm and 10.3 cm, respectively (Table 5, Figure 13). The concentration of K-IBA applied significantly affected total root length (Table 5, Figure 14). The greatest concentration tested, 15,000 mg·L\(^{-1}\) yielded cuttings with total root lengths of 16 cm while root systems which had the control (0 mg·L\(^{-1}\) K-IBA) applied were 10.8 cm per cutting. The two middle concentrations of K-IBA produced root systems of intermediate lengths. These results follow those of Copes and Randall (1993) closely and indicate that the burning (basal necrosis) which took place when cuttings were treated with higher levels of K-IBA (see chapter 2), did not affect total root length. The wounding treatments also showed significant differences in total root length (Table 5, Figure 15). The non-wounded treatment yielded cuttings of 14.5 cm in total length while the wounded cuttings only grew root systems of 10.8 cm. Findings of the current research pertaining to the wounding of the cutting contradict those of Zhou (2005) where wounding baldcypress cuttings not only significantly increased the rooting percentage but also improved root system quality. The mean root length in the current study was significantly affected \( (P \leq 0.05) \) by an interaction among the substrates and K-IBA concentrations (Table 5, Figure 16). The greatest average root lengths were found on cuttings grown in peat moss that had been treated with 15,000 mg·L\(^{-1}\) K-IBA. Cuttings
Figure 13. Total root length (cm) of cuttings in three rooting substrates tested (100% peat moss, 100% perlite and a 1:1 mix of peat moss and perlite), n = 24.
Figure 14. Total root length (cm) of cuttings in four K-IBA concentrations tested (0, 5,000, 10,000 and 15,000 mg·L⁻¹ K-IBA), n = 18.

\[ y = 1.74x + 8.3, r^2 = 0.87 \]
Figure 15. Total root length (cm) of cuttings when tested with one of two wounding treatments (wound, non-wound), n = 36.
Figure 16. Mean root length (cm) of cuttings in each of three rooting substrates (100% peat moss, 100% perlite and a 1:1 mix of peat moss and perlite), treated with one of four K-IBA concentrations (0, 5,000, 10,000 and 15,000 mg·L⁻¹), n = 6.
grown in perlite produced shorter average root lengths across K-IBA treatments while the peat moss:perlite mix had intermediate root lengths. Cuttings rooted in peat also responded more positively to lower (5,000 mg·L⁻¹) K-IBA concentrations than cuttings rooted in the mixed or perlite substrates (Figure 16). Again the greater root quality measures, in this case the average root length, were found in cuttings that were grown in the substrate with the highest water holding capacity and treated with the highest K-IBA concentration. On the whole, when callus percentage data is used as a ceiling for rooting potential and the response of callus percentage is compared to the response of the root quality measures (root:shoot ratio, total root length, average root length) there seems to be a tradeoff between achieving maximum possible rooting potential in a more aerated substrate and achieving optimal root quality in a substrate with a greater water holding capacity. This hypothesized tradeoff conflicts with results found by Copes and Randall (1993) in which rooting percentage increased with increasingly moist substrates (29%, 40% and 50% water content by weight, respectively). A significant \( P \leq 0.05 \) main effect of wounding was also found for mean root length (Table 5). The average length of a given root was 7.4 cm for non-wounded cuttings versus 6.1 cm for the wounded treatments (Figure 17), again contradicting Zhou (2005). This result could be due in part to the fact that cuttings in Zhou (2005) were taken later in the season and more mature cuttings might be less prone to the deleterious affects of wounding.

Root number per cutting was significantly affected \( P \leq 0.05 \) by a three-way interaction (Table 5). The greatest values of mean root number per cutting were just above four and were found in cuttings that were not wounded, treated with either 0 or
Figure 17. Mean root length (cm) of cuttings when tested with one of two wounding treatments (wound, non-wound), n = 36.
Figure 18. Number of roots per cutting when treated with one of four K-IBA concentrations (0, 5,000, 10,000 and 15,000 mg · L⁻¹), one of two wounding treatments (wound, non-wound) in (A) 100% perlite (B) a 1:1 mix of peat moss and perlite (C) 100% peat moss, n = 3.
5,000 mg·L\(^{-1}\) K-IBA and rooted in perlite (Figure 18A) or the mixed substrate (Figure 18B). The fewest roots per cutting were found in cuttings that had been treated with either 5,000 or 10,000 mg·L\(^{-1}\) K-IBA, planted in peat moss and either wounded or not (Fig. 18C). Linear regression models (Fig. 18C) indicated that in the peat moss treatment the wounded and non-wounded cuttings produced similar root numbers across all K-IBA concentrations while the perlite (Fig. 18A) and peat:perlite mix (Fig. 18B) treatments displayed some significant differences in mean root number. The wounded and non-wounded cuttings in the perlite treatment showed similar trends in relation to K-IBA concentrations however the non-wounded treatment produced significantly \((P \leq 0.05)\) greater numbers of roots in the 5,000 and 15,000 mg·L\(^{-1}\) K-IBA concentrations. The mix of substrates produced cuttings with numbers of roots that trended in opposite directions, where the non-wound treatment produced approximately four roots per cutting at the 0 mg·L\(^{-1}\) K-IBA concentration followed by a marked decrease at the intermediate K-IBA concentrations to an increase at the 15,000 mg·L\(^{-1}\) K-IBA concentration (Fig. 18A-C). This differs from the results reported in Pezeshki and DeLaune (1994) in which treatment with IBA did not significantly affect root number. The wounded treatment produced significantly \((P \leq 0.05)\) fewer roots per cutting at the 0 and 15,000 mg·L\(^{-1}\) K-IBA concentrations than did the non-wounded treatment with an increase in root number at the intermediate K-IBA concentrations, which again contradicts Zhou’s (2005) results.

Root dry mass was significantly affected \((P \leq 0.05)\) by an interaction between rooting substrates and wounding treatments (Table 5, Fig. 19) as well as a main effect of K-IBA concentrations (Fig. 20). The interaction appeared to be due to the root dry mass of the cuttings in the peat moss/non-wounded treatment which had an average mass 2.5 times
Figure 19. Root dry mass (g) of cuttings placed in one of three rooting substrates (100% peat moss, 100% perlite and a 1:1 mix of peat moss and perlite), when tested with one of two wounding treatments (wound, non-wound), n = 12.
Figure 20. Root dry mass (g) of cuttings when treated with one of four K-IBA concentrations (0, 5,000, 10,000 and 15,000 mg · L⁻¹), n = 18.

\[ y = 0.0003x^2 + 0.001x + 0.006, \quad R^2 = 0.60 \]
greater than any other treatment. All other treatments were not significantly different (Fig. 19). This result supports the case for higher water holding substrates yielding cuttings with higher quality root systems. The pattern of root dry mass in the K-IBA treatments was similar to those seen throughout the rest of the experiment (Fig. 20). The greatest K-IBA concentration yielded cuttings with the greatest root dry mass and cuttings in the control (0 mg·L⁻¹) had the least root dry mass with the 5,000 and 10,000 mg·L⁻¹ being intermediate, which again contradicted Pezeshki and DeLaune (1994) who found no effect of K-IBA applications on root quality measures.

A significant \(P \leq 0.05\) three-way interaction among rooting substrates, wounding treatments and K-IBA concentrations was observed in shoot dry masses (Table 5, Fig. 21A-C). The greatest shoot dry masses were found in cuttings stuck in perlite (Fig. 21A) across all K-IBA and wounding treatments while the lowest shoot dry masses were found for cuttings grown in the mixed (Fig. 21B) and peat moss (Fig. 21C) substrates with 10,000 and 15,000 mg·L⁻¹ K-IBA concentrations across wounding treatments. Linear regression models show an inverse relationship for the trends shown by wounded and non-wounded treatments in all three rooting substrates and across all K-IBA concentrations (Fig 21 A-C). Cuttings in the peat moss treatment (Fig 21C) showed trend lines for shoot dry mass in the non-wounded cuttings that were relatively flat with a small decrease for the intermediate K-IBA concentrations while the wounded treatment was almost inversely opposite with the only significant difference occurring at the 15,000 mg·L⁻¹ K-IBA concentration. The wounding treatments showed no significant difference in the mix substrate across all K-IBA concentrations but did trend in opposite directions (Fig. 21B). The perlite treatment showed significant differences \(P \leq 0.05\) at the 0
Figure 21. Shoot dry mass (g) of cuttings when treated with one of four K-IBA concentrations (0, 5,000, 10,000 and 15,000 mg · L⁻¹), one of two wounding treatments (wound, non-wound) in (A) 100% perlite (B) a 1:1 mix of peat moss and perlite (C) 100% peat moss, n = 3.

y = -0.022x² + 0.08x + 0.4, R² = 0.8

y = -0.018x² + 0.13x + 0.23, R² = 0.62

y = -0.006x² + 0.005x + 0.29, R² = 0.73

y = 0.025x² - 0.13x + 0.39, R² = 0.80

y = 0.014x² - 0.061x + 0.37, R² = 0.31

y = -0.028x² + 0.13x + 0.15, R² = 0.52
and 15,000 mg L\(^{-1}\) K-IBA concentrations with the non-wounded treatment having greater shoot dry masses at the 0 mg L\(^{-1}\) K-IBA concentration and the wounded treatment being greater at 15,000 mg L\(^{-1}\) K-IBA (Fig. 21A). This result both conflicts and confirms results found in Pezeshki and DeLaune (1994) in which shoot dry mass was significantly \((P \leq 0.05)\) affected by IBA treatment. This conflict could be explained by the fact that unlike the current research, Pezeshki and DeLaune (1994) did not include a wounding treatment or rooting substrate testing. Shoot dry masses were greatest in the perlite treatment perhaps due to the greater incidence of callus tissue formation in that treatment.

The results of the current research suggest a tradeoff between greater rooting percentages in a substrate with a greater aeration versus greater root quality in a substrate with a higher water-holding capacity. The K-IBA concentration did not affect rooting percentage as previously anticipated, but did affect cutting quality. Contrary to previous reports (Zhou, 2005), wounding the basal end of the cutting proved to be detrimental to both rooted cutting percentage and root quality.
CHAPTER IV
SECONDARY BRANCH ANGLES AND THEIR POTENTIAL TOPOPHYTIC EFFECTS ON VEGETATIVELY PROPAGATED BALDCYPRESS

Topophysis has been defined in a number of different ways. One of the first attempts to reconcile these conflicting definitions was conducted by Olesen (1978) in which the term topophysis was proposed as “the phenomenon that scions, buddings and cuttings for some time after grafting, budding or rooting maintain the branch-like growth habit they had as shoots on the ortet”. This definition was challenged by Dodd and Power (1988) which defined topophysis alternatively as “states of differentiation of meristems on the ortet that vary according to the hierarchical order of the meristem within a branched system.” The meaning of the term cyclophysis is much less controversial. The term is defined as “the process of maturation of the apical meristems” (Olesen, 1978). Power et al. (1988) discussed how the effects of topophysis and cyclophysis are often confounded in experiments which did not permit the two phenomena to be separated.

Much research has been conducted concerning topophysis and cyclophysis, but none could be found concerning the affects of either phenomena on baldcypress [Taxodium distichum (L.) L.C. Rich.]. Black (1973) discovered that in Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, cuttings branch position in the crown made a significant difference in rooting percentage of cuttings and that there was a varied response in timing for rooted cuttings to reorient from a plagiotropic (away from vertical) to an orthotropic (vertical) growth habit. Smith et al. (1956) emphasized topophysis in a vegetative propagation study of black cottonwood (Populus trichocarpa Torr. and Gray) and
Carolina poplar (*Populus canadensis* var. *eugenei* Simon-Louis). Cuttings were taken from three different positions on each donor plant. In each species there was a significant correlation between both cutting survival and growth and original orientation of the cuttings on the ortet. Average growth rates were calculated for both species. Cuttings from leaders averaged 30.5 cm of vertical growth, those taken from primary branches next to the leader averaged 20.3 cm of vertical growth and those taken from secondary branches grew 11.4 cm on average. Both of these studies were limited to measuring affects of topophysis on amount of growth. Perhaps the most closely-related study, due to its qualitative nature, was by Power et al. (1988). Topophysis was studied in coast redwood [*Sequoia sempervirens* (D. Don) S.F.L. Endlicher], cuttings. Cuttings taken from terminal branches predominantly grew in an orthotropic manner, while primary and secondary branches grew progressively more plagiotropic in habit. It was also noted that more mature donor plants rendered a cutting that was more inclined to exhibit the negative effects of topophysis.

The objective of the current research was to determine 1) whether or not topophysis or cyclophysis significantly affect the orientation and/or growth of ramets in baldcypress and 2) if branch angle divergence from the trunk of the ortet impacts the degree of topophysis observed on the ramets from rooted shoot tips.

**A. Materials and Methods**

*Documenting Field Level Variation in Branch Angles*

Branch angle measurements were taken from 4-year-old baldcypress seedlings grown in a research plot in College Station, Texas (lat. 30°37′645″N long. 96°22′319″W).
Twenty-three open-pollinated families were collected from provenances that ranged from central Mexico to Alabama as described by Denny (2007). Seedlings were planted in 18 east to west oriented rows containing two randomized replications of each of 14 families per row (504 total trees). Branch angle measurements were determined by measuring the angle with a large protractor between a randomly-selected first order secondary branch and the primary leader derived directly from the apical meristem. The protractor was oriented in such a way that a measurement of 90 degrees was perpendicular to the bole of the tree. Measurements were taken on branches which were perpendicular to the row (oriented north or south) in an effort to negate any affects of canopy shading by adjacent trees. Branch angles were measured at three different locations on the tree: a secondary branch near the terminus (but below any succulent immature growth) of the central leader (primary branch); a secondary branch near the middle of the canopy height; and a secondary branch within the bottom 30 cm of the canopy. Three similar measurements were taken on opposite sides of each tree. These six measurements were used as means for comparison of the general habit of the trees. Possible interactions between open-pollinated family, location in canopy (high, middle, low) and cardinal direction of branch (north or south) were analyzed using the General Linear Models Procedure in SAS and means were compared using least squared means comparisons (SAS 9.1 for Windows, Institute, Cary, N.C.) From this analysis the three families with the most acute branch angles on average and the three families with the most obtuse branch angles on average were determined. A tree in each family, clones MX6MC14, MX4MC17 and EP7DC29, were selected for collection of cuttings with obtuse branch angles, while clones MX2MC31, MX3MC13 and EP6DC14 were selected for acute branch angles.
Determining Topophytic Effects During Container Nursery Production

For each of the selected clones from the obtuse (MX6MC14, MX4MC17 and EP7DC29) and acute (MX2MC31, MX3MC13 and EP6DC14) branch angle families, 30 cuttings were taken from the upper portion of the canopy, near the terminal branch (more upright growth), and 30 cuttings were taken from the basal portion of the canopy (more horizontal growth). Cuttings of each of the six families were made on 18 June 2008 and kept overnight in a cooler at 8.7°C in plastic bags partially filled with water to ensure hydration. Cuttings were planted the following day, 19 June 2008. Cuttings were 11 to 14 cm long. The basal end of each cutting was submerged 5 cm deep into a 7,500 mg·L⁻¹ solution of K-IBA for five seconds. Cuttings were then placed in 10 cm x 36 cm x 51 cm black plastic nursery flats (Dyna-flat™, Kadon Corp., Dayton, Ohio) containing a 1:1 peat moss:perlite substrate. Cuttings were placed in a modified randomized completed block design in a greenhouse at the Texas A&M University Horticultural Gardens (College Station, Texas) under natural photoperiods. Reverse osmosis water was misted intermittently through high pressure mist nozzles for a period of 10 sec. on a 16 min. cycle during the daylight hours. Cuttings were allowed to root for approximately 12 weeks.

Successfully rooted cuttings were transplanted into 4.4L (#1) black plastic containers (Nursery Supplies Inc., Fairless Hills, Pa. and Orange, Ca.) on 11 September 2008. Care was taken during transplanting, and confirmed again on 15 September 2008 after the substrate settled from initial waterings, to ensure that the cuttings were oriented as vertically as possible in the containers after transplant from the propagation flats. Initial height (from soil surface to apex) and trunk diameter (5 cm above soil surface) of rooted
cuttings were measured. Rooted cuttings were maintained in a gravel-floored nursery at Texas A&M University Horticultural Gardens. Plants were grown for approximately 8 months. At cessation of the experiment, the angle that the trunk made with the substrate surface and the overall orientation of the plant was determined with a protractor, and ending height (from soil surface to apex) and trunk diameter (5 cm above soil surface) were measured and recorded. The protractor was oriented in such a way that a measurement of 90 degrees was perfectly vertical. The angle that the plant made with the substrate was then subtracted from 90 degrees. This calculation made it such that a value of 90 degrees would be perpendicular to vertical in both the field and container studies.

Possible interactions between clones propagated and cutting location in the canopy (high or low) and main effects of clones propagated and cutting location in the canopy (high or low) were analyzed using the General Linear Models Procedure in SAS and means were compared using least squared means comparisons (SAS 9.1 for Windows, Institute, Cary, N.C.). Possible correlations among growth parameters and angle deviation from vertical were analyzed using the PROC CORR function in SAS (SAS 9.1 for Windows, Institute, Cary, N.C.).

**B. Results and Discussion**

*Documenting Field Level Variation in Branch Angles*

The three-way interaction among families, canopy location, and cardinal direction of the branch and the two-way interactions among families and cardinal direction, and canopy location and cardinal direction were not significant ($P \leq 0.05$) for branch angles in the field (Table 6). However, the two-way interaction among open-pollinated families
and location in the canopy (low, middle, high) along with the main effect of cardinal
direction in which measurements were taken (north or south) were highly significant ($P \leq 0.0001$) (Table 6).

The significant ($P \leq 0.0001$) two-way interaction among open-pollinated families and
location in the canopy was principally due to a trend of branch angles to be progressively
more acute toward the apical portion of the canopy (Figure 22). On the whole, branch
angles toward the bottom of the canopy were more obtuse with branches in the middle of
the canopy growing at intermediate angles. While this general trend was apparent for
numerous families, including families MX1M, MX2M, MX3M, MX4M, MX5M, TX1D,
and EP9D, a few families such as MX6M, TX3D, TX6D, and EP7D exhibited minimal or
no differences related to canopy position on the branch angles between the primary
central leader and secondary branches (Figure 22). This result is in agreement with the
moderate heritability of branch angle reported in Velling (1988) and Haapanen et al.

There was a significant ($P \leq 0.0001$) main effect of cardinal direction of branches
(north or south). Branches which were oriented toward the south grew approximately
one degree more acute than did those oriented toward the north (Figure 23). Due to the
large sample size this difference proved to be highly significant statistically, however the
practical significance of such a slight difference in branch angle is questionable.
Determining Topophytic Effects During Container Nursery Production

Due to a lack of rooting percentages only five of the six clones initially propagated were used in the experiment and sample sizes were uneven for each of the remaining clones. Three clones (MX4MC17, MX6MC14, EP7DC29) represented families with the most obtuse branch angles observed in the field study while only two clones (MX2MC31, MX3MC13) represented those families with the most acute branch angles (Figure 22). Clone EP6DC14 was excluded from the experiment due to poor rooting percentages and a high mortality rate for those successfully rooted cuttings.

### Table 6. Levels of significance for factors in the analysis of variance for branch angles in field.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>****z</td>
</tr>
<tr>
<td>Canopy location</td>
<td>****</td>
</tr>
<tr>
<td>Family x canopy location</td>
<td>****</td>
</tr>
<tr>
<td>Cardinal direction</td>
<td>****</td>
</tr>
<tr>
<td>Family x cardinal direction</td>
<td>ns</td>
</tr>
<tr>
<td>Canopy location x cardinal direction</td>
<td>ns</td>
</tr>
<tr>
<td>Family x canopy location x cardinal direction</td>
<td>ns</td>
</tr>
</tbody>
</table>

z – significant at P ≤ 0.05, 0.01, 0.001, 0.0001 = *, **, ***, ****, respectively; ns = not significant at P ≤ 0.05.
Figure 22. Means (± standard errors) for family by canopy location effects on branch angles of field-grown trees across all families and positions in the canopy measured, n = 108.
Figure 23. Means (± standard errors) for main effect of cardinal direction on angles of branches oriented to the north or south in field-grown baldcypress, n = 108.
No significant interactions ($P \leq 0.05$) were found among the clones propagated and the location of the shoots for rooted cuttings (ramets) on the original tree (ortet) for stem angle from vertical, initial height at transplant from the mist bench, or final height or final trunk diameter (caliper) after nursery production (Table 7). The main effects of canopy location were also not significant ($P \leq 0.05$) for any of these parameters. However, the main effects of clones were highly significant ($P \leq 0.0001$) for all parameters measured, with the exception of initial caliper at transplant from the mist bench (Table 7).

A main effect of clone was significant ($P \leq 0.0001$) for the final stem angle of the ramets at the end of nursery production (Table 7). No significance ($P \geq 0.05$) was found for the cutting position in the canopy. These results confirm the non-quantified observations of clonal significance of Power et.al. (1988); however, they contradict other results of the same research in which both topophysis and cyclophysis significantly affected the stem angle of ramets. Results of the current research indicate that in baldcypress, genetic effects (clonal variation) is the most important factor influencing orthotropic and plagiotropic growth habits in the ramets. Topophytic and cyclophytic responses of ramets relative to location of origin of the cuttings to produce the ortets were not observed. These results however do not exclude the possibility of baldcypress displaying topophytic and/or cyclophytic responses when more mature ortets or different clones are tested. Ortets in the current study were only 4 years old at the time of the study. Power et al. (1988) reports the tendency of increased topophytic response with increasing maturity of the ortet in coast redwood [Sequoia sempervirens (D. Don) S.F.L. Endlicher].
Table 7.
Levels of significance factors in analysis of variance for stem angle and growth parameters in containers for rooted cuttings from clones with obtuse versus acute branch angles originating from upper versus basal portions of the ortet canopy.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Stem angle</th>
<th>Initial height</th>
<th>Final height</th>
<th>Initial caliper</th>
<th>Final caliper</th>
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<tr>
<td>Clone</td>
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<td>****</td>
<td>****</td>
<td>ns</td>
<td>****</td>
</tr>
<tr>
<td>Location</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Clone x location</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

z – significant at $P \leq 0.05, 0.01, 0.001, 0.0001 = *, **, ***, ****$, respectively; ns = not significant at $P \leq 0.05$.

The significance of the clonal effects on divergence from vertical growth suggests that it would be possible to select clones with increased chances of producing rooted cuttings with vertical leaders that would not require as much staking during production (Figure 24A). It also suggests, at least for the clones and age of materials tested, that the canopy location of obtaining branch tips for rooting cuttings would not alter staking requirements in the nursery. Interestingly, the initial angle of the branches (acute versus more obtuse, Figure 24) on the ortet in the field was not a good predictor of the ramets’ tendencies to grow vertically or not when rooted and grown in the nursery (Figure 24A), suggesting the factors controlling branch angle divergence from the main trunk and that of tendencies for topophytic responses are under different mechanisms of control.

Main effects of clones propagated were also significant ($P \leq 0.0001$) for both initial and final height of the ramets (Table 7). Since the initial heights were measured immediately after transplanting from the mist bench this measurement gives insight into
Figure 24. Means (± standard errors) for the angle at which the cuttings (ramets) grew in a container (A) and the interaction of clones by canopy location on branch angles observed in the field (B) for five families (ortets) tested for topophytic and cyclophytic response, n = 8-29 n = 72 respectively.
the amount of growth that occurred during the 12-week propagation period. Clone MX4MC17 grew to just over 9 cm (above the substrate) on average while clone EP7DC29 averaged approximately 4.1 cm per cutting (Figure 25A). This difference of approximately 5 cm is likely due to some unequal cutting sizes or the planting depth of the cutting (determined by the height of the uppermost adventitious root regenerated which was planted just below the substrate surface), but may also represent small differences in shoot elongation during rooting in the mist bench. Upon final height measurements, clone MX3MC13 had grown to approximately 40 cm while clone MX6MC14 had reached only 24 cm in height, a difference of over 33% (Figure 25B). The final height of the ramet was positively correlated with the initial height ($R^2 = 0.42$, $P \leq 0.0001$) indicating that the same clones which showed increased vigor in the mist bench were generally the most vigorous during the remainder of the experiment.

Finally, a significant ($P \leq 0.0001$) main effect of clone propagated was observed for the final trunk diameter of the ramets in the nursery. The initial trunk diameter measurements of the plants were not significant (Table 7). This non-significance in the mist bench was likely due to the care taken to make the cuttings as uniform as possible across clones and the relatively slow rate of girth growth in the mist bench when compared to height. Upon measurement of the final trunk diameter, clone MX3MC13 had grown to a trunk diameter of approximately 5.2 mm while clone MX2MC31 was approximately 2.8 mm (Figure 26). This difference of approximately 2.5 mm, though relatively small, is quite significant in light of the fact that the difference between the greatest and smallest initial trunk diameter was 0.37 mm. This confirms Denny’s (2007) previous reports of differential growth responses among these families in the nursery and
Figure 25. Means (± standard errors) for the main effects of clones on the initial (A) and final (B) cutting heights in containers, n = 8-29.
Figure 26. Means (± standard errors) for the main effects of clone on trunk diameter (caliper) of cuttings at the end of the experiment revealed significantly different ($P \leq 0.05$) growth rates, $n = 8-29$. 
field. As one might expect, final trunk diameter was also positively correlated with both final ($R^2 = 0.86, P \leq 0.0001$) and initial height ($R^2 = 0.39, P \leq 0.0001$) measurements, respectively.

In this study, branch angles of the ortet did not correspond with the stem angle that the ramets developed relative to the substrate surface during container production (Figure 24). The location in the canopy of the ortet from which cuttings were taken (high, middle, low) proved to be not significant ($P \leq 0.05$) for divergence of stems from vertical, whereas the clonal effect was highly significant ($P \leq 0.0001$) (Table 7), suggesting that clones can be selected which will present less problems with tophophytic responses during nursery production, and that branch angle of the ortet was not a good predictor of this response.
CHAPTER V
CONCLUSION

Baldcypress, *Taxodium distichum* (L.) Rich., var. *distichum* is a highly adaptable tree of significant ecological importance in the Southeastern U.S. (Arnold, 2008; Pezeshki and DeLaune, 1994). The species type's native range extends from southeast Texas to southern Illinois and Indiana in the west and from Florida to southern Delaware in the east (Wilhite and Toliver, 1990). With the addition of *Taxodium distichum* var. *imbricarium* (Pondcypress) and *Taxodium distichum* var. *mexicanum* (Montezuma cypress), the native range is extended into south Texas and Mexico (Denny and Arnold, 2007). The wide range of climates across this large geographic area has resulted in tolerances to an array of climatic conditions making it a suitable choice of plant material in other regions of the world as well.

While baldcypress exhibits durability and adaptability in landscapes, it does have limitations under some environmental conditions. It displays stress-induced foliar response symptoms of chlorosis, marginal necrosis, and/or premature senescence when exposed to high pH soils, prolonged drought or salt-laden foliar spray from irrigation (Arnold, 2008). In response to these stresses, initial screenings of baldcypress populations were made in an effort to identify more drought, salt and high pH soil tolerant selections (Denny, 2007).

The purpose of the experiments reported in this thesis were: 1) to examine the commercial viability of propagating the previously-selected clones of *Taxodium* from Denny’s (2007) work via shoot tip cuttings based on rooted cutting quantity and quality
measures 2) to determine which stages of development and hormone concentrations would yield optimum rooting percentages and highest quality adventitious root systems 3) to determine the combination of rooting substrate, wounding treatment and K-IBA concentration that yielded the optimal percentage of rooted cuttings and a root system of optimal quality 4) to determine whether or not topophysis significantly affects the orientation and/or growth of ramets in baldcypress and 5) to determine if branch angle divergence from the trunk of the ortet impacts the degree of topophysis observed on the ramets from rooted shoot tips.

In the initial study which included all of the clones selected from Denny’s (2007) work, Taxodium distichum cuttings generally rooted at a greater percentage and were higher in quality when collected as softwood cuttings from provenances in south Texas and Mexico and treated with either 7,500 or 15,000 mg·L⁻¹ K-IBA. Rooting quantity and quality from semi-hardwood cuttings were generally found to be great enough to warrant commercial propagation. Cuttings from the hardwood stage in this study rooted so poorly that the practice of taking hardwood cuttings of baldcypress is unadvisable at this time. A gradient was observed with cuttings from more southern and western provenances typically exhibiting greater rooted cutting quantity and quality than cuttings from more eastern and northern provenances. Though the clones from south Texas and Mexican provenances rooted at greater quantities and quality on the whole, the clone that performed best overall was TX8DC38, a central Texas clone. While cuttings treated with the medium (7,500 mg·L⁻¹) and high (15,000 mg·L⁻¹) concentrations of K-IBA rooted at similar quantities and quality, the cuttings treated with the high concentration often exhibited burning of the tissues that had contact with the K-IBA solution (basal necrosis).
This basal necrosis did not seem to affect the overall quality of the root system which was regenerated from above the necrotic tissues. The results suggest that the medium and high concentrations both assist in producing cuttings with optimal root systems.

A study in which the effects of three substrates (100% peat moss, 100% perlite, 1:1 peat moss:perlite), two wounding treatments (wounded, non-wounded) and four K-IBA concentrations (0, 5,000, 10,000 and 15,000 mg·L⁻¹) on softwood baldcypress cuttings were observed suggested a tradeoff between greater rooting percentages in a substrate with greater aeration (100% perlite) versus greater root quality (root number, length, and mass) in a substrate with a higher water-holding capacity (100% peat moss). The K-IBA concentration did not affect rooting percentage (unlike results found in chapter 1 of this thesis) but did affect cutting quality. Contrary to previous reports (Zhou, 2005), wounding the basal end of the cutting proved to be detrimental to both rooted cutting percentage and root quality.

The final study conducted was an effort to determine if a correlation exists between the initial branch angles on the ortets and the vertical orientation of the central leaders from cuttings of that ortet (topophytic effect) and whether topophysis would significantly affect the orientation and/or growth of ramets in baldcypress. Results indicated that branch angles of the ortet did not correlate with deviations of the central leader of rooted cuttings during container production. The location in the canopy of the ortet from which cuttings were taken (high, middle, low) proved to be not significant (P ≤ 0.05) for divergence of stems from vertical, whereas the individual genetic variation among clones was highly significant (P ≤ 0.0001), suggesting that clones can be selected which will
present less problems with topophytic responses during nursery production, however branch angle of the ortet was not a good predictor of this response.

Several clones from *Taxodium distichum var. distichum* (central Texas) or *Taxodium distichum var. mexicanum* (south Texas and Mexican provenances) could be rooted in sufficient quantity and produce rooted cuttings of sufficient quality to be commercially viable releases. These provenances could provide clones to the Texas (and perhaps outside of Texas) nursery and landscape industries which possess enhanced ability to tolerate high pH soils, withstand foliar salt exposure, and tolerate greater drought than the typical baldcypress trees derived from more eastern provenances. Future research should focus on 1) further optimizing the rooted cutting quantity and quality of select baldcypress clones by identifying intermediate rooting substrates which would enhance quantity without decreasing root quality, observing the performance of cuttings other than those found on the apical portion of a branch and continuing studies to enhance the process of rejuvenating ortets that have matured past the point of being easily vegetatively propagated by cuttings, 2) selecting clones that tend to grow more vertically in an effort to decrease production costs, 3) testing the adaptability of the candidate clones in other environments for potential use outside of Texas. Other issues such as the propensity of baldcypress to develop knees may also be under genetic control and be candidates for selection work.
LITERATURE CITED


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